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MAST CELL REGULATION OF DENDRITIC CELL MIGRATION IN VIVO

by

Dunia M. Jawdat

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at

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This thesis is dedicated to

My family

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ABSTRACT

Langerhans cells are immature dendritic cells in the skin their function is capture intruding antigen mature and migrate to the draining lymph node where they present the antigen to T lymphocytes and initiate an adaptive immune response. Mast cells are critical effector cells in innate immunity, however their role in acquired immune responses is less understood. Upon activation, mast cells produce a number of mediators known to aid in the maturation and subsequent migration of Langerhans cells to the draining lymph node. In this study, the ability of locally activated mast cells to induce Langerhans cell migration was examined. Mast cells in one ear pinna were activated using IgE and specific antigen or *S. aureus* derived peptidoglycan and saline was injected as a control in the other ear pinna. We observed a significant increase in draining lymph node total cellularity and accumulation of Langerin-positive cells in the node draining mast cell activated side compared to control. In *W/Wv* mast cell deficient mice these responses were absent. However, this response was restored after mast cell reconstitution. Histamine receptor blockade with cimetidine or pyrilamine suggested that histamine is an important mediator in inducing Langerhans cell migration in response to IgE/antigen-mediated mast cell activation but not in response to peptidoglycan. However, experiments using TNF knockout mice suggested an important role of this mediator in response to peptidoglycan. To further examine possible mechanisms of peptidoglycan induced Langerhans cell migration we examined potential molecules or receptors involved. Surprisingly, the Langerin-positive dendritic cell migration response to peptidoglycan was retained in TLR2 and TLR4 deficient mice but was abrogated in complement C3 deficient mice. These data demonstrate novel and distinct mechanisms whereby mast cells participate in both lymph node activation and dendritic cell responses and may provide new strategies for modifying host immune responses in allergic disease and in response to infection.

LIST OF ABBREVIATIONS AND SYMBOLS USED

APC	antigen presenting cell
AEC	aminoethyl carbazole
BMMC	bone marrow derived mast cells
BSA	bovine serum albumin
C	C chemokine
C3a	a degradation product of the third complement component
C3aR	complement 3a receptor
C5a	a degradation product of the fifth complement component
C5aR	complement 5a receptor
CBMC	cord blood mast cells
CC	CC chemokine
CCR	CC chemokines receptor
CD	cluster determinant
c-kit	stem cell factor receptor
CLP	cecal ligation and puncture
CR	complement receptor
CXCR	CXC chemokine receptor
DC	dendritic cells
DNA	deoxyribonucleic acid
dsRNA	double stranded ribonucleic acid
ELISA	enzyme-liked immunosorbent assay
FACS	fluorescence-activated cell sorter
FcεR	Fc receptor for IgE antibody
FCS	fetal calf serum
FITC	fluorescein isothiocyanate
GM-CSF	granulocyte-macrophage colony stimulating factor
GPI	glycosylphosphatidylinositol
GTP	guanosine triphosphate
h	hours

i.p.	intraperitoneal
i.v.	intravenous
ICAM-1	intercellular adhesion molecule
Ig	immunoglobulin
IL	interleukin
INF	interferon
IRAK	interleukin-1 receptor associated kinase
IRF3	interferon regulatory factor
kDa	kilodalton
LBP	LPS binding protein
LC	Langerhans cell
LN	lymph node
LPS	lipopolysaccharide
LT	leukotriene
MAP	mitogen activated protein
mCD14	membrane bound
MC^T	a human subset of mast cells that contain tryptase
MC^{TC}	a human subset of mast cells that contain tryptase and cymase and other proteases
MHC	major histocompatibility complex
MMP	matrix metalloproteinases
MRP-1	Multidrug resistant related protein 1
MyD88	myeloid differentiation factor 88
NFκB	nuclear factor kappa B
NGF	Nerve Growth Factor
NK	natural killer
NOD	nucleotide-binding oligomerization domain
PAF	platelet activating factor
Pam_3CSK_4	synthetic tripalmitoyl lipopeptide
PAMP	pathogen associated molecular patterns
PBS	phosphate buffer solution
PFA	paraformaldehyde

PG	prostaglandin
PGN	peptidoglycan
PGRPs	peptidoglycan recognition proteins
PRR	pattern recognition receptor
SCF	stem cell factor
SEM	standard error of the mean
TCR	T cell receptor
TGF-β	tumor growth factor
TIR	toll/IL-1 receptor domain
TLR	toll-like receptor
TNF	tumor necrosis factor
TNP	trinitrophenyl
TRIF	toll-interleukin-1 receptor interferon inducing factor
TRAF6	TNFR-associated factor 6
48/80	a polybasic compound used to activate mast cells
W/W^v	WBB6F1-KitW/KitWv (ckit deficient mice)

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CHAPTER 1

INTRODUCTION

1.1 The Immune System

Immunity is a state of protection against harmful pathogens. Our immune response to non-self antigens has long been divided into two types, an early innate and a later adaptive immune response. Microbial infections, in most situations, are detected and cleared within a short time by the innate immune response before the adaptive immune response is even activated. However, adaptive immunity is triggered and can be particularly important when the pathogen evades or escapes innate immunity. Each one of these responses is mediated by fundamental processes. In this chapter some of the main aspects of the immune system that are related to this thesis will be briefly discussed before a detailed description of the role of mast cells in the immune response is presented.

1.1.1 The Innate Immune Response

Innate immunity is the first line of defense used against intruders. It is primarily composed of four defensive barriers: (1) anatomic such as the skin and the mucous membranes; (2) physiologic such as the body temperature and low pH; (3) phagocytic and (4) inflammatory. The main features of the phagocytic and inflammatory arms of the innate immune response are the ability to rapidly recognize a danger signal or a pathogen, which may then trigger microbial internalization by phagocytic cells, such as neutrophils and macrophages, and the activation of killing mechanisms. This will also trigger the release of pro-inflammatory cytokines and chemokines that activate and recruit other immune

effector cells which in turn, will translate the danger signal to the adaptive immune system, and orchestrate its development.

The key players of the innate immune system can be divided into immune effector cells and humoral components. Key immune effector cells include cells with phagocytic and pro-inflammatory features such as neutrophils which are usually the first cells arriving at a site of acute inflammation. They are phagocytic cells that are usually able to kill ingested microorganisms either though their antimicrobial granule contents or though the production of reactive oxygen or nitrogen species. Natural killer (NK) cells are also one of the earliest cells involved in the response against certain viral and intracellular bacterial infections. Their activity is stimulated by IFN-α and IFN-β which can be primairly produced by many cell types including dendritic cells (DCs) in the blood (Ferbas et al., 1994). Once NK cells are activated they can kill infected cells and release a large amount of IFN-γ which aids in the differentiation of cytotoxic T lymphocytes and the induction of chemokine responses. Humoral innate immunity is mediated by proteins such as bacteriolytic enzymes, complement components, mannose binding proteins and soluble CD14 (sCD14). Critical innate immune effector molecules when present on the surface of cells are called pattern recognition receptors (PRRs). Toll-like receptors (TLRs), membrane bound (mCD14), Fc receptors, mannose receptors and scavenger receptors are important examples of such receptor systems.

PRRs can recognize specific structures shared between pathogens and are not normally found in normal tissues. These structures are now identified as

pathogen-associated molecular patterns (PAMPs). These receptors often function following the formation of a signalling complex which consists of several molecules that interact and bind to the main ligand-binding receptor. It is also recognized that some of the innate immune receptors can recognize multiple ligands, each of the PRRs can initiate a signaling cascade independently, which then may interact with other signals from the organism or from other aspects of the host defense mechanisms to determine the eventual cellular response.

1.1.1.1 Toll-Like Receptors

Toll receptors were first discovered in *Drosophila* (fruit fly) where they were shown to be essential for antifungal immunity (Lemaitre et al., 1996). They were subsequently shown to have a homologous family in mammals identified as TLR (Medzhitov et al., 1997). TLRs are type I transmembrane proteins identified as a family of PRRs (Anderson, 2000). They are one of the most effective receptors in innate immunity that recognize a wide spectrum of conserved PAMPs including lipopolysaccharide (LPS), peptidoglycan (PGN), lipoproteins, lipopeptides and nucleic acids including DNA and double stranded (dsRNA) (Medzhitov and Janeway, 2000).

TLRs are characterized by an extracellular domain with leucine-rich repeats as well as a cytoplasmic portion which is similar to the cytoplasmic portion of the IL-1R family, identified as the Toll/IL-1 receptor (TIR) domain. TLRs are mostly expressed on immune cells. However, other cells may also

express TLRs including endothelial cells, cardiac myocytes and intestinal epithelial cells.

Thus far eleven members of the TLR family (TLR1-11) have been identified (Akira et al., 2001; Zhang et al., 2004). Some of these receptors are expressed on the surface of cells such as TLR1, TLR2 and TLR4 whereas TLR3, TLR7, TLR8 and TLR9 are expressed in intracellular compartments such as endosomes. It has been suggested that some microbes are captured by phagocytosis then internalized and degraded, thus exposing their nucleic acid and other constituents in the lysosomal compartment in which TLRs may be expressed or recruited from other organelles. It is not well understood how surface TLRs function, but interestingly it has been shown that TLR2 was internalized and recruited following zymosan exposure (Underhill et al., 1999). This suggests that the main site of recognition might be within intracellular endosomal compartments.

Each of these TLRs responds differently to stimuli with a distinct specificity for ligands [see table 1.1 adapted from (Marshall et al., 2003)] that is also dependent on co-receptor function. Once microbial recognition occurs it facilitates the dimerization of the receptors. Most TLRs homodimerize except TLR2 which is recognized to heterodimerize with TLR1, TLR6 or TLR10. This dimerization will then lead to a distinct signalling pathway (Ozinsky et al., 2000b). Most TLRs function through the same signalling molecules including MyD88, IL-1R-associated kinase (IRAK), TNFR-associated factor 6 (TRAF6) and mitogen activated protein (MAP) kinase leading to the activation and

Tabel 1.1 Toll-like receptors and some of their activators, adapted from Marshall *et al.* 2004.

Toll-like receptors	Toll-like receptor ligans
TLR1	Lipopeptides (Pam_3Cys)
TLR2	Gram-positive bacteria, PGN, zymosan, LTA, Lipopeptides (Pam_2Cys/Pam_3Cys), Lipoarabinomannan, LPS (*P. gingivalis* and *L. interrogans*), GPI-anchors from *Trypansoma cruzi*, lipoarabinomannan from *M. tuberculosis*, porins from *Neisseria meningitides*.HSP 60, bacteria fimbriae
TLR3	PolyI:C (dsRNA)
TLR4	LPS, lipid A, RSV protein F, HSP60, fibrinogen, *M. tuberculosis*
TLR5	Flagellin
TLR6	PGN, zymosan, Lipopeptides (Pam_2Cys)
TLR7	Antiviral compound R-848, S-27609, Imiquimod, ssRNA
TLR8	Antiviral compound R-848, ssRNA
TLR9	Bacterial CpG DNA, CpG oligonucleotides
TLR10	Inhaled allergens?
TLR11	Uropathogenic bacteria

translocation of nuclear factor (NF-κB) to the nucleus. This pathway is usually referred to as the MyD88-dependent pathway and is required for the production of inflammatory cytokines (see Figure 1.1). MyD88-independent signalling pathways have also been identified that use toll-interleukin-1 receptor interferon inducing factor (TRIF) or other alternate adaptor molecules and are employed by TLR2, TLR3, TLR4, TLR7 and TLR8 in response to certain ligands (Sugawara et al., 2003; Takeda and Akira, 2004). The latter pathway induces the activation of the transcription factor interferon regulatory factor 3 (IRF-3), and can also induce NF-κB activation, but with delayed kinetics (Kawai et al., 1999). This pathway has been suggested to play a greater role in the development of tolerance, induction of apoptosis and initiation of IFN-β responses critical for anti-viral immunity, however more studies are required to further explore these issues.

TLRs are key elements in the initiation of both innate and adaptive immune responses against pathogens. TLR activation initiates innate immunity by the production of pro-inflammatory mediators such TNF and IL-6. Activation of TLRs also induces phagocytosis and microbial killing mechanisms via the production of reactive oxygen and nitrogen species (Brightbill et al., 1999). TLRs are also important initiators of the adaptive immune response through the activation of antigen presenting cells (APCs). They induce the production of pro-inflammatory cytokines such as TNF and IL-12 and up-regulate the co-stimulatory molecules CD80 and CD86. TLR activation has also been linked to phagocytosis. In the absence of TLR2, TLR4 or MyD88 phagocytosis of several pathogens including *S. aureus* was impaired (Blander and Medzhitov, 2004). In

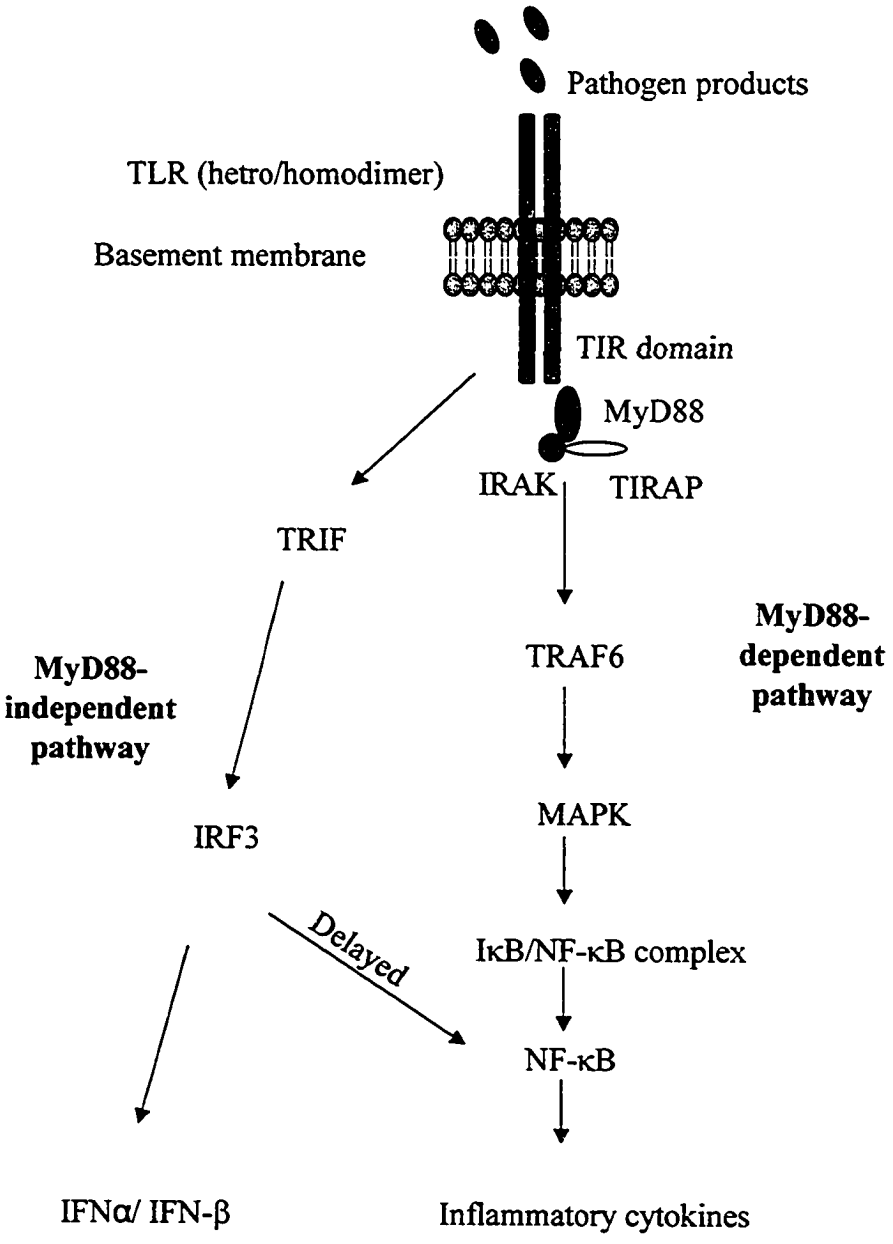

Figure 1.1 A representative signaling cascade for TLR that may differ according to the TLR being activated.

addition, TLR interaction with LPS has been shown to down regulate the expression of CCR5 and up regulate the expression of CCR7 chemokine receptors on DCs. These receptors are required for DC migration to the lymph node (LN) to initiate an adaptive immune response (Rescigno et al., 2000). TLR-mediated activation of DCs often leads to the production of IL-12 which tends to direct T-cell differentiation toward a Th1-type of response. However, it was recently suggested that, depending on the stimuli, some TLRs can selectively induce T-cell differentiation to either Th1 or Th2 immune responses via effects on the cytokine microenvironments and APC (Dabbagh et al., 2002).

TLRs are known to have an important role in recognizing pathogens and inducing the subsequent release of essential mediators, however the specific role of some TLRs in host defense is still not clear. The effect of deletion of some TLRs on microbial infection is not always severe (Hoshino et al., 1999; Qureshi et al., 1999). However, it has been suggested that this reflects redundancy of the system, in which a response may be mediated by an alternate TLR or a completely different receptor pathway.

It is important to note that TLRs usually function as a complex of molecules which consist of TLR homodimers or heterodimers in addition to co-receptors (such as CD14, Dectin-1) and intracellular (such as MyD88) and extracellular adaptor molecules (such as MD-2). Some specific TLRs have also been recognized to function in combination with other important immune receptors such as Fc receptors and complement component receptors. These interactions maybe critical since they can modify the nature of the immune

response that develops to a given organism, particularly in the context of an existing antibody response (Underhill, 2003).

1.1.1.2 Toll-Like Receptor 2

TLR2 has been shown to recognize a wide variety of microbial components (see table 1.1) including PGN from Gram-positive bacteria (*S. aureus*). It is expressed on monocytes, macrophages, neutrophils, dendritic cells and mast cells. The important role of TLR2 was recognized mostly from studies using TLR2 deficient mice which were shown to be more susceptible to Gram-positive bacteria (*S. aureus*, *Streptococcus pneumoniae* and *M. tuberculosis*) than wild type mice (Echchannaoui et al., 2002; Koedel et al., 2003; Reiling et al., 2002; Takeuchi et al., 2000). LPS, a component of Gram-negative bacteria is usually recognized by TLR4; however some LPS structural variables have been shown to be recognized by TLR2, such as LPS from *P. gingivalis*. Interestingly, TLR2 responses to such LPS requires the presence of CD14 (Werts et al., 2001).

The reason why TLR2 can recognize such a wide spectrum of pathogen components compared to most other TLRs might be explained by two proposals (Takeda and Akira, 2005). The first is their ability to form heterodimers with other TLRs, which as mentioned earlier include TLR1, TLR6 (Ozinsky et al., 2000a) and TLR10 (Hasan et al., 2005). The second proposal is their ability to cooperate with a number of other receptors and co-receptors. For example, TLR2 recognizes fungal components such as yeast zymosan in collaboration with dectin-1 (Gantner et al., 2003).

1.1.1.3 Toll-Like Receptor 4

TLR4 was the first mammalian TLR discovered and is also the best characterized member of the TLR family. Its most significant feature is the recognition of the Gram-negative bacterial cell wall component LPS (the main inducer of septic shock). Studies using knockout mice confirmed the essential role of TLR4 in LPS induced responses (Hoshino et al., 1999). TLR4 can also recognize other microbial products (see table 1.1), however, most studies of TLR4 have been performed with LPS. It has been shown that TLR4 mediated responses to LPS on DCs induce the secretion of IL-12, CXCL10/IP-10 and IFN-β (Re and Strominger, 2001). TLR4 function as a receptor requires the presence of other molecules, including CD14 and MD-2. MD-2 is a glycoprotein that appears to function as a secretory protein. MD-2 lacks a transmembrane domain, but still remains associated with cells through the interaction with TLR4 extracellular domain. MD-2 has a high affinity for LPS and has been shown to be required for TLR4 mediated responses (Nagai et al., 2002). In a recent study it was shown that in the presence of CD14, LPS can come in close proximity with TLR4 and MD-2 (da Silva Correia et al., 2001). It is important to know that even very small amounts of LPS can activate TLR4 on some cell types, thus it is important to be extremely cautious, since any contamination of ligands with LPS might give wrong information.

1.1.1.4 CD14

CD14 is a cell surface glycosylphosphatidylinositol (GPI)-liked 55 kDa glycoprotein, which contains 8-10 leucine-rich glycoprotein repeats. CD14 lacks a transmembrane or cytoplasmic domain so it may not transduce signals independently. This mCD14 is highly expressed by monocytes and macrophages. CD14 is mostly studied in the context of LPS recognition as it has a high-affinity for LPS, but it also has the ability to recognize other microbial substances including PGN, lipotechoic acid and lipopeptides (Dziarski et al., 1998) by acting as a co-receptor for both TLR4 and TLR2. Several studies using CD14 antibodies and CD14-/- mice have demonstrated the important role of CD14 for LPS responses (Haziot et al., 1995) with CD14-/- mice shown to be >100-1000 times less sensitive to LPS. It has been widely suggested that once LPS is introduced in the host it will be captured by the LPS binding protein (LBP) which will in turn transfer single LPS molecules from aggregates onto the membrane bound CD14. Consequently, CD14 will form a receptor complex with both TLR4 and MD2 molecules. Although LBP enhances CD14 responses to LPS it does not do so for PGN (Gupta et al., 1996b). In addition, CD14 may not be as important for PGN mediated responses as for LPS, since CD14-/- mice are only 5-10 times less sensitive to PGN than wild type controls. Interestingly, PGN unresponsive CD14 negative cells become responsive following transfection of these cells with CD14 (Gupta et al., 1996b). In addition, CD14 antibodies inhibit binding and activation of CD14 positive cells by PGN, demonstrating a role for this molecule in some PGN responses (Weidemann et al., 1994).

CD14 is also exists in a soluble form (sCD14), with the same sequence but no GPI anchor; it is present in serum and milk at a concentration of 2-4μg/ml (Grunwald et al., 1992). sCD14 has been shown to bind to LPS, soluble PGN, muramyl dipeptide, but not PGN pentapeptide. sCD14 has been shown to form complexes with LPS and activate mCD14 negative cells such as epithelial cells and fibroblasts. It has also been shown to form complexes with PGN, but this interaction has not been shown to enhance responses of mCD14 negative cells, although it enhances activation of mCD14 positive cells by PGN (Dziarski et al., 2000).

1.1.1.5 Dectin-1

Dectin-1 is a C-type lectin, identified as a major phagocytic receptor that has the ability to recognize the yeast product zymosan (Brown and Gordon, 2001). As mentioned earlier dectin-1 has recently been found to cooperate with TLR2 to mediate an immune response to zymosan. In this study, it was shown that zymosan-mediated TLR2 activation induces the production of TNF, whereas zymosan-mediated dectin-1 activation induces phagocytosis and the production of reactive oxygen species. However, only in the presence of both receptors will IL-12 be produced by zymosan activated cells (Gantner et al., 2003). Dectin-1 is also important in responses to other fungal pathogens such as *Candida albican* (Brown and Gordon, 2001).

1.1.1.6 Nucleotide-Binding Oligomerization Domain

The nucleotide-binding oligomerization domain (NOD) is a family of cytoplasmic proteins that function as intracellular recognition proteins independent of TLRs. Two members have been identified from this family NOD1 and NOD2 each with a nucleotide-binding site and a leucine-rich repeat (Inohara et al., 2002). Both NOD1 and NOD2 have the ability to recognize PGN, however each one recognizes a distinct structure within PGN. NOD1 recognizes "glutamyl-mesodiaminopimelic acid" and is widely expressed by several cells and tissues, whereas, NOD2 recognizes the low molecular weight PGN fragment "muramyl dipeptide" and its expression is thus far restricted to monocytes (Inohara et al., 2002; Ogura et al., 2001). Interestingly, NOD2 activation has been shown to produce inflammatory signals that amplify the TLR signalling response (Nau et al., 2002). NOD2 mutations have been associated with Crohn's disease, but its role in the disease process is still unknown. However, one interesting observation is that in the presence of NOD2 mutations TLR2–mediated activation of NF-κB is enhanced (Watanabe et al., 2004).

1.1.1.7 Ppetidoglycan Recognition Proteins

Peptidoglycan recognition proteins (PGRPs) are another important family of pattern recognition molecules of innate immunity. Thus far four human PGRPs have been identified PGRP-S, PGRP-L, PGRP-Iα and PGRP-Iβ. Originally they were thought to only bind PGN and Gram-positive bacteria. However, PGRPs have recently been shown to bind Gram-negative bacteria and some fungi which

broadens their spectrum of recognition (Dziarski, 2004). Although their role in recognizing and binding PGN has been confirmed, the exact role of these PGRPs in innate immunity and their interaction with other receptor systems such as TLRs is not known.

1.1.1.8 The Complement System

The complement system is the major effector arm of the humoral components of the innate immune system. It consists of at least 30 soluble or membrane bound components which are proteins or glycoproteins mostly synthesized by the liver. They usually circulate in the serum in an inactive form, activated with an enzymatic cascade. The complement system can be activated by three pathways: (1) the classical pathway which is mainly activated by the interaction of C1q with an immune complex and thereby requires some pre-existing antibodies. Non-immune molecules (such as small polysaccharides, C reactive proteins, serum amyloid A and bacterial, viral and fungal membrane components) have also been shown to activate this pathway early in innate immune responses; (2) the alternative pathway which is initiated by the interaction of C3 and carbohydrate particle such as yeast cell wall and bacterial cell wall; (3) the lectin pathway which is activated by mannose-binding lectin binding to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms.

Complement components and their activated products can bind to specific receptors present on a variety of cells inducing a wide range of functions. Most of

the biological activities of the complement system significantly affect both the innate and adaptive immune response. The most critical functions include: (1) protection against microbial infection either though the formation of the membrane attack complex mediating cell lysis, or through opsonization of pathogens promoting phagocytosis, or through the activation of immune effector cells; (2) bridging the innate immune response to the adaptive immune response through the effect of CR2 on B lymphocytes; and (3) immune clearance by disposing of immune complexes as well as apoptotic and toxic cellular debris reviewed in (Gasque, 2004) .

As mentioned earlier, the complement system can be activated by three different pathways. It is important to note which components are involved in each pathway. Some of the components are shared, whereas some components are specific to a particular pathway. Experimental inhibition of specific components can be particularly useful to identify the exact pathway involved in a specific response. Specific complement deficient mice, such C3 or C4 knockouts are also extremely valuable in the identification of certain pathways. Since C3 is involved in all the three pathways, using C3 knockout mice can provide evidence about the general involvement of the complement system in a specific response, which could be followed-up by a more specific knockout or blocking antibody approach.

1.1.2 The Adaptive Immune Response

Adaptive immunity is the second line of defense which recognizes specific antigens on pathogens. This system primarily involves the function of

lymphocytes and APCs. The main features of the adaptive immune response are the utilization of gene rearrangements which mediate the development of a large diversity of antigen specific cells and the ability to maintain memory cells. The process of generation of an adaptive immune response requires functional presentation of antigens most often by DCs leading to the activation and proliferation of specific T cells and B cells.

1.1.2.1 Dendritic Cells

DCs are bone marrow derived leukocytes that constantly enter and migrate through most tissues in the body. They are characterized by a dendritic morphology with constitutive expression of MHC class I and II molecules. They are found in lymphoid tissues and at sites that come in close contact with the external environment such as the skin, the intestine and the respiratory tract where they act as sentinels of the immune system. As they recognize a danger signal in the local environment they translate that signal by stimulating antigen-specific T lymphocytes to initiate an appropriate immune response. This will be discussed in more detail later. In the blood, DC precursors can directly encounter pathogens and secrete a large amount of some cytokines, specifically IFN-α, which in turn can activate other immune cells including macrophages, eosinophils and NK cells. The term "dendritic cell" has been widely used to represent all the members of this type of cell including the Langerhans cells (LCs) in the skin, the interdigitating dendritic cells (IDCs) in the T cell area of the lymphoid tissues and

the veiled cells in the lymph. It is important to note that these types of cells share some general features, however they differ in location and function.

DCs were first discovered in 1868 by Langerhans. It was the LCs in the skin that were first noted and due to their dendrites they were dismissed as cells of neuronal origin (Langerhans, 1868). Almost a century later LCs started to be of large interest and received widespread attention as accessory cells with essential roles in mediating immune responses. In 1961 Birbeck *et al.* discovered a unique marker for LC, now known as the Birbeck granules (Birbeck MS, 1961). Subsequently these organelles were found in irregular cells in the LN (Kondo, 1969; Vernon et al., 1973). At this time LCs were found to have a migratory ability (Hashimoto and Tarnowski, 1968) and dendritic shaped cells (IDCs) were demonstrated in the lymphoid tissue. In 1973 Steinman and Cohn identified DCs in mouse spleen with a distinct role as APCs (Steinman and Cohn, 1973). In 1976 LCs were shown to migrate from the skin through the lymphatics into the draining LNs and thus, highly suggested to transport antigens from the periphery into the lymphoid tissues (Silberberg et al., 1976). Rowden *et al.* then showed that DCs of all types express high levels of MHC class II which place them in the group of professional APCs (Rowden et al., 1977). Shortly after that, they were widely accepted as potent T cell activators and fundamental in the initiation of primary immune responses (Klinkert et al., 1980; Steinman and Witmer, 1978).

1.1.2.2 Dendritic Cell Heterogeneity

Four stages have been identified for DC development, the bone marrow progenitors, the blood, lymph and lymphoid organs precursors, the tissue resident immature DCs, and finally the secondary lymphoid tissue DCs. The heterogeneity of DCs differs slightly among species. In mice, two subsets were first identified, $CD8^+$ and $CD8^-$DCs which were thought to originate from lymphoid and myeloid precursors respectively. However, more recently grouping DCs into different subsets turned out to be much more complex than this. Indeed in the lymphoid tissues alone, five subsets of mature DCs have been identified thus far (see table 1.2). In addition to the LCs in the epidermis, the dermal DCs in the dermis and the $CD11c^{lo}$ plasmacytoid DCs which are present in most lymphoid tissues. Interestingly, it is now clear that both lymphoid and myeloid precursors can give rise to all DC subsets but with some bias in the subset balance (Manz et al., 2001; Traver et al., 2000; Wu et al., 2001). Although the terms lymphoid or myeloid DCs are still commonly used, they no longer indicate their origin. These subsets share some similarities such as the expression of CD11c, moderate to high MHC class II, the costimulatory molecules CD80, CD86 and CD40. However; they differ in phenotype, with the most significant markers used to segregate these subsets being CD4, CD8, CD11b and CD205 (Shortman and Liu, 2002). These subsets also differ in location and function (see Table 1.2). Some of the functional differences include the ability to determine the fate of the activated T cells. Most studies in the past suggested that $CD8^+$ DCs induce a Th1 response while $CD8^-$ DCs induce a Th2 response. However, it is now recognized that $CD8^-$ DCs can

Table 1.2 The characteristics of mouse dendritic cell subtypes.

Characteristics	**Mouse DC subtypes**				
Surface marker	MHC class II, CD11c, CD80, CD86, CD40, $CD4^-$, $CD8^+$, $DEC\text{-}205^{hi}$	MHC class II, CD11c, CD80, CD86, CD40 $CD4^+$, $CD8^-$, $CD11b^+$	MHC class II, CD11c, CD80, CD86, CD40 $CD4^-$, $CD8^-$, $CD11b^+$	MHC class II, CD11c, CD80, CD86, CD40 $CD4^-$, $CD8^-$, $CD11b^+$, $DEC\text{-}205^{mod}$ (might be the mature form of the interstitial DCs)	MHC class II^{hi}, CD11c, $CD80^{hi}$, $CD86^{hi}$, $CD40^{hi}$ $Langerine^{hi}$ $DEC\text{-}205^{hi}$ $CD8^{lo}$ (might be the emigrated LC from the skin)
Location	In the T cell rich areas of PALS in the spleen and LN Dominate in the thymus	In the marginal zone bridging channels of the spleen	In the marginal zone bridging channels of the spleen	In all LNs	In the skin derived LN

also induce a Th1 response and that this balance can change depending on the stimuli and microenvironment (Pulendran et al., 2001).

In the human, only a few studies deal with DC subsets due to the difficulty in accessing freshly isolated human tissues. The most easily available source is the blood which mostly contains immature DCs or DC precursors which have been subdivided into three subsets: [see Figure 1.2 adapted from (Banchereau et al., 2000b)]: (1) $CD14^+$, $CD11c^+$ and $CD1a^-$ monocytes, which originate from myeloid progenitors and under the influence of GM-CSF and IL-4 differentiate into immature DCs; (2) $CD14^-$, $CD11c^+$ and $CD1a^+$ cells are also from myeloid origin and differentiate into LC in response to GM-CSF, IL-4 and TGFβ. Both of these precursors can differentiate into macrophages in response to M-CSF; and (3) $CD14^-$, $CD11c^-$, $CD1a^-$ and IL-$3R\alpha^+$ cells probably originate of lymphoid progenitors and are also called IFN-α producing cells or plasmacytoid DCs. They differentiate into immature DCs in response to IL-3 (Banchereau et al., 2000a). All of the immature DCs differentiate into mature DCs in response to danger signals such as pro-inflammatory cytokines or pathogen products. Follicular DCs are another type of DC. They are present in the lymphoid organs especially, in the B cell area, but their origin is still controversial. Human DCs can also be divided based on their location into LC in the skin, interstitial DCs in the dermis, interdigitating DCs in T-cell zone area of the lymphoid tissues, germinal center DCs, splenic marginal DCs, thymic DCs, liver DCs and blood DCs. Theses cells can also be divided based on the outcome of the immune response they have

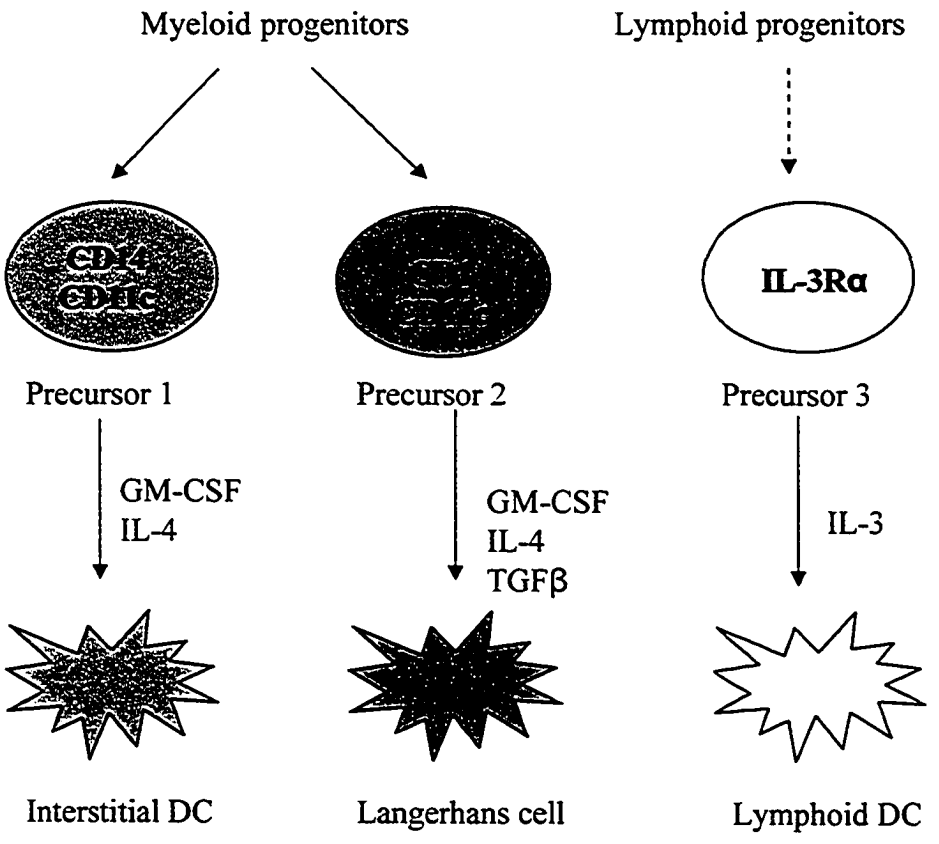

Figure 1.2 Dendritic cell precursors in human blood

mediated if it is toward a Th1 or Th2 response or whether they induce immunity or tolerance.

1.1.2.3 Langerhans Cells

LCs are important sentinels in the skin that can initiate systemic immune responses. They were the first DCs to be described (Langerhans, 1868). They are present in the epidermis where they form a continuous network of immature DCs that are specialized in capturing intruding microbes or any environmental antigen. Once they internalize and process the intruder they begin to mature and migrate to the draining LN. In the LN they present antigen fragments complexed to MHC class II molecules which allow specific selection for antigen-specific T lymphocytes from circulating T cells inducing their proliferation and differentiation. Once T cells are activated they proliferate and can migrate to the infected site under the influence of appropriate chemokine and cytokine signals (Cumberbatch et al., 2000a) (see Figure 1.3).

LCs are characterized by their dendritic morphology. They have a lobulated nucleus and clear cytoplasm containing microtubules, microfilaments and lysosomes. One of the main morphological characterization of LCs is the presence of Birbeck granules (Birbeck MS, 1961) which are rod-shaped organelles with a center zipper-like striation observed only by electron microscopy, occasionally having a vesicular dilatation at one end forming a tennis-racket shape. LCs share some similarities with other DCs, but also express some specific markers that differentiate them from other DCs. LCs express MHC

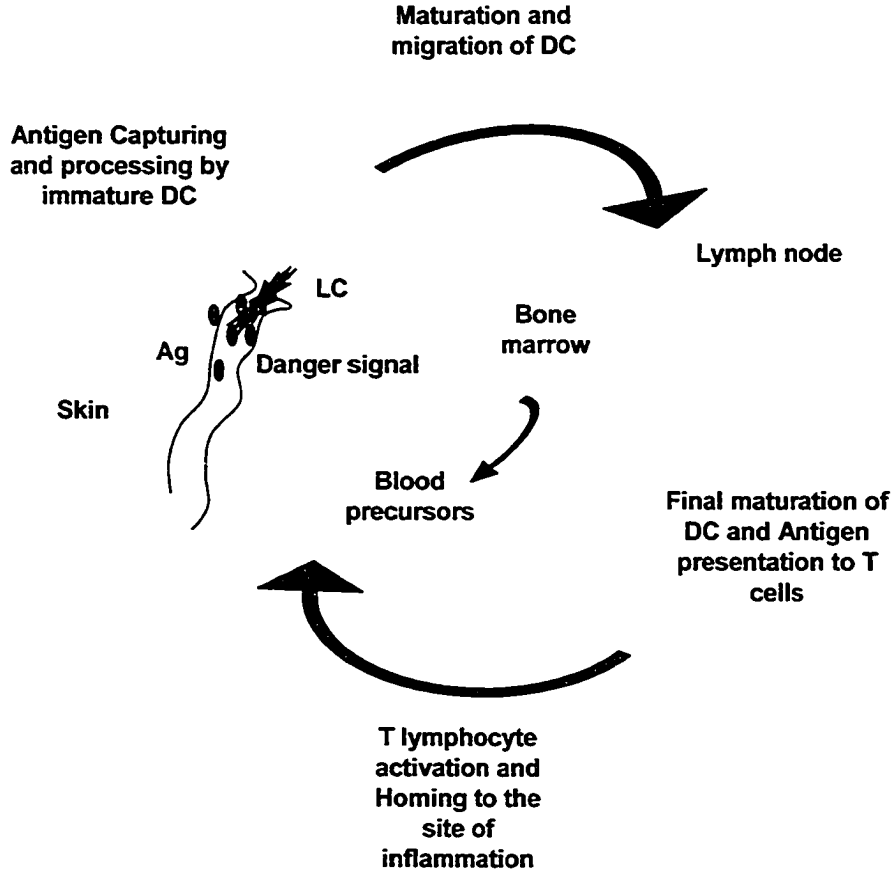

Figure 1.3 The life cycle of Langerhans cells

class I and II (Klareskog et al., 1977; Rowden et al., 1977), CD1a (Fithian et al., 1981; Murphy et al., 1981), the costimulatory molecules CD80, CD86, adhesion molecules such as ICAM-1, ICAM-3 and LFA-3 (Teunissen et al., 1990), which are important for naïve T cell interactions, complement receptors such as CR3 and Fc receptors such as FcγRII (Stingl et al., 1977). The high affinity receptor FcεRI for IgE antibodies is absent on mouse LCs, but is expressed on human LCs. LCs also express several C-type lectins which might be important for antigen uptake and internalization such as the macrophage mannose receptor (MMR), DEC-205/CD205, TLRs such as TLR1, TLR2, TLR3, TLR5, TLR6, TLR8 and TLR10 (Kadowaki et al., 2001), Dectin-1 and Dectin-2. DC-SIGN is absent on LC but has been shown to be up-regulated following their activation and to play an important role in T cell interaction. Langerin is a type II C-type lectin and its formation is associated with the BG. This molecule is very important as a LC marker because it is only expressed by LCs. A small percentage of IDC have been shown to express Langerin, since they are mainly present in LNs draining the skin it is thought that they represent the newly arrived LCs from the skin.

1.1.2.4 Antigen Capture

One of the fundamental features of immature DCs is their efficient role in antigen capture. DCs have the ability to capture antigen in multiple ways including (1) phagocytosis, which is the ingestion of particles (e.g. apoptotic and necrotic fragments, or whole microorganisms such as viruses and bacteria) through the expansion of the plasma membrane around the particulate material

forming a large vesicles called a phagosome which will eventually fuse with the lysosome; (2) endocytosis which is frequently receptor-mediated in which extracellular antigen is internalized after the binding to specific receptors on the cell surface such as the C-type lectin receptors including DEC-205, Langerin, DC-SIGN, mannose receptors and Fc receptors; and (3) a modified type of endocytosis is known as macropinocytosis in which extracellular fluid and solutes are internalized through the formation of large pinocytic vesicles (Banchereau et al., 2000b).

1.1.2.5 Antigen Processing and Presentation

Antigen processing is a way of degrading the antigen taken up by DCs into small peptides that can associate with MHC complexes to be recognized by T lymphocytes. **Exogenous antigens** are found outside the host cell and enter the cell by phagocytosis or endocytosis. The antigen is subsequently degraded in endosomes and transported to MHC class II-rich compartments (MIIC). MHC class II molecules are associated with an invariant chain which is degraded by cathepsin S after DC maturation which in immature DCs is inhibited by cystatin C. A non-classical MHC class II molecule HLA-DM plays an important role in removing the invariant chain peptide. This allows the antigenic peptides to be loaded onto the MHC class II peptide-binding cleft and subsequently transported to the plasma membrane to be presented. This peptide-MHC class II complex can then be recognized by $CD4^+$ T cells. MHC class II molecules usually have a short half life and are rapidly internalized in immature DCs. However, upon DC

maturation, there is a burst in synthesis of MHC class II that is transported to the membrane in association with antigenic peptides where it remains stable for several days (reviewed in (Banchereau et al., 2000b)).

Endogenous antigens are produced intracellularly, examples include, replicating viruses or selected proteins synthesized by cancerous cells. These proteins are degraded within the cytoplasm by proteosomes and transported into the endoplamic reticulum (ER) in an ATP-dependent manner by the transporter protein TAP (transporter associated with antigen processing). In the ER the degraded peptides associate with MHC class I and β_2-microglobulin exit the ER and move to the cell surface to be presented and recognized by $CD8^+$ cytotoxic T cells.

In addition to this classical pathway, DCs also have the ability to load MHC class I molecules with exogenous antigens. Several routes have been described, a TAP-independent route (Pfeifer et al., 1993), a phagosome to cytosol pathway which is TAP–dependent and an immune complex captured pathway (Kovacsovics-Bankowski et al., 1993; Kovacsovics-Bankowski and Rock, 1995).

1.1.2.6 Non-Peptide Antigen Presentation

MHC class I and II present antigenic peptides whereas antigenic lipid and glycolipid molecules derived from pathogenic bacteria are presented by members of the CD1 family. The CD1 family have been identified as a non-classical antigen-presenting molecule which share structural similarities with MHC class I molecules. Both endogenous and exogenous lipid antigens can be processed by

this pathway. Recent studies show that CD1 molecules process their antigen in a TAP-independent manner which suggest the existence of a third pathway for processing antigens (Burdin and Kronenberg, 1999; Porcelli and Modlin, 1999). In humans, four CD1 molecules have been identified (CD1a-d) whereas in mice only one (CD1d) has been described. Most of these molecules present antigens to a large repertoire of T cells except CD1d which activates a more restricted set of T cells as well as NK T cells (Kitamura et al., 1999).

1.1.2.7 Dendritic Cell Maturation

Maturation is a key step in DC development where immature DCs, such as LCs, undergo a number of phenotypical and morphological changes that enable them to undertake their function as APCs. This process starts upon recognition of a danger signal and terminates during DC-T cell interaction. Some of the factors that could trigger LC maturation include GM-CSF, TNF, IL-1β, CD40 ligand, LPS and other TLR ligands as well as some endogenous factors such as heat shock proteins. The main phenotypic changes that LCs undergo are the loss of the ability to effectively capture antigens through their loss of endocytic receptors, the down-regulation of many of their inflammatory associated chemokine receptors including CCR1, CCR2, CCR4, CCR5, CCR6, CXCR1, CXCR2 and CXCR4. At the same time they up-regulate the expression of the chemokine receptor CCR7 as well as the costimulatory molecules CD40, CD80 and CD86 (Dieu et al., 1998; Gunn et al., 1998; Sallusto et al., 1998). LCs also undergo morphological changes which includes the loss of adhesive structures and cytoskeleton reorganization,

shift in the lysosomal compartments and change in MHC class II compartments (Winzler et al., 1997).

1.1.2.8 Dendritic Cell Migration

In general two major forms of DC migration have been identified *in vivo*. The first form is the migration of DC precursors from the bone marrow through the blood into the tissue (Charbonnier et al., 1999; Dieu et al., 1998; Dieu-Nosjean et al., 2000). The second form is the migration of DCs from the tissues through the afferent lymph into the T cell rich area of lymphoid organs. Knowledge in DC migration mainly comes from studing LC migration from the skin into the draining LN, which is also the form of migration examined in our studies and thus the focus of the next section. It is important to know that a small percentage of LC continuously migrate out of the epidermis into the draining LN in a steady state. However, during inflammation, the rate of LC migration increases, such that up to 30% local depletion may be observed. Before discussing how LCs leave the epidermis it is also important to know what normally keeps these LC in the epidermis. LCs express high levels of E-cadherin, a family of homophilic adhesion molecules that mediate intercellular adhesion between LCs and keratinocytes they also serve to maintain tissue integrity (Tang et al., 1993). TGF-β is locally produced by keratinocytes and may be a key molecule that retains LCs in the epidermis, since TGF-β knockout mice are deficient in LCs in the skin, but not in their precursors in the bone marrow (Borkowski et al., 1996; Borkowski et al., 1997). TGF-β has also been shown to inhibit LC maturation in

response to pro-inflammatory cytokines (Geissmann et al., 1999). In addition, IL-10 and lactoferrin, which are both constitutively produced in the epidermis, down regulate LC maturation and mobilization, respectively (Cumberbatch et al., 2000b; Wang et al., 1999). It is interesting to note that any perturbation in the skin microenvironment, by physical, chemical or biological factors, may provide a danger signal and shift the balance to favor LC activation and mobilization. In the skin, following LC activation, they migrate from the epidermis to the draining LNs.

The most common factors that have been shown to induce LC migration include contact sensitizing agents, LPS, CpG DNA and the pro-inflammatory cytokines TNF and IL-1β. Interestingly, intradermal injection of either TNF or IL-1β mobilizes LC from the skin and induces the accumulation in the draining LN (Cumberbatch and Kimber, 1992; Enk et al., 1993). It is thought that in response to contact allergen IL-1β will be released mainly from LC themselves which then stimulate the production of TNF from adjacent cells such as keratinocytes, which in turn will induce LC maturation and migration through the up-regulation of CCR7 which is required for LC migration (Cumberbatch et al., 2000a).

CD40-CD40 ligand interaction in the skin has also been shown to be required for LC migration (Moodycliffe et al., 2000). In a contact hypersensitivity model using CD40 ligand deficient mice, LC failed to migrate to the draining LN. This response was then shown to be associated with decreased production of TNF. The exact source of TNF and the CD40-CD40 ligand interacting cells in the

skin have not been identified. However, some evidence suggests a role for mast cells expressing CD40 ligand.

Two lipid transporters have also been shown to be required for LC migration. Multidrug resistant transporter (MDR-1) is a membrane protein that acts as an ATP-dependent drug-efflux pump and regulates DC migration (Randolph et al., 1998). Multidrug resistant related protein 1 (MRP-1) also mediates DC migration., since DC migration is inhibited in MRP-1 knockout mice. MRP-1 mediates the secretion of LTC_4 which converts into LTD_4 and LTE_4. Injection of these leukotrienes reversed DC migration inhibition in MRP-1 knockout mice (Robbiani et al., 2000). Based on this result it was hypothesized that MRP-1 was mainly required in LC migration for the transport of secreted leukotrienes, the source of which could either be LC themselves or other neighboring cells, and to up-regulate LC CCR7 expression and function.

One of the first steps in LC migration is the down regulation of E-cadherin which has been shown to be mediated by the exposure to TNF and IL-1β. LPS has also been shown to reduce E-cadherin surface expression, but as an indirect effect through the induction of TNF (Jakob and Udey, 1998). Once LCs lose such adhesion molecule expression LCs begin to move slowly by squeezing themselves between keratinocytes. This has been nicely demonstrated by Romani *et al.* using electron microscopy of skin explant cultures (Romani et al., 2001). In order to enter the skin dermis LCs face their biggest barrier, the basement membrane which mainly consists of type IV collagen. Several studies have determined that the matrix metalloproteinases (MMP) enzymes are crucial for penetrating the

basement membrane by LCs, especially MMP9 and MMP2 (Kobayashi et al., 1999; Lebre et al., 1999; Ratzinger et al., 2002). The plasminogen activator enzyme system has also been suggested to be involved in LC penetration of the basement membrane (Chapman, 1997). However, this still needs to be confirmed. Once LCs reach the dermis they still face collagenous and elastic fibers through which they have to migrate. Integrin molecules, specifically α_6 integrin, have been indicated to be involved in the transmigration of LCs. Antibodies to α_6 integrin have been shown to significantly inhibit the migration of LCs in response to TNF (Price et al., 1997). CD44, which is a receptor for hyaluronic acid, a component of the dermal matrix, has also been suggested to aid in the penetration of the dermal basement membrane by LCs (Weiss et al., 1997). Not much is known regarding LC entry into the lymphatic vessels which are lined by a thin layer of endothelial cells. Once in the LN, LCs move to the T cell area most likely through the interaction of selectins and integrins. Chemokines and their receptors have also been shown to play an extremely important role in LC migration within the node which will be discussed later. DCs have only been seen in the afferent lymphatics, but not in the efferent, which indicates DC death or major morphological changes following their interaction and activation of antigen-specific T lymphocytes.

1.1.2.9 Chemokines and Chemokine Receptors Involved in LC Migration

Chemokines are crucial for LC migration since they are what guide and direct LCs to the appropriate site. It is well recognized that LC maturation and migration is associated with a change in their expression of chemokine receptors.

During skin inflammation, numerous chemokines are produced including RANTES/CCL5, MIP-1α/CCL3, MIP-1β/CCL4, MCP-1/CCL2, TARC/CCL17, MDC/CCL22, MIP-3α/CCL20 IL-8/CXCL8 and SDF-1/CXCL12. Immature DCs have been shown to express a wide range of inflammatory chemokine receptors including CCR1, CCR2, CCR4, CCR5, CCR6, CXCR1, CXCR2 and CXCR4 (Sozzani et al., 1999) Upon maturation, immature DCs have been shown to down-regulate the expression of these chemokine receptors allowing them to leave the site of inflammation. It has been suggested that during inflammation LC precursors are being recruited from the blood through the chemotactic effect of these chemokines to replace the LC that have left the epidermis to migrate to the draining LN.

Subsequent to the down-regulation of these chemokine receptors, LCs up-regulate the chemokine receptor CCR7 (Forster et al., 1999) and acquire responsiveness to its ligands CCL19/MIP-3β (also known as Ebstein-Barr virus-induced molecule 1 ligand chemokine (ELC)/ Exodus3) and CCL21/6Ckine (also known as secondary lymphoid-tissue chemokine (SLC)/ Exodus2). Consequently, LCs will enter the lymph in response to the chemotactic effect of CCL21 constitutively expressed on lymphatic vessels (Saeki et al., 1999). This will lead them into the subcapsular region of the LN. LCs will then be attracted into the paracortical area of the LN in response to both CCL19 and/or CCL21 which are produced in the T cell area of the nodes (Gunn et al., 1998). Interestingly, the newly arrived DCs might become a source of these chemokines themselves, which in turn attract naïve T lymphocytes into this area (Gunn et al., 1999; Stein

et al., 2000). The essential role of CCR7 was noted in CCR7 knock-out mice studies in which LCs failed to migrate into the LN (Forster et al., 1999). CCR2 the receptor for the chemokine MCP1-4 (macrophage chemoattractant protein) has also been shown to play an important role in DC migration but the exact role will require further investigations (Sato et al., 2000). Upon DC-T cell interaction DCs will undergo the final stage of maturation in response to maturation signals received from CD40 ligand, OX40 ligand, RANK/TRANCE and 4-1BB molecules which will induce the production of other chemokines such as IL-8/CXCL8 and fractalkine that attract T lymphocytes (Adema et al., 1997; Kanazawa et al., 1999). CD40-CD40 ligand interaction has also been reported to extend DC survival through the up-regulation of anti-apoptotic genes.

1.1.2.10 T Cell Priming

While all APC such as B cells and macrophages can activate previously primed T lymphocytes, one of the most crucial features of DCs is their ability to prime naïve T lymphocytes. It has been recognized that in order for DCs to find the rare naïve T cells bearing T cell receptors (TCRs), specific for the particular antigen they have taken up, they form cell aggregates. This allows efficient antigen sampling which in the event of exact recognition will lead into an even stronger DC-T cell interaction. The formation of these aggregates is mediated by several molecules such as the intracellular adhesion molecule-1 (ICAM-1) which interacts with its T cell ligand LFA-1. Antibodies directed to either one of these molecules have been shown to inhibit aggregate formation and the consequent

signalling between DC and T lymphocytes (Scheeren et al., 1991). ICAM-3 also interacts with LFA-1 and is constitutively expressed by immature DCs. Since the expression of ICAM-1 is low, but increases following interaction, it is thought that ICAM-3 is involved in the early interaction while ICAM-1 is more important in stabilizing the antigen-dependent events (McBride and Fathman, 2002). DC-SIGN/CD209 which is up-regulated on LCs following antigen capture has a high affinity for ICAM-2 and ICAM-3 on T lymphocytes and is thought to be one of the main molecules facilitating DC-T cell aggregate formation (Geijtenbeek et al., 2002; Geijtenbeek et al., 2000). This interaction allows the TCR to sample for specific antigen-MHC class II complexes. In the absence of antigen recognition, T cells dissociate from DC and continue to circulate to other LNs to sample other DCs. It is believed that even in the absence of antigen specific TCR, forming antigen-independent aggregates induces a weak signal that mediates some level of T cell survival. However, the recognition of antigen-MHC class II complex, by TCR induces "signal one" for T cell activation. This will then facilitate the further interaction between costimulatory molecules on DCs CD86 (B7-2) and CD80 (B7-1) and their ligand CD28 on T lymphocytes which will provide "signal two". CD2 interaction with LFA-3 on DCs can also induce "signal two", but T cell activation and proliferation is thought to be much weaker than with classical CD28 and its ligands interaction. Several other molecules have also been shown to be involved in the DC-T cell interaction such as OX40 expressed on activated lymphocytes interacting with OX40 ligand expressed on DCs, 4-1BB on activated

lymphocytes acting as a co-stimulatory molecule through the interaction with 4-1BB ligand present on mature DCs (McBride and Fathman, 2002).

It is also widely recognized that during DC-T cell interaction, T cells provide essential signals for DCs through the interaction of several molecules, most critically CD40-CD40 ligand. It is thought that this will condition DCs to perform specific functions such as priming naïve $CD8^+$ T cells directly in the absence of $CD4^+$ T helper cells. It was initially thought that $CD4^+$ T cells and $CD8^+$ T cells must recognize the antigen on the same DC for full activation. However, it is now recognized that DCs can become licensed to activate $CD8^+$ T cells in the absence of T helper cells (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998). CD40-CD40 ligand interaction also induces increased up-regulation of the costimulatory molecules CD80 and CD86 on DCs mediating the final stage of maturation and enhancing their antigen presentation capability.

The interaction of naïve T cells and DCs is complex. For instance in order to interact and induce the appropriate signals, both cells have to undergo cytoskeletal reorganization of surface receptors and signalling molecules. Al-Alwan et al. demonstrated that DCs actively polarize their actin cytoskeleton when they interact with T cells, which is important for both clustering and activation of resting T cells (Al-Alwan et al., 2001). Thus, receptors involved in DC-T cell interaction accumulate preferentially at one pole of the cells coming in close proximity to their ligands on the other cell. This type of interaction is referred to as the "immunological synapse" which only lasts for a short time but is critical for optimal T cell activation (Grakoui et al., 1999).

DCs can prime naïve $CD4^+$ T cells through the interaction with MHC class II and release of IL-12 and IFN-γ that induce the production of Th1 helper cells, which in turn activates macrophages and promotes the differentiation of cytotoxic T cells. In contrast, in the presence of IL-12 and IL-4 DCs induce the production of Th2 helper cells which secrete IL-4 and IL-5, which in turn activates eosinophils and B lymphocytes promoting the production of appropriate antibodies (Maldonado-Lopez and Moser, 2001). The ability of DCs, activated in different ways, to influence the nature of the ensuing T-cell mediated response has lead to increased interest in the mechanisms of DC activation particularly in the context of vaccine development.

1.2 Mast Cells

Mast cells are immune effector cells, best known as the histamine producers in allergic disease. However, in this chapter substantial evidence will be provided to the reader to show the good side of mast cells. For example, they are important for host defense against pathogens, mostly in innate immunity but also with high potential in modulating adaptive immunity. Furthermore, not only are these cells normally beneficial to the host but also represent a key cell for manipulating immune responses and are therefore great targets for therapeutic use.

Mast cells were first recognized by Paul Ehrlich who identified them as "mastzellen" which means well-fed cells due to the large amounts of granules present in their cytoplasm (Ehrlich, 1878). Mast cells are single nucleated cells

with a round, oval or elongated shape ranging from 10-18 microns in size. They contain several types of granules which vary according to their location and differentiation status (Dvorak, 1986). They are long lived cells as they can live in tissues for up to several months.

Mast cells are bone marrow derived cells expressing CD34, c-kit (CD117) and CD13 in humans whereas in mice they express low levels of Thy-1 and c-kit. They also characteristically express the high affinity receptor for IgE (FcεRI). They enter the blood as undifferentiated cells which then migrate into the tissues to undergo maturation under the influence of local factors provided by stromal cells. The most important factor required for mast cell differentiation is stem-cell factor (SCF) which is the ligand for the c-kit tyrosine kinase associated growth factor receptor on mast cells (Tsai et al., 1991). This factor not only mediates mast cell differentiation, but also regulates mast cell proliferation and survival. IL-3 is considered crucial for mouse mast cell growth and development in response to SCF based on *in vitro* studies, however, the role of IL-3 in human mast cell development is still controversial. During immunological responses, mast cells can undergo changes in number and phenotype which can be regulated, in addition to SCF and IL-3, by IL-4, IL-9, IL-10, IL-12 and Nerve Growth Factor (NGF) (Marone et al., 2002).

Mast cells express a number of receptors that are essential for their role in immune responses including multiple Fc receptors, complement receptors and TLR. Mast cells can be activated by a wide range of stimuli which lead to the

release of pharmacologically active mediators most of which play essential roles in immune responses (to be discussed).

1.2.1 Mast Cell Distribution

Mast cells are widely distributed in the body. They are frequently located at sites often exposed to the external environment such as the skin where they have been estimated to reach up to 7000-10000 cell per mm^3 (Mikhail and Miller-Milinska, 1964). They are resident in the connective tissues of various organs and in the mucosal epithelial tissues of the respiratory, urinary and digestive tract, in addition to their close association with blood vessels and peripheral nerves (Marshall, 1975; Selye, 1966). Galli *et al.* have described them as sentinel cells due to their anatomical location and ability to influence immune responses (Galli et al., 1999).

1.2.2 Mast Cell Heterogeneity

A spectrum of mast cell types exist in both mice and humans which vary in terms of mediator content and activation profile. However, to simplify these types one can divide them into two general classes. In both species these mast cells differ in their anatomical location, granule content and activities. In mice, mast cells have traditionally been divided into the connective tissue mast cells and the mucosal mast cells. Connective tissue mast cells are found in the skin and contain large amounts of histamine and heparin. Mucosal mast cells are found on the mucosa of the lung and intestine. These are high in chondroitin sulfate

proteoglycan, but contain on average only one tenth the amount of histamine of connective tissue mast cells (Schwartz, 1998).

In humans, mast cell types are less well characterized. However in general they are divided based on their contents of neutral proteases. MC^T, are mostly identified by the presence of the tryptase enzyme, but not other neutral proteases such as chymase. Most of the mast cells found in the intestinal mucosa and alveolar spaces of the lung are of this type. In contrast MC^{TC} contain both tryptase and chymase as well as other neutral proteases. Most of the mast cells found at connective tissue sites such as the skin and intestinal submucosa are of this type (Irani et al., 1986). It is also recognized that IL-3 is important for MC^T hyperplasia and not MC^{TC} during parasitic infection (Madden et al., 1991).

As mentioned earlier these mast cell types differ not only in location and granule content, but also in their activities and response to stimuli. For example, a single stimulus can be a powerful inducer of degranulation of one type of mast cell, whereas the other type will remain unresponsive. Alternatively, the same stimulus may induce the production of specific mediators by one type of mast cell and different mediators by the other type (Selye, 1966). Examples of this include, the responses of connective tissue type mast cells to polybasic stimuli such as compound 48/80 which are not observed in mucosal mast cells, as well as the ability of Disodium cromoglycate to stabilize only some mast cell subsets (Marshall, 1972; Pearce et al., 1984). It is also important to note that both types of mast cells can be found at one site. However, one type usually predominates. Some pathological conditions have been recognized to be

associated with alterations in this ratio (Gotis-Graham and McNeil, 1997; Irani et al., 1990).

1.2.3 Mast Cell Mediators

Upon mast cell activation, mast cells can release a wide variety of mediators with biological activity that can act on the local tissues as well as on effector cells (Compton et al., 1998; Huang et al., 1998). When released in response to pathogens, these mediators can initiate beneficial activities that protect the host. However, when they are released in response to an allergen, these mediators will induce unnecessary responses, which usually harm the host and mediate pathological damage. It is also been suggested that inappropriate activation of mast cells by pathogens can exacerbate a specific infection or a disease such as allergic asthma (McCurdy et al., 2003) and atopic dermatitis (Abeck and Mempel, 1998; Masenga et al., 1990). However, this connection still requires further investigation.

It is believed that mast cells can re-enter a degranulation/regranulation cycle and thus participate in several rounds of activation and release of mediators which can vary depending on the stimuli. In general, mast cell mediators are classified into preformed; newly generated lipids; and newly synthesized cytokines and chemokines (reviewed in (Marshall, 2004)). Interestingly, depending on the stimuli, mast cell will release only the mediators that are appropriate for the specific condition. Thus, mast cells have the ability to discriminate between different stimuli and respond accordingly (King et al., 2002;

Lin et al., 2003; McCurdy et al., 2003). In this context it is important to know that mast cell mediator production can occur in the absence of classical degranulation (Leal-Berumen et al., 1994).

1.2.3.1 Preformed Mediators

Preformed mediators are those already found and stored in the cytoplasmic granules. Upon mast cell activation, these cytoplasmic granules move toward and fuse with the plasma membrane where they release their contents into the extracellular environment (Metcalfe et al., 1997). The term "degranulation" is usually used to describe this process. These granule-associated mediators include histamine, serotonin, proteoglycans, proteases such as tryptase and chymase, eosinophil chemotactic factor, neutrophil chemotactic factor and acid hydrolases such as β–hexosaminidase which is used in many studies to measure mast cell degranulation (Schwartz and Austen, 1984). Some cytokines are also present in the granules including TNF and lower amounts of IL-4, IL-5 and IL-6 (Bradding et al., 1993). The effect of these mediators can be seen within seconds of mast cell degranulation, which is most frequently induced through the cross-linking of the FcεRI on the surface of the mast cell. However, other stimuli can also induce mast cell degranulation including the cross-linking of the FcγRs (Tkaczyk et al., 2002), complement components such as C3a and C5a (Nilsson et al., 1996), substance P (Marshall and Waserman, 1995), pathogens and pathogen products (Lin et al., 2000) calcium ionophore, and basic components such as 48/80 (Pearce et al., 1984). The effect of these mediators includes increased vascular permeability,

smooth muscle contraction, mucus secretion and the initiation of the complement cascade.

1.2.3.2 Lipid Mediators

Lipid mediators are generated and released through the breakdown of plasma membrane phospholipids, followed by the action of several enzymes including cyclooxygenase and 5-lipoxygenase. Mast cell produced lipid mediators include leukotrienes (LT) specifically LTB_4 (Freeland et al., 1988) and LTC_4 (Razin et al., 1982) which can be further metabolized to form LTD_4 and LTE_4, prostaglandins specifically PGD_2 and PGE_2 (Heavey et al., 1988) and PAF (Mencia-Huerta et al., 1983). These mediators are usually released within minutes of mast cell activation. Their release induces increased vascular permeability, vasodilation, smooth muscle contraction and platelet aggregation (McMillan, 2001). It is interesting to note, that in some cases the biological effects of lipid mediators such as the induction of bronchoconstriction by LTC_4 may be 1000 times more potent as well as longer lasting than the effect of histamine (Drazen, 1998). The profile of lipid mediators produced can vary substantially with mast cell type and microenvironment. For example, mucosal mast cells produce mainly LTC_4 while connective tissue mast cells produce mainly PGD_2 (Heavey et al., 1988). This mediator profile can vary with microenvironment for example, if rodent mast cells are treated with NGF they can begin to produce increased amount of PGE_2 (Marshall et al., 1999).

1.2.3.3 Newly Synthesized Mediators

Mast cells can synthesize a wide variety of both cytokine and chemokines which are released within hours of mast cell activation. These mediators can be classified into pro-inflammatory cytokines including TNF, IL-1α, IL-1β, IL-6 GM-CSF, IFNα and IFNβ (Burd et al., 1989; Gordon et al., 1990; Gordon and Galli, 1990; Marshall et al., 1993; Plaut et al., 1989; Williams and Coleman, 1995); Th1 associated cytokines including IFNγ, IL-2 and IL-12 (Gupta et al., 1996a; Smith et al., 1994); Th2 associated cytokines including IL-3, IL-4, IL-5, IL-9, IL-13, IL-15 and IL-16 (Rumsaeng et al., 1997; Stassen et al., 2001); immune regulatory and anti-inflammatory cytokines such as IL-10, TGFβ and vascular endothelial growth factor (VEGF) (Bissonnette et al., 1997). In addition, a large number of chemokines are also produced by activated mast cells including CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, CCL11, CCL20/MIP-3, SDF-1, CXCL1, CXCL2, CXCL8/IL-8 CXCL9, CXCL10/IP-10 and CXCL11 (Jia et al., 1996; King et al., 2002; Lin et al., 2003; Rajakulasingam et al., 1997; Selvan et al., 1994). The most critical effects of these chemokines have been associated with the recruitment of neutrophils and eosinophils to sites of inflammation including those induced in allergic diseases. However, many of these chemokines also have the potential to recruit other cell types such as T-cells and immature DCs.

1.2.3.4 Antimicrobial Peptides

In addition to the above mentioned significant mediator release by mast cells, several antimicrobial peptides have been also been shown to be produced by activated mast cells such as cathelicidins and defensins (Marshall and Jawdat, 2004). In mice, mast cell release of the cathelicidin-related antimicrobial peptide (CRAMP) in response to group A streptococci has been shown to be critical in host defense, since mast cell deficient in CRAMP suffered from a reduced killing of the organism (Di Nardo et al., 2003).

1.2.3.5 Histamine and its Receptors

Histamine is a major component of mast cell granules, accounting for approximately 10% of the granule weight. Histamine is a biogenic amine formed by the decarboxylation of the amino acid histidine by the enzyme L–histidine decarboxylase (Barnes, 2001). Its biological effect can be seen within minutes of mast cell activation in response to various stimuli including immunological (IgE/antigen, cytokines) and non-immunological (calcium ionophore, substance 48/80 and substance P) factors. Although mast cells and basophils are the main source of histamine, other sources have been described. In human there are two additional sources, one in the gastric enterochromafin-like cells and one in the histaminergic nerves in the brain. There is also a less significant source of histamine from some circulating cells including monocytes, lymphocytes and platelets in addition to some tissues like skeletal muscles (MacGlashan, 2003). However, the biological significance of these sources needs further investigation.

The effects of histamine are mediated through binding to specific surface receptors present on various target cells. In humans, almost every cell expresses at least one type of histamine receptor, except mast cells. In rodents even mast cells express histamine receptors (Antohe et al., 1988; Kohno et al., 1993). So far four types of histamine receptor have been identified, H1, H2, H3 and H4, all of which are members of the seven transmembrane-spanning family of receptors coupled to guanosine triphosphate (GTP)-binding proteins. In addition, there is an intracellular receptor identified as H_{IC} (MacGlashan, 2003). Most histamine studies are associated with H1 and H2 receptors as the other receptors have only more recently been identified. The effect of histamine binding to H1 receptors mediates an increase in the intracellular calcium. Its biological effects include smooth muscle contraction, increased venular permeability and increased mucus secretion (Schwartz and Austen, 1984). Thus, most of the biological effects of histamine in allergic reactions seem to be mediated through H1 which can be blocked by mepyramine/pyrilamine. Histamine binding to H2 mediates an intracellular increase of cyclic AMP (Hill, 1990). Its effects include inducing vascular permeability and dilation, as well as the stimulation of exocrine glands. However, binding of histamine to H2 receptors on murine mast cells seem to have a negative feedback on the release of mediators since it suppresses degranulation. The effects mediated through H2 receptors have been shown to be blocked by selective antagonists such as cimetidine and ranitidine (Jin et al., 1986). H3 receptors were identified in the brain and peripheral neurons (Lovenberg et al.,

1999). Studies of its function are very limited but it is suggested to act as an inhibitory autoreceptor.

A large number of anti-histamine drugs have been identified which are specific receptor antagonists. However, it is increasingly being considered that drugs that inhibit histamine production by altering the cell function, in a way that does not involve histamine receptor binding, may have some advantages. However, this type of inhibition requires a much higher concentration of the drug than to block the receptor. It is also important to consider that since mast cells are required in host defense, altering mast cell function to block histamine release may have bad effects on the host's ability to combat infections.

1.2.3.6 TNF and its Role in Immune Responses

TNF is a pro-inflammatory cytokine that can be released by various cells. However, what makes this cytokine specifically important in mast cells is its existence as a preformed mediator stored in the granules which could be released within seconds of mast cell activation. In addition to its rapid generation and release as newly synthesized proteins (Gordon and Galli, 1991), TNF is an essential mediator for a large number of immune responses, specifically in the induction and maintenance of inflammatory reactions. For instance, TNF induces up-regulation of adhesion molecules such as E-selectin on endothelial cells to recruit immune effector cells into the site of inflammation. TNF can also stimulate the production of other pro-inflammatory cytokines including IL-1β and IL-6. Each of these three cytokines can induce vascular permeability resulting in

edema in the tissue and extravasation of leukocytes including neutrophils, eosinophils and monocytes. TNF together with IFN-γ increases the phagocytic activity of macrophages and neutrophils (Papadakis and Targan, 2000; Walsh et al., 1991). Mast cell-derived TNF has been shown of particular importance in host defense against bacterial infections. In a mouse model of cecal ligation and puncture (CLP) induced bacterial peritonitis the rate of bacterial clearance and mice survival was dependent on mast cell production of TNF (Echtenacher et al., 1996b; Malaviya et al., 1996b). The role of TNF in these studies was mainly associated with the recruitment of neutrophils.

1.2.4 Mast Cells Relationship to Basophils

Basophils are bone marrow derived $CD34^+$ cells that account for 0.5-1% of the circulating white blood cells. Thus, they are one of the least common cells in the peripheral blood. They are granulocytes with metachromatic granules and a segmented nucleus and highly condensed chromatin. Basophils share several similarities with mast cells specifically their role as effector cells in IgE-associated immune responses. Their site of maturation differs from mast cells. As mentioned earlier mast cells mature after they enter the tissues whereas basophils mature within the bone marrow and enter the blood where they have a half-life of several days (Kepley et al., 1998). Mast cells are not identifiable in the blood whereas basophils can also be identified in the peripheral tissues following inflammation. Like mast cells, basophils express the high affinity FcεRI, FcγRII, complement receptors (CD11b, CD11c, CD35 and CD88) (Agis et al., 1996;

Bochner and Schleimer, 2001). Basophil activation can be mediated by the crosslinking of IgE and specific antigen and the complement components C3a and C5a. Basophil activation will lead to the release of mediators including histamine and proteoglycans, and the generation of lipid mediators, primarily LTC_4, and cytokines which mainly include IL-4 and IL-13 (Schroeder et al., 2001). The high and rapid expression of IL-4 by basophils suggests its role in the Th2 cell type differentiation. Moreover, the expression of IL-4 in addition to CD40 ligand has been shown to induce IgE switching by B lymphocytes *in vitro*, which may suggest an alternate mechanism of IgE class switching *in vivo* (Prussin and Metcalfe, 2003). Although a role for basophils in allergic reactions is highly suggested, their exact function in asthma pathogenesis is not well understood. In asthmatic airway tissues, basophils predominate as the IL-4-expressing cells following allergen exposure. In addition, during a late phase response in both cutaneous and pulmonary tissues there is often an increased number of basophils (Buckley et al., 2002; Kepley et al., 2001). Similar to mast cells, basophils have also been suggested to play a role in the inflammatory response to parasites (Falcone et al., 2001). Parasitic infections are often associated with an increased number of mast cells and basophils (Wedemeyer et al., 2000) and basophils have been shown to be required for immune resistance to ticks (Brown et al., 1982).

1.2.5 How Do Mast Cells Become Activated?

Mast cells can be activated by a danger signal which is provided either through a direct interaction with a pathogen or indirect through the effect of other

molecules. Both of these methods will be discussed in more detail in the next section. One of the most important features of mast cells is their rapid and selective response to pathogens. Direct interaction of mast cells with a pathogen or its products is specifically important in primary infections, since it will provide early signals to alarm the host to fight and clear that specific microbe before it can mediate any further damage. This direct interaction requires the presence of specific receptors that can recognize the pathogen or its molecular patterns. Some of the mast cell receptors that are thought to directly interact with a pathogen are discussed below.

1.2.5.1 Mast Cells and Toll-Like Receptors

Since the discovery of TLRs a number of studies have examined the expression and function of TLR on mast cells. Several TLRs have been shown to be expressed by mast cells including TLR1, TLR2, TLR3, TLR4, TLR6, TLR7 and TLR9, but not TLR5. However, differences between species have been recognized. In this section only the murine and human system will be discussed since other species are out of the scope of this thesis.

Rodent mast cells were initially shown to produce a significant amount of IL-6 without degranulation in response to LPS (Leal-Berumen et al., 1994). At that time the nature of the LPS receptor had not been identified. In 2001 McCurdy *et al.* linked this response to TLR4 expression on mast cells. In this study both murine bone marrow-derived mast cells and the murine mast cell line MC/9 were activated with LPS from Gram-negative *E.coli*. Mast cells were shown to produce

significant levels of both TNF and IL-6. When TLR4 deficient mast cells were used this response was completely inhibited (McCurdy et al., 2001). These valuable observations were confirmed at the same time by another group (Supajatura et al., 2001). In 2002 Supajatura *et al.* examined the functional role of both TLR2 and TLR4 *in vivo* in response to microbial challenge using TLR2 and TLR4 deficient mice (Supajatura et al., 2002). Mast cells stimulated with PGN from *S. aureus* produced TNF, IL-6, IL-4, IL-5, IL-13 and degranulation. In addition, intradermal injection of PGN induced vasodilation and inflammation. All of these responses were TLR2-dependent, which may suggest a contribution of TLR2 in allergic responses, but this needs to be further examined. Mast cells stimulated with LPS from *E. coli* produced TNF, IL-6, IL-1β, IL-13 and no degranulation. This response was TLR4 dependent.

Human mast cells have been shown to express TLR1, TLR2, TLR3, TLR6, TLR7 and TLR9 (Kulka et al., 2004; McCurdy et al., 2003; Sundstrom et al., 2004). TLR4 has been shown to be expressed by some groups, but not by others, which might reflect the presence of cytokines that seems to be required for TLR4 expression. For example IL-4 primed CBMC and IFNγ treated peripheral blood derived mast cells were shown to express TLR4 (Okumura et al., 2003; Varadaradjalou et al., 2003). A study using CBMC have shown that stimulation of mast cells with LPS or PGN induce the release of several cytokines including TNF, IL-5, IL-10 and IL-13 with the exception of histamine which was only released in response to PGN (Varadaradjalou et al., 2003). This study suggests a role of these ligands in causing mast cells to induce a Th2 type immune response.

However, another study shows that PGN stimulated CBMC to produce IL-1β and GM-CSF without significant degranulation (McCurdy et al., 2003).

Interestingly, both PGN and zymosan have been shown to stimulate CBMC to produce LTC_4 independently of degranulation. However, the tripalmytoylated lipopeptide ($Pam_3CysSerLyse_4$) did not induce LTC_4, but was able to induce degranulation. Since all of these pathogen products are recognized as TLR2 ligands, this suggests the ability of mast cells to respond differently according to the specific ligand even for TLR2 mediated events. These different responses may result from the use of different heterodimers (for example TLR2/TLR1 for lipopeptide and TLR2/TLR6 for PGN) or from the use of alternative co-receptors (for example Dectin-1 for zymosan).

In addition to TLRs, mast cells have other important innate immune receptors. CD48 has been recognized to have crucial functions on mast cells in innate immunity. CD48 is a GPI-anchored protein and a receptor for FimH, a protein on fimbriated Gram-negative bacteria such as *E.coli*. FimH interaction with CD48 have been shown to induce mast cell degranulation, TNF and leukotriene production (Malaviya et al., 1999). Mast cells also express complement receptors and a number of lectin-like molecules such DEC-205 which may play a role in their host defense function.

1.2.5.2 Indirect Mast Cell Activation

Mast cells can also be activated indirectly by numerous stimuli. This is biologically important as it broadens the mast cell's opportunity to mediate

responses to a variety of pathogens without the requirement for receptors specific for a particular organism. Some of the molecules involved in this type of activation are described below. These indirect interactions are particularly important in the context of secondary infections.

1.2.5.2.1 Fc-Receptor Mediated Mast Cell Activation

One of the main characteristics of mast cells is their expression of the high-affinity receptor for IgE antibodies (FcεRI) (Kent et al., 1994) which is the fundamental receptor for type I hypersensitivity. Mast cells also express the low-affinity receptor for IgG antibodies (FcγRII). Both of these receptors are continuously expressed on mast cells. Interestingly, mast cells can up regulate the expression of other Fc receptors including the high affinity receptor for IgG (FcγRI) and also FcγRIII under the influence of IFNγ exposure (Woolhiser et al., 2001).

So far there are two existing models showing mast activation through Fc-receptors in response to pathogens. The first model is antigen dependent, which means only antigens that are specific for the antibodies bound to the cell surface can interact and activate the cell. An example of this model is classical IgE-mediated mast cell activation which will be discussed in more detail next. This type of response is important in host response against helminthes in which the antigen binds to the IgE antibodies on the surface of the mast cell leading to degranulation and the release newly sensitized mediators important for secondary infections (Jarrett and Miller, 1982). Another example is IgE-mediated mast cell

activation in response to an allergen which induces degranulation and harmful effects. The second model is both antigen and specific antibody independent. However, this model requires specific pathogen-derived proteins that can bind non-specifically to antibodies on the surface of mast cells. An example of this model is protein A from *S. aureus* which has been shown to bind to IgE antibodies or IgE/IgG complexes on mast cells inducing their activation (Genovese et al., 2000).

1.2.5.2.2 IgE-Mediated Mast Cell Activation/Degranulation

IgE-mediated activation is the most studied naturally occurring mechanism of mast cell activation. It is mainly studied in the context of allergy, since it is the classical model of type I hypersensitivity in response to environmental allergens. However, it is also the model of parasite-mediated mast cell activation as parasites also induce specific IgE antibody production. Since this mode of IgE-mediated mast cell activation is used in this study it will be discussed in more detail herein. It begins with the organism's first exposure to a specific allergen/antigen that induces a Th2 type response and the activation of B lymphocytes. This activation will then lead to the generation of IgE-secreting plasma cells. These secreted IgE antibodies can then bind to their high affinity receptors (FcεRI) on the surface of mast cells. At this stage, mast cells are identified as sensitized. Following a second exposure, the allergen/antigen will bind to the IgE fixed to its receptor inducing the cross-linking of the IgE-receptor complex which will activate the mast cell and lead to degranulation and the

release of its mediators (Williams and Galli, 2000). [For signalling events see Figure1.4, adapted from (Blank and Rivera, 2004; Siraganian, 2003)]. These mediators usually have variable effects on the host ranging from annoying, to serious, to life-threatening depending on the extent of the mediators released.

In general the effects of classical IgE-mediated mast cell activation can be divided into an early-phase reaction followed by a late-phase reaction. The early-phase starts with the release of preformed mediators, specifically histamine. This is followed within minutes by the release of lipid mediators, mainly LTC_4 and PGD_2. Some cytokines are also released at this stage (e.g. TNF) while many others are newly synthesized over the next several hours. Cytokines such as TNF and IL-1β and multiple chemokines enhance the reaction through the recruitment of effector cells while others such as G-CSF and IL-5 may help to mobilize cells from the bone marrow. Some of the effects of the early phase include vasodilation, increased vascular permeability, smooth muscle contraction and the up-regulation of adhesion molecules on endothelial cells. These events will in turn lead to the late-phase reaction. This phase is characterized by the infiltration of neutrophils, eosinophils, macrophages, lymphocytes and basophils. Eosinophils play a critical role in the late phase-reaction. Their activation can lead to degranulation and the further release of leukotrienes, PAF, major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin, which have been shown to contribute to extensive tissue damage as well as chronic inflammation. Neutrophils are also a major participant of this reaction. Their mediators include

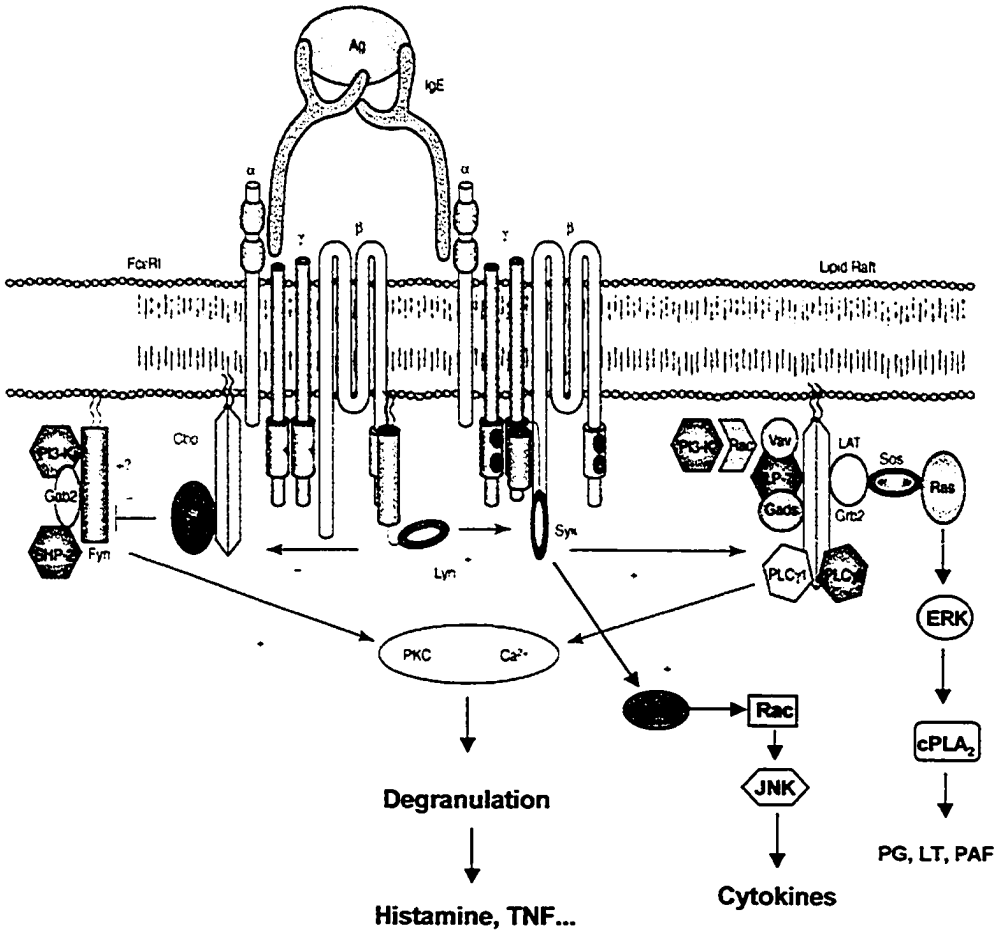

Figure 1.4 FcεRI-mediated signaling in mast cells. Adapted from Blank *et al.* 2004 and Siraganian 2003.

leukotrienes, PAF and lytic enzymes all of which can intensify the tissue damaging inflammatory response (Leung, 1998).

1.2.5.2.3 Complement Receptor Mediated Mast Cell Activation

The activation of mast cells through complement components is very significant since the complement cascade can be activated by a very wide range of pathogens. This increases the mast cell's contribution to immune responses, either directly, or indirectly through the recruitment of other immune effector cells. Mast cell complement receptor expression varies between species and also between mast cell types within the same host (see Table1.3). Some types express them continuously where others do not, and some can only up-regulate their expression in response to specific microenvironmental factors. Human mast cells have been shown to express several complement receptors including CR1/CD35 CR3/CD11b-CD18, CR4/CD11c-CD18 (Fureder et al., 1995). Human mast cells also express the receptors for the anaphylatoxins C3aR and C5aR/CD88, which can activate the mast cell inducing degranulation or could act as chemotactic factors to recruit the mast cell to the site of complement activation (Nilsson et al., 1996).

In mice, Gommerman *et al.* have shown that CR1 and CR2 are expressed by serosal type mast cells and that mouse survival was decreased following CLP in CR1 and CR2 deficient mice (Nilsson et al., 1996; Rosenkranz et al., 1998) Serosal type mast cells have also been shown to express CR3. In the absence of this receptor mast cell development was impaired and mice survival was again

Table 1.3 Complement receptor distribution on mast cells

Complement receptors	**Murine mast cells**	**Human mast cells**
CR1(CD35)/CR2(CD21)	+ (Serosal)	+
CR2 (CD21)	+ (Serosal and mucosal)	NA
CR3 (CD11b/CD18)	+ (Serosal)	+
CR4 (CD11c/CD18)	NA	+
C3aR (CD11b/CD18)	+ (Serosal)	+
C5aR (CD88)	+ (Serosal)	+

decreased following CLP (Rosenkranz et al., 1998), a model of bacterial infection in which mouse survival is known to be dependent on mast cells (Prodeus et al., 1997). None of the complement receptors were identified on mucosal type mast cells except CR2. However, the function of this receptor is not well understood and needs further investigations. Serosal mast cells also express the receptors for the anaphylatoxins C3a and C5a and can be activated to degranulate via this route (Erdei et al., 2004; Kimura et al., 1998). Interestingly, the receptor for C3a (CR3a) was not expressed on mucosal mast cells. However, C3a was still shown to be able to bind to mucosal mast cells through interaction with the β subunit of the FcεRI. This interaction has been recognized as a regulatory effect, since it inhibits IgE-mediated degranulation. It is believed that C3a may activate serosal mast cells only for a short time and then act as an inhibitor to regulate the release of mediators (Erdei et al., 1999).

1.2.5.2.4 Microenvironmental Factors

One of the most important factors that can indirectly regulate mast cell responses is the local cytokine milieu. As mentioned earlier, pretreatment of mast cells with certain cytokines induces the up-regulation of TLR4. In addition, mast cell exposure to IFN-γ up-regulates the expression of FcγRI. Moreover, the mediators released from adjacent cells, in response to infection or tissue damage, can induce the release of specific mediators by mast cells. For instance, treating mast cells with IL-5 and IgE increases the release of TNF and the production of CCL3 and GM-CSF (Ochi et al., 2000). Treating mast cells with IL-4 mediates

the increase of LTC_4 synthase in mast cells as well as Th2 type cytokines (Bischoff et al., 2001; Hsieh et al., 2001). Treating mast cells with IL-1β induces the release of IL-6 (Kandere-Grzybowska et al., 2003). There are many other examples of these types of responses that are outside the scope of this chapter. However, the interesting point to note is the ability to alter a mast cell response to a specific stimulus through the effect of the microenvironment. These types of changes in mast cell function may be particularly important in the context of tissue injury or infection.

1.3 The Role of Mast Cells in Innate Immunity

One of the first observations exploring the potential role of mast cells in host defense was the discovery of mast cell production of pro-inflammatory cytokines, without degranulation, in responses to the bacterial product LPS (Leal-Berumen et al., 1994). Subsequently, two major *in vivo* studies verified the essential role of mast cells in murine host defense in response to certain bacterial infections (Echtenacher et al., 1996b; Malaviya et al., 1996b). One of these studies showed that when *Klebsiela pneumoniae* is introduced into the peritoneal cavity of mice, their survival is mast cell dependent. They also showed that the limited bacteria clearance in mast cell deficient mice correlates with the impaired TNF production that is required for neutrophil influx into the infected site. The production of TNF was mainly shown to be a response dependent upon mast cell interaction with the FimH component of the pathogen (Malaviya et al., 1996b). The other major study at this time used a mouse bacterial peritonitis model of

CLP. Following infection, mast cell deficient mice died whereas mast cell containing mice were able to survive. Adding anti-TNF antibodies during the infection suppressed the protection in mast cell containing mice (Echtenacher et al., 1996b). Interestingly, both of these studies have linked the important role of mast cells to the production of TNF and the recruitment of neutrophils. In addition, leukotrienes, specifically LTB_4 and LTC_4, have been shown to be important in response to FimH bacteria (Malaviya and Abraham, 2000a).

The role of mast cells in host defense against parasites has been widely implicated for many years (Grencis, 1997; Lantz et al., 1998; Woodbury et al., 1984). Many parasitic infections especially by helminthes are associated with an increase of mast cell numbers. However, some recent studies have questioned the role of mast cells as effector cells in some parasitic infections (Wershil et al., 1994). Although others have highly suggested an important role of mast cells or their granule mediators such as proteases in response to intestinal parasitic infection as well as in response to Shistosomas and Filaria (Bleiss et al., 2002; Cutts and Wilson, 1997) the relative importance of mast cells depends upon the nature of the parasite, the type of host and whether primary or subsequent infections are being considered.

The role of mast cells in viral infections is not very clear. There are only a few studies showing an association of mast cells with viral infections. For example, the inflammatory responses in the brain of Sindbis virus infection have been shown to be mast cell dependent (Mokhtarian and Griffin, 1984). Several studies have shown that mast cells can produce important mediators in host

defense in response to a number of viruses and viral products. For example, mast cells infected with dengue virus induced the production of IL-6, MIP-1α/CCL3, MIP-1β/CCL4 and RANTES/CCL5 all of which have a critical function in inflammation and chemotaxis (King et al., 2002; King et al., 2000). In addition, it is well recognized that viral infections can exacerbate the symptoms of asthma, which suggest a potential role for mast cells in linking the two responses (Corne and Holgate, 1997). However, this needs further investigations. It is interesting to note that although mast cells may have a potential role in host defense against viruses most *in vivo* studies rather suggest a role in the pathology associated with the infection (Mokhtarian and Griffin, 1984; Sorden and Castleman, 1995).

1.3.1 Mast Cell Effector Mechanisms

Based on most mast cell-pathogen studies it is apparent that one of their main effector roles is the recruitment of immune effector cells. This function is partially facilitated by their close proximity to blood vessels and could be mediated by three main mechanisms (Marshall and Jawdat, 2004). One is the effect of mast cell mediators including histamine, TNF, LTC_4 and PGD_2 on the endothelial cells by inducing vascular permeability (Drazen, 1998). Another is the effect of mast cell mediators on the expression of adhesion molecules. This could be induced by a number of mediators including histamine which has been shown to induce the up-regulation of P-selectin that is important for neutrophil adhesion and recruitment (Torres et al., 2002). Early production of TNF, IL-1α, IL-1β as well as IFNγ has also been shown to be important in this context. Finally, several

mast cell mediators have been well recognized to be crucial in recruiting effector cells to the site of inflammation through their chemotactic effect including CCL3, CCL4 (King et al., 2002), CCL5 (Rajakulasingam et al., 1997), CCL11 (Hogaboam et al., 1998), CCL20 (Lin et al., 2003), CXCL8 (Moller et al., 1993) as well as LTC_4, LTB_4 (Malaviya and Abraham, 2000b) and proteases (Huang et al., 1998).

Another important effector mechanism of mast cells is their production of antimicrobial peptides. These peptides can disrupt the integrity of the microbial membrane by their cationic and amphipathic properties which enable them to bind negative-charged microbes and insert into their membrane. As mentioned earlier, two types of antimicrobial peptides have been shown to be expressed by mast cells cathelicidins and defensins. In addition to mediators released, mast cells may have more direct anti-bacterial effects through phagocytosis of organisms, as well as killing via reactive oxygen and nitrogen species (Malaviya et al., 1994).

1.4 The Role of Mast Cells in Adaptive Immunity

Several studies have shown interesting features of mast cells that may reflect their potential role in adaptive immune responses. However, most of these studies are *in vitro*. Thus, further *in vivo* studies have to be done to further confirm these findings. The effect of mast cells on the adaptive immune response may be mediated directly through the interaction with lymphocytes or through their effect on DCs. Both of these areas will be discussed and the later forms the basis for the major studies described in the thesis.

1.4.1 Mast Cell Effects on T Lymphocytes

Mast cells are not classified as a classical APC. However, several studies have suggested that mast cells can present antigens to T lymphocytes. For example, *in vitro* studies show that MHC class I molecules on mast cells have the ability to interact directly with TCR to induce antigen-specific clonal expansion (Henz et al., 2001; Mekori and Metcalfe, 1999). MHC class I molecules were also expressed on mouse bone marrow cultured mast cells and mediated T cell activation through direct bacterial antigen presentation (Malaviya et al., 1996c). Several factors such as LPS, TNF and IFNγ have been shown to up-regulate MHC class II molecule expression on both human and murine mast cells (Henz et al., 2001). In addition to the up-regulation of co-stimulatory molecules such as CD80 and CD86 on murine mast cells in response to GM-CSF exposure (Frandji et al., 1996). Mast cells can also express OX40 ligand and 4-1BB ligand which enhance T cell activation following the interactions with OX40 and 4-1BB on the surface of T cells (Kashiwakura et al., 2004).

Mast cell exosomes have been shown to induce T cell activation and proliferation and the release of IL-2 and IFN-γ (Skokos et al., 2001). McLachlan *et al.* showed that mast cell responses to Gram-negative bacteria (*E. coli*) induced LN activation through the accumulation of lymphocytes in the node. This effect of mast cells was shown to be mainly dependent on the release of TNF (McLachlan et al., 2003). Mast cells themselves were also shown to have the ability to migrate to the skin draining LN in response to antigen (Wang et al.,

1998) which may suggest a potential role for mast cells in influencing not only T lymphocytes at the site of antigen challenge but also those in the draining LN.

Mast cells can also promote T cell recruitment into the site of antigen entry either directly through the release of chemotactic factors such as LTB_4, MCP-1/CCL2, MIP-1α/CCL3, MIP-1β/CCL4, RANTES/CCL5, MIP-3α/CCL20, IP-10/CXCL10 and IL-16 (Lin et al., 2003; Nakajima et al., 2002; Ott et al., 2003), or indirectly through the up-regulation of adhesion molecules on endothelial cells such as ICAM-1 and E-selectin (Marshall, 2004). The wide range of mast cell cytokines which include both Th1 (IL-12 and IFN-γ) and Th2 (IL-4, IL-5, IL-9 and IL-13) associated signals, may suggest profound influence of mast cell activation on T cell differentiation.

1.4.2 Mast Cell Effect on Dendritic Cells

Each primary immune response requires the activation of DCs which like mast cells are considered sentinel cells in host defense. Having an effect on these cells would be of great value for mast cells to enhance the activation of the adaptive immune response. Mast cells, as previously described, can produce numerous chemokines. Among these chemokines is MIP-3α/CCL20 the ligand for CCR6 expressed on immature DCs. Thus, mast cells might play an important role in recruiting immature DCs into the site of inflammation following antigen exposure. This potential interaction has been demonstrated with *P. aeruginosa* in response to which mast cells release of MIP-3α/CCL20 (Lin et al., 2003). Moreover, mast cell mediators have been implicated in DC maturation and

migration including GM-CSF, TNF, IL-1β (Cumberbatch et al., 2000a), IL-16 (Kaser et al., 1999), IL-18 (Cumberbatch et al., 2001), and PGE_2 (Kabashima et al., 2003). TNF and IL-1β are of particular importance as they are widely recognized as potent inducers of DC maturation and migration specifically following the application of contact sensitizers. Blocking either one of these cytokines has been shown to inhibit LC migration from the skin into the draining LN (Cumberbatch et al., 1997c).

Histamine has been shown to have significant effects on DCs. It induces DC maturation through the up-regulation of MHC class II, CD86, CD80, CD40, CD49d and CD54, in addition to its effect on DC production and/or up-regulation of IL-1, IL-6 cytokines and several chemokines that are essential for the recruitment of several immune effector cells. However, histamine by itself does not induce full maturation of DCs, which clearly suggests the contribution of other mediators (Caron et al., 2001a). Histamine has also been shown to have the ability to alter the cytokine and chemokine profile of mature DCs to polarize naïve $CD4^+$ T lymphocytes towards a Th2 phenotype (Mazzoni et al., 2001).

Exosomes are extracellular vesicles secreted during exocytosis from the lumen of cells into the extracellular environment by variable cells. They are primarily implicated in the transport of materials between cells (Denzer et al., 2000). Mast cell-derived exosomes have been shown to induce DC maturation through the up-regulation of MHC class II, CD80, CD86 and CD40 molecules and eliciting effective immune responses *in vivo* (Skokos et al., 2003). Moreover, mast cell mediators such as histamine (Caron et al., 2001a; Mazzoni et al., 2001),

and PGD_2 or PGE_2 are able to inhibit IL-12 production of DCs in response to LPS leading to a Th2 type response instead of the expected Th1 immune response (Faveeuw et al., 2003; Gosset et al., 2003; Kalinski et al., 1997). Thus, mast cells not only may alter DC activation, but also may change the nature of the subsequent immune response.

1.5 Objectives

Both mast cells and LC are present in the skin, one of the major interfaces with the external environment. The function of LC's is to capture local antigen, process it and migrate from the skin to the draining LN. Having arrived in the LN they migrate to the T cell rich area to present the antigen to T lymphocytes. As mentioned earlier, LC migration can be induced and regulated by a number of stimuli. Interestingly, mast cells have the ability to produce most of the key molecules involved such as TNF and IL-1β. Based on these observations we hypothesized that local mast cell activation in the skin and the release of its mediators can induce LC migration into the draining LN to initiate an immune response.

In the next chapter we will provide the reader with our experimental procedures to examine our hypothesis. In chapter 3, we examine the ability of IgE-mediated mast cell activation to induce LC migration from the skin into the draining LN. These results were published in the Journal of Immunology (Jawdat et al., 2004). In chapter 4, we examine the role of mast cells in inducing LC maturation and migration in response to the bacterial product PGN from *S.*

aureus. In this study we also examine the potential mechanisms responsible for the responses we have observed. These studies are currently submitted for publication. Overall, these studies have provided an opportunity to take a number of *in vitro* observations on DC and mast cell interactions and examine their importance *in vivo*.

CHAPTER 2

MATERIALS AND METHODS

2.1 Mice

C57BL/6 mice, genetically mast cell-deficient WBB6F$_1$-*W/W^v* (*W/W^v*) mice and their normal congenic WBB6F$_1$-+/+ (+/+) mice, TLR4 deficient C57BL/10ScNJ mice and their wild type control C57BL/10J, C3 deficient B6;129S4-C3 mice and their littermates, genetically TNF deficient B6;129S6-TNF mice and their wild type control B6;129SF2 mice, CD14 deficient mice (Jackson Laboratory, Bar Harbor, Maine) and TLR2 deficient mice backcrossed on to a C57Bl/6 background (a kind gift from Dr. S. Akira, Osaka, Japan) were used. All mice were males, 6-10 weeks of age. Mice were housed under conventional conditions with food and water provided *ad libitum*. All experiments were approved by the animal research ethics boards of Dalhousie University.

2.2 Induction of local Skin Reactions Using IgE/Antigen

Mice received an intradermal (i.d.) injection into the ear pinna of 70μg purified mouse anti-TNP IgE prepared from the B cell line TIB-141 (ATCC, Manassas, VA, USA) in 25μl of phosphate buffer solution (PBS), mouse anti-dansyl IgE (Pharmingen, San Diego, CA) in 25μl PBS or diluent control (as stated). After a resting period, to allow excess IgE to dissipate (7-14 days), mice were challenged with an intravenous (i.v.) injection of 5mg/ml trinitrophenyl BSA (TNP-BSA) (Biosearch Technologies, Inc. CA, USA) in 250μl of saline. Mice were sacrificed and their ear pinnae were removed after 18h or draining auricular LN excised after 24h. For experiments using locally mast cell reconstituted *W/Wv* mice both ear pinnae were injected with anti-TNP IgE 14 days prior to i.v. injection of TNP-

BSA. In further experiments, one ear pinna was injected with anti-TNP IgE and the other with anti-dansyl IgE 14 days prior to i.v. TNP administration. Some animals were also examined 14 days after i.d. IgE injection, but in the absence of i.v. TNP-BSA administration.

2.3 Examining The Role of Histamine Receptors

To determine the role of histamine H1 and H2 receptors in the IgE-mediated response, 14 days following IgE or control injection in the ear pinnae mice were treated with either the H1 antagonist pyrilamine 50mg/kg i.p. or the H2 antagonist cimetidine 50mg/kg i.p (Sigma, St. Louis, Mo,USA). The doses of histamine receptor antagonists used were based on previous reports of a single dose *in vivo* used in mice (Chen et al., 2001; Jin et al., 1986; Kurihara et al., 2003). 90 minutes later the animals were injected i.v. with TNP-BSA and responses evaluated as described below following harvest of LN cells 24h later.

To determine the role of histamine H1 and H2 receptors in the PGN-mediated response, PGN or control diluent were injected in to the ear pinnae 90 minutes before either H1 antagonist pyrilamine 50mg/kg i.p. or the H2 antagonist cimetidine 50mg/kg i.p. were injected. 18h later mice were sacrificed and LNs were harvested and responses were evaluated as described below.

2.4 Examining The Role of Serotonin

To determine the role of serotonin (5-HT), 14 days following IgE or control injection in the ear pinnae mice were injected i.p. with 1.6mg/kg of ketanserin, a 5-HT receptor antagonist (RBI, ON, Canada). 90 minutes later the animals were

injected i.v. with TNP-BSA and responses evaluated as described below following harvest of LN cells 24h later.

2.5 Induction of Local Responses to Bacterial Products

To examine the effect of lipopolysaccharide (LPS) or peptidoglycan (PGN), mice received an intradermal (i.d.) injection into the right ear pinna of 0.25μg of *Escherichia coli* LPS serotype 055:B5 (Sigma, St. Louis, MO) or 2.5μg purified *Staphylococcus aureus* derived PGN (Fluka BioChemika distributed by Sigma St. Louis, Mo, USA) in 25μl and as a control 25μl of diluent in the left ear pinna. After 18h mice were sacrificed and their draining auricular LN were excised. A LN derived single cell suspension (LNC) was prepared, counted, washed and resuspended in RPMI 1640 medium. Either cytocentrifuge preparations were made or flow cytometric analysis of the LNC performed as described below. For experiments using locally mast cell reconstituted *W/W^v* mice both ear pinnae were injected with PGN.

2.6 Mast Cell Reconstitution

Bone marrow-derived mast cells (BMMCs) were generated from the bone marrow of WBB6F$_1$-+/+ (+/+) mice according to the method of Tertian *et al.* (Tertian et al., 1981), a minimum of 98% pure cells were used. *W/Wv* mice were locally reconstituted as previously described (Nakano et al., 1985). Cells derived from +/+ mice ($0.5x10^6$) were locally injected into the right ear pinna of *W/Wv* mice and allowed to mature for 10 weeks.

2.7 Preparation and Analysis of Epidermal Sheets

Epidermal sheets were prepared as previously described by Baker *et. al* (Baker et al., 1983). Briefly, mice were sacrificed and their ears were split into dorsal and ventral halves by forceps. The dorsal halves were incubated for 2h at 37°C with 10mM disodium ethylenediamine tetraacetic acid (BDH, Inc., Toronto, Canada) dissolved in PBS. Ears were washed with PBS, and then under a dissecting microscope the epidermis was pealed off using forceps. The epidermis was then fixed in cold acetone (–20°C) for 2 minutes, washed in PBS, and stored at 4°C until immunohistochemical staining.

2.8 Preparation of Auricular Lymph Node Cells

After the mice were challenged with TNP-BSA, the draining (auricular) LNs were excised and, in some experiments (as stated), pooled for each animal. A LN derived single cell suspension (LNC) was prepared, washed and resuspended in RPMI 1640 medium. Viable cell counts were performed using trypan blue, and the cell concentration was adjusted to $1x10^6$ cells/ml. Cytocentrifuge preparations of the LNC were made and cells were stained with anti-Langerin/CD207 or control antibody according to the immunohistochemical procedure described below.

2.9 Immunohistochemistry and Cell Counts

Immunostaining was preformed on epidermal skin sheets, LN frozen sections, and on cytocentrifuge preparation of LN cell suspensions. Following fixation, sheets

or slides were washed with PBS, and incubated with 3% BSA (Boehringer Mannheim, Germany) for 1h. Sheets or slides were then incubated with either anti-Langerin antibody/CD207, clone 959F3 rat IgG1 (DCGM4) (0.75μg/ml) (courtesy of Dr. S. Saeland, Schering-Plough, France) or in some experiments (as stated) with an anti-mouse MHC class II antibody MRC OX-3 (Cedarlane, Hornby, ON, Canada) (12.5μg/ml) or an isotype matched control antibody for 16-18h at room temperature. Sheets or slides were washed and incubated with biotinylated goat anti-mouse antibody, then detected using streptavidin horseradish peroxidase (DAKO LSAB-2 System, Mississauga, Ontario) and aminoethylcarbazole (AEC) substrate (Zymed Laboratories, Inc. San Francisco, USA). A randomly chosen 10 fields ($0.25mm^2$) of epidermal sheets were counted and at least 2000 cells were assessed/slide for examination of cell suspensions. All counts were performed on coded slides with the observer being unaware which slides belonged to which experimental group.

2.10 Flow Cytometry

Cells were washed twice with 2% FBS/PBS, fixed with 1% PFA/PBS for 30 minutes and washed with 2% FBS/PBS. The cells were permeabilized with 0.1% saponin/2% FBS/PBS for 20 minutes and stained with anti-Langerin (CD207) Ab, anti-fascin Ab or an isotype control for 20 minutes. Following staining the cells were washed twice by gentle shaking in 0.1% saponin/2% FBS/PBS for 5 minutes before centrifugation. Cells were incubated with the secondary Ab, an FITC-labeled mouse anti-rat IgG Ab and a PE-labeled goat anti-mouse IgG Ab for 20

minutes, followed by the same washing procedure. To block free Ab binding sites, the cells were incubated with 5% rat serum and 20μg/ml hamster IgG. Cells were washed twice with 0.1% saponin/2% FBS/PBS, once with 2% FBS/PBS and then stained for cell surface markers with a PE-conjugated anti-CD86, CD40 or isotype control as well as an APC-conjugated CD11c or isotype control. Finally, the cells were washed twice with 2% FBS/PBS. The specificity of Langerin/CD207 staining was confirmed using isotype control Ab in place of anti-Langerin/CD207, no positively stained DC were observed under these conditions (data not shown). For flow cytometric analysis 3 $x10^4$ cells were acquired with a FACScalibur (BD Bioscience Pharmingen, CA, USA) (BD) and analyzed with Cellquest (BD). All antibodies were purchased from (BD) except for anti-fascin, which was purchased from Dako Cytomation (Mississauga, ON, Canada) and the PE-labeled goat anti-mouse IgG Ab which was purchased from Molecular Probes (Leiden, Netherlands).

2.11 Evaluation of Anti-TNP Antibody Responses

Serum antibody responses against TNP were evaluated by ELISA. Briefly, the wells of ELISA plates were coated with TNP-KLH (Calbiochem) and non-specific binding blocked by incubation with 1% BSA. A series of dilutions of serum samples from immunized and non-immunized mice were added to the washed plate and incubated for 16-18h. Binding of antibodies was detected using a biotinylated goat anti-mouse IgG/IgA/IgM antibody (Cedarlane) followed by a commercial amplification substrate system (Life Technologies).

2.12 Statistical Methods

Following confirmation of appropriate data distribution, differences between left and right epidermal sheets or left and right LNs were evaluated using a paired Student's t-test. Differences between groups of animals were assessed using an unpaired Students's t-test.

CHAPTER 3

"IGE-MEDIATED MAST CELL ACTIVATION INDUCES LANGERHANS CELL MIGRATION *IN VIVO*"

3.1 Introduction

In atopic individuals, IgE antibodies are observed to a large number of environmental antigens (Leung, 1993), which sensitize mast cells and basophils, for subsequent responses to allergen. Sensitization to environmental antigens is dependent upon effective antigen presentation within the draining LNs, a process in which the migration of resident tissue dendritic cells plays a key role.

Langerhans cells (LC) are immature dendritic cells found in the epidermal layer of the skin. They are characterized by the presence of Birbeck granules, expression of high levels of MHC class II and a characteristic dendritic morphology. Recently, Langerin/CD207, a transmembrane C-type lectin and an unconventional endocytic receptor associated with the formation of the Birbeck granules, has been identified as a LC marker (Valladeau et al., 2002). LC act as sentinels for immune responses by capturing antigen in the epidermis in response to appropriate signals, and by maturing and migrating into the draining LN where they become very potent in antigen presentation (Banchereau and Steinman, 1998; Streilein and Grammer, 1989). A number of cytokines are important inducers of LC maturation and/or migration including GM-CSF (Heufler et al., 1988), TNF and IL-1β (Cumberbatch et al., 1997c; Roake et al., 1995). Recombinant TNF and IL-1β induce a significant migration of LC from the epidermis and a consequent accumulation of dendritic cells in the draining LN (Cumberbatch et al., 1997a; Cumberbatch et al., 1994; Cumberbatch and Kimber, 1992).

Mast cells are also present in the skin and are mostly studied in the context of allergy, but also have an important role in host defence. They express high affinity FcεRI and IgE-mediated activation leads to the release of multiple preformed, newly formed and lipid mediators. Preformed mediators, include histamine and granule-associated TNF, which induce dendritic cell maturation (Caron et al., 2001b; Mazzoni et al., 2001). Mast cells produce a number of cytokines in the hours following IgE-mediated activation with known effects on dendritic cells including TNF, IL-1β and GM-CSF (Burd et al., 1989; Gordon and Galli, 1990; Plaut et al., 1989). CD40 ligand, is also expressed by mast cells, and is important in regulating dendritic cell migration *in vivo* (Moodycliffe et al., 2000). Exosomes from IgE-activated human mast cells have recently been shown to induce human dendritic cell maturation *in vitro* (Skokos et al., 2003). IL-1β, TNF and GM-CSF have all been clearly demonstrated to have the ability to induce dendritic cell maturation *in vivo* or *in vitro* (Cumberbatch et al., 1997c; Cumberbatch et al., 1994; Cumberbatch and Kimber, 1992; Heufler et al., 1988; Rupec et al., 1996). A study by Alard *et al.* suggested that mast cell derived TNF might inhibit contact sensitization through effects on dendritic cells in mice (Alard et al., 1999). A recent report has also demonstrated, that mast cell derived TNF is primarily responsible for LN swelling following bacterial or compound 48/80 induced mast cell activation (McLachlan et al., 2003), while histamine did not contribute significantly to this response. Dendritic cells have, however, been shown to express H1, H2 and H3 receptors and to undergo significant

maturational changes as a result of histamine treatment (Caron et al., 2001a; Caron et al., 2001b; Gutzmer et al., 2002; Idzko et al., 2002).

Herein, we examined the role of mast cells in LC migration from the skin to the draining auricular LN using a mouse model. We demonstrate that local IgE-mediated mast cell degranulation provides an effective signal for LC migration to the draining LN *in vivo,* in the absence of additional local antigen or Toll-like receptor activator administration.

3.2 Results

3.2.1 Mast cell deficient mice exhibit a normal distribution of LC in the epidermis

The consequences of functional c-kit deficiency on the normal distribution of LC in the skin of *W/Wv* mice were examined to confirm the appropriateness of this mouse model for these studies. The number of Langerin/CD207 positive cells in the epidermis of untreated mast cell deficient *W/Wv* mice was compared to those in the wild type control +/+ mice. No significant differences in LC distribution or morphology were observed (Figure 3.1).

3.2.2 IgE-mediated mast cell activation induces a reduction of LC in the skin and an increase in Langerin/CD207 positive dendritic cells in the draining LN

IgE-mediated mast cell activation was employed to selectively activate mast cells in one ear pinna. C57BL/6 mice were injected with anti-TNP IgE antibody into the right ear pinna and saline into the left ear pinna 14 days prior to antigen challenge with an i.v. injection of TNP-BSA. After a further 18h time, selected on the basis of pilot experiments, the number of LC in the epidermal sheets were assessed. A significant ($p<0.05$) reduction in the number of Langerin/CD207 positive LC was consistently observed in the right (IgE-injected) ears of the mice compared to their left (control) ears (Figure 3.2.A and B). These results were confirmed using MHC class II as a second marker of LC (data not shown). The mean reduction in LC in the IgE injected skin, compared with the control skin

Figure 3.1 Mast cell deficient W/Wv mice exhibit a normal distribution of LC in the epidermis. Immunohistochemical staining of LC using anti-Langerin/CD207 in ear pinna epidermal sheets obtained from mast cell deficient W/Wv mice (A) compared to (B) their wild type control +/+ mice. (C) The mean $\pm$ SEM numbers of LC (n=4). Note similar numbers of LC and similar distribution in W/Wv compared to wild type control +/+ mice.

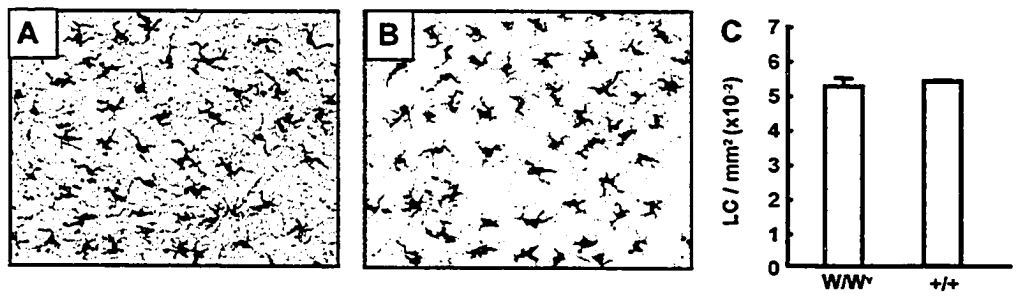

Figure 3.1

Figure 3.2 LC numbers decrease in the epidermis and increase in the draining LN following IgE-mediated mast cell activation. Saline or anti-TNP IgE were given i.d. in ear pinnae 1 week prior to the i.v. TNP-BSA injection and tissues harvested after 18h for examination of ear tissue or 24h for LN. All tissues were stained using anti-Langerin/ CD207 antibody. **(A)** LC in epidermal sheet of saline injected mouse ear pinna. **(B)** Anti-TNP IgE injected mouse ear pinna. **(C)** LC density in epidermal sheets from individual mice (n=6). **(D)** Mean ± SEM number of total cells/draining LN. Frozen section of draining LN from the nodes draining the **(E)** saline injected side and **(F)** anti-TNP IgE injected side. **(G)** Mean ± SEM numbers of Langerin/CD207 positive dendritic cells/draining LN. **(H)** Cytocentrifuge preparation of draining LN cell suspension showing Langerin/CD207 positive dendritic cells. * represents $P<0.05$

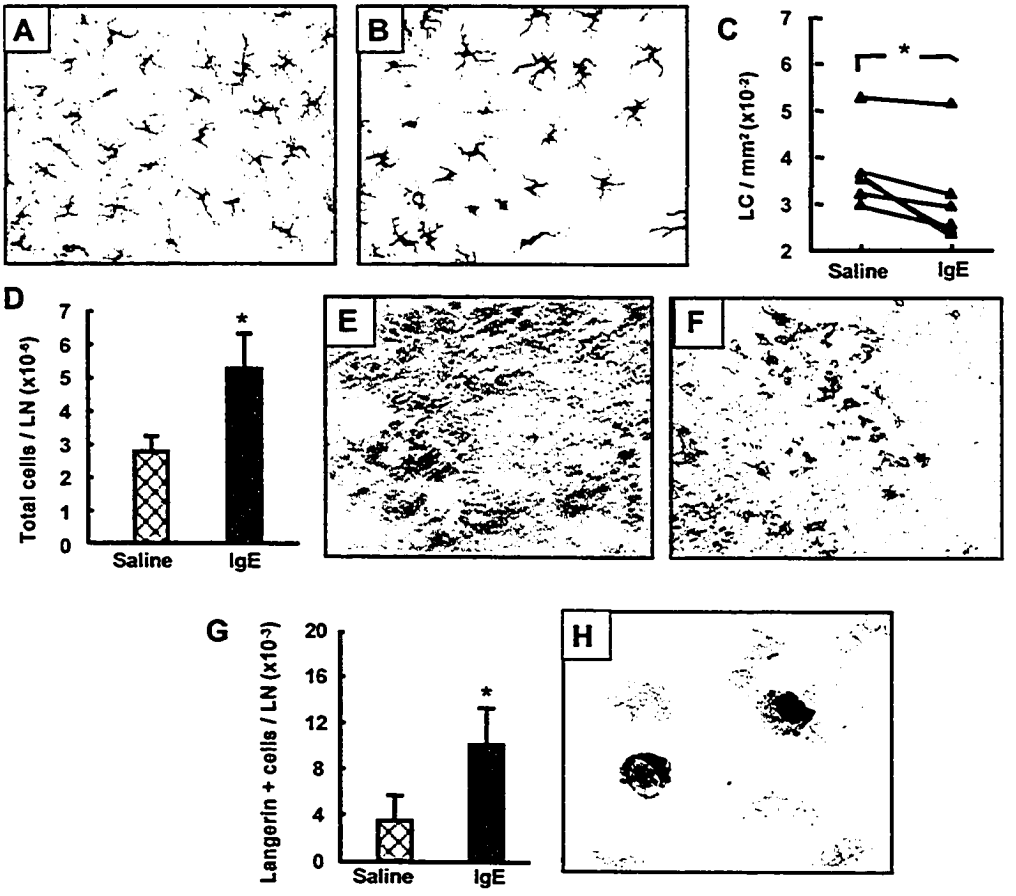
A
B
C
LC / mm² (x10⁻²)
7
6
5
4
3
2
*
Saline
IgE
D
Total cells / LN (x10⁻⁶)
7
6
5
4
3
2
1
0
*
Saline
IgE
E
F
G
Langerin + cells / LN (x10⁻³)
20
16
12
8
4
0
*
Saline
IgE
H

Figure 3.2

was 16.4% (Figure 3.2.C) using Langerin/CD207 staining and 16.7% using MHCII staining.

In separate animals, the auricular LNs draining ear pinnae that had been previously sensitized with anti-TNP IgE (as above) or with saline as control were excised 24h following i.v. antigen challenge and the total cellularity and number of Langerin positive dendritic cells determined. A 2.1 fold increase ($p<0.02$) was observed in the total cell numbers recovered from nodes draining sites of IgE-mediated mast cell activation compared to control sites (Figure 3.2.D).

The decrease in LC at skin sites of IgE-mediated activation was mirrored by an increase in the number of Langerin positive dendritic cells within the draining LNs (Figure 3.2.E and F). A 2.3 fold increase ($p<0.05$) was observed in the number of Langerin/CD207 positive cells/LN draining sites of IgE-mediated activation compared to control sites (Figure 3.2.G and H). The specificity of Langerin/CD207 staining was confirmed using isotype control antibody in place of anti-Langerin/CD207, no positively stained dendritic cells were observed under these conditions (data not shown).

3.2.3 LC migration in *W/Wv* mast cell deficient mice

To determine the role of mast cells in IgE-mediated LC migration, both mast cell-deficient W/Wv mice and their wild type control +/+ mice were injected with anti-TNP IgE antibody and challenged with i.v. antigen as described above. No significant decrease in the numbers of Langerin positive cells was observed in the IgE injected ear pinnae of W/Wv mice (Figure 3.3.A). However, a small but

Figure 3.3 Mast cells are required for changes in LC distribution following local IgE-mediated activation. Genetically mast cell-deficient W/Wv mice, and normal congenic +/+ (mast cell containing) mice received a saline injection as a control in one ear pinna and an anti-TNP IgE injection in the contralateral ear pinna. 7 days later, mice were injected with TNP-BSA i.v. and the numbers of their LC examined after 18h using anti-Langerin/CD207 immunohistochemistry. The density of LC stained with anti-Langerin in epidermal sheets from both W/Wv mice (A) (n=8), and from +/+ mice (B) (n=6) was determined. Cells from the draining LN were also examined 24h after the i.v. challenge. Numbers of total cells/draining LN from W/Wv mice (C) (n=10) and +/+ mice (D) (n=10). Numbers of Langerin/CD207 positive dendritic cells/draining LN from W/Wv mice (E) (n=10) and from +/+ mice (F) (n=10). All numbers represent the mean values ± S.E.M. * represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$.

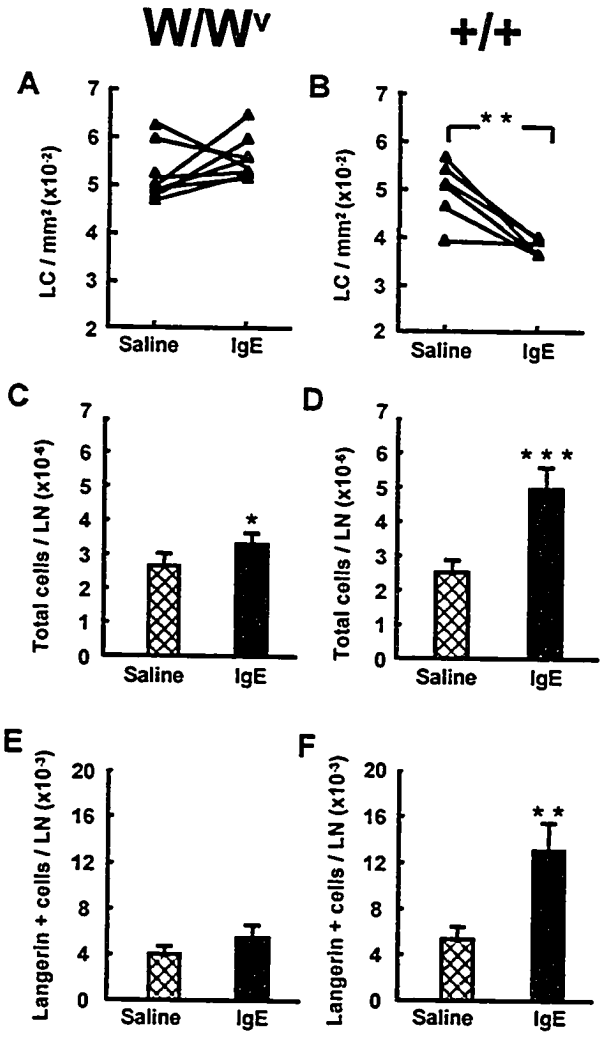
W/Wv
+/+
A
B
C
D
E
F
LC / mm² (x10⁻²)
Total cells / LN (x10⁻⁴)
Langerin + cells / LN (x10⁻³)
Saline
IgE
*
**
***

Figure 3.3

consistent increase in the number of Langerin/CD207 positive LC cells was observed. In control +/+ mice, a 23% decrease in Langerin/CD207 positive LC cells ($P<0.01$) was observed in the IgE injected ear pinna compared to the control site (Figure 3.3.B). Consistent with these data, only a 25% increase ($P<0.05$) in total LN cellularity was observed in *W/Wv* mice in the LNs draining the IgE activated ear pinnae compared with controls (Figure 3.3.C). In contrast, a 2 fold increase ($P<0.0001$) in total cellularity was observed in +/+ mice in the LNs draining the IgE activated side compared to control (Figure 3.3D). No significant increase was observed in the numbers of Langerin/CD207 positive cells in the LN of *W/Wv* mice (Figure 3.3.E). However, a 2.5 fold increase ($P<0.01$) in the numbers of Langerin/CD207 positive cells was observed in control +/+ mice (Figure 3.3.F). These observations suggested that the induction of LC migration following local IgE-mediated activation is mast cell dependent.

The anti-TNP antibody (IgG and IgM) response in *W/Wv* and control mice was determined 2 weeks after i.v. antigen injection in a separate set of animals (n=5/group). There was no significant difference in the anti-TNP titre between the mast cell deficient and mast cell containing mice (data not shown). These observations confirm the ability of mast cell deficient mice to mount a normal immune response following systemic antigen administration.

3.2.4 LC cells observed in draining LNs have undergone maturation

To examine the maturation status of LC within LNs draining sites of IgE-mediated mast cell activation a flow cytometric approach was employed. The dual

Langerin positive and CD11c positive cells were examined for their expression of the well-defined maturation markers CD86, fascin and CD40. LC at these sites were found to be on average (n=6) 85% CD86 positive, 87% fascin positive and 45% CD40 positive (data not shown). This marker profile is in keeping with a matured LC phenotype. The marker profile of LC was not significantly different between nodes draining sites of IgE mediated mast cell activation and control sites.

3.2.5 LC migration in *W/Wv* mice with local mast cell reconstitution

To more directly address the role of the mast cell, *W/Wv* mice were locally reconstituted by intradermal injection with BMMC in the right ear only. After a 10-week resting period, to allow mast cells to mature *in vivo,* mice were injected with anti-TNP IgE antibody in both ear pinnae. One week later, mice were challenged with TNP-BSA antigen i.v. and both epidermal sheets (18h) and auricular nodes (24h) were examined for their numbers of Langerin/CD207 positive dendritic cells. Examination of the epidermal sheets from the ear pinnae revealed that Langerin/CD207 positive cell numbers were significantly ($P<0.01$) decreased in the mast cell reconstituted site compared with non-reconstituted site (Figure 3.4.A). A significant ($P<0.01$) increase in total cellularity was observed in the LNs draining the mast cell-reconstituted ear pinna (Figure 3.4.B). A significant ($p<0.02$) increase in the number of Langerin/CD207 positive dendritic cells/LN was also observed at this site (Figure 3.4.C).

Figure 3.4 Local mast cell reconstitution restores LC migration. Mast cell deficient *W/Wv* mice were injected with cultured bone marrow derived mast cells in the right ear only. After 10weeks, both ears were injected with anti-TNP IgE and after a further 7 days, the mice were challenged with TNP-BSA i.v. After 18h the number of LC in the ear pinna epidermis for individual mice (n=5) was determined **(A)**. After 24h, cells from the draining auricular LN (n=5) were examined. **(B)** Numbers of total cells/draining LN. **(C)** Numbers of Langerin/CD207 positive dendritic cells/draining LN. All numbers represent the mean values ± S.E.M.* represents $P<0.05$, ** represents $P<0.01$.

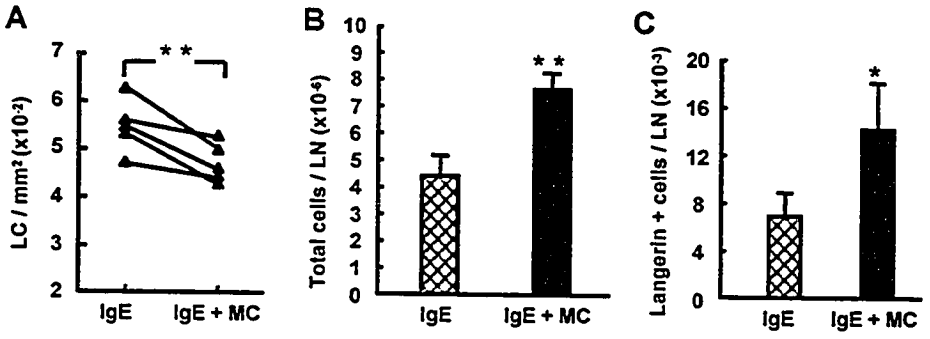
A
LC / mm² (x10⁻²)
7
6
5
4
3
2
* *
IgE
IgE + MC
B
Total cells / LN (x10⁻⁶)
10
9
8
7
6
5
4
3
2
1
0
* *
IgE
IgE + MC
C
Langerin + cells / LN (x10⁻³)
20
16
12
8
4
0
*
IgE
IgE + MC

Figure 3.4

3.2.6 LPS induces LC migration in mast cell deficient *W/Wv* mice

LPS is a known inducer of dendritic cell migration (Roake et al., 1995) via interactions with Toll-like receptors, but does not induce mast cell degranulation (Leal-Berumen et al., 1994). Both *W/Wv* mice and wild type control +/+ mice were injected with LPS into their right ear and saline into the left. After 18h, mice were sacrificed and LN examined. In the draining LN, there was a similar significant ($p<0.01$) increase in the number of total cells in the LPS draining node compared to the control in both *W/Wv* mice (2 fold) (Figure 3.5.A) and control +/+ mice (2.6 fold) (Figure 3.5.B). In addition, the numbers of Langerin/CD207 positive dendritic cells showed a significant ($p<0.05$ and $p<0.01$) increase in the sites draining LPS injected skin compared to control in both *W/Wv* mice (3.4 fold) (Figure 3.5.C) and +/+ mice (4 fold) (Figure 3.5.D).

3.2.7 Changes in LC numbers are dependent upon specific antigen administration

In order to investigate the mechanism of these responses in more detail we focused on the changes in Langerin positive cells and total cellularity observed in the auricular LNs draining sites of IgE-mediated mast cell activation. Murine LC do not express FcεRI (Hayashi et al., 1999), however they do express the low affinity FcεRII/CD23. There was no significant difference in the number of Langerin/CD207 positive cells found within the LNs draining IgE injected or control (saline injected) ear pinnae (Figure 3.6.A and B). As further confirmation that the substantial dendritic cell responses observed in the LNs were the result of

Figure 3.5 LPS induces similar levels of LC migration in *W/Wv* and control mice. Genetically mast cell-deficient *W/Wv* mice (*n*=5) and their normal congenic +/+ mice (*n*=5) received saline as a control in the left ear pinna and LPS (0.75μg) in the right ear pinna. 18h later, mice were sacrificed and the total number of cells and Langerin/CD207 positive dendritic cells in the draining auricular LN were examined. Mean ± SEM numbers of total cells/draining LN from *W/Wv* mice **(A)** and from +/+ mice **(B)**. Mean ± SEM numbers of Langerin$^+$ dendritic cells/draining LN from *W/Wv* mice **(C)** and from +/+ mice **(D)**. * represents $P<0.05$, ** represents $P<0.01$.

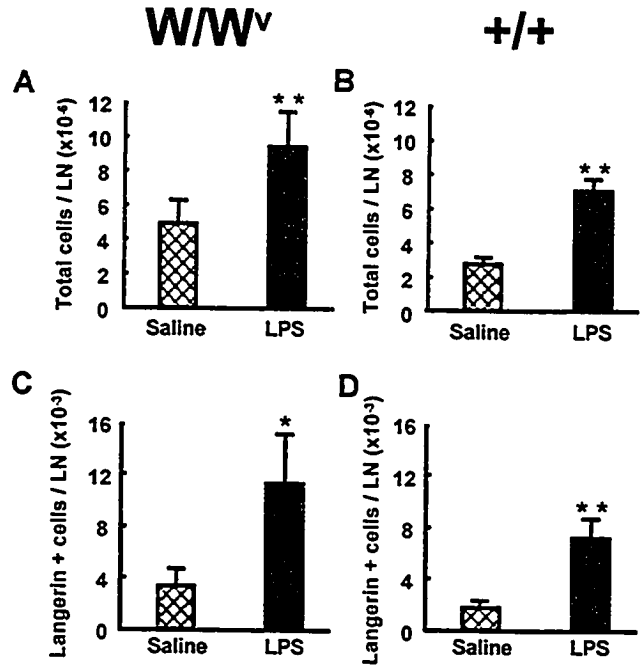
W/Wv
+/+
A
B
C
D
Total cells / LN (x10⁴)
Langerin + cells / LN (x10³)
0
2
4
6
8
10
12
16
Saline
LPS
*
**

Figure 3.5

Figure 3.6 Changes in LC numbers are antigen and specific IgE dependent. **A and B**, Saline or anti-TNP IgE were given i.d. in the ear pinnae alone without further antigen challenge. LN tissue were harvested at day 14 and total cells and Langerin/CD207 positive cells were counted. **(A)** Numbers of total cells per draining LN. **(B)** Numbers of Langerin/CD207 positive dendritic cells per draining LN. **C and D,** anti-dansyl IgE or anti-TNP IgE were given i.d. in the ear pinnae 14 days prior the i.v. TNP-BSA challenge injection. LN tissues were harvested and total cells and Langerin/CD207 positive cells were counted. **(C)** Numbers of total cells per draining LN. **(D)** Numbers of Langerin/CD207 positive dendritic cells per draining LN. All values represent groups of 6-8 animals, mean values ± S.E.M. * represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$.

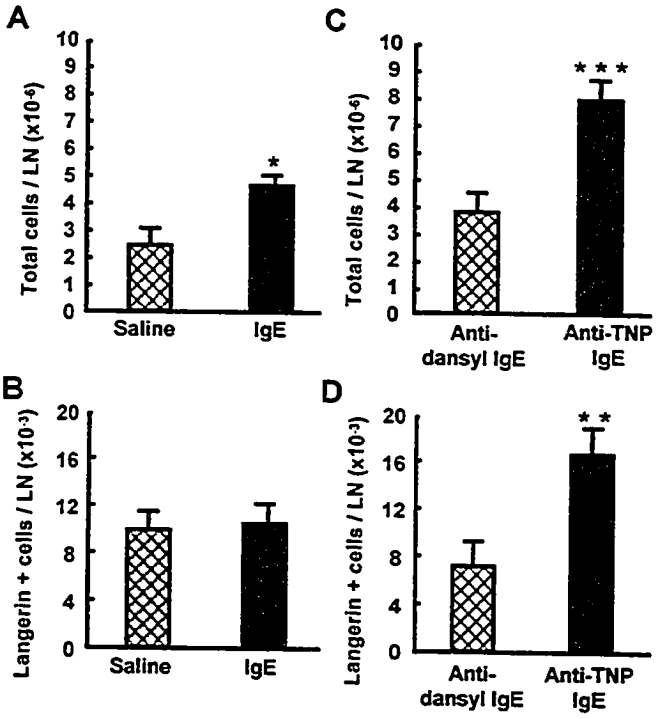
A
Total cells / LN (x10⁶)
10
9
8
7
6
5
4
3
2
1
0
*
Saline
IgE
C
Total cells / LN (x10⁶)
* * *
Anti-dansyl IgE
Anti-TNP IgE
B
Langerin + cells / LN (x10⁻³)
20
16
12
8
4
0
Saline
IgE
D
Langerin + cells / LN (x10⁻³)
* *
Anti-dansyl IgE
Anti-TNP IgE

Figure 3.6

antigen specific IgE-mediated mast cell activation, one ear pinna of a group of 6 C57Bl/6 mice was injected i.d. with anti-dansyl IgE while the other was injected with anti-TNP IgE. 14 days later the mice were injected i.v. with TNP-BSA and after 24h the draining auricular LNs were excised and examined for the number of Langerin/CD207 positive cells. Again, the LNs draining the site of IgE-mediated mast cell activation (injected with anti-TNP IgE) showed significantly increased numbers of Langerin/CD207 positive cells compared with the site that received anti-dansyl IgE injection alone (Figure 3.6.C and D).

3.2.8 Consequences of H1 and H2 histamine receptor blockade

In view of the known effects of histamine on dendritic cell maturation and function the consequences of pharmacologic blockade of either H1 receptors using pyrilamine or H2 receptors using cimetidine were examined. LNs draining sites of IgE-mediated mast cell activation were examined in C57Bl/6 mice that had been pre-treated with cimetidine, pyrilamine or with diluent (control) injection i.p. 90 minutes prior to i.v. antigen challenge of IgE sensitized mice (anti-TNP IgE 14 days prior to antigen injection). The numbers of Langerin/CD207 positive cells migrating to the LN draining sites of IgE mediated activation were similar in diluent treated animals and following H1 blockade (Figure 3.7.B and D). In marked contrast, following H2 blockade, increases in Langerin/CD207 positive cells in the auricular node draining sites of IgE-mediated activation, were almost completely blocked (Figure 3.7.F) (90.5% inhibition compared with diluent treated control group). The increase in total LN

Figure 3.7 Histamine effect through H2 receptor is required for LC migration. Saline or anti-TNP IgE were injected i.d. in the ear pinnae. After 7 days mice were injected i.p. with water (diluent control), pyrilamine (H1 antagonist), cimetidine (H2 antagonist) or a combination of cimetidine and pyrilamine 90 minutes prior to i.v. TNP-BSA antigen challenge injection. LN tissues were harvested and total cells and Langerin/CD207 positive cells were counted. **(A)** Numbers of total cells/draining LN in diluent control treated animals. **(B)** Numbers of Langerin positive dendritic cells/draining LN of diluent control treated animals. **(C)** Numbers of total cells/draining LN of pyrilamine treated animals. **(D)** Numbers of Langerin/CD207 positive dendritic cells/draining LN of pyrilamine treated animals. **(E)** Numbers of total cells/draining LN of cimetidine treated animals. **(F)** Numbers of Langerin/CD207 positive dendritic cells per draining LN of cimetidine injected animals. **(G)** Numbers of total cells/draining LN of cimetidine and pyrilamine treated animals. **(H)** Numbers of Langerin/CD207 positive dendritic cells/draining LN of cimetidine and pyrilamine treated animals. All values represent groups of 6-12 animals, mean values ± S.E.M. * represents $P<0.05$, ** represents $P<0.01$, *** represents $P<0.001$.

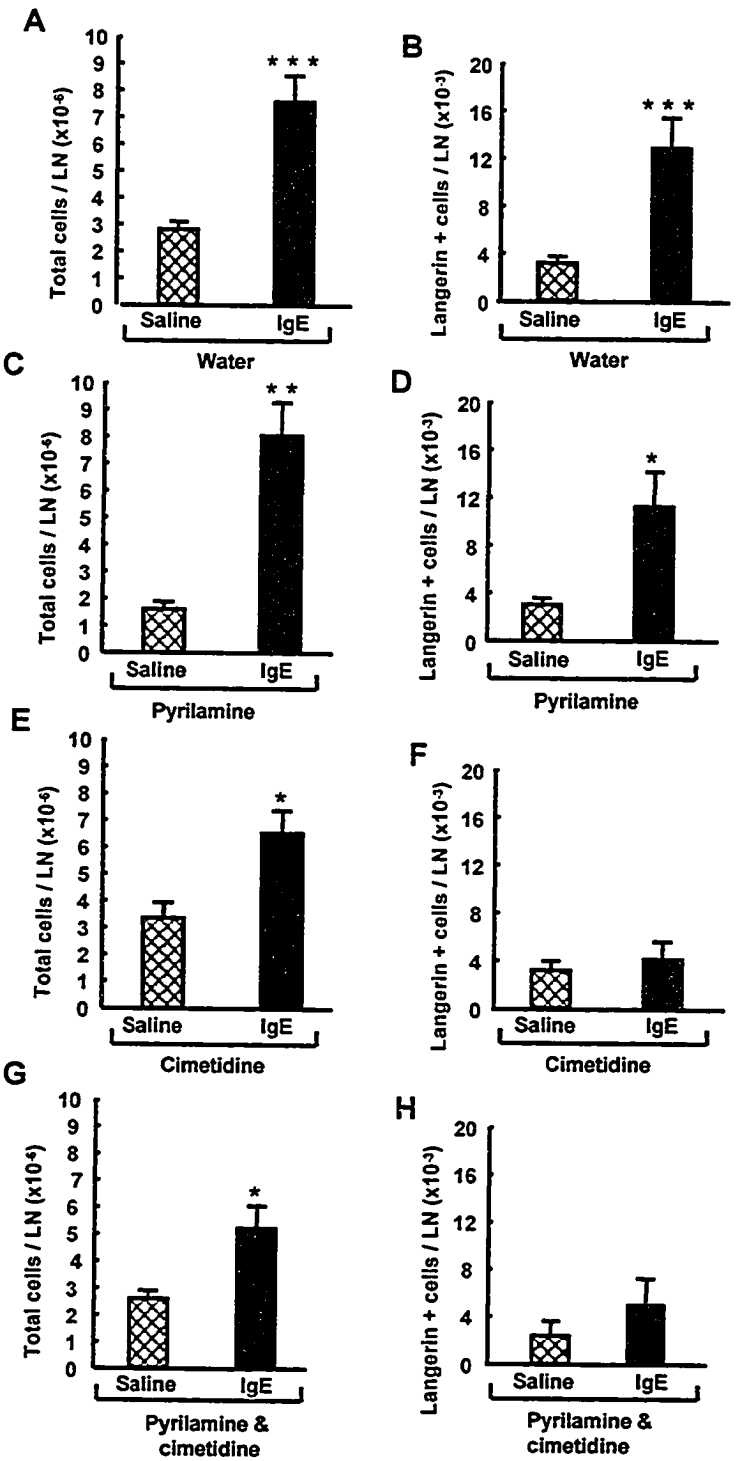
A
Total cells / LN (x10⁶)
Saline
IgE
Water
B
Langerin + cells / LN (x10⁻³)
Saline
IgE
Water
C
Total cells / LN (x10⁶)
Saline
IgE
Pyrilamine
D
Langerin + cells / LN (x10⁻³)
Saline
IgE
Pyrilamine
E
Total cells / LN (x10⁶)
Saline
IgE
Cimetidine
F
Langerin + cells / LN (x10⁻³)
Saline
IgE
Cimetidine
G
Total cells / LN (x10⁶)
Saline
IgE
Pyrilamine & cimetidine
H
Langerin + cells / LN (x10⁻³)
Saline
IgE
Pyrilamine & cimetidine

Figure 3.7

cellularity, as a result of IgE-mediated mast cell activation, was not significantly inhibited by cimetidine pre-treatment of the animals. The effects of combined cimetidine and pyrilmaine treatment were similar to those of cimetidine alone (Figure 3.7.G and H).

To further evaluate the ability of histamine alone to mimic the effects of local mast cell degranulation of LC, separate groups of C57Bl/6 mice were injected i.d. with 2.75μg or 27.5μg of histamine. 24h later the presence of LC in the draining LNs were evaluated. No significant LC increase in the draining LNs was observed following such histamine injection compared with diluent injected controls (data not shown).

Serotonin is also found in large amounts in rodent mast cells. In order to examine the ability of this mediator to participate in the observed LC response, the serotonin receptor antagonist ketanserin was employed. Ketanserin was completely ineffective in blocking LC migration in response to mast cell activation when employed at *in vivo* doses that have been shown to be highly effective in blocking other serotonin dependent events (Matsuda et al., 1997). Ketanserin was injected 90 minutes before antigen challenge, and both the number of total cells and Langerin positive cells were determined. Total cells in the IgE injected side were $5.6x10^6$ cells verses $3.2x10^6$ cells in the control side. Langerin positive cells were $17x10^3$ cells in the IgE injected side verses $8.3x10^3$ cells in the control injected side with a p value <0.0001.

3.3 Discussion

This study directly demonstrates the role of mast cells *in vivo* in the regulation of LC migration. Both decreases in LC in the epidermis following IgE/antigen challenge and increases in Langerin/CD207 positive cells in the draining auricular LNs were mast cell dependent using the *W/Wv* mast cell deficient mouse model. Results of experiments using the H2 receptor blocker cimetidine strongly implicate histamine as the major mast cell mediator involved via interaction with the H2 receptors known to be present on dendritic cells.

Several different approaches confirmed that changes in LC populations were the result of mast cell activation and not IgE administration alone or IgE/antigen interactions on other cell types. First, when *W/Wv* mice were selectively reconstituted in one ear pinna with mast cells and then injected in both ear pinnae with IgE, prior to i.v. antigen challenge a significant ($p<0.01$) difference was observed between the mast cell containing site and the non-reconstituted site. Both a decrease in Langerin/CD207 positive cells in the epidermal sheets where mast cells were present and an increase in Langerin/CD207 positive cells in the draining auricular LN were observed when compared with the mast cell deficient ear sites within the same animals (Figure 3.4). Second, we confirmed that IgE injection into the ear pinna alone, in the absence of i.v. antigen challenge did not induce changes in the number of Langerin/CD207 positive cells within the draining LNs when compared with saline injected control ears in the same animals (Figure 3.6.B). As a further

control for potential effects of IgE injection alone on LC migration, some animals were injected in both ears with IgEs of differing specificity. When antigen was injected i.v., only the LC populations from sites injected with IgE of appropriate specificity were observed to substantially migrate from the ear epidermis to the draining node (Figure 3.6.D).

It is notable that the observed changes in Langerin/CD207 positive cells within the epidermal sheets were lower than might have been expected given the substantial increases in Langerin/CD207 positive cells within the draining auricular node. The scale of change in both the epidermal site and the draining node are consistent with that observed by others using direct injection of TNF and/or IL-1β into the skin (Cumberbatch et al., 1997a; Cumberbatch et al., 1994). The small overall reduction in LC numbers may, in part, be explained by recruitment of new LC to the site of mast cell activation (Cyster, 1999). Henry *et al.* have demonstrated that the numbers of Langerin positive cells in LN draining the skin are normally regulated by a steady state of migration, consistent with the presence of some Langerin/CD207 positive cells in the control LN (Henri et al., 2001). In addition, Stoitzner *et al.* have shown that Langerin/CD207 positive cells in the skin-draining LN are mostly mature (Stoitzner et al., 2003). Changes in LC numbers in both the ear epidermis and draining LN were consistent with IgE-mediated mast cell activation inducing LC migration in the context of a background level of continuous recruitment of immature LC to the skin and subsequent migration of cells to the draining LN. The appropriateness of the

specific auricular node selected for our study was confirmed by pilot experiments in which India ink was injected i.d. into the ear pinnae of mice (data not shown). This enabled us to isolate a single LN for study, reducing any contribution from other tissues to the changes in LC populations we examined. However, we cannot completely rule out the possibility that Langerin/CD207 positive cells from neighboring sites might contribute to the increase in Langerin/CD207 positive cells within the node.

The LC that migrated to draining LNs following IgE-mediated mast cell activation appear to have a similar phenotype to those found normally at this site and to have undergone some degree of maturation. A high percentage of Langerin/CD207 positive cells in these LNs were fascin and CD86 positive, a profile typical of a mature dendritic cell. It has been suggested that as LC mature in the LN they will lose their expression of Langerin/CD207 so that both the numbers of LC migrating to the draining nodes and the degree of maturity, we are reporting, might be underestimates.

The ability of LPS administration to induce LC migration in both *W/Wv* and control +/+ mice, to a similar extent, illustrates that mast cell activation is not required for LC migration in response to all stimuli. Although murine mast cells express the TLR4 receptor and have been shown to respond functionally by producing multiple cytokines in response to LPS (Ikeda and Funaba, 2003; McCurdy et al., 2003; Supajatura et al., 2002; Supajatura et al., 2001), the mast

cell contribution to LC mobilization, under our experimental conditions was not significant. These data also illustrate that the LC in *W/Wv* mice are capable of being mobilized normally following appropriate stimulation, further confirming that the functional c-kit deficiency in *W/Wv* mice has not substantially altered the ability of LC to migrate normally. It is also notable, that while changes in auricular LN cellularity in response to IgE/antigen mediated activation were highly mast cell dependent, LPS injection induced similar changes in LN cellularity in both mast cell containing and mast cell deficient mice. These data suggest that mast cell independent mechanisms for LN activation are mobilized following LPS injection in addition to the mast cell-derived TNF-dependent mechanisms that have been recently described (McLachlan et al., 2003).

The mechanism by which mast cells induce LC migration appears to be highly dependent upon histamine and H2 receptors. Our data demonstrates a critical role for H2 receptors in the migration of LC to draining LNs following IgE-mediated mast cell activation. It is notable, however, that H2 blockade was not effective as an inhibitor of mast cell dependent increases in total LN cellularity. These data are in keeping with recent studies suggesting that this process is mainly TNF-dependent (McLachlan et al., 2003). H1 blockade did not significantly alter total LN cellularity or LC migration to draining LNs at sites of IgE-mediated activation. However, there was a slight trend towards reduced LC numbers in the activated LNs, which may reflect some cross inhibition of H2 receptors by the relatively high doses of pyrilamine employed in this study.

Histamine up-regulates the expression of CD86, CD40, CD54, CD80 and MHC class II, as well as inducing pro-inflammatory cytokine and chemokine production by human immature dendritic cells through H1 and H2 receptors (Caron et al., 2001b). However, histamine alone does not up-regulate CCR7, which is thought to be crucial for dendritic cell migration and can be regulated by IL-1β or TNF (Forster et al., 1999; Yanagihara et al., 1998). While histamine is clearly required, it is probably not the only necessary mast cell derived factor involved in the process of mast cell-dependent LC mobilization. Indeed, administration of histamine alone into ear skin did not induce LC migration, suggesting a role for other factors induced from mast cells or neighboring cells as a result of IgE-mediated mast cell activation. One potentially important factor is TNF, which is found both preformed in mast cells and newly synthesized following activation (Gordon and Galli, 1990). Intradermal administration of TNF induces LC migration and dendritic cell accumulation in the draining LN in mice (Cumberbatch et al., 1994; Cumberbatch and Kimber, 1992). Other additional candidate inducers of mast cell mediated LC migration include IL-1β, GM-CSF and CD40 ligand, which have been implicated in dendritic cell migration or maturation and are expressed by activated human and rodent mast cells (Galli et al., 1989; Gauchat et al., 1993). Mast cell exosomes may provide many of these mediators, or additional signals and have been shown to up-regulate dendritic cell MHC class II, CD80, CD86, and CD40 molecules. Mast cell exosomes, containing histamine, also induce dendritic cells to become efficient antigen-

presenting cells, eliciting both IgG1 and IgG2a antibodies in mice following immunization with exosome-associated antigens (Skokos et al., 2003).

Complement activation has also been implicated as an important signal for mast cell activation and subsequent interaction with dendritic cells. Tsuji *et al.* have shown that C5 is required early for the elicitation phase of contact sensitivity and delayed-type hypersensitivity. C5 was required for macrophage chemotactic activity and the late production of IFN-γ (Tsuji et al., 1997). C5a has also been shown to induce serotonin release from mast cells which induces endothelial cells activation and Th1 cells extravasation (van Loveren et al., 1983). The design of the current studies largely preclude an important role for complement locally within one ear as compared with the contralateral ear. Mouse IgE, particularly when bound to FcεRI is not considered an effective complement activator. Free IgE has a relatively short (12h) half-life in rodents and is injected 7-14 days prior to i.v. antigen administration in our experiments.

The data reported here, do not directly implicate mast cells in the process of antigen presentation or contact sensitization, which has previously been implied (Askenase et al., 1983; Galli and Hammel, 1984). For example, Bryce *et al.* have shown that mast cells are required for contact sensitivity and dendritic cell reduction in the epidermis following hapten exposure (Bryce et al., 2004). However, our results indicate that LC migration to draining LNs can be induced by IgE-mediated mast cell activation. Further signals may then be required to

allow such LC to present antigen effectively. The process of mast cell induced LC migration, suggests a potential role in the perpetuation of chronic allergic disease. Once an IgE response to a specific environmental antigen has been established, subsequent exposure may lead to mast cell activation and release of mediators. Potentially, these mediators may enhance the migration of further local LC, carrying further environmental antigens to the draining LN. This process could increase the possibility that additional IgE responses would occur in response to common environmental antigens. Histamine has been demonstrated to enhance Th2 responses through effects on dendritic cells (Caron et al., 2001a). Our current studies demonstrate that mast cell derived signals, including histamine, also provide key stimuli for inducing LC migration *in vivo*. The importance of mast cell dependent mobilization of LC to both allergic sensitization and the rapid development of effective immunity to pathogens will be important topics for future studies.

CHAPTER 4

LANGERHANS CELL MIGRATION IN RESPONSE TO BACTERIAL PEPTIDOGLYCAN *IN VIVO* IS MAST CELL AND COMPLEMENT (C3) DEPENDENT

4.1 Introduction

Early innate and later adaptive immune responses are closely related and interdependent. One compelling example is the mechanisms whereby dendritic cells, necessary for many adaptive immune responses to develop, mature and migrate into the draining LN under the influence of potent signals provided by the innate immune response. Mast cells, complement activation and Toll-like receptors (TLR) have all been implicated in this process.

Mast cells have been widely studied in allergy, however, they also have a critical role as sentinel cells in host defence (Echtenacher et al., 1996a; Malaviya et al., 1996a). Strategically located at host-environmental interfaces, such as the skin, they produce a wide range of mediators including histamine, arachidonic acid derivatives and numerous cytokines and chemokines which can be released selectively depending on the stimuli (Burd et al., 1989; Plaut et al., 1989). Mast cells also express a wide range of immune receptors that aid in pathogen recognition, including TLR, complement receptors, CD48 and many others (reviewed in (Marshall and Jawdat, 2004)).

Langerhans cells (LC) are immature DC present in the skin. They express high levels of MHC class II and are characterized by the presence of Birbeck granules and characteristic dendritic morphology. Their major function is to capture antigen and, in response to appropriate signals, mature and migrate to the draining LN and present the antigen to T lymphocytes (Banchereau and Steinman,

1998; Streilein and Grammer, 1989). Recently, a monoclonal antibody (Ab) specific for LC (Langerin/207) was identified (Valladeau et al., 2002) and shown to enable the tracing of LC from the epidermis to the draining LN (Stoitzner et al., 2003). Since LC down-regulate Langerin following arrival in the LN. Langerin-positive DC within LN represent recent immigrant LC from the skin.

We have previously shown that LC migration from the skin into the draining auricular LN in response to a classic IgE mediated allergic immune response is mast cell dependent (Jawdat et al., 2004). IgE antibodies on the surface of mast cells have also been demonstrated to enhance contact sensitization to irrelevant antigens possibly as a result of mast cell effects on DC migration (Bryce et al., 2004). Mast cells have also been implicated in the process of LN activation following bacterial challenge (McLachlan et al., 2003).

Atopic dermatitis is a common chronic inflammatory skin disease. The skin of over 90% of atopic dermatitis patients is colonized with *Staphylococcus aureus* (*S. aureus)* a Gram positive bacteria, that may exacerbate the severity of the disease (Leung et al., 2004). The major cell wall component of this bacteria is peptidoglycan (PGN) which can activate innate immune responses via interaction with TLR2 (Schwandner et al., 1999). PGN has also been shown to activate the complement system through the classical, alternative and lectin pathways (Greenblatt et al., 1978; Ma et al., 2004; Verbrugh et al., 1979). Some studies have correlated increased numbers or activation of mast cells with the severity of

the atopic dermatitis (Damsgaard et al., 1997; Mihm et al., 1976), however their role in this disease is unclear. DCs have also altered function in atopic dermatitis. Sensitization to a large number of environmental antigens and very high serum IgE levels are commonly observed in this patient population (reviewed in (Leung et al., 2004)).

Cultured murine mast cells have been shown to respond to PGN from *S. aureus* via TLR2 leading to mast cell degranulation and the release of IL-4 and IL-5. In addition they facilitate increased neutrophil recruitment via a TLR2 dependent mechanism (Supajatura et al., 2002). In the current study, we examined the role of mast cells in inducing LC migration in response to *S. aureus* derived PGN. Our results demonstrate a critical role for both mast cells and complement activation in this process.

4.2 Results

4.2.1 PGN-induced LC migration is mast cell dependent

To determine the role of mast cells in LC migration, in response to *S. aureus* derived PGN, mast cell deficient *W/W^v* mice and mast cell containing +/+ mice were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18h, the draining auricular LNs were excised and the total cellularity and number of Langerin-positive DC were determined. In mast cell containing +/+ mice a significant increase in the number of Langerin-positive cells was observed in the LN draining the PGN-injected site compared to LN draining the saline injected side. In contrast, no increase in the number of Langerin-positive cells was observed in the LN draining the PGN-injected site compared to saline injected sites in *W/W^v* mice (Figure 4.1). The total number of cells within the draining LN increased in concert with the numbers of Langerin-positive DCs.

To more specifically address the role of mast cells in this response, mast cell deficient *W/W^v* mice were locally reconstituted by intradermal injection with BMMC in the right ear only. After a 10-week resting period, to allow mast cells to mature *in vivo*, mice were injected with PGN into both ear pinnae. After 18h, the ear pinna draining auricular LN were excised and the total cellularity and number of Langerin-positive DC were determined. A significant increase in the number of Langerin-positive cells was observed in the mast cell reconstituted site compared with non-reconstituted site confirming the requirement of mast cells to induce LC migration. Changes in total LN cellularity were also examined. The

Figure 4.1 PGN induced LC migration is mast cell dependent. Genetically mast cell-deficient *W/W^v* mice, and their normal congenic +/+ mice received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear. *W/W^v* reconstituted with mast cells in one ear pinna only received PGN injection into both ear pinnae. After 18h draining LN tissues were harvested and total cells and Langerin-positive cells were counted using anti-Langerin/CD207 immuno-histochemistry. A, Mean ± SEM numbers of Langerin/CD207 positive cells /draining LN. B, Mean ± SEM number of total cells/draining LN. * represents $P<0.05$

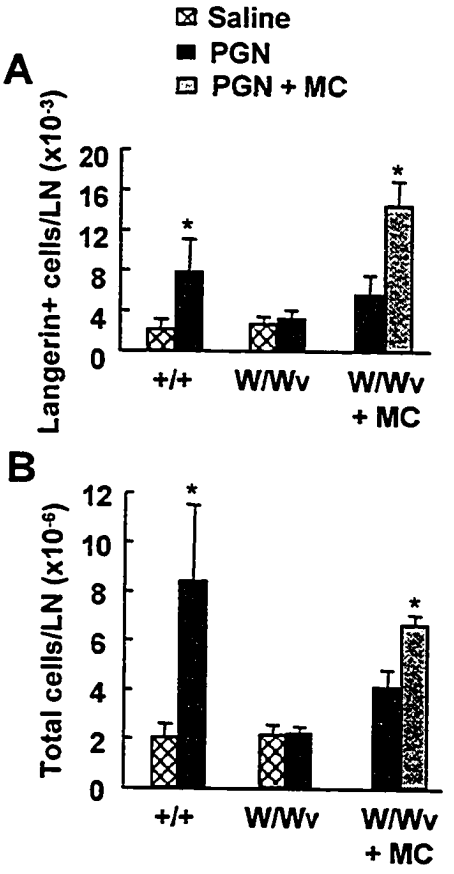
Saline
PGN
PGN + MC
A
Langerin+ cells/LN (x10-3)
20
16
12
8
4
0
+/+
W/Wv
W/Wv + MC
B
Total cells/LN (x10-6)
12
10
8
6
4
2
0
+/+
W/Wv
W/Wv + MC

Figure 4.1

increase in LN cellularity observed 18h after PGN administration was substantial and mast cell dependent.

4.2.2 PGN-induced LC migration is TLR2 and TLR4 independent but requires complement

To examine the contribution of TLR2 to the mast cell dependent increase in Langerin-positive DC in draining LN, C57BL/6 and TLR2 deficient mice (TLR2-/-) (Takeuchi et al., 1999) on a similar genetic background were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18h, the ear pinna draining auricular LNs were excised and the total LN cellularity and number of Langerin-positive DC were determined. Surprisingly, both TLR2-/- mice and their control C57BL/6 mice showed a significant increase in the number of Langerin-positive cells in the PGN injected side compared to saline injected side, with the TLR2-/- mice having a significantly ($p<0.001$) greater response to PGN. In view of the possibility that TLR4-mediated activation might contribute to the PGN response, both TLR4 deficient C57BL10ScNJ mice that have a gene deletion of TLR4 and their appropriate control C57BL/10J were injected with PGN into the right ear pinna and saline into the left ear pinna and examined as previously. Both TLR4 deficient and wild type control mice showed a similar significant increase in the number of Langerin-positive DC in the LN draining the PGN injected ear pinna compared to saline injected side. Notably, the total LN cellularity increases observed as a result of PGN administration were also

Figure 4.2 PGN induced LC migration is not TLR2 or TLR4 dependent but requires complement C3. C57BL/6, TLR2-/-, TLR4-/- and WT control, C3-/- and WT control received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear. After 18h draining LN tissues were harvested and total cells and Langerin positive cells were counted using anti-Langerin/CD207 flowcytometry. A, Mean ± SEM numbers of Langerin/CD207 positive cells /draining LN. B, Mean ± SEM number of total cells/draining LN. * represents $P<0.05$ ** represents $P<0.01$, *** represents $P<0.001$

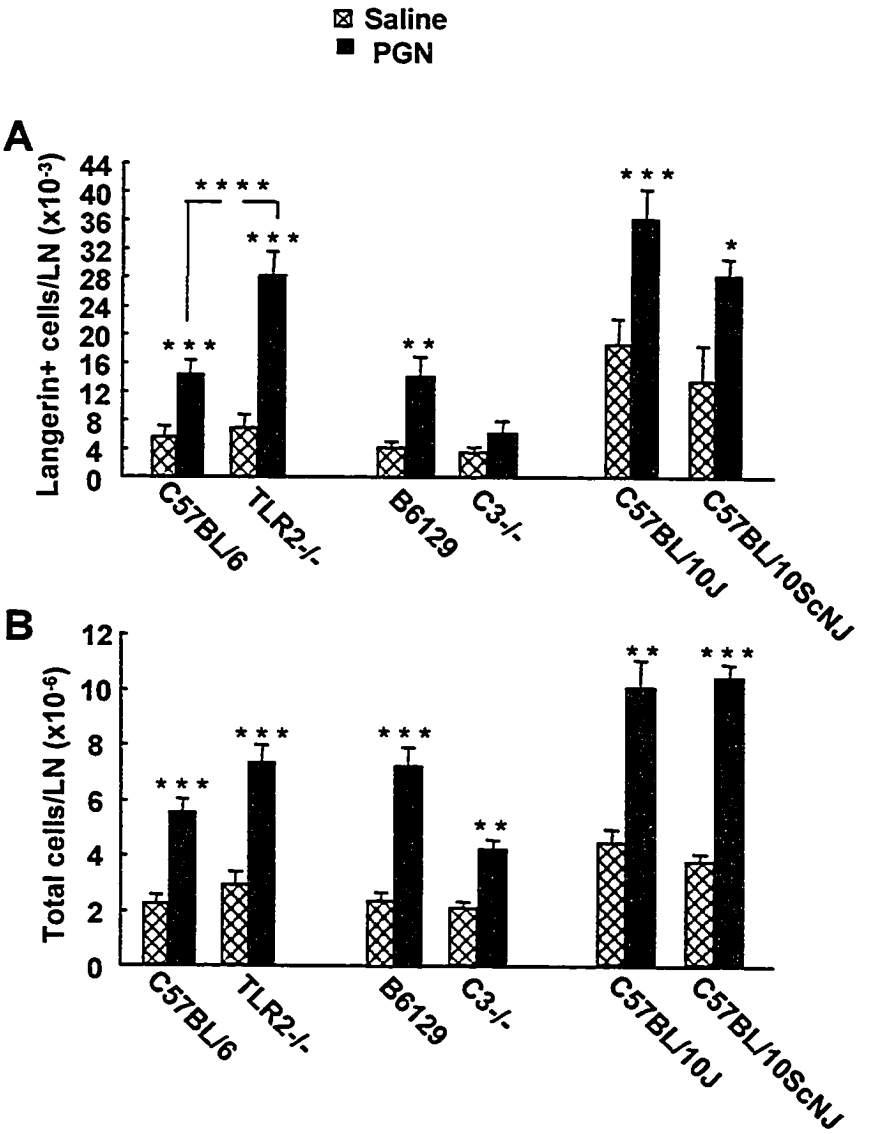
Saline
PGN
A
Langerin+ cells/LN (x10-3)
44
40
36
32
28
24
20
16
12
8
4
0
C57BL/6
TLR2-/-
B6129
C3-/-
C57BL/10J
C57BL/10ScNJ
B
Total cells/LN (x10-6)
12
10
8
6
4
2
0
C57BL/6
TLR2-/-
B6129
C3-/-
C57BL/10J
C57BL/10ScNJ

Figure 4.2

unchanged in TLR2 or TLR4 deficient animals when compared with control animals.

To examine the possibility of a contribution from complement activation by PGN, both C3-/- mice and their wild type control mice were injected with PGN into the right ear pinna and saline into the left ear pinna. After 18h, the ear pinna draining auricular LNs were excised and the total cellularity and number of Langerin-positive DC was determined. Interestingly, unlike their congenic controls C3-/- mice did not show a significant increase in the number of Langerin-positive cells in the LN draining the PGN injected ear pinna compared to the saline injected site (Figure 4.2). Notably, the increase in draining LN total cellularity was abrogated following PGN injection in C3-/- mice compared with control mice.

4.2.3 Histamine receptors H1 and H2 do not block LC migration in response to PGN

Langerhans cell migration in response to IgE mediated mast cell activation has been previously shown to be histamine dependent (Jawdat et al., 2004) therefore C57BL/6 mice were pretreated with either pyrilamine (H1 antagonist) , cimetidine (H2 antagonist) or diluent control 90 min before PGN and saline ear pinna injections. After 18h, the draining auricular LNs were examined. Surprisingly, neither H1 nor H2 blockade inhibited the increase in LC in the draining LNs after PGN administration. The increase in the total cellularity of LNs was also unaffected by anti-histamine treatment (Figure 4.3).

Figure 4.3 Blocking histamine receptor H1 or H2 does not inhibit LC migration. C57BL/6 mice were injected with diluent, pyrilamine 20 mg/Kg or cimetidine 50mg/Kg i.p. 90 min prior to PGN and saline injection into the ear pinnae. After 18h LN tissue were harvested and total cells and Langerin-positive cells were counted using anti-Langerin/CD207 flowcytometry. A, Mean ± SEM numbers of Langerin /CD207 positive cells /draining LN. B, Mean ± SEM number of total cells/draining LN. * represents $P<0.05$, *** represents $P<0.001$

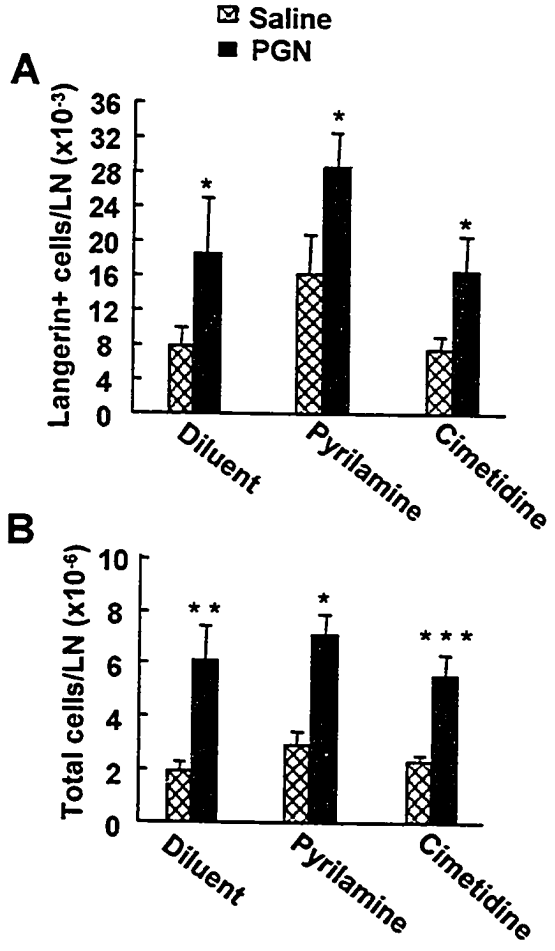
Saline
PGN
A
Langerin+ cells/LN (x10-3)
36
32
28
24
20
16
12
8
4
0
Diluent
Pyrilamine
Cimetidine
B
Total cells/LN (x10-6)
10
8
6
4
2
0
Diluent
Pyrilamine
Cimetidine

Figure 4.3

4.2.4 TNF contributes to LC migration in response to PGN

To examine the role of TNF in PGN induced LC response, TNF-/- mice and their wild type controls were treated with PGN. TNF-/- mice showed a significantly ($p<0.01$) reduced Langerin-positive cell response in LNs draining sites of PGN injection when compared with control mice. However, the absence of TNF did not completely block LC migration. In contrast to the effects of TNF deficiency on the increase in Langerin-positive DC within the draining node, lack of TNF had no significant impact on the PGN-induced and mast cell dependent increase in total LN cellularity (Figure 4.4).

4.2.5 The Langerin-positive DC observed in the skin draining LN have undergone maturation.

In order to examine the maturational status of LC within auricular nodes following PGN treatment LN cells were stained with the maturation marker CD86. The proportion of CD11c+ cells that were CD86+ remained high (>80%) in all groups, TLR2, TLR4, C3 and TNF deficient mice all demonstrated similar proportions of CD86+/ Langerin-positive cells within the nodes following saline or PGN treatment of the ear pinnae (Table 4.1).

The proportion of CD11c+ LN DC that were Langerin-positive and the overall number of CD11c+ cells/node were also determined (Table 4.1). C57BL/6 mice, TLR4 and TLR2 deficient mice showed significant increases in the number of CD11c+ cells in the LN draining PGN injected sites, with the TLR2-/- mice having a significantly ($p<0.05$) greater response to PGN than the

Figure 4.4 TNF significantly contributes to degree of LC migration. TNF-/- mice and their WT control received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear. After 18h draining LN tissues were harvested and total cells and Langerin-positive cells were counted using anti-Langerin/CD207 flow cytometry. A, Mean ± SEM numbers of Langerin/CD207 positive cells/draining LN. B, Mean ± SEM number of total cells/draining LN. * represents $P<0.05$ ** represents $P<0.01$, *** represents $P<0.001$

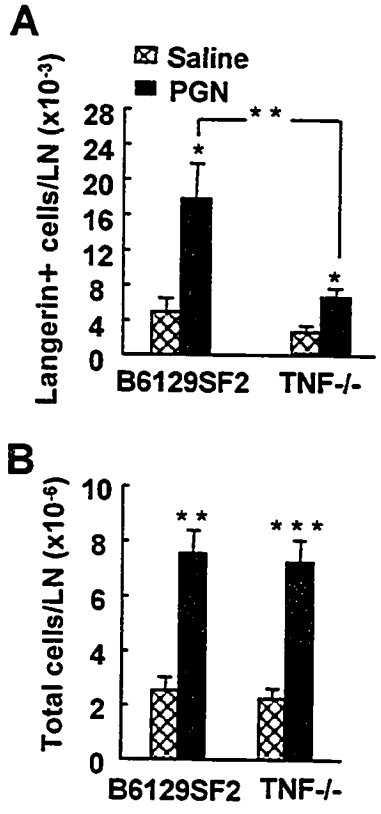
A
Saline
PGN
Langerin+ cells/LN (x10-3)
28
24
20
16
12
8
4
0
* *
*
*
B6129SF2
TNF-/-
B
Total cells/LN (x10-6)
10
8
6
4
2
0
* *
* * *
B6129SF2
TNF-/-

Figure 4.4

Table 4.1. CD11c and CD86 positive cell populations in the draining auricular nodes of various mouse strains 18h after PGN or saline injection of the ear pinna

Mouse Strain	Number of CD11c$^+$ cells (Thousands)		% of CD11c$^+$ cells that are CD86$^+$		% of Langerin-positive cells that are CD86$^+$	
	PGN	Saline	PGN	Saline	PGN	Saline
C57BL/6	86.0 ± 10.0 ***	27.6 ± 4.8	54.1 ± 3.5	51.2 ± 4.1	87.1 ± 1.9	85.9 ± 2.8
TLR2-/-	121.8± 15.9 ***	35.0 ± 4.9	63.6 ± 2.8	59.7 ± 5.2	88.7 ± 2.8	90.6 ± 2.4
C57BL/10J	150.5 ± 19.0 **	79.0 ± 9.5	66.2 ± 2.9	71.8 ± 2.1	92.0 ± 1.4	96.7 ± 1.2
C57BL/10ScNJ	135.6 ± 29.0 *	62.2 ± 13.2	62.4 ± 1.2	67.8 ± 3.3	90.7 ± 1.7	93.6 ± 0.9
B6/129	38.3 ± 10.2 **	10.6 ± 1.93	69.9 ± 2.3	60.4 ± 4.1	93.9 ± 1.7	94.7 ± 2.4
C3-/-	11.4 ± 4.3 #	5.6 ± 1.5	59.9 ± 6.6	67.9 ± 3.3	93.8 ± 2.9	92.2 ± 0.9
C57BL/6 + diluent	117.1 ± 34.7 *	36.1 ± 8.21	56.3 ± 3.9	56.3 ± 5.7	94.5 ± 2.9	88.2 ± 5.2
C57BL/6+ pyrilamine	155.6 ± 23.5 *	54.1 ± 10.7	65.1 ± 4.1	65.8 ± 4.1	99.7 ± 0.1	97.1 ± 2.8
C57BL/6 + cimetidine	95.6 ± 14.4 ***	36.2 ± 5.1	60.7 ± 5.5	57.3 ± 5.9	94.0 ± 3.6	92.3 ± 4.6
B6/129SF2	52.8 ± 15.9 *	14.8 ± 2.2	54.5 ± 2.8	51.5 ± 2.9	97.5 ± 1.4	99.6 ± 0.4
TNF-/-	37.4 ± 3.7 ***	12.3 ± 1.7	45.8 ± 1.7	42.6 ± 5.7	97.2 ± 1.1	98.3 ± 1.1

In each group LN draining PGN injected site was compared to contralateral saline injected site. * represents $P<0.05$ ** represents $P<0.01$, *** represents $P<0.001$. # denotes significantly ($P<0.05$) reduced number of CD11c +ve cells compared to PGN injected site in B6/129 control animals.

C57Bl/6 mice. In contrast, C3 deficient mice showed significantly ($P<0.05$) lower numbers of CD11c+ cells in the LN draining PGN injected sites. C57BL/6 mice injected with diluent, pyrilamine or cimetidine had similar responses to control C57Bl/6 mice. TNF deficient mice and their congenic controls both showed similar increases in the number of CD11c+ cells in LN draining PGN injection sites.

Discussion

In this study the role of mast cells in inducing LC migration in response to the bacterial product PGN was examined using mast cell deficient *W/W^v* mice. Our results demonstrate a high degree of mast cell dependency to this response. LC migration in response to PGN was also TLR2 independent whereas a contribution from the complement system, specifically C3, was shown to be required. This study also demonstrates that histamine might not be required based on the application of H1 and H2 blockade. However, TNF showed a significant role as an important mediator in inducing LC migration in response to PGN.

The requirement for mast cells indicated by comparison of responses in *W/W^v* and +/+ mice was confirmed by reconstituting *W/W^v* mice with mast cells in one ear only which was able to restore the ability of LC to migrate in response to PGN as well as the ability of PGN to induce an increase in total LN cellularity . These findings are consistent with McLachlan *et al.* who showed that mast cells are required for LN activation in response to certain whole bacteria (McLachlan et al., 2003).

TLR2 is a major recognition receptor for PGN, studies by Supajatura *et al.* have suggested that murine mast cells become activated via a TLR2 dependent mechanism following PGN administration in the skin (Supajatura et al., 2002). Interestingly, in the current study it was shown that the absence of TLR2 had no inhibitory effect on LC migration in response to PGN suggesting that TLR-

independent pathways play a major role in regulating this response. Indeed a greater LC response to PGN was observed in TLR2 deficient mice than control mice. To role out any potential effects of endotoxin contamination of the PGN, TLR4 deficient mice were examined. Again both TLR4 containing and TLR4 deficient animals showed similar responses. Taken together, these data indicate that neither TLR2 nor TLR4 are essential for the observed mast cell-dependent DC response following PGN administration.

Complement components can play an important role in mast cell activation especially through the actions of the anaphylatoxins C3a and C5a. Mast cells also express other complement product receptors C3R (CD11b/CD18) and C4R (CD11c/CD18). Complement activation has previously been demonstrated to be important for effective, mast cell dependent anti-bacterial responses (Prodeus et al., 1997). Thus, the requirement for complement activation in PGN-induced LC migration was examined. PGN administered *in vivo* might activate any of the three major complement pathways (Greenblatt et al., 1978; Ma et al., 2004; Verbrugh et al., 1979), therefore C3-/- mice were selected for this study. C3-/- mice did not show a significant increase in the number of Langerin-positive cells in the LN draining the PGN injected ear pinna compared to the saline injected site. In addition, the increase in draining LN total cellularity was abrogated following PGN injection in C3-/- mice compared with control mice. These results clearly demonstrate the importance of complement activation in inducing mast cell-dependent LC migration.

PGN has previously been suggested to induce mast cell degranulation *in vivo* and using an IgE –mediated mast cell degranulation model we have previously demonstrated that mast cell medicated LC migration can occur via a histamine H2 receptor dependent mechanism (Jawdat et al., 2004). Histamine has also been demonstrated to have profound effects on DC maturation *in vitro* (Mazzoni et al., 2001). However, our current data suggest that histamine is not a major mediator for PGN induced mast cell dependent LC migration. since both pyrilamine and cimetidine histamine blockade had no effect on LC migration, although cimetidine was employed at a dose previously demonstrated to be effective in blocking LC migration *in vivo* following antigen/IgE mast cell activation (Jawdat et al., 2004).

Mast cells are known to produce multiple mediators capable of inducing DC maturation and migration, these include the potent pro-inflammatory cytokine TNF, which is found preformed within mast cells and can also be newly generated in response to multiple forms of activation (Gordon and Galli, 1990). TNF has been shown to be required for mast cell dependent increases total LN cellularity in response to Gram-negative bacteria (*E. coli*) (McLachlan et al., 2003).

TNF-/- mice showed a significantly reduced Langerin-positive cell response in LNs draining sites of PGN injection compared with that observed in control mice. However, the absence of TNF did not completely block LC migration suggesting additional contributions by other mediators. The ability of

PGN to induce changes in LN cellularity however, was largely unnaffected by the absence of TNF. These latter observations are in marked contrast to the results of studies using Gram-negative bacteria *in vivo*, which suggested that mast cell derived TNF was a critical contributor to LN activation (McLachlan et al., 2003). These observations suggest alternate mechanisms whereby mast cells can enhance LN activation, dependent on the type of activating stimulus encountered. Notably, TNF deficient mice and their congenic controls both showed similar increases in the number of CD11c+ cells in LN draining PGN injection sites consistent with changes in the total cellularity of the auricular LN.

The LC migrating to the draining LNs varied in number following PGN administration but appeared to undergo appropriate maturational changes regardless of the type of mice used for the studies as indicatd by the high proportion of $CD86^{+}$ Langerin-positive dendritic cells observed in each of the animal groups (Table 4.1). It is also notable that increased numbers of Langerin positive DCs were accompanied by substantial overall increases in the numbers of CD11c positive cells observed within the draining nodes.

The ability of local immature DC to respond to mast cell activation by undergoing maturation and relocation to draining LNs has some important implications for the role of mast cell in the development of acquired immune responses. Bryce *et al.* have recently demonstrated an important role for mast cells in contact hypersensitivity responses related to decreases in LC numbers

within the skin. The microenvironment in which DC mature can have a profound impact on their ability to interact with T-cells and the polarization of the T-cell response that ensues. Both histamine and TNF have been demonstrated to regulate these processes. It remains to be investigated, whether alternate routes of mast cell dependent LC maturation and migration to draining LNs lead to different types of immune responses. The ability of PGN to act via mast cells to enhance LC maturation and migration may also be of importance in diseases such as atopic dermatitis where high levels of infection with *S. aureus* are observed. The activities of mast cells activated by PGN or other bacterial products could enhance the process of sensitization to multiple environmental antigens by increasing the turnover of LC under conditions in which the epidermal integrity may already be reduced.

Overall, our data demonstrate a novel complement and TNF-dependent mechanism of mast cell mediated LC maturation and accumulation in draining LNs in response to PGN. This mechanism does not require TLR2 or TLR4 and is mechanistically distinct from the histamine (H2) dependent LC migration that occurs as a result of IgE/antigen mediated mast cell activation. One potential model for this response would involve PGN activation of the complement pathway, subsequent activation of mast cells to produce TNF as a result of interactions with complement split products and subsequent TNF-dependent maturation and migration of resident skin LC. Since LC also express complement receptors including C5a (Morelli et al., 1996), it is possible that complement

induce LC maturation directly, however other signals such TNF from mast cells are also required. Multiple mast cell dependent and independent mechanisms contribute to this process in response to different pathogen associated stimuli. Further studies of these pathways should provide novel opportunities to modulate the earliest stages in the development of immune responses.

CHAPTER 5

CONCLUSION

5.1 Summary of Major Findings

Originally it was thought that the immune system consisted of two separate responses, an early non-specific innate and a late specific adaptive immune response. However, over the last several years, a number of studies have provided evidence that both responses function together rather than separately and there is a requirement for an appropriate innate immune response for an optimal adaptive response to occur. It is also now recognized that the innate immune response can be activated selectively by certain pathogens through PRRs expressed by many cells. Activation of these cells can then act on DCs, inducing maturation and other events and thus link innate to adaptive immunity. The important role of DCs as professional APC has made them a subject of interest to many scientists. Not only are they the major initiators of adaptive immune responses, but also, thus far, the only known cells that can activate naïve T lymphocytes. The main function of DCs is to capture intruding antigen, process it migrate to the draining lymphoid tissues and present the antigen, complexed to MHC class II, to antigen-specific T lymphocytes to activate them and initiate an immune response. It is hoped that by understanding what regulates DC function, it will be possible to better manipulate the immune response. Of particular interest are LCs, which are classical immature DCs in the skin. These are particularly amenable to study in view of our ability to trace their migration from the skin to the draining LN, in response to a particular stimulus. Several mediators have been shown to be involved in LC maturation and /or migration including TNF, IL-1β (Cumberbatch et al., 1997b), GM-CSF, CD40 ligand (Moodycliffe et al., 2000),

LTC_4 and histamine (Caron et al., 2001b; Mazzoni et al., 2001). Interestingly, mast cells are also present in the skin in large numbers. Following activation, mast cells can produce all of these mediators to a greater or lesser extent depending on the stimulus. Thus, we hypothesized that locally activated mast cells can induce LC migration from the skin to the draining LN. Our preliminary objective was to locally activate mast cells in the skin and then observe LC responses to such activation. Using a specific antibody for LC "anti-Langerin" (CD207) it was possible to count their numbers in the epidermis and, based on widely recognized previous studies (Stoitzner et al., 2003), the same antibody was used to identify LC that had emigrated from the skin into the draining LN.

In our initial study (Chapter 3), the ability of locally activated mast cells to induce LC migration was examined. IgE and antigen was chosen as the mast cell activating stimulus to mimic a classical allergic response. Using anti-Langerin/CD207 antibody, it was possible to count the numbers of LC both in the skin epidermis and draining LN, following local IgE-mediated activation. Following this type of mast cell activation a significant decrease in the number of LC was observed in the skin, where as a significant increase in Langerin-positive cells were observed in the LN. As this response was not seen in mast cell deficient *W/W^v* mice it was suggested that mast cells played a critical role in this process. To further confirm the importance of mast cells, *W/W^v* mice were locally reconstituted with mast cells. This reconstitution was able to restore the LC migration response and confirmed that LC migration in response to IgE-mediated

activation was mast cell dependent. Notably IgE injection alone did not induce LC migration.

It was also shown that histamine was required for this response as no significant increase in Langerin-positive cells in the draining node was observed following blockade of histamine H2 receptors using cimetidine. However, injecting histamine alone, at doses similar to those likely to be generated by local mast cells, was not sufficient to induce LC migration. Thus, it was suggested that in addition to histamine, other mediators may also be required. These data indicate an important role for mast cells in inducing LC migration in response to IgE and antigen activation, mainly through the effect of histamine with the help of other still unknown mediators or cell-cell interactions.

In our second study (Chapter 4), we were interested in examining the role of mast cells in LC migration in a different model, such as would be observed in host defense against pathogens. We were particularly interested in bacterial infections since mast cells have been shown to play an essential role in host defense against certain types of bacteria. In particular, we wanted to examine the effect of the bacterial cell-wall component PGN from Gram-positive bacteria *S. aureus*. This is also relevant to certain types of allergic disease since more than 90% of patients suffering from atopic dermatitis are colonized on the skin with *S. aureus* (Leung et al., 2004). Patients with this disease usually have a high level of IgE antibodies to different environmental antigens in their serum. However, the role of mast cells in skin disease in these patients is still not clear. Thus, we

wanted to study the effects of PGN injection on LC migration and the role of mast cells in this process. Both mast cell deficient mice and their wild type controls were used. Surprisingly, only wild type mice and not mast cell deficient mice had a significant increase of Langerin-positive cells in the skin draining LN that was injected with PGN compared to saline which suggests a mast cell requirement for inducing such a response. To further confirm mast cell dependency, we reconstituted mast cell deficient mice with mast cells in one ear only and then injected PGN into both ears. The side that received both mast cells and PGN was completely restored in its ability to induce significant LC migration.

We then considered the mechanisms that could be involved. PGN is one of the PAMPs that have been shown to be recognized by PRRs such as TLRs. Numerous studies have implicated the requirement for TLR2 for mediating PGN responses. Since mast cells express TLR2, we wanted to examine the role of TLR2 in response to PGN in inducing LC migration. We used TLR2 knockout mice and their wild type controls. Surprisingly, both groups of mice had a significant increase in the number of LC in the draining LN in the PGN injected side compared to saline which suggest that TLR2 may not be required. Moreover, TLR2 knockout mice had a significantly higher LC migration response than wild type mice. More recent observations have suggested that NOD-1 and NOD-2 may be more important for responses to PGN than previously recognized and this may provide some explanations for these results (Royet and Reichhart, 2003; Tanabe et al., 2004; Viala et al., 2004). Interestingly, another TLR2 ligand (*Borrelia burgdorferi* lipoproteins) has been shown by another group to induce acquired

humoral responses in TLR2 knockout mice (Leung et al., 2004). They suggested that TLR2 was required for innate host defense, but not for some inflammatory responses or specific antibody development. We also examined the role of TLR4 which is widely recognized as a receptor for LPS. Both TLR4 deficient mice and their wild type control had a significant increase in the number of Langerin-positive cells in the draining LN in the PGN injected side compared to saline, which suggested that TLR4 was also not required. We then considered the role of complement since PGN has the ability to activate all three major complement pathways. We decided to use C3 knockout mice since C3 is involved in all three complement pathways. Interestingly, C3 knockout mice compared to their wild type controls had no significant increase in the number of Langerin-positive cells in the PGN injected side compared to saline, which suggested an important role of complement activation in inducing LC migration in response to PGN.

In order to determine the key mediators that could be involved, we considered histamine since we had already seen an important role for histamine in inducing LC in response to IgE-mediated mast cell activation. Thus, we used histamine receptor blockade (pyrilamine and cimetidine) as used in our first model. Surprisingly, neither of the two histamine receptors appeared to mediate the response to PGN. These observations suggest the requirement of different mediators in two mast cell dependent models of LC migration. We then examined the role of TNF since TNF is a key inducer of LC migration when injected into mice directly. In addition, mast cells can release TNF within seconds of activation. When we compared TNF knockout mice and their wild type controls,

TNF knockout mice had a much lower LC migration response than the control mice, which suggested an important role for TNF in inducing LC migration in response to PGN.

Overall, these data indicate an important role of mast cells in inducing LC migration in response to the bacterial cell-wall product PGN. Surprisingly, this response was shown to be TLR2 and TLR4 independent while C3 was required. In addition, neither H1 nor H2 receptor blockade, were able to block this response. However, TNF was a major contributor in inducing mast cell dependent LC migration in response to PGN. Our proposed model suggests that when a specific bacteria such as *S. aureus* enters the skin it may activate the complement system through one of the complement activating pathways. This in turn will activate the mast cells in the skin either through the anaphylatoxins (C3a or C5a), which are widely recognized to induce mast cell degranulation, or through binding to specific complement receptors expressed by mast cells, such as CR3 and CR4. Mast cell activation will then lead to the release of mediators such as TNF, which may provide a danger signal for LCs. This will then lead to LC activation and migration from the skin into the draining LN. Emigrated LCs will then present the antigen to T lymphocytes and initiate an immune response (see Figure 5.1).

5.2 Relationship to Previous Studies of Mast Cell and DC Interactions

Initial studies, in the literature, examining LC migration used mainly contact sensitizers such as fluorescein isothiocyanate (FITC) and traced their

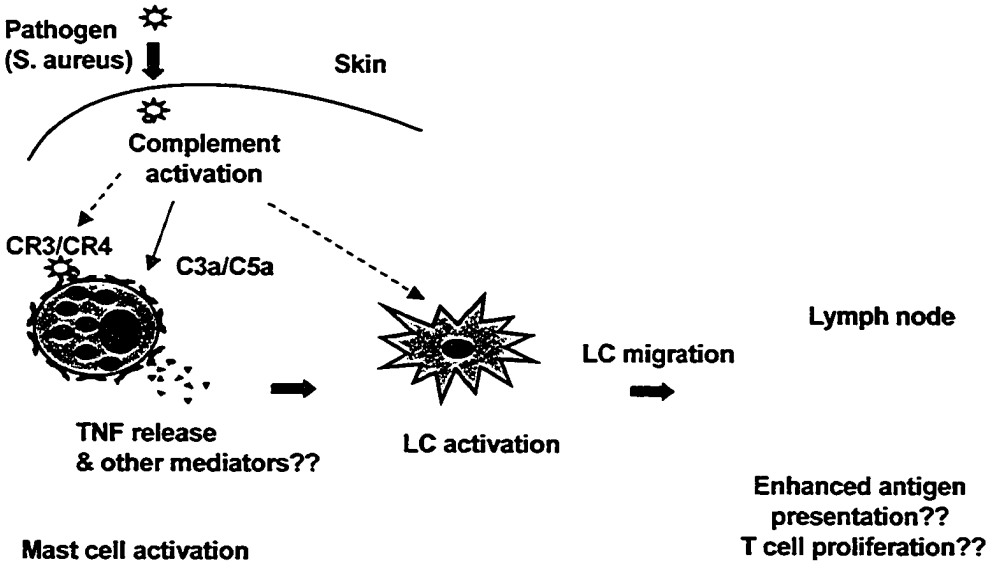

Figure 5.1 Proposed model for mast cell inducing Langerhans cell migration in response to PGN.

migration into the draining LN where they will present FITC to T lymphocytes. This method was widely appreciated as it allows studies of the role of LC in delayed hypersensitivity to various chemicals (Kinnaird et al., 1989; Macatonia et al., 1987). Using this method also permits the tracing of the newly emigrated LC as they will express the easily detectable chemical in the draining node. However, it is important to know that these chemicals can be transported via the lymph from the skin to the LN and then be picked up by resident DCs and thus, the number of the chemical positive expressing DCs may not always represent a true number of emigrated LC. Following the discovery of LC function in response to numerous chemicals and contact sensitizers a great deal of interest shifted to studying the mechanisms regulating LC migration. TNF and IL-1β were among the first essential mediators to be discovered (Cumberbatch et al., 1997d). It was thought that the TNF is released by keratinocytes and the IL-1β by the LCs themselves. Although mast cells are also in the skin and thus far the only cell known to store a large a mount of TNF in their granules, no specific role was mentioned for mast cells in these responses. However, when the role of CD40 ligand was studied which is required for LC migration and expressed by mast cells, it was suggested that mast cells might be important in this process (Moodycliffe et al., 2000).

More recent studies have examined the role of histamine on DC maturation and activation (Caron et al., 2001b; Mazzoni et al., 2001). Interestingly, histamine was shown to be able to up-regulate several maturation markers on DCs. However, histamine did not induce full maturation of DCs *in vitro*. These observations were consistent with our initial study of IgE-mediated

mast cell activation inducing LC migration which was shown to require histamine. We also injected histamine alone in the ears which did not induce LC migration. Thus, both our study and the *in vitro* study of histamine suggest that histamine plays an important role in this response. Histamine did not induce full maturation of DCs nor LC migration by itself, indicating a requirement of other mediators or cellular interactions. Surprisingly, in our second study where LC migration was mast cell dependent in response to PGN histamine receptor blockade had no significant effect. This suggests the ability of mast cells to induce LC migration via two independent mechanisms. This is consistent with that IgE and antigen induced mast cell degranulation and the release of histamine while PGN induces cytokines without degranulation. A study by Supajatoura *et al.* previously showed that PGN can induce murine mast cell degranulation, but this was not shown in our lab *in vitro* or *in vivo*. These experiments however only assess classical or compound degranulation which is measured within minutes of mast cell activation but not piecemeal degranulation which is another type of degranulation in which mediators are released slower over a long time. Thus, we cannot role out the possibility the PGN induces piecemeal degranulation of mast cells which could be examined by electron microscopy.

Mast cell exosomes as mentioned earlier have also shown by Skokos *et al.* to induce immature DCs to up-regulate the expression of MHC class II and co-stimulatory molecules. They demonstrated that mast cell exosomes can harbor exogenous antigens and are able to elicit specific antibody immune responses (Skokos et al., 2003). They also showed that antigen associated with mast cell

exosomes can be delivered to DCs *in vivo* inducing efficient antigen presentation. Whether mast cell exosomes play an important role in our system is not known. However, it is worth considering that some antigens such as PGN might be engulfed by mast cells and then delivered to LC through exocytosis.

A study by Mclachlan *et al.* showed that mast cells are also required in inducing LN hypertrophy in response to bacterial infection such as *E. coli* (McLachlan et al., 2003). This response was also shown to be TNF dependent. Although we have also shown that mast deficiency almost completely abrogated LC migration and total LN cellularity and that TNF is a major contributor in inducing this response, the absence of TNF, in TNF knockout mice, did not alter increase in total LN cellularity. Whether the difference between the two studies reflects the differences between Gram-positive versus Gram-negative bacteria is not known. Since we have only used a bacteria cell-wall component and they have used whole bacteria other differences in the system used might alter the relative importance of TNF.

One of the latest studies on mast cells and DCs by Bryce *et al.* was published around the same time as our first study. They showed that mast cells are required for efficient contact sensitization through their FcεRI. They also concluded that the dependency on mast cells varies among chemical haptens used (Bryce et al., 2004).

All these studies implicate an important role for mast cells in modulating DCs, which might have significant effects on manipulating immune responses to a variety of stimuli.

5.3 Clinical Implications

5.3.1 Atopy/Sensitization

In our initial study (Chapter 3) we have shown an important role for mast cells in inducing LC migration from the skin to the draining LN in response to IgE and antigen. It is possible that this may have implications for individuals suffering from allergic diseases specifically skin allergies. Those individuals often produce a large amount of IgE antibodies when exposed to environmental antigens. These IgE molecules will then bind to their high affinity receptors (FcεRI) on mast cells in the skin. Subsequent exposure to the same antigen will then lead to mast cell activation and degranulation. If the model that we have developed from our studies is correct, mast cell mediators will then activate LC in the skin and induce their migration to the draining LN where they can present additional antigens to T lymphocytes and maybe initiate an immune response to such additional environmental stimuli. Thus, it is possible that the activation of mast cells by a particular antigen provides a maturational signal for LCs which might at the same time be exposed to normal environmental antigens. In the absence of mast cell activation, LC will not get activated by contact with those harmless environmental antigens alone and thus no immune response will be developed. Individuals suffering from type I hypersensitivity mediated disease in the skin will continuously have mast cell activation and degranulation, which then in turn activate the LC carrying other environmental antigens and thus increase the chances of having more IgE responses and allergies to different antigens working as a continuous circle. This may explain the generation of antibodies to

an increased range of environmental antigens and contribute to the chronicity of some allergic diseases.

5.3.2 Immunity or Tolerance?

One of the most important decisions made during the generation of a response to a given antigen is whether it induces immunity or tolerance? DCs were originally thought of as the natural "adjuvants" for inducing primary immunity to foreign-antigens. However, they are also becoming widely recognized as the initiators of tolerance to self-antigens. DCs not only play a key role in central tolerance by inducing apoptosis of potentially self-reactive T lymphocytes, but also are the main inducers of peripheral tolerance. However, the exact mechanisms of peripheral tolerance are not understood. Two mechanisms have been proposed (1) the existence of a specific subset of DCs that is responsible for inducing tolerance "tolerogenic DCs"; and (2) the ability of one subset to induce either immunity or tolerance depending on its stage of maturation or activation. The initial and simple model was that immature DCs induce tolerance while mature DCs induce immunity (Dhodapkar et al., 2001; Steinman et al., 2000). However, it is widely recognized that DCs even in the steady state have to mature in order to be able to migrate out of the tissue into the lymphoid organs. Thus, this concept was modified and it is now believed that mature but quiescent DCs induce tolerance whereas, mature but "fully activated" DCs induce immunity. The "fully activated" DCs might be induced by receiving a danger signal which could either be an additional signal to what quiescent DCs receive,

or a much stronger signal than what is normally present (Albert et al., 2001; Shortman and Heath, 2001). Since mast cells can produce both T cell activator cytokines as well as immune regulatory cytokines, their close proximity to DCs, together with their ability to both recruit T cells as well as migrate to lymphoid tissues it is tempting to suggest that mast calls can manipulate an immune response toward immunity or tolerance. Whether this occurs *in vivo* is not known and will be hard to determine, however trying to induce such a route might be worthwhile for clinical therapies. Tolerogenic DCs are suggested to be an effective mechanism to treat graft rejection, autoimmune disease and allergic disease. Much more work is required to define the role of mast cells in such models.

5.3.3 Vaccine Development

Immunization against pathogens is a powerful weapon and in high demand. However, despite antigen availability and characterization, the development of effective immunity is not always successful. Since mast cells have been shown to have such an important role in inducing LC migration and perhaps the initiation of an immune response we have considered the possibility that these cells could be exploited in novel vaccine development approaches in particular by enhancing the development of adaptive immunity through the activation of LC. A number of current adjuvants may have mast cell activating activity, especially those based upon known TLR activators, such as lipid A or CBG-DNA. Through targeted mast cell activation, in the absence of

degranulation, effective new adjuvant approaches might be developed. The ability of mast cells to selectively produce either type I (e.g. IFN-γ) or type II (e.g. IL-4, IL-13) cytokines dependent on the stimuli used, adds to their attractiveness as targets in adjuvant approaches.

5.3.4 Atopic Dermatitis

PGN is a part of the cell-wall of Gram-positive bacteria including *S. aureus*. Infection with this organism is observed in more than 90% of patients with atopic dermatitis. These patients also have increased numbers of mast cells however their role in atopic dermatitis is still not understood. In our initial study (chapter 3) we suggested that repeated activation of mast cells may contribute to the chronicity of some allergic diseases such as atopic dermatitis. Since PGN induction of LC migration is also mast cell dependent, it is tempting to assume that this type of activation may occur in those patients. However, this requires further investigation using whole *S. aureus* in place of purified PGN. Other *S. aureus* associated molecules such as the IgG binding protein A may contribute to mast cell activation in this context. The potential use of blockade of mast cell mediators or mast cell activation, early in disease, as a mechanism to reduce sensitization to additional environmental antigens, also needs to be considered.

5.4 Limitations of The Current Work

5.4.1 Species Differences

One of the greatest criticisms of this work is the use of a murine model instead of a human system. The use of *in vivo* models is often of high impact since it reflects what happens in a host as a whole instead of a single cell in culture. However, the fact that cell function in different species can be varied is widely recognized. For instance, in our system, murine mast cells differ from human mast cells in their granule contents, cytokine expression and most importantly to their responses to different stimuli. Thus, there are limitations in the conclusions that could be drawn without knowing if specific events happen in the human system. It would be nice to have both systems to examine in parallel. Thus, showing these responses using human mast cells might add some confirmation to our system. *In vitro* studies using cultured mast cells and monocyte-derived DCs have been done by other groups and suggest that mast cells are capable of providing maturational signals to human DCs (Caron et al., 2001b). The consequences of these responses for human immune activation *in vivo* remain to be determined.

5.4.2 Redundancy

Using knockout mice is an extremely important approach to identify the role of a specific cells or molecules within a particular response. It is often more efficient than using blocking antibodies in which its always a challenge to block completely and specifically. In addition to the side effects a blocking antibody

may have on the host if injected systemically. However, even though the use of knockout mice seems optimal, one should not forget the possibility of redundancy. Showing that the absence of a specific cell or molecule is important, in a particular response, may not always reflect the real picture of their role in a normal animal. Several knockouts were used in this study. The most striking result was the discovery that TLR2 was not required for mast cell induced LC migration in response to PGN. Although several studies indicate the importance of TLR2 for mediating responses to PGN this was not the case in our system. The question is does this reflect what naturally occurs in the host thus PGN activates mast cells through a different receptor, in the case of LC migration, or is it that the TLR2 knockout host is able to compensate for the lost molecule with another that can mediate the same response or maybe even better? Additional controls using selective TLR2 deficient mast cells reconstituted in a TLR2 containing host or use of blocking antibodies locally could help clarify some of these issues.

5.4.3 LC Function in Knockout and Deficient Mice

Since several knockout mice have been used in our studies, one of our major concerns was whether LCs are responding normally in these animals. An initial question was whether the negative response we have seen in mast cell deficient *W/W^v* mice was due to a the absence of mast cells or due to another abnormality of the LC in these mice. In *W/W^v* mice we counted the number of LC in both epidermis and draining LN which were similar to their wild type control. We also tested LC function in migration in response to LPS which appeared to be

independent of mast cells which suggests that these LCs in *W/W^v* are able to respond normally to some stimuli. Similarly, in the case of C3-/- or TNF-/- mice there remains the possibility that deficiency could alter LC development or responses or mast cell functional development such that our observations are over-interpretated due to a functional role of these molecules. Thus, further experiments challenging the ability of LC in such mice to respond to skin LPS or IgE-mediated mast cell activation would be particularly useful to clarify this issue.

5.4.4 Langerin/CD207 As a Marker

Anti-Langerin antibody has been well recognized as an important tool to trace LC migration from the skin to the draining LN in the literature (Romani et al., 2001; Stoitzner et al., 2003). Therefore, Langerin was used as a specific marker of LC both in the skin and the draining LN throughout the current study. However, since we are not able to see the migrating LC *per se*, there is a possibility that the response to mast cell challenge may down-regulate Langerin expression in the skin and up-regulate its expression in the draining LN. However, the chance of this providing an explanation for our results is very minimal. In epidermal sites, we also used MHC class II as a marker for LC. Consistent with our anti-Langerin staining results, anti MHC class II positive cells were significantly fewer in the mast cell activated epidermal sites compared to control sites. As for the draining LN, we also counted the number of CD11c$^+$ cells and the overall total number of cells. These were in concert with CD207 positive cells and

consistently increased in nodes draining the mast cell activated side compared to the control side. However, there remains the possibility that some of the treatments used in our study might also modify the length of time of Langerin expression within the node. More detailed studies of DC subsets within the LN following mast cell activation will help to clarify these issues.

5.4.5 The Use of Pharmacological Agents

The use of pharmacological agents always has some potential risks as we don't know if there are any non-specific effects caused by particular drugs that are responsible for the blockade observed. In the current study, several drugs have been used including pyrilamine and cimetidine. However, no further studies such as antibody blockade or receptor knockout mice were used to confirm the function of these drugs. It would be important to evaluate alternate strategies to block the effect of histamine such as the use of mast cells from histidine decarboxylase knockout mice in reconstitution experiments. Alternatively, other H1 and H2 blocking agents should be employed

5.4.6 The Lack of Identifying a Specific Complement Component Pathway

In our second study (chapter 4) the involvement of complement components in inducing LC migration in response to PGN was significant. This was shown with mice lacking C3 which is involved in all three complement activating pathways. Since PGN can also activate all the three pathways we were not able to determine which particular pathway is involved without using

additional blocking antibodies or knockout mice as discussed later. However, the high cost and difficulty in obtaining these mice have limited additional studies in this area.

5.4.7 The Lack of Functional Consequences of The Migrating LC

We have demonstrated an essential role for mast cells in inducing LC migration from the skin to the draining LN in two different models and yet we have not shown any consequences of these events. It is possible that through our models we enhanced the migration of LC but not their function in T cell activation. Or, as previously mentioned, we may induce tolerance via this type of LC activation. In some experiments we have examined some maturation markers such CD86 and CD40 which were consistently up-regulated in the emigrated cells. However, additional studies are required to examine T cell activation as discussed later.

5.5 Future Directions

In our first study (chapter 3) we demonstrated that IgE-mediated mast activation induces LC migration from the skin to the draining LN. It is possible that this activation of mast cells may lead to the migration of LC that have simultaneously taken up environmental antigens. These antigens will be carried into the LN and an immune response will be initiated, which is ultimately dependent on the mast cell activation. In the absence of such mast cell activation, LC activation would not occur and thus environmental antigens will be carried by

quiescent or not fully activated LC which when they arrive in the LN will not initiate an immune response. If this theory is correct, then it may explain aspects of the chronicity of some allergic diseases. In order to examine this theory, one could test several harmless allergens or haptens known not to normaly induce immune responses. For example, most studies examining LC migration have used contact sensitizers such as FITC or oxazolone which normally induced LC migration from the skin to the draining LN. However, some sensitizing antigens have no effect on LC migration. Thus, by using one of these non-LC migration inducers as a surrogate environmental antigen and injecting it simultaneously with IgE-mediated mast cell activation, it would be interesting to see whether the host will develop an immune response to this particular antigen.

In our second study (chapter 4) we have shown that complement activation is required to induce LC migration in response to PGN. Specifically C3 which is involved in all three complement pathways. To identify which complement activation pathway is involved additional, knockout or antibody studies might be used. C4 knockout mice would allow us to determine the involvement of the classical pathway. Factor B knockout mice could permit studies of the involvement of the alternative pathway. MBL knockout mice would allow us to determine the involvement of the lectin pathway. Once the exact pathway is identified, we may examine which molecule or receptor is required. We know that mast cells express several complement receptors including CR3, CR4, C3a and C5a. Blocking any of these receptors may provide information on

which exact receptor is involved. In addition, to determine if this complement component and receptor interact directly on the mast cells, we could reconstitute W/W^v mast cells deficient mice with mast cells deficient in that particular receptor. In addition, factor H is the main inhibitor of the alternative pathway and factor H deficient mice tend to have uncontrolled C3 activation. Thus, it would also be interesting to see wheather factor H deficient mice have more LC migration compared to wild type in response to PGN. These observations might also be important for potential clinical use for therapies.

We have also shown that PGN inducing LC migration is mast cell dependent and requires TNF production. However, we do not know if the TNF is directly released from the mast cells or other cells. Since keratinocytes are also present in the skin and can also produce large amounts of TNF, it is possible that the TNF might be released from other cells, but yet requires specific mast cell-mediator to induce this production. However, it is still tempting to assume that the TNF is released from the mast cells since they are the only cell that can release it within seconds of activation while other cells need longer. In addition, TNF might be required early at the time of PGN exposure. In order to examine whether the TNF is required directly from the mast cells, we can reconstitute mast cell deficient W^{sh}/W^{sh} mice with cultured TNF knockout BMMC and see whether this will restore or inhibit LC migration in response to PGN.

In general the most essential study regarding both models is the examination of the functional consequences of the emigrated LC from the skin to the draining LN in response to the IgE-mediated mast cell activation and also in response to PGN. It would be of great interest to see whether the responses of LC migration initiate specific immune responses or whether they induce a tolerogenic effect and whether this varies depending on the mode of mast cell activation. For instance, looking at the expression of IL-2. In the case of initiating immunity it would be interesting to see whether it is a type 1 or type 2 immune response by looking at the expression of IL-4 versus IFN-γ. It would also be interesting to see if the outcome response varies between different mouse strains such as C57Bl/6 and BALB/c, which may reflect the differences between normal individuals and atopic individuals.

One of our most interesting observations was the independence of LC migration in response to PGN on TLR2. This was striking, as PGN is a known ligand for TLR2 and numerous studies indicate the requirement of TLR2 to mediate cellular responses such as cytokine release. Now the question is whether TLR2 is truly not required for LC migration in response to PGN or whether TLR2 is usually required, but that knockout mice have developed redundancy by using another receptor which could be another TLR or another PRR. Regarding the question of whether another TLR is required, MyD88 knockout mice could be used. However, this will only tell us if any TLR that uses an MyD88 dependent

pathway is involved. Using one of the MyD88 independent pathway involved signalling molecules knockouts such as IRF-3 will be potentially useful here.

New studies are emphasizing the importance of NOD in recognizing PGN instead of TLR2. It would also be interesting to examine the involvement or requirement of NOD1/2 in our system by using NOD knockout mice and see whether this will abrogate LC migration in response to PGN.

5.6 Concluding Remarks

Overall, our studies have provided a novel discovery about the function of mast cells. In response to specific stimuli, mast cells can induce LC migration from the epidermis into the draining LN. Since LCs are professional APCs, this suggests an important role for mast cells in manipulating immune responses following the encounter with specific pathogens or allergens.

It is important to understand that the purpose of this study is not just to introduce the key roles of mast cell in response to pathogens or allergens, but rather to show the ability of mast cells to interact with the initial stages of adaptive immune functions, which could potentially be of great benefit if used therapeutically to manipulate the immune response.

APPENDIX

PRELIMINARY STUDIES OF THE ROLE OF CD14 IN PEPTIDOGLYCAN INDUCED LANGERHANS CELL MIGRATION

6.1 Introduction

PGN is an essential cell wall component of all bacteria especially Gram-positive bacteria where it accounts for almost half of the cell wall mass. PGN consists of a polymer of N-acetylglucosamine and N-acetylmuramic acid cross-liked by short peptides which surrounds the cytoplasmic membrane of the bacteria and is important for maintaining the shape and osmotic pressure of the cell (Schleifer and Kandler, 1972). Since PGN is present in almost all bacteria it is an excellent target for studies of bacterial infection especially those involving Gram-positive bacteria.

Atopic dermatitis is a chronic inflammatory skin disease associated with hyperreactivity to environmental allergens. It is mostly characterized by skin lesions, pruritus, dry skin and high levels of IgE antibodies to a variety of environmental antigens (Johnson et al., 1974). Atopic dermatitis mostly effect children, often developing during infancy. Interestingly, atopic dermatitis patients often suffer from serious skin infection, more than 90% of the patients skin are colonized with the Gram-positive organism *S. aureus* in their skin lesions and suffer from relapsing disease (Leung, 2002). However, the mechanisms that result in bacterial infection in atopic dermatitis and consequences for disease progression are not well understood.

DCs are the most potent APCs. They are specialized to capture antigen, process it into peptides and migrate to the draining LN where they present the

antigenic peptides to T lymphocytes to initiate an immune response (Banchereau and Steinman, 1998). One of the subtypes of immature DCs in the skin are the LCs. These cells have a characteristic dendritic morphology and their migration to the LN has recently been shown to be traceable, through their specific expression of Langerin/CD207 that can be detected by anti-Langerin antibodies (Stoitzner et al., 2003). The role of DCs in atopic dermatitis has been widely recognized. Interestingly, increased numbers of LCs have been reported in the epidermis of patients with atopic dermatitis in addition to another type of LC which differ form the classical type identified as inflammatory dendritic epidermal cells (IDECs). These LCs have been shown to possess an abnormal phenotype with increased expression of the FcεRI and enhanced ability to capture normal environmental antigens, which were believed to activate $CD4^+$ T cells to induce a Th2 type cytokine response (Leung et al., 2004). Although DCs are the focus of attention in atopic dermatitis to many researchers, the exact role and mechanism by which they enhance the disease process and interact with ongoing bacterial infection in this disease is not completely understood.

Mast cells are sentinel cells of the immune system, although initially associated with allergic immune responses, more recently their function in host defense has become widely recognized. Their effects are not limited to innate immunity, since mast cells have also proven to be important in LC migration necessary for adaptive immune responses. Recently we have shown that IgE-mediated mast cell activation induces LC migration from the skin into the

draining LN (Jawdat et al., 2004). Whether this process has a link to atopic dermatitis is not known. We have also shown that LPS-induced LC migration from the skin into the draining LN is mast cell independent. Whereas, PGN-induced LC migration is mast cell dependent, whereas mast cell deficient (*W/W*^{v}) mice subcutaneously challenged with PGN, showed almost completely abrogated LC migration response.

CD14 is a cell surface GPI-liked glycoprotein expressed by several cells including LCs, but also exists in a soluble form. CD14 does not possess a transmembrane or cytoplasmic domain, so it may not transduce signals independently. CD14 is widely recognized as a coreceptor for LPS from Gram-negative bacteria through association with TLR4 (da Silva Correia et al., 2001). More recently it was shown that CD14 also binds to PGN (Gupta et al., 1996b). Interestingly, although TLR4-mediated responses to LPS are CD14 dependent, TLR2-mediated responses to PGN do not require CD14. However, CD14 has been reported to enhance TLR2-mediated responses to PGN and induce the production of pro-inflammatory mediators. Mast cell expression of CD14 is low to absent in our hands. However, a study by Ikeda and colleagues shows mast cell expression of CD14 (Ikeda and Funaba, 2003). Since CD14 can bind PGN and LCs express CD14 but only weakly express TLR2, the role of CD14 in a mast cell-dependent PGN-induced LC migration was studied.

6.2 Results and Discussion

CD14 is not required for mast cell dependent IgE-mediated Langerhans cell migration.

CD14 deficient mice and wild type control mice were injected with IgE for the hapten TNP-BSA subcutaneously in the right ear pinne and as a control with saline in the left ear pinne. After 14 days mice were intravenously challenged with TNP-BSA. After 24h mice were sacrificed and their ear skin draining LNs were harvested and the number of Langerin-positive cells were determined as well as the total LN cellularity. As demonstrated in (Figure 6.1) the LC migration response was normal in CD14 deficient mice compared to controls. In addition, more than 80% of the Langerin–positive cells were $CD86^{+}$. This suggest that CD14 is not required for inducing LC migration in response to IgE-mediated mast cell activation.

CD14 is required for PGN induced Langerhans cell migration.

CD14 deficient mice and wild type control mice were injected subcutaneously with PGN in the right ear pinna. And as a control with saline in the left ear pinna. After 18h mice were sacrificed and their ear draining LNs were harvested and the number of Langerin-positive cells and the total LN cellularity were determined. Interestingly, CD14 was shown to be required for inducing LC migration in response to PGN (Figure 6.2), which we found mast cell dependent but TLR2 independent. Since LC express CD14 but only weakly express TLR2 it is possible that CD14 interacts with another receptor to induce response to PGN which could

Figure 6.1 CD 14 is not required for inducing LC migration in response to IgE-mediated mast cell activation. CD14-/- mice and WT control. Saline or anti-TNP IgE were given i.d. in ear pinnae 7-14 days prior to the i.v. TNP-BSA injection. After 18h cells from draining LN were harvested and total cells and Langerin-positive cells were counted. A, Mean ± SEM numbers of Langerin/CD207 positive DC/draining LN. B, Mean ± SEM number of total cells/draining LN. * represents $P<0.05$ ** represents $P<0.01$

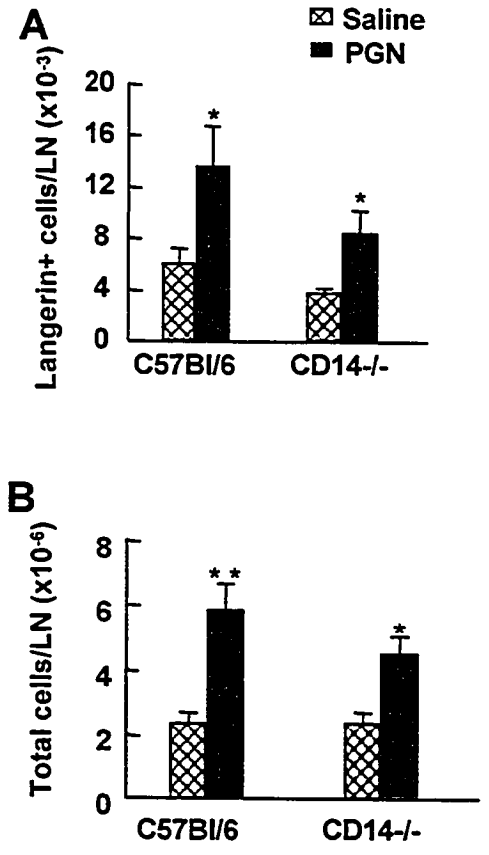
A
Saline
PGN
Langerin+ cells/LN (x10-3)
20
16
12
8
4
0
C57Bl/6
CD14-/-
B
Total cells/LN (x10-6)
8
6
4
2
0
C57Bl/6
CD14-/-

Figure 6.1

Figure 6.2 CD14 might be required for mast cell induction of LC migration in response to PGN. CD14-/- mice and WT control received a saline injection as a control in one ear pinna and PGN injection in the contralateral ear. After 18h draining LN tissues were harvested and total cells and Langerin-positive DC were counted using anti-Langerin/CD207 flow cytometry. A, Mean ± SEM numbers of Langerin/CD207 positive DC/draining LN. B, Mean ± SEM number of total cells/draining LN. * represents $P<0.05$

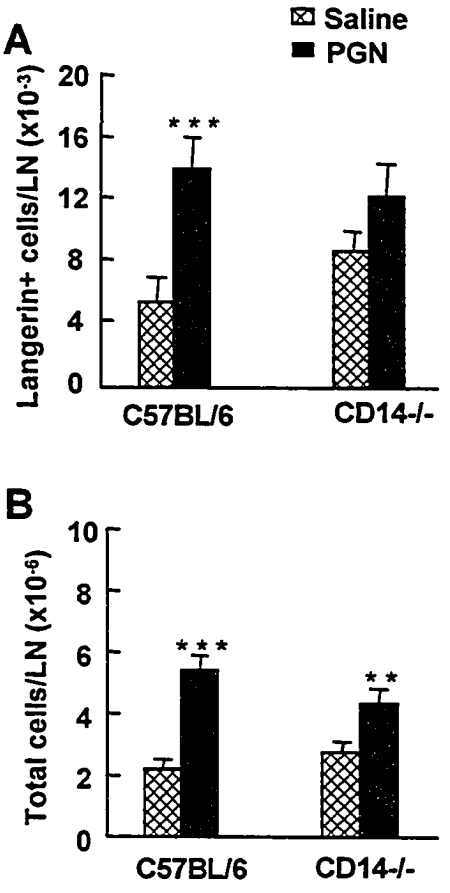
A
Saline
PGN
Langerin+ cells/LN (x10-3)
20
16
12
8
4
0
* * *
C57BL/6
CD14-/-
B
Total cells/LN (x10-6)
10
8
6
4
2
0
* * *
* *
C57BL/6
CD14-/-

Figure 6.2

be another TLR. However, this is unlikely to be TLR4 since the role of TLR4 in response to PGN in inducing Langerhans cell migration was ruled out using TLR4 deficient mice (chapter 4). Since complement components were shown to be required for inducing this response it is extremely interesting how these different molecules interact with PGN simultaneously to induce LC migration.

Mast cells may not express CD14, however PGN can bind to both soluble and membrane CD14. Interestingly, it has been shown that soluble CD14-PGN complexes have the ability to indirectly activate CD14-negative endothelial cells through the secretion of TNF and IL-1β by monocytes (Jin et al., 1998). Thus, CD14 possesses the ability for both direct and indirect activation of cells. Since both mast cells and LCs express complement component receptors and specifically C3 is required to induce LC migration it is not impossible to suggest that CD14 interacts with complement system. Indeed, it has been shown that CD14 and CR3 and CR4 were required in response to whole bacteria (group B streptococci) to induce NF-κB activation and the release of TNF (Medvedev et al., 1998). This study demonstrates the complexity of our immune system and the importance of multiple receptor interactions. It also demonstrates the importance of CD14 not only for enhancing pro-inflammatory reactions but in the induction of DC migration which is the first step for primary adaptive immune responses.

References

Abeck D. and Mempel M. (1998) Staphylococcus aureus colonization in atopic dermatitis and its therapeutic implications. *Br J Dermatol* **139 Suppl 53**, 13-6.

Adema G. J., Hartgers F., Verstraten R., de Vries E., Marland G., Menon S., Foster J., Xu Y., Nooyen P., McClanahan T., Bacon K. B. and Figdor C. G. (1997) A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. *Nature* **387**, 713-7.

Agis H., Fureder W., Bankl H. C., Kundi M., Sperr W. R., Willheim M., Boltz-Nitulescu G., Butterfield J. H., Kishi K., Lechner K. and Valent P. (1996) Comparative immunophenotypic analysis of human mast cells, blood basophils and monocytes. *Immunology* **87**, 535-43.

Akira S., Takeda K. and Kaisho T. (2001) Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* **2**, 675-80.

Al-Alwan M. M., Rowden G., Lee T. D. and West K. A. (2001) The dendritic cell cytoskeleton is critical for the formation of the immunological synapse. *J Immunol* **166**, 1452-6.

Alard P., Niizeki H., Hanninen L. and Streilein J. W. (1999) Local ultraviolet B irradiation impairs contact hypersensitivity induction by triggering release of tumor necrosis factor-alpha from mast cells. Involvement of mast cells and Langerhans cells in susceptibility to ultraviolet B. *J Invest Dermatol* **113**, 983-90.

Albert M. L., Jegathesan M. and Darnell R. B. (2001) Dendritic cell maturation is required for the cross-tolerization of CD8+ T cells. *Nat Immunol* **2**, 1010-7.

Anderson K. V. (2000) Toll signaling pathways in the innate immune response. *Curr Opin Immunol* **12**, 13-9.

Antohe F., Dobrila L. N. and Heltianu C. (1988) Histamine receptor on mast cells. *Microcirc Endothelium Lymphatics* **4**, 469-88.

Askenase P. W., Van Loveren H., Kraeuter-Kops S., Ron Y., Meade R., Theoharides T. C., Nordlund J. J., Scovern H., Gerhson M. D. and Ptak W. (1983) Defective elicitation of delayed-type hypersensitivity in W/Wv and SI/SId mast cell-deficient mice. *J Immunol* **131**, 2687-94.

Baker K. W., McKee-Protopapas S. and Habowsky J. E. (1983) The Langerhans cell in hairless mouse epidermis. *Scan Electron Microsc*, 457-65.

Banchereau J., Briere F., Caux C., Davoust J., Lebecque S., Liu Y. J., Pulendran B. and Palucka K. (2000a) Immunobiology of dendritic cells. *Annu Rev Immunol* **18**, 767-811.

Banchereau J., Briere F., Caux C., Davoust J., Lebecque S., Liu Y. J., Pulendran B. and Palucka K. (2000b) Immunobiology of dendritic cells [In Process Citation]. *Annu Rev Immunol* **18**, 767-811.

Banchereau J. and Steinman R. M. (1998) Dendritic cells and the control of immunity. *Nature* **392**, 245-52.

Barnes P. J. (2001) Histamine and serotonin. *Pulm Pharmacol Ther* **14**, 329-39.

Bennett S. R., Carbone F. R., Karamalis F., Flavell R. A., Miller J. F. and Heath W. R. (1998) Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* **393**, 478-80.

Birbeck MS B. A., Everall JD. (1961) An elctron microscopic study of basal melanocytes and high level clear cells (Langerhans cells) in vitilogo. *J Invest Dermatol* **37**, 51-64.

Bischoff S. C., Sellge G., Manns M. P. and Lorentz A. (2001) Interleukin-4 induces a switch of human intestinal mast cells from proinflammatory cells to Th2-type cells. *Int Arch Allergy Immunol* **124**, 151-4.

Bissonnette E. Y., Enciso J. A. and Befus A. D. (1997) TGF-beta1 inhibits the release of histamine and tumor necrosis factor-alpha from mast cells through an autocrine pathway. *Am J Respir Cell Mol Biol* **16**, 275-82.

Blander J. M. and Medzhitov R. (2004) Regulation of phagosome maturation by signals from toll-like receptors. *Science* **304**, 1014-8.

Blank U. and Rivera J. (2004) The ins and outs of IgE-dependent mast-cell exocytosis. *Trends Immunol* **25**, 266-73.

Bleiss W., Oberlander U., Hartmann S., Adam R., Marko A., Schonemeyer A. and Lucius R. (2002) Protective immunity induced by irradiated third-stage larvae of the filaria Acanthocheilonema viteae is directed against challenge third-stage larvae before molting. *J Parasitol* **88**, 264-70.

Bochner B. S. and Schleimer R. P. (2001) Mast cells, basophils, and eosinophils: distinct but overlapping pathways for recruitment. *Immunol Rev* **179**, 5-15.

Borkowski T. A., Letterio J. J., Farr A. G. and Udey M. C. (1996) A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. *J Exp Med* **184**, 2417-22.

Borkowski T. A., Letterio J. J., Mackall C. L., Saitoh A., Farr A. G., Wang X. J., Roop D. R., Gress R. E. and Udey M. C. (1997) Langerhans cells in the TGF beta 1 null mouse. *Adv Exp Med Biol* **417**, 307-10.

Bradding P., Feather I. H., Wilson S., Bardin P. G., Heusser C. H., Holgate S. T. and Howarth P. H. (1993) Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* **151**, 3853-65.

Brightbill H. D., Libraty D. H., Krutzik S. R., Yang R. B., Belisle J. T., Bleharski J. R., Maitland M., Norgard M. V., Plevy S. E., Smale S. T., Brennan P. J., Bloom B. R., Godowski P. J. and Modlin R. L. (1999) Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* **285**, 732-6.

Brown G. D. and Gordon S. (2001) Immune recognition. A new receptor for beta-glucans. *Nature* **413**, 36-7.

Brown S. J., Galli S. J., Gleich G. J. and Askenase P. W. (1982) Ablation of immunity to Amblyomma americanum by anti-basophil serum: cooperation between basophils and eosinophils in expression of immunity to ectoparasites (ticks) in guinea pigs. *J Immunol* **129**, 790-6.

Bryce P. J., Miller M. L., Miyajima I., Tsai M., Galli S. J. and Oettgen H. C. (2004) Immune sensitization in the skin is enhanced by antigen-independent effects of IgE. *Immunity* **20**, 381-92.

Buckley M. G., McEuen A. R. and Walls A. F. (2002) The return of the basophil. *Clin Exp Allergy* **32**, 8-10.

Burd P. R., Rogers H. W., Gordon J. R., Martin C. A., Jayaraman S., Wilson S. D., Dvorak A. M., Galli S. J. and Dorf M. E. (1989) Interleukin 3-dependent and -independent mast cells stimulated with IgE and antigen express multiple cytokines. *J Exp Med* **170**, 245-57.

Burdin N. and Kronenberg M. (1999) CD1-mediated immune responses to glycolipids. *Curr Opin Immunol* **11**, 326-31.

Caron G., Delneste Y., Roelandts E., Duez C., Bonnefoy J. Y., Pestel J. and Jeannin P. (2001a) Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol* **167**, 3682-6.

Caron G., Delneste Y., Roelandts E., Duez C., Herbault N., Magistrelli G., Bonnefoy J. Y., Pestel J. and Jeannin P. (2001b) Histamine induces CD86 expression and chemokine production by human immature dendritic cells. *J Immunol* **166**, 6000-6.

Chapman H. A. (1997) Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. *Curr Opin Cell Biol* **9**, 714-24.

Charbonnier A. S., Kohrgruber N., Kriehuber E., Stingl G., Rot A. and Maurer D. (1999) Macrophage inflammatory protein 3alpha is involved in the constitutive trafficking of epidermal langerhans cells. *J Exp Med* **190**, 1755-68.

Chen Z., Chen J. Q. and Kamei C. (2001) Effect of H1-antagonists on spatial memory deficit evaluated by 8-arm radial maze in rats. *Acta Pharmacol Sin* **22**, 609-13.

Compton S. J., Cairns J. A., Holgate S. T. and Walls A. F. (1998) The role of mast cell tryptase in regulating endothelial cell proliferation, cytokine release, and adhesion molecule expression: tryptase induces expression of

mRNA for IL-1 beta and IL-8 and stimulates the selective release of IL-8 from human umbilical vein endothelial cells. *J Immunol* **161**, 1939-46.

Corne J. M. and Holgate S. T. (1997) Mechanisms of virus induced exacerbations of asthma. *Thorax* **52**, 380-9.

Cumberbatch M., Dearman R. J., Antonopoulos C., Groves R. W. and Kimber I. (2001) Interleukin (IL)-18 induces Langerhans cell migration by a tumour necrosis factor-alpha- and IL-1beta-dependent mechanism. *Immunology* **102**, 323-30.

Cumberbatch M., Dearman R. J., Griffiths C. E. and Kimber I. (2000a) Langerhans cell migration. *Clin Exp Dermatol* **25**, 413-8.

Cumberbatch M., Dearman R. J. and Kimber I. (1997a) Interleukin 1 beta and the stimulation of Langerhans cell migration: comparisons with tumour necrosis factor alpha. *Arch Dermatol Res* **289**, 277-84.

Cumberbatch M., Dearman R. J. and Kimber I. (1997b) Langerhans cells require signals from both tumour necrosis factor alpha and interleukin 1 beta for migration. *Adv Exp Med Biol* **417**, 125-8.

Cumberbatch M., Dearman R. J. and Kimber I. (1997c) Langerhans cells require signals from both tumour necrosis factor-alpha and interleukin-1 beta for migration. *Immunology* **92**, 388-95.

Cumberbatch M., Dearman R. J. and Kimber I. (1997d) Stimulation of Langerhans cell migration in mice by tumour necrosis factor alpha and interleukin 1 beta. *Adv Exp Med Biol* **417**, 121-4.

Cumberbatch M., Dearman R. J., Uribe-Luna S., Headon D. R., Ward P. P., Conneely O. M. and Kimber I. (2000b) Regulation of epidermal Langerhans cell migration by lactoferrin. *Immunology* **100**, 21-8.

Cumberbatch M., Fielding I. and Kimber I. (1994) Modulation of epidermal Langerhans' cell frequency by tumour necrosis factor-alpha. *Immunology* **81**, 395-401.

Cumberbatch M. and Kimber I. (1992) Dermal tumour necrosis factor-alpha induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. *Immunology* **75**, 257-63.

Cutts L. and Wilson R. A. (1997) Elimination of a primary schistosome infection from rats coincides with elevated IgE titres and mast cell degranulation. *Parasite Immunol* **19**, 91-102.

Cyster J. G. (1999) Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. *J Exp Med* **189**, 447-50.

da Silva Correia J., Soldau K., Christen U., Tobias P. S. and Ulevitch R. J. (2001) Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem* **276**, 21129-35.

Dabbagh K., Dahl M. E., Stepick-Biek P. and Lewis D. B. (2002) Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* **168**, 4524-30.

Damsgaard T. E., Olesen A. B., Sorensen F. B., Thestrup-Pedersen K. and Schiotz P. O. (1997) Mast cells and atopic dermatitis. Stereological quantification of mast cells in atopic dermatitis and normal human skin. *Arch Dermatol Res* **289**, 256-60.

Denzer K., van Eijk M., Kleijmeer M. J., Jakobson E., de Groot C. and Geuze H. J. (2000) Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. *J Immunol* **165**, 1259-65.

Dhodapkar M. V., Steinman R. M., Krasovsky J., Munz C. and Bhardwaj N. (2001) Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J Exp Med* **193**, 233-8.

Di Nardo A., Vitiello A. and Gallo R. L. (2003) Cutting edge: mast cell antimicrobial activity is mediated by expression of cathelicidin antimicrobial peptide. *J Immunol* **170**, 2274-8.

Dieu M. C., Vanbervliet B., Vicari A., Bridon J. M., Oldham E., Ait-Yahia S., Briere F., Zlotnik A., Lebecque S. and Caux C. (1998) Selective

recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* **188**, 373-86.

Dieu-Nosjean M. C., Massacrier C., Homey B., Vanbervliet B., Pin J. J., Vicari A., Lebecque S., Dezutter-Dambuyant C., Schmitt D., Zlotnik A. and Caux C. (2000) Macrophage inflammatory protein 3alpha is expressed at inflamed epithelial surfaces and is the most potent chemokine known in attracting Langerhans cell precursors. *J Exp Med* **192**, 705-18.

Drazen J. M. (1998) Leukotrienes as mediators of airway obstruction. *Am J Respir Crit Care Med* **158**, S193-200.

Dvorak A. M. (1986) Morphologic expression of maturation and function can effect the ability to identify mast cells and basophils in man, guinea pig, and mouse. *In: "Mast cell differentiation and heterogenety"(A.D Befus, J. Bienenstock, and J. Denburg, Eds) Raven Press, New York*, p. 95.

Dziarski R. (2004) Peptidoglycan recognition proteins (PGRPs). *Mol Immunol* **40**, 877-86.

Dziarski R., Tapping R. I. and Tobias P. S. (1998) Binding of bacterial peptidoglycan to CD14. *J Biol Chem* **273**, 8680-90.

Dziarski R., Viriyakosol S., Kirkland T. N. and Gupta D. (2000) Soluble CD14 enhances membrane CD14-mediated responses to peptidoglycan: structural requirements differ from those for responses to lipopolysaccharide. *Infect Immun* **68**, 5254-60.

Echchannaoui H., Frei K., Schnell C., Leib S. L., Zimmerli W. and Landmann R. (2002) Toll-like receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningitis because of reduced bacterial clearing and enhanced inflammation. *J Infect Dis* **186**, 798-806.

Echtenacher B., Mannel D. N. and Hultner L. (1996a) Critical protective role of mast cells in a model of acute septic peritonitis. *Nature* **381**, 75-7.

Echtenacher B., Mannel D. N. and Hultner L. (1996b) Critical protective role of mast cells in a model of acute septic peritonitis [see comments]. *Nature* **381**, 75-7.

Ehrlich P. (1878) Beitrage zur Theorie und Histologichen Farbung. *University of Leipzig. Ref Type: Thesis/Dissertation.*

Enk A. H., Angeloni V. L., Udey M. C. and Katz S. I. (1993) An essential role for Langerhans cell-derived IL-1 beta in the initiation of primary immune responses in skin. *J Immunol* **150**, 3698-704.

Erdei A., Andrasfalvy M., Peterfy H., Toth G. and Pecht I. (2004) Regulation of mast cell activation by complement-derived peptides. *Immunol Lett* **92**, 39-42.

Erdei A., Toth G. K., Andrasfalvy M., Matko J., Bene L., Bajtay Z., Ischenko A., Rong X. and Pecht I. (1999) Inhibition of IgE-mediated triggering of mast cells by complement-derived peptides interacting with the Fc epsilon RI. *Immunol Lett* **68**, 79-82.

Falcone F. H., Pritchard D. I. and Gibbs B. F. (2001) Do basophils play a role in immunity against parasites? *Trends Parasitol* **17**, 126-9.

Faveeuw C., Gosset P., Bureau F., Angeli V., Hirai H., Maruyama T., Narumiya S., Capron M. and Trottein F. (2003) Prostaglandin D2 inhibits the production of interleukin-12 in murine dendritic cells through multiple signaling pathways. *Eur J Immunol* **33**, 889-98.

Ferbas J. J., Toso J. F., Logar A. J., Navratil J. S. and Rinaldo C. R., Jr. (1994) CD4+ blood dendritic cells are potent producers of IFN-alpha in response to in vitro HIV-1 infection. *J Immunol* **152**, 4649-62.

Fithian E., Kung P., Goldstein G., Rubenfeld M., Fenoglio C. and Edelson R. (1981) Reactivity of Langerhans cells with hybridoma antibody. *Proc Natl Acad Sci U S A* **78**, 2541-4.

Forster R., Schubel A., Breitfeld D., Kremmer E., Renner-Muller I., Wolf E. and Lipp M. (1999) CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* **99**, 23-33.

Frandji P., Tkaczyk C., Oskeritzian C., David B., Desaymard C. and Mecheri S. (1996) Exogenous and endogenous antigens are differentially presented by mast cells to CD4+ T lymphocytes. *Eur J Immunol* **26**, 2517-28.

Freeland H. S., Schleimer R. P., Schulman E. S., Lichtenstein L. M. and Peters S. P. (1988) Generation of leukotriene B4 by human lung fragments and purified human lung mast cells. *Am Rev Respir Dis* **138**, 389-94.

Fureder W., Agis H., Willheim M., Bankl H. C., Maier U., Kishi K., Muller M. R., Czerwenka K., Radaszkiewicz T., Butterfield J. H. and et al. (1995) Differential expression of complement receptors on human basophils and mast cells. Evidence for mast cell heterogeneity and CD88/C5aR expression on skin mast cells. *J Immunol* **155**, 3152-60.

Galli S. J. and Hammel I. (1984) Unequivocal delayed hypersensitivity in mast cell-deficient and beige mice. *Science* **226**, 710-3.

Galli S. J., Maurer M. and Lantz C. S. (1999) Mast cells as sentinels of innate immunity. *Curr Opin Immunol* **11**, 53-9.

Galli S. J., Wershil B. K., Gordon J. R. and Martin T. R. (1989) Mast cells: immunologically specific effectors and potential sources of multiple cytokines during IgE-dependent responses. *Ciba Found Symp* **147**, 53-65; discussion 65-73.

Gantner B. N., Simmons R. M., Canavera S. J., Akira S. and Underhill D. M. (2003) Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J Exp Med* **197**, 1107-17.

Gasque P. (2004) Complement: a unique innate immune sensor for danger signals. *Mol Immunol* **41**, 1089-98.

Gauchat J. F., Henchoz S., Mazzei G., Aubry J. P., Brunner T., Blasey H., Life P., Talabot D., Flores-Romo L., Thompson J. and et al. (1993) Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature* **365**, 340-3.

Geijtenbeek T. B., Engering A. and Van Kooyk Y. (2002) DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. *J Leukoc Biol* **71**, 921-31.

Geijtenbeek T. B., Krooshoop D. J., Bleijs D. A., van Vliet S. J., van Duijnhoven G. C., Grabovsky V., Alon R., Figdor C. G. and van Kooyk Y. (2000) DC-

SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat Immunol* **1**, 353-7.

Geissmann F., Revy P., Regnault A., Lepelletier Y., Dy M., Brousse N., Amigorena S., Hermine O. and Durandy A. (1999) TGF-beta 1 prevents the noncognate maturation of human dendritic Langerhans cells. *J Immunol* **162**, 4567-75.

Genovese A., Bouvet J. P., Florio G., Lamparter-Schummert B., Bjorck L. and Marone G. (2000) Bacterial immunoglobulin superantigen proteins A and L activate human heart mast cells by interacting with immunoglobulin E. *Infect Immun* **68**, 5517-24.

Gordon J. R., Burd P. R. and Galli S. J. (1990) Mast cells as a source of multifunctional cytokines. *Immunol Today* **11**, 458-64.

Gordon J. R. and Galli S. J. (1990) Mast cells as a source of both preformed and immunologically inducible TNF-alpha/cachectin. *Nature* **346**, 274-6.

Gordon J. R. and Galli S. J. (1991) Release of both preformed and newly synthesized tumor necrosis factor alpha (TNF-alpha)/cachectin by mouse mast cells stimulated via the Fc epsilon RI. A mechanism for the sustained action of mast cell-derived TNF-alpha during IgE-dependent biological responses. *J Exp Med* **174**, 103-7.

Gosset P., Bureau F., Angeli V., Pichavant M., Faveeuw C., Tonnel A. B. and Trottein F. (2003) Prostaglandin D2 affects the maturation of human monocyte-derived dendritic cells: consequence on the polarization of naive Th cells. *J Immunol* **170**, 4943-52.

Gotis-Graham I. and McNeil H. P. (1997) Mast cell responses in rheumatoid synovium. Association of the MCTC subset with matrix turnover and clinical progression. *Arthritis Rheum* **40**, 479-89.

Grakoui A., Bromley S. K., Sumen C., Davis M. M., Shaw A. S., Allen P. M. and Dustin M. L. (1999) The immunological synapse: a molecular machine controlling T cell activation. *Science* **285**, 221-7.

Greenblatt J., Boackle R. J. and Schwab J. H. (1978) Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. *Infect Immun* **19**, 296-303.

Grencis R. K. (1997) Th2-mediated host protective immunity to intestinal nematode infections. *Philos Trans R Soc Lond B Biol Sci* **352**, 1377-84.

Grunwald U., Kruger C., Westermann J., Lukowsky A., Ehlers M. and Schutt C. (1992) An enzyme-linked immunosorbent assay for the quantification of solubilized CD14 in biological fluids. *J Immunol Methods* **155**, 225-32.

Gunn M. D., Kyuwa S., Tam C., Kakiuchi T., Matsuzawa A., Williams L. T. and Nakano H. (1999) Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J Exp Med* **189**, 451-60.

Gunn M. D., Tangemann K., Tam C., Cyster J. G., Rosen S. D. and Williams L. T. (1998) A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci U S A* **95**, 258-63.

Gupta A. A., Leal-Berumen I., Croitoru K. and Marshall J. S. (1996a) Rat peritoneal mast cells produce IFN-gamma following IL-12 treatment but not in response to IgE-mediated activation. *J Immunol* **157**, 2123-8.

Gupta D., Kirkland T. N., Viriyakosol S. and Dziarski R. (1996b) CD14 is a cell-activating receptor for bacterial peptidoglycan. *J Biol Chem* **271**, 23310-6.

Gutzmer R., Langer K., Lisewski M., Mommert S., Rieckborn D., Kapp A. and Werfel T. (2002) Expression and function of histamine receptors 1 and 2 on human monocyte-derived dendritic cells. *J Allergy Clin Immunol* **109**, 524-31.

Hasan U., Chaffois C., Gaillard C., Saulnier V., Merck E., Tancredi S., Guiet C., Briere F., Vlach J., Lebecque S., Trinchieri G. and Bates E. E. (2005) Human TLR10 is a functional receptor, expressed by B cells and plasmacytoid dendritic cells, which activates gene transcription through MyD88. *J Immunol* **174**, 2942-50.

Hashimoto K. and Tarnowski W. M. (1968) Some new aspects of the Langerhans cell. *Arch Dermatol* **97**, 450-64.

Hayashi S., Matsuda H., Okumura K. and Ra C. (1999) Mouse Langerhans cells do not express the high-affinity receptor for IgE. *Arch Dermatol Res* **291**, 241-3.

Haziot A., Ferrero E., Lin X. Y., Stewart C. L. and Goyert S. M. (1995) CD14-deficient mice are exquisitely insensitive to the effects of LPS. *Prog Clin Biol Res* **392**, 349-51.

Heavey D. J., Ernst P. B., Stevens R. L., Befus A. D., Bienenstock J. and Austen K. F. (1988) Generation of leukotriene C4, leukotriene B4, and prostaglandin D2 by immunologically activated rat intestinal mucosa mast cells. *J Immunol* **140**, 1953-7.

Henri S., Vremec D., Kamath A., Waithman J., Williams S., Benoist C., Burnham K., Saeland S., Handman E. and Shortman K. (2001) The dendritic cell populations of mouse lymph nodes. *J Immunol* **167**, 741-8.

Henz B. M., Maurer M., Lippert U., Worm M. and Babina M. (2001) Mast cells as initiators of immunity and host defense. *Exp Dermatol* **10**, 1-10.

Heufler C., Koch F. and Schuler G. (1988) Granulocyte/macrophage colony-stimulating factor and interleukin 1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. *J Exp Med* **167**, 700-5.

Hill S. J. (1990) Distribution, properties, and functional characteristics of three classes of histamine receptor. *Pharmacol Rev* **42**, 45-83.

Hogaboam C., Kunkel S. L., Strieter R. M., Taub D. D., Lincoln P., Standiford T. J. and Lukacs N. W. (1998) Novel role of transmembrane SCF for mast cell activation and eotaxin production in mast cell-fibroblast interactions. *J Immunol* **160**, 6166-71.

Hoshino K., Takeuchi O., Kawai T., Sanjo H., Ogawa T., Takeda Y., Takeda K. and Akira S. (1999) Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* **162**, 3749-52.

Hsieh F. H., Lam B. K., Penrose J. F., Austen K. F. and Boyce J. A. (2001) T helper cell type 2 cytokines coordinately regulate immunoglobulin E-dependent cysteinyl leukotriene production by human cord blood-derived mast cells: profound induction of leukotriene C(4) synthase expression by interleukin 4. *J Exp Med* **193**, 123-33.

Huang C., Friend D. S., Qiu W. T., Wong G. W., Morales G., Hunt J. and Stevens R. L. (1998) Induction of a selective and persistent extravasation of neutrophils into the peritoneal cavity by tryptase mouse mast cell protease 6. *J Immunol* **160**, 1910-9.

Idzko M., la Sala A., Ferrari D., Panther E., Herouy Y., Dichmann S., Mockenhaupt M., Di Virgilio F., Girolomoni G. and Norgauer J. (2002) Expression and function of histamine receptors in human monocyte-derived dendritic cells. *J Allergy Clin Immunol* **109**, 839-46.

Ikeda T. and Funaba M. (2003) Altered function of murine mast cells in response to lipopolysaccharide and peptidoglycan. *Immunol Lett* **88**, 21-6.

Inohara N., Ogura Y. and Nunez G. (2002) Nods: a family of cytosolic proteins that regulate the host response to pathogens. *Curr Opin Microbiol* **5**, 76-80.

Irani A. A., Schechter N. M., Craig S. S., DeBlois G. and Schwartz L. B. (1986) Two types of human mast cells that have distinct neutral protease compositions. *Proc Natl Acad Sci U S A* **83**, 4464-8.

Irani A. M., Butrus S. I., Tabbara K. F. and Schwartz L. B. (1990) Human conjunctival mast cells: distribution of MCT and MCTC in vernal conjunctivitis and giant papillary conjunctivitis. *J Allergy Clin Immunol* **86**, 34-40.

Jakob T. and Udey M. C. (1998) Regulation of E-cadherin-mediated adhesion in Langerhans cell-like dendritic cells by inflammatory mediators that mobilize Langerhans cells in vivo. *J Immunol* **160**, 4067-73.

Jarrett E. E. and Miller H. R. (1982) Production and activities of IgE in helminth infection. *Prog Allergy* **31**, 178-233.

Jawdat D. M., Albert E. J., Rowden G., Haidl I. D. and Marshall J. S. (2004) IgE-mediated mast cell activation induces Langerhans cell migration in vivo. *J Immunol* **173**, 5275-82.

Jia G. Q., Gonzalo J. A., Lloyd C., Kremer L., Lu L., Martinez A. C., Wershil B. K. and Gutierrez-Ramos J. C. (1996) Distinct expression and function of the novel mouse chemokine monocyte chemotactic protein-5 in lung allergic inflammation. *J Exp Med* **184**, 1939-51.

Jin Y., Gupta D. and Dziarski R. (1998) Endothelial and epithelial cells do not respond to complexes of peptidoglycan with soluble CD14 but are activated indirectly by peptidoglycan-induced tumor necrosis factor-alpha and interleukin-1 from monocytes. *J Infect Dis* **177**, 1629-38.

Jin Z. W., Kumar A., Cleveland R. P., Murray D. L. and Kaufman D. B. (1986) Inhibition of suppressor cell function by cimetidine in a murine model. *Clin Immunol Immunopathol* **38**, 350-6.

Johnson E. E., Irons J. S., Patterson R. and Roberts M. (1974) Serum IgE concentration in atopic dermatitis. Relationship to severity of disease and presence of atopic respiratory disease. *J Allergy Clin Immunol* **54**, 94-9.

Kabashima K., Sakata D., Nagamachi M., Miyachi Y., Inaba K. and Narumiya S. (2003) Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. *Nat Med* **9**, 744-9.

Kadowaki N., Ho S., Antonenko S., Malefyt R. W., Kastelein R. A., Bazan F. and Liu Y. J. (2001) Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. *J Exp Med* **194**, 863-9.

Kalinski P., Hilkens C. M., Snijders A., Snijdewint F. G. and Kapsenberg M. L. (1997) IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. *J Immunol* **159**, 28-35.

Kanazawa N., Nakamura T., Tashiro K., Muramatsu M., Morita K., Yoneda K., Inaba K., Imamura S. and Honjo T. (1999) Fractalkine and macrophage-derived chemokine: T cell-attracting chemokines expressed in T cell area dendritic cells. *Eur J Immunol* **29**, 1925-32.

Kandere-Grzybowska K., Letourneau R., Kempuraj D., Donelan J., Poplawski S., Boucher W., Athanassiou A. and Theoharides T. C. (2003) IL-1 induces vesicular secretion of IL-6 without degranulation from human mast cells. *J Immunol* **171**, 4830-6.

Kaser A., Dunzendorfer S., Offner F. A., Ryan T., Schwabegger A., Cruikshank W. W., Wiedermann C. J. and Tilg H. (1999) A role for IL-16 in the cross-talk between dendritic cells and T cells. *J Immunol* **163**, 3232-8.

Kashiwakura J., Yokoi H., Saito H. and Okayama Y. (2004) T cell proliferation by direct cross-talk between OX40 ligand on human mast cells and OX40 on human T cells: comparison of gene expression profiles between human tonsillar and lung-cultured mast cells. *J Immunol* **173**, 5247-57.

Kawai T., Adachi O., Ogawa T., Takeda K. and Akira S. (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* **11**, 115-22.

Kent U. M., Mao S. Y., Wofsy C., Goldstein B., Ross S. and Metzger H. (1994) Dynamics of signal transduction after aggregation of cell-surface receptors: studies on the type I receptor for IgE. *Proc Natl Acad Sci U S A* **91**, 3087-91.

Kepley C. L., McFeeley P. J., Oliver J. M. and Lipscomb M. F. (2001) Immunohistochemical detection of human basophils in postmortem cases of fatal asthma. *Am J Respir Crit Care Med* **164**, 1053-8.

Kepley C. L., Pfeiffer J. R., Schwartz L. B., Wilson B. S. and Oliver J. M. (1998) The identification and characterization of umbilical cord blood-derived human basophils. *J Leukoc Biol* **64**, 474-83.

Kimura T., Andoh A., Fujiyama Y., Saotome T. and Bamba T. (1998) A blockade of complement activation prevents rapid intestinal ischaemia-reperfusion injury by modulating mucosal mast cell degranulation in rats. *Clin Exp Immunol* **111**, 484-90.

King C. A., Anderson R. and Marshall J. S. (2002) Dengue virus selectively induces human mast cell chemokine production. *J Virol* **76**, 8408-19.

King C. A., Marshall J. S., Alshurafa H. and Anderson R. (2000) Release of vasoactive cytokines by antibody enhanced dengue virus infection of a human mast cell/basophil line. *J. Virol* **74**, 7146 -7150.

Kinnaird A., Peters S. W., Foster J. R. and Kimber I. (1989) Dendritic cell accumulation in draining lymph nodes during the induction phase of contact allergy in mice. *Int Arch Allergy Appl Immunol* **89**, 202-10.

Kitamura H., Iwakabe K., Yahata T., Nishimura S., Ohta A., Ohmi Y., Sato M., Takeda K., Okumura K., Van Kaer L., Kawano T., Taniguchi M. and Nishimura T. (1999) The natural killer T (NKT) cell ligand alpha-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J Exp Med* **189**, 1121-8.

Klareskog L., Tjernlund U., Forsum U. and Peterson P. A. (1977) Epidermal Langerhans cells express Ia antigens. *Nature* **268**, 248-50.

Klinkert W. E., LaBadie J. H., O'Brien J. P., Beyer C. F. and Bowers W. E. (1980) Rat dendritic cells function as accessory cells and control the production of a soluble factor required for mitogenic responses of T lymphocytes. *Proc Natl Acad Sci U S A* **77**, 5414-8.

Kobayashi Y., Matsumoto M., Kotani M. and Makino T. (1999) Possible involvement of matrix metalloproteinase-9 in Langerhans cell migration and maturation. *J Immunol* **163**, 5989-93.

Koedel U., Angele B., Rupprecht T., Wagner H., Roggenkamp A., Pfister H. W. and Kirschning C. J. (2003) Toll-like receptor 2 participates in mediation of immune response in experimental pneumococcal meningitis. *J Immunol* **170**, 438-44.

Kohno S., Ogawa K., Nabe T., Yamamura H. and Ohata K. (1993) Dimaprit, a histamine H2-agonist, inhibits anaphylactic histamine release from mast cells and the decreased release is restored by thioperamide (H3-antagonist), but not by cimetidine (H2-antagonist). *Jpn J Pharmacol* **62**, 75-9.

Kondo Y. (1969) Macrophages containing langerhans cell granules in normal lymph nodes of the rabbit. *Z Zellforsch Mikrosk Anat* **98**, 506-11.

Kovacsovics-Bankowski M., Clark K., Benacerraf B. and Rock K. L. (1993) Efficient major histocompatibility complex class I presentation of exogenous antigen upon phagocytosis by macrophages. *Proc Natl Acad Sci U S A* **90**, 4942-6.

Kovacsovics-Bankowski M. and Rock K. L. (1995) A phagosome-to-cytosol pathway for exogenous antigens presented on MHC class I molecules. *Science* **267**, 243-6.

Kulka M., Alexopoulou L., Flavell R. A. and Metcalfe D. D. (2004) Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. *J Allergy Clin Immunol* **114**, 174-82.

Kurihara H., Fukami H., Asami S., Shibata H., Kiso Y., Tanaka T. and Yao X. S. (2003) Influence of histamine in a liver injury model induced by Propionibacterium acnes and lipopolysaccharide. *Biol Pharm Bull* **26**, 1393-7.

Langerhans P. (1868) Ueber die Nerven der Menschlichen Haut. *Virchows Arch path Anat physio* **44**, 325-337.

Lantz C. S., Boesiger J., Song C. H., Mach N., Kobayashi T., Mulligan R. C., Nawa Y., Dranoff G. and Galli S. J. (1998) Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites. *Nature* **392**, 90-3.

Leal-Berumen I., Conlon P. and Marshall J. S. (1994) IL-6 production by rat peritoneal mast cells is not necessarily preceded by histamine release and can be induced by bacterial lipopolysaccharide. *J Immunol* **152**, 5468-76.

Lebre M. C., Kalinski P., Das P. K. and Everts V. (1999) Inhibition of contact sensitizer-induced migration of human Langerhans cells by matrix metalloproteinase inhibitors. *Arch Dermatol Res* **291**, 447-52.

Lemaitre B., Nicolas E., Michaut L., Reichhart J. M. and Hoffmann J. A. (1996) The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. *Cell* **86**, 973-83.

Leung D. Y. (1993) Role of IgE in atopic dermatitis. *Curr Opin Immunol* **5**, 956-62.

Leung D. Y. (1998) Molecular basis of allergic diseases. *Mol Genet Metab* **63**, 157-67.

Leung D. Y. (2002) Role os Staphylococcus aureus in atopic dermatitis. *In atopic dermatitis, Edited by Leung DYM, Bieber T. New York: Marcel Dekker, Inc*, 401-418.

Leung D. Y., Boguniewicz M., Howell M. D., Nomura I. and Hamid Q. A. (2004) New insights into atopic dermatitis. *J Clin Invest* **113**, 651-7.

Lin T. J., Maher L. H., Gomi K., McCurdy J. D., Garduno R. and Marshall J. S. (2003) Selective early production of CCL20, or macrophage inflammatory protein 3alpha, by human mast cells in response to Pseudomonas aeruginosa. *Infect Immun* **71**, 365-73.

Lin T.-J., Zhu F.-G. and Marshall J. S. (2000) Differential regulation of mast cell mediaotr secretion by bacterial products. In *Mast cells and basophils* (Edited by Galli S. J.), p. 419

432. Academic Press, London.

Lovenberg T. W., Roland B. L., Wilson S. J., Jiang X., Pyati J., Huvar A., Jackson M. R. and Erlander M. G. (1999) Cloning and functional expression of the human histamine H3 receptor. *Mol Pharmacol* **55**, 1101-7.

Ma Y. G., Cho M. Y., Zhao M., Park J. W., Matsushita M., Fujita T. and Lee B. L. (2004) Human mannose-binding lectin and L-ficolin function as specific pattern recognition proteins in the lectin activation pathway of complement. *J Biol Chem* **279**, 25307-12.

Macatonia S. E., Knight S. C., Edwards A. J., Griffiths S. and Fryer P. (1987) Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. *J Exp Med* **166**, 1654-67.

MacGlashan D., Jr. (2003) Histamine: A mediator of inflammation. *J Allergy Clin Immunol* **112**, S53-9.

Madden K. B., Urban J. F., Jr., Ziltener H. J., Schrader J. W., Finkelman F. D. and Katona I. M. (1991) Antibodies to IL-3 and IL-4 suppress helminth-induced intestinal mastocytosis. *J Immunol* **147**, 1387-91.

Malaviya R. and Abraham S. N. (2000a) Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J Leukoc Biol* **67**, 841-6.

Malaviya R. and Abraham S. N. (2000b) Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis [In Process Citation]. *J Leukoc Biol* **67**, 841-6.

Malaviya R., Gao Z., Thankavel K., van der Merwe P. A. and Abraham S. N. (1999) The mast cell tumor necrosis factor alpha response to FimH-expressing Escherichia coli is mediated by the glycosylphosphatidylinositol-anchored molecule CD48. *Proc Natl Acad Sci U S A* **96**, 8110-5.

Malaviya R., Ikeda T., Ross E. and Abraham S. N. (1996a) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature* **381**, 77-80.

Malaviya R., Ikeda T., Ross E. and Abraham S. N. (1996b) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha [see comments]. *Nature* **381**, 77-80.

Malaviya R., Ross E. A., MacGregor J. I., Ikeda T., Little J. R., Jakschik B. A. and Abraham S. N. (1994) Mast cell phagocytosis of FimH-expressing enterobacteria. *J Immunol* **152**, 1907-14.

Malaviya R., Twesten N. J., Ross E. A., Abraham S. N. and Pfeifer J. D. (1996c) Mast cells process bacterial Ags through a phagocytic route for class I MHC presentation to T cells. *J Immunol* **156**, 1490-6.

Maldonado-Lopez R. and Moser M. (2001) Dendritic cell subsets and the regulation of Th1/Th2 responses. *Semin Immunol* **13**, 275-82.

Manz M. G., Traver D., Miyamoto T., Weissman I. L. and Akashi K. (2001) Dendritic cell potentials of early lymphoid and myeloid progenitors. *Blood* **97**, 3333-41.

Marone G., Galli S. J. and Kitamura Y. (2002) Probing the roles of mast cells and basophils in natural and acquired immunity, physiology and disease. *Trends Immunol* **23**, 425-7.

Marshall J. S. (2004) Mast-cell responses to pathogens. *Nat Rev Immunol* **4**, 787-99.

Marshall J. S., Gauldie J., Nielsen L. and Bienenstock J. (1993) Leukemia inhibitory factor production by rat mast cells. *Eur J Immunol* **23**, 2116-20.

Marshall J. S., Gomi K., Blennerhassett M. G. and Bienenstock J. (1999) Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism. *J Immunol* **162**, 4271-6.

Marshall J. S. and Jawdat D. M. (2004) Mast cells in innate immunity. *J Allergy Clin Immunol* **114**, 21-7.

Marshall J. S., McCurdy J. D. and Olynych T. (2003) Toll-like receptor-mediated activation of mast cells: implications for allergic disease? *Int Arch Allergy Immunol* **132**, 87-97.

Marshall J. S. and Waserman S. (1995) Mast cells and the nerves--potential interactions in the context of chronic disease. *Clin Exp Allergy* **25**, 102-10.

Marshall R. (1972) Protective effect of disodium cromoglycate on rat peritoneal mast cells. *Thorax* **27**, 38-43.

Marshall R. (1975) Disruption of isolated peritoneal mast cells of the rat by human serum and that by other species. *Int Arch Allergy Appl Immunol* **49**, 647-57.

Masenga J., Garbe C., Wagner J. and Orfanos C. E. (1990) Staphylococcus aureus in atopic dermatitis and in nonatopic dermatitis. *Int J Dermatol* **29**, 579-82.

Matsuda H., Ushio H., Geba G. P. and Askenase P. W. (1997) Human platelets can initiate T cell-dependent contact sensitivity through local serotonin release mediated by IgE antibodies. *J Immunol* **158**, 2891-7.

Mazzoni A., Young H. A., Spitzer J. H., Visintin A. and Segal D. M. (2001) Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest* **108**, 1865-73.

McBride J. M. and Fathman C. G. (2002) A complicated relationship: fulfilling the interactive needs of the T lymphocyte and the dendritic cell. *Pharmacogenomics J* **2**, 367-76.

McCurdy J. D., Lin T. J. and Marshall J. S. (2001) Toll-like receptor 4-mediated activation of murine mast cells. *J Leukoc Biol* **70**, 977-84.

McCurdy J. D., Olynych T. J., Maher L. H. and Marshall J. S. (2003) Cutting edge: distinct Toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. *J Immunol* **170**, 1625-9.

McLachlan J. B., Hart J. P., Pizzo S. V., Shelburne C. P., Staats H. F., Gunn M. D. and Abraham S. N. (2003) Mast cell-derived tumor necrosis factor induces hypertrophy of draining lymph nodes during infection. *Nat Immunol* **4**, 1199-205.

McMillan R. M. (2001) Leukotrienes in respiratory disease. *Paediatr Respir Rev* **2**, 238-44.

Medvedev A. E., Flo T., Ingalls R. R., Golenbock D. T., Teti G., Vogel S. N. and Espevik T. (1998) Involvement of CD14 and complement receptors CR3 and CR4 in nuclear factor-kappaB activation and TNF production induced by lipopolysaccharide and group B streptococcal cell walls. *J Immunol* **160**, 4535-42.

Medzhitov R. and Janeway C., Jr. (2000) Innate immune recognition: mechanisms and pathways. *Immunol Rev* **173**, 89-97.

Medzhitov R., Preston-Hurlburt P. and Janeway C. A., Jr. (1997) A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* **388**, 394-7.

Mekori Y. A. and Metcalfe D. D. (1999) Mast cell-T cell interactions. *J Allergy Clin Immunol* **104**, 517-23.

Mencia-Huerta J. M., Lewis R. A., Razin E. and Austen K. F. (1983) Antigen-initiated release of platelet-activating factor (PAF-acether) from mouse bone marrow-derived mast cells sensitized with monoclonal IgE. *J Immunol* **131**, 2958-64.

Metcalfe D. D., Baram D. and Mekori Y. A. (1997) Mast cells. *Physiol Rev* **77**, 1033-79.

Mihm M. C., Jr., Soter N. A., Dvorak H. F. and Austen K. F. (1976) The structure of normal skin and the morphology of atopic eczema. *J Invest Dermatol* **67**, 305-12.

Mikhail G. R. and Miller-Milinska A. (1964) Mast Cell Population in Human Skin. *J Invest Dermatol* **43**, 249-54.

Mokhtarian F. and Griffin D. E. (1984) The role of mast cells in virus-induced inflammation in the murine central nervous system. *Cell Immunol* **86**, 491-500.

Moller A., Lippert U., Lessmann D., Kolde G., Hamann K., Welker P., Schadendorf D., Rosenbach T., Luger T. and Czarnetzki B. M. (1993) Human mast cells produce IL-8. *J Immunol* **151**, 3261-6.

Moodycliffe A. M., Shreedhar V., Ullrich S. E., Walterscheid J., Bucana C., Kripke M. L. and Flores-Romo L. (2000) CD40-CD40 ligand interactions in vivo regulate migration of antigen-bearing dendritic cells from the skin to draining lymph nodes. *J Exp Med* **191**, 2011-20.

Morelli A., Larregina A., Chuluyan I., Kolkowski E. and Fainboim L. (1996) Expression and modulation of C5a receptor (CD88) on skin dendritic cells. Chemotactic effect of C5a on skin migratory dendritic cells. *Immunology* **89**, 126-34.

Murphy G. F., Bhan A. K., Sato S., Harrist T. J. and Mihm M. C., Jr. (1981) Characterization of Langerhans cells by the use of monoclonal antibodies. *Lab Invest* **45**, 465-8.

Nagai Y., Akashi S., Nagafuku M., Ogata M., Iwakura Y., Akira S., Kitamura T., Kosugi A., Kimoto M. and Miyake K. (2002) Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol* **3**, 667-72.

Nakajima T., Inagaki N., Tanaka H., Tanaka A., Yoshikawa M., Tamari M., Hasegawa K., Matsumoto K., Tachimoto H., Ebisawa M., Tsujimoto G., Matsuda H., Nagai H. and Saito H. (2002) Marked increase in CC chemokine gene expression in both human and mouse mast cell transcriptomes following Fcepsilon receptor I cross-linking: an interspecies comparison. *Blood* **100**, 3861-8.

Nakano T., Sonoda T., Hayashi C., Yamatodani A., Kanayama Y., Yamamura T., Asai H., Yonezawa T., Kitamura Y. and Galli S. J. (1985) Fate of bone marrow-derived cultured mast cells after intracutaneous, intraperitoneal, and intravenous transfer into genetically mast cell-deficient W/Wv mice. Evidence that cultured mast cells can give rise to both connective tissue type and mucosal mast cells. *J Exp Med* **162**, 1025-43.

Nau G. J., Richmond J. F., Schlesinger A., Jennings E. G., Lander E. S. and Young R. A. (2002) Human macrophage activation programs induced by bacterial pathogens. *Proc Natl Acad Sci U S A* **99**, 1503-8.

Nilsson G., Johnell M., Hammer C. H., Tiffany H. L., Nilsson K., Metcalfe D. D., Siegbahn A. and Murphy P. M. (1996) C3a and C5a are chemotaxins for human mast cells and act through distinct receptors via a pertussis toxin-sensitive signal transduction pathway. *J Immunol* **157**, 1693-8.

Ochi H., De Jesus N. H., Hsieh F. H., Austen K. F. and Boyce J. A. (2000) IL-4 and -5 prime human mast cells for different profiles of IgE-dependent cytokine production. *Proc Natl Acad Sci U S A* **97**, 10509-13.

Ogura Y., Inohara N., Benito A., Chen F. F., Yamaoka S. and Nunez G. (2001) Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* **276**, 4812-8.

Okumura S., Kashiwakura J. I., Tomita H., Matsumoto K., Nakajima T., Saito H. and Okayama Y. (2003) Identification of specific gene expression profile in human mast cells via Toll-like receptor 4 and Fc{epsilon}RI. *Blood.*

Ott V. L., Cambier J. C., Kappler J., Marrack P. and Swanson B. J. (2003) Mast cell-dependent migration of effector CD8+ T cells through production of leukotriene B4. *Nat Immunol* **4**, 974-81.

Ozinsky A., Smith K. D., Hume D. and Underhill D. M. (2000a) Co-operative induction of pro-inflammatory signaling by Toll-like receptors. *J Endotoxin Res* **6**, 393-6.

Ozinsky A., Underhill D. M., Fontenot J. D., Hajjar A. M., Smith K. D., Wilson C. B., Schroeder L. and Aderem A. (2000b) The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci U S A* **97**, 13766-71.

Papadakis K. A. and Targan S. R. (2000) The role of chemokines and chemokine receptors in mucosal inflammation. *Inflamm Bowel Dis* **6**, 303-13.

Pearce F. L., Befus A. D. and Bienenstock J. (1984) Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. *J Allergy Clin Immunol* **73**, 819-23.

Pfeifer J. D., Wick M. J., Roberts R. L., Findlay K., Normark S. J. and Harding C. V. (1993) Phagocytic processing of bacterial antigens for class I MHC presentation to T cells. *Nature* **361**, 359-62.

Plaut M., Pierce J. H., Watson C. J., Hanley-Hyde J., Nordan R. P. and Paul W. E. (1989) Mast cell lines produce lymphokines in response to cross-linkage of Fc epsilon RI or to calcium ionophores. *Nature* **339**, 64-7.

Porcelli S. A. and Modlin R. L. (1999) The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* **17**, 297-329.

Price A. A., Cumberbatch M., Kimber I. and Ager A. (1997) Alpha 6 integrins are required for Langerhans cell migration from the epidermis. *J Exp Med* **186**, 1725-35.

Prodeus A. P., Zhou X., Maurer M., Galli S. J. and Carroll M. C. (1997) Impaired mast cell-dependent natural immunity in complement C3-deficient mice. *Nature* **390**, 172-5.

Prussin C. and Metcalfe D. D. (2003) 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* **111**, S486-94.

Pulendran B., Banchereau J., Maraskovsky E. and Maliszewski C. (2001) Modulating the immune response with dendritic cells and their growth factors. *Trends Immunol* **22**, 41-7.

Qureshi S. T., Lariviere L., Leveque G., Clermont S., Moore K. J., Gros P. and Malo D. (1999) Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J Exp Med* **189**, 615-25.

Rajakulasingam K., Hamid Q., O'Brien F., Shotman E., Jose P. J., Williams T. J., Jacobson M., Barkans J. and Durham S. R. (1997) RANTES in human allergen-induced rhinitis: cellular source and relation to tissue eosinophilia. *Am J Respir Crit Care Med* **155**, 696-703.

Randolph G. J., Beaulieu S., Pope M., Sugawara I., Hoffman L., Steinman R. M. and Muller W. A. (1998) A physiologic function for p-glycoprotein (MDR-1) during the migration of dendritic cells from skin via afferent lymphatic vessels. *Proc Natl Acad Sci U S A* **95**, 6924-9.

Ratzinger G., Stoitzner P., Ebner S., Lutz M. B., Layton G. T., Rainer C., Senior R. M., Shipley J. M., Fritsch P., Schuler G. and Romani N. (2002) Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin. *J Immunol* **168**, 4361-71.

Razin E., Mencia-Huerta J. M., Lewis R. A., Corey E. J. and Austen K. F. (1982) Generation of leukotriene C4 from a subclass of mast cells differentiated in vitro from mouse bone marrow. *Proc Natl Acad Sci U S A* **79**, 4665-7.

Re F. and Strominger J. L. (2001) Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells. *J Biol Chem* **276**, 37692-9.

Reiling N., Holscher C., Fehrenbach A., Kroger S., Kirschning C. J., Goyert S. and Ehlers S. (2002) Cutting edge: Toll-like receptor (TLR)2- and TLR4-mediated pathogen recognition in resistance to airborne infection with Mycobacterium tuberculosis. *J Immunol* **169**, 3480-4.

Rescigno M., Granucci F. and Ricciardi-Castagnoli P. (2000) Molecular events of bacterial-induced maturation of dendritic cells. *J Clin Immunol* **20**, 161-6.

Ridge J. P., Di Rosa F. and Matzinger P. (1998) A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell. *Nature* **393**, 474-8.

Roake J. A., Rao A. S., Morris P. J., Larsen C. P., Hankins D. F. and Austyn J. M. (1995) Dendritic cell loss from nonlymphoid tissues after systemic administration of lipopolysaccharide, tumor necrosis factor, and interleukin 1. *J Exp Med* **181**, 2237-47.

Robbiani D. F., Finch R. A., Jager D., Muller W. A., Sartorelli A. C. and Randolph G. J. (2000) The leukotriene C(4) transporter MRP1 regulates CCL19 (MIP-3beta, ELC)-dependent mobilization of dendritic cells to lymph nodes. *Cell* **103**, 757-68.

Romani N., Ratzinger G., Pfaller K., Salvenmoser W., Stossel H., Koch F. and Stoitzner P. (2001) Migration of dendritic cells into lymphatics-the Langerhans cell example: routes, regulation, and relevance. *Int Rev Cytol* **207**, 237-70.

Rosenkranz A. R., Coxon A., Maurer M., Gurish M. F., Austen K. F., Friend D. S., Galli S. J. and Mayadas T. N. (1998) Impaired mast cell development and innate immunity in Mac-1 (CD11b/CD18, CR3)-deficient mice. *J Immunol* **161**, 6463-7.

Rowden G., Lewis M. G. and Sullivan A. K. (1977) Ia antigen expression on human epidermal Langerhans cells. *Nature* **268**, 247-8.

Royet J. and Reichhart J. M. (2003) Detection of peptidoglycans by NOD proteins. *Trends Cell Biol* **13**, 610-4.

Rumsaeng V., Cruikshank W. W., Foster B., Prussin C., Kirshenbaum A. S., Davis T. A., Kornfeld H., Center D. M. and Metcalfe D. D. (1997) Human mast cells produce the CD4+ T lymphocyte chemoattractant factor, IL-16. *J Immunol* **159**, 2904-10.

Rupec R., Magerstaedt R., Schirren C. G., Sander E. and Bieber T. (1996) Granulocyte/macrophage-colony-stimulating factor induces the migration of human epidermal Langerhans cells in vitro. *Exp Dermatol* **5**, 115-9.

Saeki H., Moore A. M., Brown M. J. and Hwang S. T. (1999) Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J Immunol* **162**, 2472-5.

Sallusto F., Schaerli P., Loetscher P., Schaniel C., Lenig D., Mackay C. R., Qin S. and Lanzavecchia A. (1998) Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol* **28**, 2760-9.

Sato N., Ahuja S. K., Quinones M., Kostecki V., Reddick R. L., Melby P. C., Kuziel W. A. and Ahuja S. S. (2000) CC chemokine receptor (CCR)2 is required for langerhans cell migration and localization of T helper cell type 1 (Th1)-inducing dendritic cells. Absence of CCR2 shifts the Leishmania major-resistant phenotype to a susceptible state dominated by Th2 cytokines, b cell outgrowth, and sustained neutrophilic inflammation. *J Exp Med* **192**, 205-18.

Scheeren R. A., Koopman G., Van der Baan S., Meijer C. J. and Pals S. T. (1991) Adhesion receptors involved in clustering of blood dendritic cells and T lymphocytes. *Eur J Immunol* **21**, 1101-5.

Schleifer K. H. and Kandler O. (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407-77.

Schoenberger S. P., Toes R. E., van der Voort E. I., Offringa R. and Melief C. J. (1998) T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40L interactions. *Nature* **393**, 480-3.

Schroeder J. T., MacGlashan D. W., Jr. and Lichtenstein L. M. (2001) Human basophils: mediator release and cytokine production. *Adv Immunol* **77**, 93-122.

Schwandner R., Dziarski R., Wesche H., Rothe M. and Kirschning C. J. (1999) Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* **274**, 17406-9.

Schwartz L. B. and Austen K. F. (1984) Structure and function of the chemical mediators of mast cells. *Prog Allergy* **34**, 271-321.

Schwartz L. B. a. H. T. F. (1998) Biology of mast cells. *In: Allergy. Principles and Practice*, 261-276.

Selvan R. S., Butterfield J. H. and Krangel M. S. (1994) Expression of multiple chemokine genes by a human mast cell leukemia. *J Biol Chem* **269**, 13893-8.

Selye H. (1966) Mast cells and necrosis. *Science* **152**, 1371-2.

Shortman K. and Heath W. R. (2001) Immunity or tolerance? That is the question for dendritic cells. *Nat Immunol* **2**, 988-9.

Shortman K. and Liu Y. J. (2002) Mouse and human dendritic cell subtypes. *Nat Rev Immunol* **2**, 151-61.

Silberberg I., Baer R. L. and Rosenthal S. A. (1976) The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. *J Invest Dermatol* **66**, 210-7.

Siraganian R. P. (2003) Mast cell signal transduction from the high-affinity IgE receptor. *Curr Opin Immunol* **15**, 639-46.

Skokos D., Botros H. G., Demeure C., Morin J., Peronet R., Birkenmeier G., Boudaly S. and Mecheri S. (2003) Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol* **170**, 3037-45.

Skokos D., Le Panse S., Villa I., Rousselle J. C., Peronet R., David B., Namane A. and Mecheri S. (2001) Mast cell-dependent B and T lymphocyte activation is mediated by the secretion of immunologically active exosomes. *J Immunol* **166**, 868-76.

Smith T. J., Ducharme L. A. and Weis J. H. (1994) Preferential expression of interleukin-12 or interleukin-4 by murine bone marrow mast cells derived in mast cell growth factor or interleukin-3. *Eur J Immunol* **24**, 822-6.

Sorden S. D. and Castleman W. L. (1995) Virus-induced increases in bronchiolar mast cells in Brown Norway rats are associated with both local mast cell

proliferation and increases in blood mast cell precursors. *Lab Invest* **73**, 197-204.

Sozzani S., Allavena P., Vecchi A. and Mantovani A. (1999) The role of chemokines in the regulation of dendritic cell trafficking. *J Leukoc Biol* **66**, 1-9.

Stassen M., Muller C., Arnold M., Hultner L., Klein-Hessling S., Neudorfl C., Reineke T., Serfling E. and Schmitt E. (2001) IL-9 and IL-13 production by activated mast cells is strongly enhanced in the presence of lipopolysaccharide: NF-kappa B is decisively involved in the expression of IL-9. *J Immunol* **166**, 4391-8.

Stein J. V., Rot A., Luo Y., Narasimhaswamy M., Nakano H., Gunn M. D., Matsuzawa A., Quackenbush E. J., Dorf M. E. and von Andrian U. H. (2000) The CC chemokine thymus-derived chemotactic agent 4 (TCA-4, secondary lymphoid tissue chemokine, 6Ckine, exodus-2) triggers lymphocyte function-associated antigen 1-mediated arrest of rolling T lymphocytes in peripheral lymph node high endothelial venules. *J Exp Med* **191**, 61-76.

Steinman R. M. and Cohn Z. A. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* **137**, 1142-62.

Steinman R. M., Turley S., Mellman I. and Inaba K. (2000) The induction of tolerance by dendritic cells that have captured apoptotic cells. *J Exp Med* **191**, 411-6.

Steinman R. M. and Witmer M. D. (1978) Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. *Proc Natl Acad Sci U S A* **75**, 5132-6.

Stingl G., Wolff-Schreiner E. C., Pichler W. J., Gschnait F., Knapp W. and Wolff K. (1977) Epidermal Langerhans cells bear Fc and C3 receptors. *Nature* **268**, 245-6.

Stoitzner P., Holzmann S., McLellan A. D., Ivarsson L., Stossel H., Kapp M., Kammerer U., Douillard P., Kampgen E., Koch F., Saeland S. and Romani N. (2003) Visualization and characterization of migratory Langerhans

cells in murine skin and lymph nodes by antibodies against Langerin/CD207. *J Invest Dermatol* **120**, 266-74.

Streilein J. W. and Grammer S. F. (1989) In vitro evidence that Langerhans cells can adopt two functionally distinct forms capable of antigen presentation to T lymphocytes. *J Immunol* **143**, 3925-33.

Sugawara I., Yamada H., Mizuno S., Takeda K. and Akira S. (2003) Mycobacterial infection in MyD88-deficient mice. *Microbiol Immunol* **47**, 841-7.

Sundstrom J. B., Little D. M., Villinger F., Ellis J. E. and Ansari A. A. (2004) Signaling through toll-like receptors triggers HIV-1 replication in latently infected mast cells. *J Immunol* **172**, 4391-401.

Supajatura V., Ushio H., Nakao A., Akira S., Okumura K., Ra C. and Ogawa H. (2002) Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* **109**, 1351-9.

Supajatura V., Ushio H., Nakao A., Okumura K., Ra C. and Ogawa H. (2001) Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4. *J Immunol* **167**, 2250-6.

Takeda K. and Akira S. (2004) TLR signaling pathways. *Semin Immunol* **16**, 3-9.

Takeda K. and Akira S. (2005) Toll-like receptors in innate immunity. *Int Immunol* **17**, 1-14.

Takeuchi O., Hoshino K. and Akira S. (2000) Cutting Edge: TLR2-Deficient and MyD88-Deficient Mice Are Highly Susceptible to Staphylococcus aureus Infection. *J Immunol* **165**, 5392-5396.

Takeuchi O., Hoshino K., Kawai T., Sanjo H., Takada H., Ogawa T., Takeda K. and Akira S. (1999) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* **11**, 443-51.

Tanabe T., Chamaillard M., Ogura Y., Zhu L., Qiu S., Masumoto J., Ghosh P., Moran A., Predergast M. M., Tromp G., Williams C. J., Inohara N. and

Nunez G. (2004) Regulatory regions and critical residues of NOD2 involved in muramyl dipeptide recognition. *Embo J* **23**, 1587-1597.

Tang A., Amagai M., Granger L. G., Stanley J. R. and Udey M. C. (1993) Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature* **361**, 82-5.

Tertian G., Yung Y. P., Guy-Grand D. and Moore M. A. (1981) Long-term in vitro culture of murine mast cells. I. Description of a growth factor-dependent culture technique. *J Immunol* **127**, 788-94.

Teunissen M. B., Wormmeester J., Krieg S. R., Peters P. J., Vogels I. M., Kapsenberg M. L. and Bos J. D. (1990) Human epidermal Langerhans cells undergo profound morphologic and phenotypical changes during in vitro culture. *J Invest Dermatol* **94**, 166-73.

Tkaczyk C., Okayama Y., Woolhiser M. R., Hagaman D. D., Gilfillan A. M. and Metcalfe D. D. (2002) Activation of human mast cells through the high affinity IgG receptor. *Mol Immunol* **38**, 1289-93.

Torres R., de Castellarnau C., Ferrer L., Puigdemont A., Santamaria L. and de Mora F. (2002) Mast cells induce upregulation of P-selectin and intercellularadhesion molecule 1 on carotid endothelial cells in a new invitro model of mast cell to endothelial cell communication. *Immunol Cell Biol* **80**, 170-7.

Traver D., Akashi K., Manz M., Merad M., Miyamoto T., Engleman E. G. and Weissman I. L. (2000) Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. *Science* **290**, 2152-4.

Tsai M., Shih L. S., Newlands G. F., Takeishi T., Langley K. E., Zsebo K. M., Miller H. R., Geissler E. N. and Galli S. J. (1991) The rat c-kit ligand, stem cell factor, induces the development of connective tissue-type and mucosal mast cells in vivo. Analysis by anatomical distribution, histochemistry, and protease phenotype. *J Exp Med* **174**, 125-31.

Tsuji R. F., Geba G. P., Wang Y., Kawamoto K., Matis L. A. and Askenase P. W. (1997) Required early complement activation in contact sensitivity with generation of local C5-dependent chemotactic activity, and late T cell interferon gamma: a possible initiating role of B cells. *J Exp Med* **186**, 1015-26.

Underhill D. M. (2003) Toll-like receptors: networking for success. *Eur J Immunol* **33**, 1767-75.

Underhill D. M., Ozinsky A., Hajjar A. M., Stevens A., Wilson C. B., Bassetti M. and Aderem A. (1999) The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* **401**, 811-5.

Valladeau J., Clair-Moninot V., Dezutter-Dambuyant C., Pin J. J., Kissenpfennig A., Mattei M. G., Ait-Yahia S., Bates E. E., Malissen B., Koch F., Fossiez F., Romani N., Lebecque S. and Saeland S. (2002) Identification of mouse langerin/CD207 in Langerhans cells and some dendritic cells of lymphoid tissues. *J Immunol* **168**, 782-92.

van Loveren H., Meade R. and Askenase P. W. (1983) An early component of delayed-type hypersensitivity mediated by T cells and mast cells. *J Exp Med* **157**, 1604-17.

Varadaradjalou S., Feger F., Thieblemont N., Hamouda N. B., Pleau J. M., Dy M. and Arock M. (2003) Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* **33**, 899-906.

Verbrugh H. A., Van Dijk W. C., Peters R., Van Der Tol M. E. and Verhoef J. (1979) The role of Staphylococcus aureus cell-wall peptidoglycan, teichoic acid and protein A in the processes of complement activation and opsonization. *Immunology* **37**, 615-21.

Vernon M. L., Fountain L., Krebs H. M., Horta-Barbosa L., Fuccillo D. A. and Sever J. L. (1973) Birbeck granules (Langerhans' cell granules) in human lymph nodes. *Am J Clin Pathol* **60**, 771-9.

Viala J., Chaput C., Boneca I. G., Cardona A., Girardin S. E., Moran A. P., Athman R., Memet S., Huerre M. R., Coyle A. J., DiStefano P. S., Sansonetti P. J., Labigne A., Bertin J., Philpott D. J. and Ferrero R. L. (2004) Nod1 responds to peptidoglycan delivered by the Helicobacter pylori cag pathogenicity island. *Nat Immunol* **5**, 1166-74.

Walsh L. J., Trinchieri G., Waldorf H. A., Whitaker D. and Murphy G. F. (1991) Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocyte adhesion molecule 1. *Proc Natl Acad Sci U S A* **88**, 4220-4.

Wang B., Zhuang L., Fujisawa H., Shinder G. A., Feliciani C., Shivji G. M., Suzuki H., Amerio P., Toto P. and Sauder D. N. (1999) Enhanced epidermal Langerhans cell migration in IL-10 knockout mice. *J Immunol* **162**, 277-83.

Wang H. W., Tedla N., Lloyd A. R., Wakefield D. and McNeil P. H. (1998) Mast cell activation and migration to lymph nodes during induction of an immune response in mice. *J Clin Invest* **102**, 1617-26.

Watanabe T., Kitani A., Murray P. J. and Strober W. (2004) NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol* **5**, 800-8.

Wedemeyer J., Tsai M. and Galli S. J. (2000) Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* **12**, 624-31.

Weidemann B., Brade H., Rietschel E. T., Dziarski R., Bazil V., Kusumoto S., Flad H. D. and Ulmer A. J. (1994) Soluble peptidoglycan-induced monokine production can be blocked by anti-CD14 monoclonal antibodies and by lipid A partial structures. *Infect Immun* **62**, 4709-15.

Weiss J. M., Sleeman J., Renkl A. C., Dittmar H., Termeer C. C., Taxis S., Howells N., Hofmann M., Kohler G., Schopf E., Ponta H., Herrlich P. and Simon J. C. (1997) An essential role for CD44 variant isoforms in epidermal Langerhans cell and blood dendritic cell function. *J Cell Biol* **137**, 1137-47.

Wershil B. K., Theodos C. M., Galli S. J. and Titus R. G. (1994) Mast cells augment lesion size and persistence during experimental Leishmania major infection in the mouse. *J Immunol* **152**, 4563-71.

Werts C., Tapping R. I., Mathison J. C., Chuang T. H., Kravchenko V., Saint Girons I., Haake D. A., Godowski P. J., Hayashi F., Ozinsky A., Underhill D. M., Kirschning C. J., Wagner H., Aderem A., Tobias P. S. and Ulevitch R. J. (2001) Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* **2**, 346-52.

Williams C. M. and Coleman J. W. (1995) Induced expression of mRNA for IL-5, IL-6, TNF-alpha, MIP-2 and IFN-gamma in immunologically activated rat peritoneal mast cells: inhibition by dexamethasone and cyclosporin A. *Immunology* **86**, 244-9.

Williams C. M. and Galli S. J. (2000) The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. *J Allergy Clin Immunol* **105**, 847-59.

Winzler C., Rovere P., Rescigno M., Granucci F., Penna G., Adorini L., Zimmermann V. S., Davoust J. and Ricciardi-Castagnoli P. (1997) Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J Exp Med* **185**, 317-28.

Woodbury R. G., Miller H. R., Huntley J. F., Newlands G. F., Palliser A. C. and Wakelin D. (1984) Mucosal mast cells are functionally active during spontaneous expulsion of intestinal nematode infections in rat. *Nature* **312**, 450-2.

Woolhiser M. R., Okayama Y., Gilfillan A. M. and Metcalfe D. D. (2001) IgG-dependent activation of human mast cells following up-regulation of FcgammaRI by IFN-gamma. *Eur J Immunol* **31**, 3298-307.

Wu L., D'Amico A., Hochrein H., O'Keeffe M., Shortman K. and Lucas K. (2001) Development of thymic and splenic dendritic cell populations from different hemopoietic precursors. *Blood* **98**, 3376-82.

Yanagihara S., Komura E., Nagafune J., Watarai H. and Yamaguchi Y. (1998) EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. *J Immunol* **161**, 3096-102.

Zhang D., Zhang G., Hayden M. S., Greenblatt M. B., Bussey C., Flavell R. A. and Ghosh S. (2004) A toll-like receptor that prevents infection by uropathogenic bacteria. *Science* **303**, 1522-6.