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EFFECTOR T CELL HOMING TO THE
SMALL INTESTINAL MUCOSA

Hanna Stenstad

This thesis will be defended on
Friday the 30th of March 2007 at 9.00 am
in Segerfalksalen, BMC, Sölvegatan 19, Lund



LUND UNIVERSITY

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ORIGINAL PAPERS

This thesis is based on the following papers:

Paper I

Gut-associated lymphoid tissue primed CD4⁺ T cells display CCR9-dependent and -independent homing to the small intestine

Hanna Stenstad, Anna Ericsson, Bengt Lindbom-Johansson, Marcus Svensson, Jan Marsal, Matthias Mack, Dulce Soler, Gabriel Marquez, Mike Briskin, and William W. Agace

Blood. 2006 May 1;107(9):3447-54

Paper II

Differential homing mechanisms regulate regionalized effector CD8 $\alpha\beta$ ⁺ T cell accumulation within the small intestine

Hanna Stenstad, Marcus Svensson, Helena Cukak, Knut Kotarsky and William W. Agace

Submitted.

ABBREVIATIONS

APC	antigen presenting cell	LTIC	lymphoid tissue inducer cell
CD	Crohn's disease	NK	natural killer
CP	cryptopatches	M cell	microfold cell
DC	dendritic cell	MHC	major histocompatibility complex
FAE	follicle-associated epithelium	MLN	mesenteric lymph node
FDC	follicular dendritic cell	MPM	multiphoton microscopy
FRC	fibroblastic reticular cell	OVA	ovalbumin
GALT	gut-associated lymphoid tissue	PP	Peyer's patches
HEV	high endothelial venule	RA	retinoic acid
IBD	inflammatory bowel disease	RALDH	retinaldehydehydrogenase
IEC	intestinal epithelial cell	SED	subepithelial dome
IEL	intestinal epithelial lymphocyte	SILT	solitary isolated lymphoid tissue
Ig	immunoglobulin	SLO	secondary lymphoid organ
JAM	junctional adhesion molecules	TCR	T cell receptor
ILF	isolated lymphoid follicle	TGF	transforming growth factor
LN	lymph node	TLR	toll-like receptor
LP	lamina propria	UC	ulcerative colitis
LPL	lamina propria lymphocyte		

INTRODUCTION TO THE IMMUNE SYSTEM

The immune system has the capacity to recognize and protect us from invading pathogens like viruses, bacteria, fungi and parasites. To meet this challenge the immune system is built up of lymphoid organs, specialized immune cells and a lymph system that work together in the initiation of immune responses for pathogen clearance.

The immune system can be subdivided into the innate and adaptive immune system. Macrophages and neutrophils are together with mast cells the major acting immune cells in an innate immune response. Mast cells are prominent near surfaces exposed to the environment where pathogens and other environmental agents are frequently encountered. Thus the positioning of these cells together with their cytokine producing properties make them to good actors in a first line of innate immune defense. The innate system uses Toll-like receptors (TLRs) to sense invasion of microbial pathogens. Recognition of microbial components by TLRs mediates cytokine production and subsequent recruitment of phagocytosing cells from the blood into the inflamed tissue. Phagocytosis is an important event for the host defense since it mediates both degradation and presentation of pathogen antigens by antigen presenting cells (APCs). Presentation of pathogen structures together with cytokine production instructs the development of the adaptive immune response. Thus during an innate immune response pathogens are rather nonspecifically eliminated. In contrast, the adaptive immune system consists of cells that have the capacity to recognize antigens specifically. The adaptive system also provides an immunologic memory that remembers past infections and thus can generate a rapid and enhanced immune response during re-infection. The main players of the adaptive immune system are T and B cells.

Immature lymphocytes develop into T and B cells in primary lymphoid organs (thymus and bone marrow). The maturation process generates cells that bear antigen receptors with the ability to recognize a specific antigen, for T cells this receptor is termed T cell receptor (TCR). After maturation, T cells enter into the blood stream as naïve T cells and start to re-circulate between secondary lymphoid organs (SLOs), blood and spleen in search for its specific antigen. SLOs are specialized to initiate adaptive immune responses by bringing antigen or antigen presenting cells (APCs) draining from tissues together with T cells entering from the blood. Naïve T cells that do not find their specific APC, will eventually leave the SLO and re-enter into the blood circulation. In contrast, a naïve T cell that finds its specific antigen will become activated and start to proliferate and differentiate into an effector T cell. During this differentiation process, the T cell will start to display a different profile of cell adhesion molecules and chemokine receptors on the cell surface. Thus in contrast to naïve T cells, the effector T cells will gain access to peripheral tissues upon re-entry into the blood circulation.

T cell entry into peripheral tissues is mediated by selective expression of adhesion molecules and chemokine receptors on the T cell surface and their ligands on vascular endothelial cells within the tissue. In this thesis, I have examined mechanisms regulating effector T cell homing to the intestinal tissue. This work has been focused on chemokine receptor CCR9 and its role in the regional localization of effector T cell subsets into the small intestinal mucosa.

THE INTESTINAL IMMUNE SYSTEM

Overview of the intestinal mucosa

The small intestine is the part of the gastrointestinal tract that connects the stomach with the large intestine. The vast majority of food digestion as well as absorption of fluids and nutrients take place in the small intestine. To complete this task, the intestinal surface is convoluted into finger-like structures that in turn is covered by microvilli, to even further increase the surface area and hence, facilitate the absorption of nutrients.

The large surface area of the gastrointestinal tract also serves as a major site for pathogen entry. Thus it is of great importance that the intestinal immune system maintain unresponsive to food antigens, while generating effective immune responses to harmful pathogens within the gut mucosa. The ability to remain non-responsiveness to oral fed antigens is termed oral tolerance, and the mechanism underlying this process remains elusive. The initiation of chronic intestinal inflammation has classically been seen as a result of impaired oral tolerance mechanisms that lead to active immunity against nonpathogenic intestinal bacteria (Shanahan, 2002). However more recent evidence propose that an impaired innate immunity initiates the cascade of event that result in the disease (Korzenik, 2007). Taken together the intestinal immune system has to maintain tolerance against food antigens and commensal bacteria, while mounting efficient responses against invading pathogens.

A singled layered epithelium separates the intestinal lumen from the lamina propria (LP). The epithelial cell layer creates a strict barrier together with tight junctions, that regulates the permeability between the intestinal epithelial cells (Turner et al., 1997). The intestinal epithelium consists primarily of specialized nutrient absorbing enterocytes and mucus secreting goblet cells. The mucus layer has good bacteria capture capacity and form an additional barrier against the intestinal lumen. Moreover paneth cells reside in the crypts and produce protecting antimicrobial peptides (Sansonetti, 2004). The epithelium is replaced every 2-3 days by cells arising from stem cells present in the intestinal crypts (MacDonald, 2003). The antimicrobial enzymes are therefore suggested to protect the crypt cells from invading bacteria.

The intestinal immune system has been divided into inductive and effector sites. The inductive sites consist of the mesenteric lymph nodes (MLN) and organized lymphoid structures in the intestinal mucosa. Intestinal effector sites are composed of the immune cells that are distributed throughout the intestinal epithelium and LP. The small intestinal inductive and effector sites (Figure 1) will be discussed in more detail below.

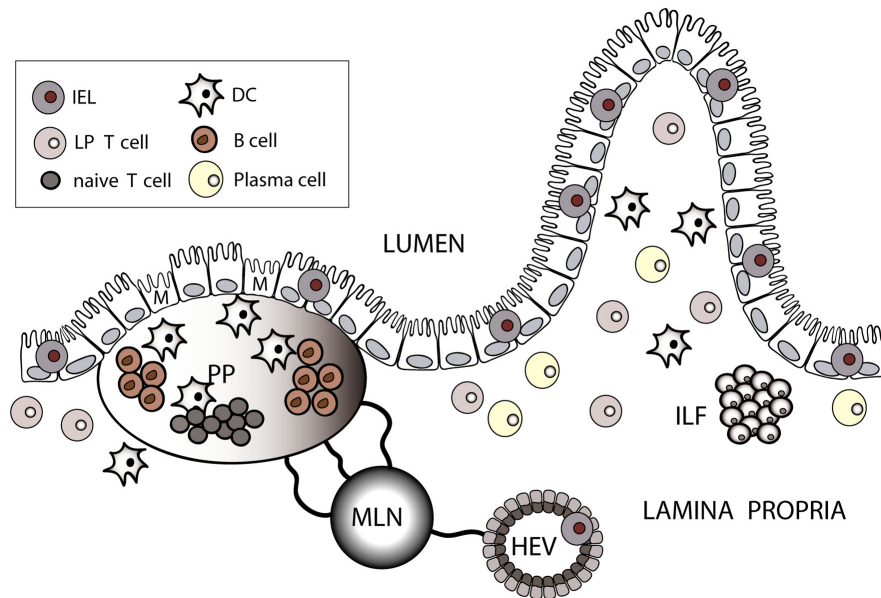


Figure 1. Sketch over the small intestinal mucosa.

Intestinal inductive sites

SLOs provide a meeting point and an environment where interactions between different subsets of immune cells can take place in order to initiate immune responses. The SLO of the intestine consists of the MLN and Peyer's patches (PPs). Apart from PPs, solitary isolated lymphoid tissue (SILT) provides the murine small intestinal mucosa with additional lymphoid structures. SILT comprise lymphoid aggregates that are heterogeneous in size and cell composition, ranging from small cluster of cryptopatches (CPs) to larger isolated lymphoid follicles (ILFs).

Mesenteric lymph nodes

MLN are the first intestinal lymphoid structures to develop during embryogenesis and located as a string of pearls in the mesentery of the intestine. MLN consists of an inner medulla and an outer cortex that are surrounded by a fibrous capsule. The medulla is rich of macrophages and plasma cells. The cortex contains a T cell area and B cell follicles where adaptive T and B cell responses are initiated. Circulating lymphocytes in the blood enter into the MLN via high endothelial venules (HEVs). MLN are connected to the PPs and LP of the small intestinal and proximal colon via afferent lymphatics. Thus intestinal antigens from PPs and intestinal LP enter

MLN via the lymphatics, either freely distributed or associated with dendritic cells (DCs) (Mowat, 2003). In the LN, soluble antigens use the conduit system, that connect the subcapsular sinus with HEV, to come in contact with resident DCs associated to the conduit network (Sixt et al., 2005). Lymphocytes leave the MLN via efferent lymphatic vessels in the medulla that are connected to the blood circulation by the thoracic duct.

Peyer's patches

The most prominent inductive sites within the small intestinal mucosa are the Peyer's patches (PPs). These lymphoid structures can be detected by the naked eye on the anti-mesenteric side of the murine small intestinal mucosa. The formation of PPs are initiated during gestation and induced by lymphotoxin- β receptor and CXCR5 signaling dependent interactions between lymphoid-tissue inducer cells (LTIC) and mesenchymal cells (Newberry and Lorenz, 2005). PPs consist of several aggregated B cell follicles and intervening T cell areas that are overlaid by a follicle-associated epithelium (FAE). Characteristic for the FAE are the microfold (M) cells that actively samples antigens from the intestinal lumen for transport into the beneath lymphoid structures (Neutra et al., 2001). In PPs antigen is transported across M cells into the subepithelial dome (SED) where it is taken up by DCs. Upon stimulation antigen presenting DCs will migrate into the interfollicular T cell zone or into B cell follicles of PPs (Shreedhar et al., 2003). This will induce T cell priming and B cell maturation into antigen producing cells.

Isolated lymphoid follicles

The architecture of isolated lymphoid follicles (ILFs) is similar to that of a single follicle in the PPs (Hamada et al., 2002). ILFs have, like the PPs, an overlaying FAE containing M cells for luminal antigen transfer into the lymphoid network. Thus according to the structural similarities, ILFs and PPs may serve common functions. Indeed, like PPs, ILFs support immunoglobulin (Ig) class switching to IgA, although its contribution to the total levels of intestinal IgA is less prominent (Newberry et al., 2002; Shikina et al., 2004). Although the generation of ILFs and PPs require common features, such as lymphotoxin- β receptor signaling, ILFs develop postnatally (Lorenz et al., 2003). Thus the generation of ILFs seems to be more flexible compared to that of PPs, since it is not restricted to the prenatal development of the organism.

Cryptopatches

Cryptopatches (CPs) are detected at the base of the intestinal crypts, and consist of small clusters of mostly lineage negative (lin⁻) cells expressing the stem cell factor receptor c-kit and the IL-7R

α -chain (Kanamori et al., 1996). It has been suggested that CP cells represent extrathymic progenitors of intestinal T cells (Saito et al., 1998; Suzuki et al., 2000). However more recent work have questioned that, since CP cells lack RAG gene activity (Guy-Grand et al., 2003) and normal IEL populations present at similar frequency are present in the absence of CPs (Pabst et al., 2005; Taylor et al., 2004). Furthermore fate-mapping analysis using a transgene knock-in mouse expressing a reporter gene under the control of retinoic acid orphan receptor (ROR γ t) demonstrated that IEL are progeny of CD4⁺CD8⁺ thymocytes (Eberl and Littman, 2004). Thus the role for CP in intestinal T cell development is controversial and recent data suggests that CP is not an important site for T cell development.

The lymphoid aggregates in the murine small intestine demonstrate highly dynamic morphologies, dependent on for example age, mouse strain and the presence of intestinal commensal bacteria (Pabst et al., 2006; Pabst et al., 2005). Moreover the similarity between lin⁻c-kit⁺ cells in CP and LTIC suggest CP as precursors to ILF (Eberl and Littman, 2004). Thus intestinal lymphoid aggregates are dynamic lymphoid structures that demonstrate plasticity and that allow structural changes in response to environmental changes.

Antigen uptake in intestinal mucosa

Antigen uptake from the intestinal lumen has been demonstrated to involve M cells in the PP or epithelial cells that transfer luminal antigens to the underlying DCs in the LP (Iwasaki and Kelsall, 2001; Vallon-Eberhard et al., 2006). LP DCs located under the villus epithelium also have the capability to extend dendrites through the epithelium for luminal antigen capture (Rescigno et al., 2001b). These extensions was shown to express tight junction proteins to preserve the epithelial barrier integrity (Rescigno et al., 2001a; Rescigno et al., 2001b). The formation of transepithelial dendrites was initially suggested to be fractalkine dependent (Niess et al., 2005), however a recent study by Chieppa et al demonstrated that fractalkine deficient DCs have the capacity to send out protrusions through the epithelium in response to inflammatory signals (Chieppa et al., 2006). Moreover this study demonstrated that DC extensions is dependent on luminal bacteria exposure and that the epithelial cells can alert DCs to form extensions via TLR dependent mechanisms (Chieppa et al., 2006).

Intraepithelial lymphocytes

The intestinal epithelium contains a large number of lymphocytes termed intraepithelial lymphocytes (IEL). Practically all IEL are T cells, and can be divided into two subpopulations expressing TCR $\alpha\beta$ or TCR $\gamma\delta$. The TCR $\alpha\beta$ population can be further divided into those expressing the conventional CD8 $\alpha\beta$ co-receptor or the CD8 $\alpha\alpha$ homodimer. The majority of the

TCR $\gamma\delta$ IEL expresses CD8 $\alpha\alpha$. Minor populations of TCR $\alpha\beta$ IEL co-expressing CD4 and CD8 $\alpha\alpha$ together with CD4CD8⁺ TCR $\gamma\delta$ IEL are also present in the IEL compartment (Lefrancois, 1991). Thus IEL consist primarily of CD8⁺ T cells that can be divided into the following three major subsets: TCR $\alpha\beta$ ⁺ CD8 $\alpha\beta$ ⁺, TCR $\alpha\beta$ ⁺ CD8 $\alpha\alpha$ ⁺ and TCR $\gamma\delta$ ⁺ CD8 $\alpha\alpha$ ⁺ IEL. The relative contribution of these IEL populations differs between human and mice. In humans the majority are TCR $\alpha\beta$ ⁺ CD8 $\alpha\beta$ ⁺ IEL and between 5-30% TCR $\gamma\delta$ ⁺ CD8 $\alpha\alpha$ ⁺ IEL (Jarry et al., 1990), in contrast to mice where up to 80% are TCR $\gamma\delta$ ⁺ CD8 $\alpha\alpha$ ⁺ IEL (Lefrancois, 1991).

The TCR $\alpha\beta$ ⁺ CD8 $\alpha\beta$ ⁺ IEL are suggested to be derived from conventional thymus derived T cells that have entered into the small intestinal epithelium after activation in SLO (Kim et al., 1997). The TCR β clonotypes of memory TCR $\alpha\beta$ ⁺ CD8 $\alpha\beta$ ⁺ IEL are comparable with memory T cells found in the LP (Arstila et al., 2000), indicating a common differentiation pathway for these populations. Recently a GFP knockin mouse has been generated in which GFP expression has been determined to correspond to IL-10 protein expression by the cell (Kamanaka et al., 2006). In these mice GFP⁺ cells were preferentially found in the intestine (Kamanaka et al., 2006). Furthermore higher frequencies of GFP⁺ CD8 $\alpha\beta$ ⁺ IEL than that of GFP⁺ CD8 $\alpha\alpha$ ⁺ IEL was detected (Kamanaka et al., 2006). Together this demonstrates that conventional CD8 $\alpha\beta$ ⁺ T cells possess a greater potential than CD8 $\alpha\alpha$ ⁺ IEL to produce IL-10 in the IEL compartment.

The ontogeny of non-conventional TCR $\alpha\beta$ ⁺ CD8 $\alpha\alpha$ ⁺ and TCR $\gamma\delta$ ⁺ CD8 $\alpha\alpha$ ⁺ IEL is controversial, however recent data indicate that they are thymic derived (Eberl and Littman, 2004; Guy-Grand et al., 2003). The non-conventional CD8 $\alpha\alpha$ ⁺ IEL can be further characterized by the lack of markers like CD2, CD28, cytotoxic T lymphocyte antigen-4 (CTLA-4) and Thy-1 (Guy-Grand et al., 1994; Ohteki and MacDonald, 1993). The CD8 $\alpha\alpha$ ⁺ TCR $\alpha\beta$ ⁺ IEL show oligoclonal TCR repertoires that do not resembles with conventional CD8 $\alpha\beta$ ⁺ TCR $\alpha\beta$ ⁺ IELs (Regnault et al., 1994), which suggest that CD8 $\alpha\alpha$ ⁺ TCR $\alpha\beta$ ⁺ IEL have a distinct differentiation pathway. Expression of CD8 $\alpha\alpha$ has been shown to promote survival of effector cells and further differentiation into mature effector cells (Madakamutil et al., 2004). This suggests that CD8 $\alpha\alpha$ is a signature for an effector/memory IEL phenotype rather than a marker for origin or TCR specificity.

Recently a naïve population of IELs was demonstrated to enter into the small intestinal epithelium without prior activation in LNs (Staton et al., 2006). These cells were demonstrated to be recent thymic emigrants (RTEs) that subsequent to intestinal entry start to proliferate in

response to antigens (Staton et al., 2006). With their diverse TCR repertoires, RTEs may play a role in maintaining TCR diversity in the intestine.

Although the IEL subpopulations have been well characterized, their function remains largely unknown. IEL have demonstrated potent cytotoxic capacity (Goodman and Lefrancois, 1989; Lefrancois and Goodman, 1989; Muller et al., 2000). An additional feature of some IEL is their expression of NK receptors (Guy-Grand et al., 1996; Tagliabue et al., 1982). The ligand for one of these receptors (NKG2D) is the human non-conventional MHC class I molecule MICA that is induced by epithelial cells under stress (Bauer et al., 1999). The TCR $\gamma\delta$ recognizes the same MICA molecule (Das et al., 2001; Groh et al., 1998), thus IEL can use both NKG2D and TCR $\gamma\delta$ to detect damaged epithelial cells. IEL have also been demonstrated to have immunoregulatory function. For example upon a systemic virus infection activated CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IEL do not show virus specific cytotoxicity, instead these cells started to secrete enhanced levels of TGF- β (Saurer et al., 2004). This result suggests that CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IEL exert regulatory functions rather than promoting immune protection against specific antigens. Direct evidence for CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IEL regulator capacity was demonstrated when CD8 $\alpha\alpha^+$ TCR $\alpha\beta^+$ IEL together with CD45RB^{hi} CD4⁺ T cells could prevent CD4⁺ mediated colitis (Poussier et al., 2002). Cell transfer studies have demonstrated that CD8 $\alpha\alpha^+$ TCR $\gamma\delta^+$ IEL only have a minor pathogen specific activity (Muller et al., 2000), however the protection of *Toxoplasma gondii* infection by CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ IEL appear, in part, to be dependent on CD8 $\alpha\alpha^+$ TCR $\gamma\delta^+$ IEL (Lepage et al., 1998). Although with minimal pathogen specific activity, CD8 $\alpha\alpha^+$ TCR $\gamma\delta^+$ IEL seems to control the activity of non-self reactive CD8 $\alpha\beta^+$ TCR $\alpha\beta^+$ IEL and thereby provide a first line of defense against invading pathogens. Mice deficient for the TCR α chain show increased number of TCR $\gamma\delta^+$ IEL after environmental microbial challenge (Viney et al., 1994), and enhanced B cell maturation and Ig secretion is demonstrated in these mice (Mizoguchi et al., 1996). Thus TCR $\gamma\delta^+$ IEL also appear to play a role in the generation and maintenance of humoral immune responses in the intestine. TCR $\gamma\delta^+$ IEL have also been implicated to be important for the integrity and healing of the epithelium (Chen et al., 2002; Komano et al., 1995).

Lamina propria lymphocytes

The LP is the connective tissue layer between the epithelium and muscularis mucosa. The intestinal LP consists of smooth muscle cells, fibroblasts, lymphatics and blood vessels. Large

numbers of immune cells are distributed throughout the LP, including T cells, B cells, macrophages, DCs and mast cells.

The intestinal LP T cells consist mainly of conventional TCR $\alpha\beta$ ⁺ T cells. About 60% of the cells express CD4 and 40% express CD8 $\alpha\beta$, similar proportions as for T cells in the peripheral blood (Ullrich et al., 1990). LP T cells display an previously activated phenotype (Schieferdecker et al., 1992; Schieferdecker et al., 1990; Ullrich et al., 1990), indicating prior activation in LNs subsequent to localization into the LP (Kim et al., 1997). CD4⁺ LPL produce large amounts of cytokines, including IL-5, IL-10 and IFN- γ (Braunstein et al., 1997; Cheroutre et al., 2004; Taguchi et al., 1990). Moreover a subpopulation of IL-17 producing CD4⁺ LPL have recently been demonstrated (Ivanov et al., 2006). These cells show proinflammatory characteristics and mediate extracellular bacteria defense (Harrington et al., 2005; Park et al., 2005). The function of LP T cells is largely unknown, however the populations of CD4⁺ LPL have been suggested to have important regulatory functions in their interactions with other T cells and B cells. For example LP T cells have been demonstrated to provide help for IgA producing plasma cells (Abreu-Martin and Targan, 1996).

The dominant plasma cell subset in the intestinal mucosa is IgA expressing (Crago et al., 1984). The secreted IgA is mostly dimeric (Kett et al., 1988), and transported to the lumen via binding of the J-chain and secretory component to the poly Ig receptor on the basolateral side of the epithelial cells. Luminal IgA binds to bacteria and virus and thereby prevents them from attaching to the intestinal epithelium (Kraehenbuhl and Corbett, 2004). However the origin of the intestinal IgA plasma blasts is controversial and yet not determined. It has been proposed that most of the IgA plasma blasts present in the LP are derived from IgA B cells generated in the PPs. In addition B1 cells are suggested to be recruited from the peritoneal cavities into the LP, where they differentiate to IgA secreting plasma blasts (Lamm and Phillips-Quagliata, 2002; Macpherson et al., 2000). However detectable levels of IgA plasma blasts is present in mice lacking PP, MLN or ILF (Eberl and Littman, 2004; Kang et al., 2002), suggesting that B cells can localize to the LP in the absence of activation in organized lymphoid tissues in the intestine. Furthermore isotype switching from IgM⁺ B cells to IgA⁺ plasma cells have been demonstrated to occur in situ in direct contact with TGF- β producing LP stroma cells (Suzuki et al., 2005). Nevertheless aly/aly mice (with a point mutation in NF- κ B inducing kinase (NIK)) that lack ILF, PP and lymph nodes have been demonstrated to completely lack IgA plasma blasts in the intestine (Fagarasan et al., 2001; Hamada et al., 2002). In addition peritoneal B1 cells have show an impaired migration from the peritoneal cavity in aly/aly mice (Fagarasan et al., 2001). In agreement with the latter observations, a recent study have demonstrated NIK sufficient stroma

cells to be essential for the recruitment of naïve B cells into the LP for subsequent generation of IgA plasma blasts at this site (Suzuki et al., 2005). Thus stroma cells appear to be an important player in the generation of IgA producing plasma cells via their NIK dependent recruitment of naïve B cells into the intestinal compartments.

CHEMOKINES AND CHEMOKINE RECEPTORS

Lymphocyte homing is one of the fundamental prerequisites for well-functioning immune responses. Homing occurs via a series of lymphocyte-endothelial interactions regulated by multiple cellular adhesion molecules. Here chemokines and chemokine receptors will be discussed, since they are key players in the activation of some of these adhesion molecules.

Chemokines

In the late 1970's, the first chemokines were described as small peptides with a size of 8-14kDa (Deuel et al., 1977). The chemokine CXCL8 (previously known as IL-8) was cloned for approximately 15 years ago (Holmes et al., 1991; Murphy and Tiffany, 1991) and since this time the number of chemokines that have been identified has expanded dramatically. Today the family contains over 50 members.

Chemokines are a family of structurally related proteins that can be sub-classified on the basis of the number and the position of cysteine residues in the N-terminal region of the molecule. There are four subclasses of chemokines designated C, CC, CXC and CX3C in which X represents any amino acid other than a cysteine (Zlotnik and Yoshie, 2000). The C subclass, containing a single cysteine residue, consists of two members XCL1 (lymphotactin- α) and XCL2 (lymphotactin- β) (Kelner et al., 1994). The CC family, which is the largest subclass, consists of 28 members. There are seventeen CXC chemokines and in the CX3C family, CX3CL1 (fractalkine) is the only member (Zlotnik and Yoshie, 2000). CX3CL1 along with CXCL16 contain, in addition to their chemokine domains, a mucin-like stalk and a transmembrane domain, that enable these chemokines to function as cell adhesion receptors (Fong et al., 1998). Proteolytical cleavage from the cell membrane allows both CX3CL1 and CXCL16 to also function as soluble chemoattractants (Garton et al., 2001; Matloubian et al., 2000).

In terms of genomic organization, chemokines can be divided into two alternative groups where one group consists of chemokines whose genes are located in large clusters on the chromosome and the other of non-cluster chemokines whose genes are located separately at unique chromosomal locations. Two major CC and CXC chemokine gene clusters together with non-clustered chemokines are found both in human and mouse. The major CC chemokine cluster is present on human chromosome 17 and mouse chromosome 11 while the CXC chemokine cluster is present on human chromosome 4 and mouse chromosome 5 (Zlotnik et al., 2006). Chemokines within the large gene clusters commonly bind to multiple receptors and display limited similarity between human and mouse. Chemokine clusters are believed to

originate from gene duplications. Thus cluster chemokines with a common evolutionary origin tend to share functional properties such as for example the CXC cluster of chemokines (including CXCL1-8) that primarily promote neutrophil recruitment to inflammatory sites (Patel et al., 2001). In contrast, non-cluster chemokines are relatively conserved between species likely because of their pivotal role in the development of the organism. For example deletion of the non-cluster chemokine CXCL12 or its receptor CXCR4 cause perinatal death, demonstrating their critical function for developmental processes (Zou et al., 1998).

All chemokines have a common three-dimensional structure consisting of a carboxy (C) terminal α -helix, a core region with three anti-parallel β -strands and a short cysteine residue containing amino (N) terminal region (Figure 2).

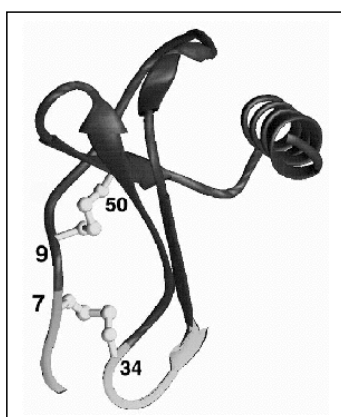


Figure 2. The monomeric structure of CXCL8 consists of a C terminal α -helix, three β -strands and a cysteine residue containing N terminal region. Here arrows represent β strands and cylinders α helices. The two disulphide bonds (between amino acids 9-50 and 7-34) are indicated in the structure.

Adapted from Rojo D et al, Biol Res 1999;32(4)263-72.

Chemokines have also been grouped based on their functional properties in the immune system into homeostatic and inflammatory chemokines. Inflammatory chemokines are induced in response to inflammatory stimuli and play a role in recruitment of effector cells to inflamed tissues, while homeostatic chemokines are constitutively expressed in distinct tissue locations and regulate lymphocyte localization under homeostatic/non-inflammatory conditions (Moser and Loetscher, 2001). There are clear overlaps between homeostatic and inflammatory chemokines. One example is CCL20 that is expressed in the intestine and may promote lymphorganogenesis there but is also highly induced on epithelial cells in response to inflammatory stimuli (Fujiie et al., 2001; Rumbo et al., 2004).

Lymphotoxin β receptor (LT β R) signaling is essential for the expression of homeostatic chemokines in lymphoid organs and the intestine (Kang et al., 2002; Ngo et al., 1999; Rumbo et al., 2004). Ligand binding to LT β R activates an alternative NF- κ B signaling pathway required for

the expression of multiple homeostatic chemokines including CCL19, CCL21, CXCL12 and CCL20 (Dejardin et al., 2002; Rumbo et al., 2004). This signaling pathway involves the translocation of the p52:RelB transcription factor complex into the cell nucleus where it associates with promoters of homeostatic chemokines (Dejardin et al., 2002; Rumbo et al., 2004).

Chemokine receptors

Chemokines exert their function by binding to G protein coupled cell surface receptors. Nineteen chemokine receptors have been identified so far and these have been classified according to the family of chemokines they bind (Murphy et al., 2000). Thus the C chemokines bind CXCR1, CC chemokines bind CCR1-10, CXC chemokines bind CXCR1-6 and CX3CL1 binds receptor CX3CR1 (Murphy et al., 2000).

Chemokine and chemokine receptor interactions vary in terms of selectivity. Some chemokines only interact with one receptor (and vice versa) i.e. CXCL16 only activates CXCR6 and CCL25 only CCR9. Other chemokines activate more than one chemokine receptor, and some receptors recognize more than one chemokine.

Furthermore there are chemokine receptor like proteins (the Duffy antigen receptor for chemokines (DARC), D6 and CCXCKR) that bind a range of CC and CXC chemokines. Chemokine binding to these receptors leads to chemokine/chemokine receptor internalization. However no classical receptor activation following chemokine binding has been demonstrated for these receptors and therefore not classified as chemokine receptors. It has been proposed that these receptors serve a function as scavenger receptors for chemokines i.e. D6 bound chemokines are targeted to endosomes and degraded under acidic conditions within the endosomes (Comerford et al., 2006; Neote et al., 1993; Nibbs et al., 1997; Weber et al., 2004). Hence their suggested function has been to reduce levels of inflammatory chemokines at sites of inflammation and thereby play a role in switching off the inflammatory response (Jamieson et al., 2005).

Chemokine receptors are seven transmembrane spanning receptors with an extracellular N-terminal portion and intracellular C-terminal tail. Initial studies using different CXCL8 analogs demonstrated the N terminal region as essential for receptor binding as well as receptor activation (Clark-Lewis et al., 1991). Ligand binding to chemokine receptors is proposed to occur in steps. For example, for ligand binding to CCR5 the initial interaction takes place between the chemokine core and the N-terminal receptor domain and the second extracellular loop of the receptor (Samson et al., 1997). The free N terminal domain of the chemokine then interacts with the transmembrane receptor helices causing receptor activation (Blanpain et al., 2003; Govaerts et al., 2003). However different chemokines seem to interact with different sites of CCR5 for ligand

binding and receptor activation (Navenot et al., 2001). Thus despite similarities in chemokine receptor as well as chemokine structures, the way chemokines interact with their chemokine receptors is likely to vary from one receptor the other.

As with chemokines, the genes of many chemokine receptors are clustered. The majority of human chemokine receptors are located on chromosome 3, a smaller cluster on chromosome 2 and single chemokine receptor genes are located on chromosomes 6, 11, 17 and X (Fredriksson et al., 2003). The genomic chemokine receptor locations is conserved between human and mouse. Mouse chemokine clusters are positioned on chromosome 9 and 1 (DeVries et al., 2006). Chemokine receptor genes clustered closely together are often most closely related, indicating that they originate from more recent gene duplications (DeVries et al., 2003).

Chemokine receptor signaling

All chemokine receptors are coupled to G proteins that initiate the intracellular signaling cascade upon ligand binding. G proteins consist of α , β and γ subunits and can be subdivided on the basis of the α -subunit into four distinct G protein families; $G\alpha_s$, $G\alpha_q$, $G\alpha_i$ and $G\alpha_{12/13}$. Most chemokine receptors signal via $G\alpha_i$. Upon ligand binding, chemokine receptors mediate activation of $G\alpha_i$ via GDP-GTP exchange followed by the release of the $\beta\gamma$ subunit. Freely distributed GTP bound $G\alpha_i$ and $\beta\gamma$ subunits activate downstream intracellular signaling pathways. Internal GTPases hydrolyse active GTP binding $G\alpha_i$ into inactive GDP binding $G\alpha_i$ that regain a high affinity for the $\beta\gamma$ subunit (Offermanns, 2003). Pertussis toxin (PTX) catalyzes the ADP-ribosylation of the $G\alpha_i$ subunit, preventing it from interacting with the receptor unit (Stryer, 1988). Thus most chemokine receptor signaling are sensitive to PTX (Curnock et al., 2002).

Chemokine receptor signaling initiates cellular responses crucial for effective homing of lymphocytes during homeostatic as well as inflammatory conditions. The best-characterized signaling cascades initiated upon ligand ligation are depicted in Figure 3.

Chemokine stimulated chemotaxis

Lymphocytes responding to chemokine gradients display rapid cell polarization with the generation of a pseudopod at the leading edge and a tail-like projection at the trailing edge (uropod), orientated to the gradient (Sanchez-Madrid and del Pozo, 1999). In fibroblast models, the Rho family small GTPases, including Rho, Rac or Cdc42, have been demonstrated to regulate actin polymerization and the formation of membrane protrusions that are important for cell migration (Servant et al., 2000).

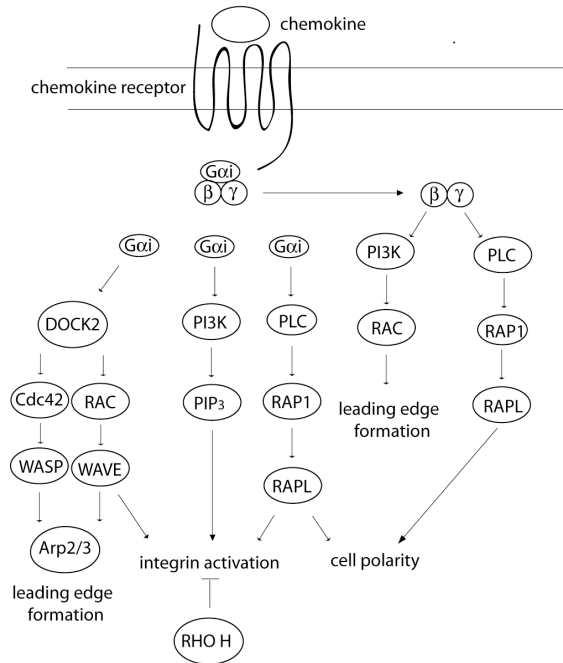


Figure 3. Network of signaling pathways demonstrated to be involved in chemokine receptor mediated chemotaxis and integrin activation.

The mechanisms controlling chemokine induced cytoskeleton remodeling during lymphocyte chemotaxis are not fully understood. Rho family GTPases are expressed by lymphocytes, and activating mutants of Rho, Rac or Cdc42 impair polarization of T cell lines (del Pozo et al., 1999). In lymphocytes chemokine mediated Rac activation is dependent on the interaction of ELMO1 and DOCK2 through its SH3 domain, followed by DOCK activation of Rac and cytoskeleton reorganization (Fukui et al., 2001; Sanui et al., 2003). DOCK2 is essential for maintain leading edge formation necessary for lymphocyte locomotion along the endothelium but not transmigration across the endothelial barrier (Kunisaki et al., 2006; Shulman et al., 2006).

The *WASP* gene is critically involved in chemotaxis. The Wiskott-Aldrich syndrome is associated with mutations in this gene (Derry et al., 1994). Since the resulting WAS protein is implicated in actin cytoskeleton rearrangements and cell mobility, T cells from Wiskott-Aldrich patients and healthy controls have been examined in migration assays. WASP deficient lymphocytes showed impaired chemotaxis to CXCL12 compared to controls and the abnormal chemotaxis correlated with the severity of disease (Haddad et al., 2001). Introduction of a fusion

protein antagonist to active Cdc42 prevented endogenous binding of WASP to Cdc42 and thereby inhibited chemotaxis to CXCL12 (Haddad et al., 2001). Rac activated WAVE together with Cdc42 activated WASP have been shown to act downstream on the Arp2/3 complex that is required for actin polymerization (Cory and Ridley, 2002; Welch and Mullins, 2002). The role for WAVE in lymphocyte migration remains to be assessed. Together these results suggest that the interaction between Cdc42 and WASP plays an essential role in lymphocyte chemotaxis.

Thus Rho family GTPases and their effector proteins control cytoskeleton remodeling that is essential for chemokine stimulated lymphocyte chemotaxis.

Chemokine receptor regulation of integrin binding

Integrins are cell surface adhesion molecules composed of two transmembrane heterodimeric glycoproteins that are non-covalently linked. Integrins can be found in multiple conformational forms on the lymphocyte surface, that vary in their affinity for their ligands (Alon and Dustin, 2007). The best characterized integrin family member is $\alpha_1\beta_2$ (LFA-1) and serves as a model for our understanding of integrin activation and signaling. In its low affinity form, LFA-1 is bent like a sheath knife and the headpiece resides in close proximity to the cell membrane (Takagi et al., 2002). Upon activation the integrin is straightened and in the extended conformation the headpiece becomes available for ligand binding (Nishida et al., 2006). The position of the integrin headpiece and the conformation of the ligand binding site thus determines integrin affinity. The intracellular signaling cascades that mediate changes in integrin affinity are referred to as inside out signaling (Dustin and Springer, 1989). The importance of a dynamic regulation of integrin affinity for integrin function is poorly understood. To study this, Lfa-1^{dl/dl} mice that express constitutively active LFA-1 were generated and found to have reduced LN and PP cell numbers compared to WT mice (Semmrlich et al., 2005). Lymphocytes isolated from Lfa-1^{dl/dl} mice show normal LFA-1 signaling but an impaired translocation through endothelial monolayers *in vitro* (Semmrlich et al., 2005). Thus the demonstrated LN hypoplasia in Lfa-1^{dl/dl} mice likely reflects a defect in the ability of lymphocytes with constitutively active LFA-1 to transmigrate through HEV into lymphoid organs. Together these results highlight the importance for integrin binding affinity changes mediating sequential adhesion and de-adhesion for functional lymphocyte migration.

Both increased affinity for the ligand and integrin clustering are thought to contribute to the total strength of the integrin mediated adhesion. However the relative role of integrin affinity and clustering, referred to as integrin avidity, in mediating integrin dependent adhesion may differ depending on ligand densities. At high ligand densities integrin affinity changes are

sufficient for binding while adhesion at low ligand densities requires both increased integrin affinity and clustering (Constantin et al., 2000a).

Chemokine binding to their receptors have been shown to mediate both integrin affinity and avidity changes (Constantin et al., 2000a; Giagulli et al., 2004). However the signaling pathways that control these processes upon lymphocyte contacts with endothelial cells are yet not fully characterized.

The small GTPase RhoA has been linked to chemokine induced lateral motility and clustering of LFA-1 and enhanced LFA-1 affinity (Giagulli et al., 2004). RhoA and its downstream effector Rho kinase (ROCK) operate at the trailing edge of the lymphocyte controlling de-attachment of the cell during migration (Smith et al., 2003). Rap1 is another small GTPase activated by phospholipase C (PLC) (Katagiri et al., 2004b). Rap1 has been shown to mediate chemokine mediated LFA-1 activation and clustering associated with transendothelial migration (Sebzda et al., 2002; Shimonaka et al., 2003). The Rap1 binding molecule RAPL has been demonstrated to mediate chemokine induced LFA-1 clustering and mediate lymphocyte migration into draining LNs (Katagiri et al., 2004a; Sebzda et al., 2002). Finally, the small GTPase RhoH has been shown to promote a low avidity state of LFA-1, potentially by inhibiting the activity of Rap1 (Cherry et al., 2004).

Regulation of chemokine receptor signaling

Chemokine receptor desensitization and internalization are important mechanisms for regulating chemokine induced lymphocyte chemotaxis. Heterologous receptor desensitization refers to processes where the activation of one chemokine receptor can result in the inhibition of another. On the other hand, heterologous desensitization does not involve agonist occupancy of the receptor and does not involve receptor internalization. Homologous desensitization is a process where only the activated receptor is desensitized. Protein families of GPCR kinases (GRKs) and arrestins appear to play a central role in regulating homologous chemokine receptor desensitization processes of ligand activated chemokine receptors. GRKs are recruited to the C terminal tail of the chemokine receptor. Opperman et al identified serine residues in the chemokine receptor CCR5 that are phosphorylated by GRKs following ligand stimulation (Oppermann, 2004). As a result the receptor binding affinity for cytosolic arrestins is enhanced and the interaction of G proteins with the receptor is inhibited. Consequently receptor signaling is prevented (Oppermann, 2004). GRKs and arrestins also mediate receptor internalization. For example, arrestin-2 promotes clathrin mediated internalization of CCR5 to endosomal compartments for subsequent recycling to the cell surface (Mueller et al., 2002).

Thus the chemokine receptor desensitization establishes a continuous redistribution of chemokine receptors for maintaining an updated directional migration.

LYMPHOCYTE HOMING

Lymphocyte entry into LNs and peripheral tissues is a directed process mediated by cellular adhesion molecules expressed on the lymphocyte and their respective ligands on the vascular endothelium. Lymphocyte interactions with the vascular endothelium have been classically divided into distinct events, namely rolling, activation, firm adhesion and transendothelial migration.

Lymphocyte interactions with vascular endothelium

The initial rolling event is mediated by low-affinity interactions between lymphocytes and the vascular endothelium. Rolling is primarily mediated by members of the selectin family including L-selectin (CD62L), expressed by leukocytes, and P- and E-selectins expressed by endothelial cells (Ley and Kansas, 2004). All selectins are capable of binding to appropriately glycosylated P-selectin glycoprotein ligand 1 (PSGL-1) (Moore, 1998), though E-selectin appears to recognize different glycosylated epitopes on PSGL-1 compared to L- and P-selectins (Moore et al., 1994). L-selectin binds several carbohydrate structures displayed on proteins on HEV, together known as peripheral node adessins (PNAd) (Ley and Kansas, 2004). The integrins $\alpha_4\beta_1$ and $\alpha_4\beta_7$ have also been demonstrated to support lymphocyte rolling via loose reversible interactions to their endothelial ligands (Bargatze et al., 1995; Berlin et al., 1995; Bradley et al., 1998). During rolling, chemokine receptors on the lymphocyte surface come in contact with chemokines presented by glycosaminoglycans on the vascular endothelium. The chemokine- chemokine receptor interaction is a key event as it induces a conformational change to a high affinity state of the integrin together with integrin clustering (Constantin et al., 2000b). The enhanced integrin avidity for Ig superfamily endothelial ligands mediates firm adhesion and lymphocyte arrest on the vascular endothelium (Springer, 1994). Lymphocytes are now ready to transmigrate the endothelium into the underlying tissue. This migration has been shown to occur either by passing between adjacent endothelial cells (Burns et al., 2000; Carman and Springer, 2004; Cinamon et al., 2001) or through the body of endothelial cells (Carman and Springer, 2004; Feng et al., 1998). The paracellular route involves leukocyte interactions with endothelial junctional structures. Junctional adhesion molecules (JAMs) and PECAM-1 are all members of the Ig superfamily and positioned at intercellular junctions of endothelial and epithelial membranes (Matter and Balda, 2003). JAMs have been demonstrated to interact with leukocyte integrins (Ebnet et al., 2004). These kinds of interactions have been suggested to unlock intercellular junctions and promote lymphocyte transendothelial migration (Johnson-Leger et al., 2002; Ostermann et al., 2002). PECAM-1 has been implicated as a mediator of lymphocyte

transmigration via upregulation of integrins involved in crossing the perivascular basement membrane (Dangerfield et al., 2002). Chemokines associated to the luminal endothelial surface have also been described to promote integrin dependent arrest and lymphocyte transendothelial migration under shear flow (Cinamon et al., 2001). Recently CXCL12 presented on the apical endothelial surface was demonstrated to promote integrin mediated lymphocyte transmigration towards subendothelial presented CCL5 under shear stress. During this migration process, lymphocytes generate protrusions into the interendothelial junctions that potentially contribute to concentrating chemokine receptors for sensing the guiding abluminal CCL5 (Schreiber et al., 2006).

Thus lymphocyte interactions with vascular endothelium involve multiple families of cellular adhesion molecules and signaling receptors. It is the selective expression of cell surface molecules on subsets of lymphocytes and their interaction with responding vascular endothelial ligands that underlie tissue specific lymphocyte homing.

Naïve lymphocyte entry into LNs as an example

Naïve lymphocyte rolling on peripheral LN HEVs is dependent on L-selectin interactions to PNA^d prior to firm adhesion mediated by lymphocyte function-associated antigen-1 (LFA-1) binding to ICAM-1 (Warnock et al., 1998). Ligand binding to the chemokine receptor CCR7 is a key event in lymphocyte migration into LNs as it induces LFA-1 activation (Forster et al., 1999; Stein et al., 2000; Warnock et al., 1998). The CCR7 ligands CCL21 (expressed by endothelial cells in HEVs (Gunn et al., 1998)), and CCL19 (produced by LN DCs and stroma cells (Luther et al., 2000; Ngo et al., 1998)), are both present on the endothelial surfaces of HEV (Stein et al., 2000; Warnock et al., 1998). Here it is worth mentioning that the first *in vivo* relevance for specific chemokines in their activation of integrins were noted in plt mice with a mutation in the region of chromosome 4 that maps to the genes encoding CCL19 and CCL21 (Nagira et al., 1997). These mice were found to have impaired leukocyte homing to LNs because of reduced expression of CCL19 and CCL21 in SLO (Gunn et al., 1999).

Naïve lymphocyte entry to intestinal LNs differs somewhat from their entry into PLN. In contrast to PLN HEV, MLN and PP HEV express the Ig superfamily MAdCAM-1, and glycosylated MAdCAM-1 acts as a key L-selectin ligand in mediating rolling on intestinal LN HEV. In addition arrest on intestinal LN HEV, and in particular PP HEV, is dependent on MAdCAM-1 binding to the integrin $\alpha_4\beta_7$ (Bargatze et al., 1995).

Thus L-selectin, CCR7 and LFA-1 are essential for naïve lymphocyte homing into LNs together with specific L-selectin and $\alpha_4\beta_7$ interactions with MAdCAM-1 in intestinal associated lymphoid tissues.

Lymphocyte migration within LNs

After LN entry T cells remain in the paracortical T cell area while B cells leave this area and localize to B cell follicles that constitute the B cell area. How this migration is controlled within LNs, and what attractants and structural networks that guide the compartmentalization of lymphocytes into distinct B and T cell areas is still not completely understood. Multiphoton microscopy (MPM) has been a good tool for studying these kinds of events. MPM is a relative new technique where infrared excitations are used to detect fluorescent signals deep below the surface of solid organs (up to 450 μm) (Miller et al., 2002). This imaging technique makes it possible to track diverse immune cell populations in intact tissues in live animals. For example, T cells, B cells and DCs can be labeled with different fluorophors to investigate their migration patterns and interactions within LNs.

T cell that have entered a LN via HEV are highly motile and appear to move randomly within the T cell area of the LN (Bajenoff et al., 2006; Bousso and Robey, 2003; Miller et al., 2004a; Miller et al., 2004b; Miller et al., 2003; Miller et al., 2002). Thus in contrast to the chemokine driven lymphocyte entry into LNs, the lymphocyte migration pattern within the different LN areas has been described as a “random walk”. Theoretical calculations based on random walking T cells and stationary DCs state that if the DC density is more than 35 cells/ mm^3 , then T cells have more than 50% chance to meet a DC within a 24 hour time period (Preston et al., 2006).

The LN fiber network is build up by fibroblastic reticular cells (FRCs) in the T cell area and follicular dendritic cells in the B cell area. Recent results suggest that these networks play an important role in promoting more directional movements of T and B cells within LNs (Bajenoff et al., 2006; Castellino et al., 2006). Bajenoff et al demonstrated the importance of the FRC network in guiding (I) T cells to resident DCs in close contact with the network within the T cell areas and (II) B cells passing the T cell area on their way to the B cell follicles (Bajenoff et al., 2006). In the B cell area the follicular dendritic cell (FDC) network stroma cells produce CXCL13 promoting CXCR5⁺ B cells entry into the follicles (Ansel et al., 2000; Gunn et al., 1998). Within the follicles, B cells have been shown to migrate along FDC fibers (Bajenoff et al., 2006). The mechanisms promoting lymphocyte migration along the fiber is not clear, but chemokines or adhesion ligands presented by the network cells may be one potential way of mediating this migratory process.

Lymphocyte egress from LNs

Naïve lymphocytes that do not interact with antigen bound to MHC on the surface of antigen presenting cells in LNs eventually return to the circulation. Lymphocyte exit LNs by entering lymphatic sinuses that are connected to the efferent lymphatics. Lymphocyte egress into the lymphatic sinuses occurs across stroma endothelial cells and the mechanisms regulating this process are slowly becoming clearer.

The G protein coupled receptor, sphingosine 1-phosphate receptor 1 (S1P₁) is expressed on both lymphocytes and LN endothelial cells (Graeler and Goetzl, 2002; Mandala et al., 2002), and T cell egress from LN is thought to be mediated by a higher S1P concentrations in the lymph compared to the LN tissues (Lo et al., 2005b; Schwab et al., 2005). FTY720 (a S1P₁ agonist) blocks lymphocyte egress from LNs, and lymphocytes get densely packed against the sinus endothelium of LNs (Mandala et al., 2002). The mechanisms mediating T cell sequestration in lymphoid organs following treatment with FTY720 are controversial. One hypothesis is that FTY720 mediate S1P₁ internalization on T cells making them unresponsive to S1P in lymph (Matloubian et al., 2004). However FTY720 binding to S1P₁ on endothelial cells has also been suggested to cause reorganized endothelial barrier functions and blocked lymphocyte egress (Mandala et al., 2002; Wei et al., 2005).

Thus by investigating the mechanisms of FTY720, LN egress has been demonstrated to be an active and complex event suggested to be controlled by S1P₁ signaling in both T cells and endothelial cells within lymphoid tissues.

Generation of tissue tropic effector T cell subsets

T cells acquire their ability to enter peripheral tissues after activation in LNs. This was demonstrated by Campbell et al when two distinct CD4⁺ T cell populations (characterized by their expression of CXCR5 or CXCR3) localized to different effector sites as B helper cells or tissue inflammatory cells after *in vivo* activation in LNs (Campbell et al., 2001) The same authors highlighted the importance of the local LN environment in the generation of tissue tropic effector T cells when CD4⁺ T effector cells activated in cutaneous LNs were shown to up regulate P-selectin ligand and preferentially localized to cutaneous tissues while cells activated in intestinal LN expressed $\alpha_4\beta_7$ and migrated into intestinal tissues (Campbell and Butcher, 2002b). Selective expression of chemokine receptors is also dependent on the LN environment, and CD8 $\alpha\beta^+$ cells activated in MLN retain and upregulated CCR9 upon activation *in vivo*, while CCR9 is actively downregulated on cells in PLN (Johansson-Lindbom et al., 2003; Svensson et al., 2002). Thus the induction of chemokine receptors and adhesion molecules on T cells is dependent on the LN environment where they have been activated.

Accumulating data suggest DCs to play an important role in the regulation of T cell homing profiles, and the expression of homing receptors have been suggested to correspond to the tissue origin of the DC. Modulation of CD8⁺ T cell homing molecules by DCs have been demonstrated *in vitro* for DCs isolated from skin, PLN, MLN and PP (Dudda et al., 2004; Johansson-Lindbom et al., 2003; Mora et al., 2003; Stagg et al., 2002). T cells activated by PLN DC or Langerhans cells start to express CCR4 and E- and P-selectin ligands (Dudda and Martin, 2004; Mora et al., 2005), adhesion molecules that have been implicated in T cell localization to inflamed skin (Reiss et al., 2001; Tietz et al., 1998). In contrast Stagg et al demonstrated an enhanced expression of $\alpha_4\beta_7$ on CD8⁺ T cells activated by anti-CD3 in the presence of MLN DCs compared to PLN DCs (Stagg et al., 2002). In another set of experiments, PP DCs (in contrast to PLN and spleen DCs) were shown to induce CD8⁺ T cells with high levels of $\alpha_4\beta_7$ and responsiveness to CCL25 (Mora et al., 2005). Also, CD8⁺ T cells activated by PP DCs had an advantage to PLN activated cells in the localization to the small intestinal mucosa demonstrating a functional role for $\alpha_4\beta_7$ and CCR9 in this process (Mora et al., 2003).

Finally MLN DCs were demonstrated to be critical for the generation of CCR9⁺ $\alpha_4\beta_7$ ⁺ CD8⁺ T cells *in vitro*. In an adoptive CD8⁺ T cell transfer model CCR9⁺ $\alpha_4\beta_7$ ⁺ CD8⁺ T cells were selectively generated in MLN after activation with adjuvants and CCR9 was found to play a critical role in the localization of effector CD8⁺ T cells to the small intestinal epithelium (Johansson-Lindbom et al., 2003).

Together these results demonstrate the importance of LN DC subsets in the establishment of tissue specific effector cells, and point out PP and MLN DCs as critical players in the generation of gut tropic $\alpha_4\beta_7$ ⁺ CCR9⁺ T cells.

Mechanisms of DC induction of gut homing T cells

The molecular mechanisms by which intestinal DCs selectively induce gut homing receptors on responding T cells are currently unclear.

Recently the vitamin A metabolite retinoic acid (RA) was demonstrated to be involved in the generation of gut homing T cells (Iwata et al., 2004). RA is generated from vitamin A (retinol) via a series of intracellular oxidation events, where the final conversion from retinal to RA is catalyzed by retinaldehydrogenases (RALDHs). RA signaling is mediated by two families of RA nuclear receptors (RARs and RXRs) that function as ligand-induced transcription factors when binding as heterodimers to the DNA.

In a study by Iwata et al RA was determined to have the ability to induce $\alpha_4\beta_7$ and CCR9 T cells activated by anti-CD3 and anti-CD28 *in vitro*. MLN and PP DCs (but not spleen DCs)

were shown to express unique isoforms of RALDH and thereby have the capacity to convert retinol to RA. Inhibition of RALDH prevented the ability of these DCs to induce $\alpha_4\beta_7$ on responding T cells. Vitamin A deficient mice show less CD4⁺ and CD8⁺ T cells in the small intestinal lamina propria indicating an *in vivo* relevance for RA in the generation of gut tropic T cells (Iwata et al., 2004). Together these results demonstrate that the selective generation of RA by intestinal DCs plays a critical role in the generation of gut homing T cells.

Expression of $\alpha_4\beta_7$ and CCR9 is not always linked, for example naïve CD8⁺ T cells are CCR9 single positive and the expression of $\alpha_4\beta_7$, but nor CCR9, is downregulated on effector CD8⁺ T cells subsequent to their entry into the small intestinal epithelium (Ericsson et al., 2004; Svensson et al., 2002). This suggests additional ways of inducing independent T cell expression of $\alpha_4\beta_7$ or CCR9. Spleen DCs have the ability to induce $\alpha_4\beta_7^+$ expressing T cells and pan-RAR antagonists can block this induction demonstrating RA production by spleen DCs (M. Svensson unpublished results). Thus low levels of RA seem to be sufficient for the selective expression of $\alpha_4\beta_7$.

Imprinting of DCs

Mechanisms underlying imprinting of DCs with the capacity to generate tissue tropic T cells are still not known.

About one third of MLN DCs and the majority of SI DCs express the α_e integrin (CD103) while most spleen DCs are negative for CD103 (Johansson-Lindbom et al., 2005). DCs expressing CD103 are, as opposed to CD103⁻ DCs, potent inducers of CCR9⁺ T cell after activation *in vitro* (Johansson-Lindbom et al., 2005).

Chemokine receptor CCR7 has been demonstrated to be crucial for the recruitment of DCs from peripheral tissues into LNs (Forster et al., 1999; Hintzen et al., 2006; Ohl et al., 2004). Thus, the presence of normal numbers of CD103⁺ LP DCs but reduced CD103⁺ MLN DCs in CCR7 deficient mice (Johansson-Lindbom et al., 2005), suggest that CD103⁺ MLN DCs derive from the LP. This was further supported by the inability of adoptive transferred OT-I cells in MLN to express CCR9 upon activation in CCR7 deficient mice (Johansson-Lindbom et al., 2005).

DCs isolated from the small intestinal LP are potent inducers of $\alpha_4\beta_7$ and CCR9 on activated CD8⁺ T cells *in vitro* (Johansson-Lindbom et al., 2005). Moreover CD103⁺ have in contrast to CD103⁻ MLN DCs the capacity to generate $\alpha_4\beta_7^+$ CCR9⁺ CD8⁺ T cells (Johansson-Lindbom et al., 2005). Together these results suggest that imprinting occur prior to DC entry into MLN.

In a study by Calzascia et al tumor specific CD8⁺ T cells with skin or gut homing phenotypes were demonstrated in the same LN after injections of tumor cells subcutaneously and intraperitoneally (i.p.). Here the generation of multiple homing phenotypes in the same LN was explained to be a consequence of different sites of antigen capture by DCs (Calzascia et al., 2005).

Together these findings suggest the nature of the antigen presenting DC to be a critical parameter for determining T cell homing phenotypes. Thus targeting specific DC populations and thereby direct the phenotype of the generated lymphocytes may be a useful method for the generating of new vaccination strategies

Effector T cell homing plasticity

There is increasing evidence for plasticity in the expression of tissue homing molecules on effector T cell. For example CD8⁺ T cells with a skin tropic phenotype can be reprogrammed *in vitro* to a gut homing T cells (or vice versa) in the presence of PLN DCs or PP DCs respectively (Dudda et al., 2005; Mora et al., 2005). Thus the reprogramming of T cell homing profile seems to correspond to the phenotype of the last activating DC.

Plasticity in tissue-homing receptor expression is also present *in vivo*. In a recent paper, Liu et al demonstrated reprogramming of skin tropic effector T cells to $\alpha_4\beta_7$ expressing T cells subsequent after entry into MLN (Liu et al., 2006). This may be explained by the ability of tissue tropic factors (such as RA) to act in trans with non-intestinal DCs in the generation of $\alpha_4\beta_7$ expressing T cells within the LN.

The flexibility in the generation of homing phenotypes support the generation of tissue-specific immune control at the site of pathogen entry as well as systemic distribution of antigen experienced T cells for protection against pathogen dissemination. This plasticity could potentially be used therapeutically by target DCs that generate disease causing effector T cells and redirect these cells from chronically inflamed tissues.

EFFECTOR T CELL HOMING TO THE INTESTINAL MUCOSA

Following their activation in SLO, subsets of effector/memory T cells re-enter the circulation and enter the small intestinal mucosa via postcapillary blood vessels in the intestinal LP. Two cellular receptors, the integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9, have been demonstrated to play a particularly important role in effector T cell localization to the intestinal mucosa.

MAdCAM-1/ $\alpha_4\beta_7$

The ligand for $\alpha_4\beta_7$ is MAdCAM-1 that is a member of the Ig superfamily of cell adhesion molecules (Berlin et al., 1993; Wagner et al., 1996), and constitutively expressed on murine and human small intestinal microvascular endothelial cells (Berlin et al., 1995; Berlin et al., 1993; Briskin et al., 1997). The importance of $\alpha_4\beta_7$ and MAdCAM-1 in effector T cell localization to the murine small intestinal mucosa was demonstrated in experiments using neutralizing antibodies to $\alpha_4\beta_7$ or MAdCAM-1 and β_7 deficient mice (Haddad et al., 2003; Hamann et al., 1994; Lefrancois et al., 1999b).

MAdCAM-1 is also expressed on large bowel vascular endothelial cells (Streeter et al., 1988), and lymphocyte entry to the colonic mucosa has been demonstrated to be dependent on β_7 (Lefrancois et al., 1999a).

Lymphocyte migration to the intestinal mucosa is not strictly dependent on $\alpha_4\beta_7$ (Kuklin et al., 2000; Lefrancois et al., 1999a). This was demonstrated when rota virus infections were equally well cured by $\beta_7^{-/-}$ and WT CD8⁺ T cells recruited to the intestinal mucosa (Kuklin et al., 2000).

CCL25/CCR9

The chemokine receptor CCR9 is expressed on a subset of circulating β_7^{+} memory T cells in human blood (Zabel et al., 1999) and on the majority of T cells in the small intestinal epithelium and LP both in human and mouse (Kunkel et al., 2000; Marsal et al., 2002; Svensson et al., 2002; Zabel et al., 1999). Its sole functional ligand CCL25 is constitutively and selectively expressed by epithelial cells in the human and mouse small intestine but not on epithelial cells in other mucosal sites (Ericsson et al., 2006; Kunkel et al., 2000; Papadakis et al., 2000; Vicari et al., 1997; Wurbel et al., 2000; Zabel et al., 1999). In the human CCL25 appears to be expressed primarily by crypt epithelium (Kunkel et al., 2000; Papadakis et al., 2000), while in the mouse CCL25 mRNA is expressed at similar levels by both villous and crypt epithelial cells (Ericsson et al., 2006; Wurbel et al., 2000). Immunohistochemical studies have also detected CCL25 protein

on human and murine microvascular endothelium in the small intestinal LP (Hieshima et al., 2004; Papadakis et al., 2000), indicating a potential role for this chemokine in mediating lymphocyte recruitment from the vasculature. Nevertheless laser capture microscopy samples of the small intestinal LP containing MAdCAM-1 positive vessels do not contain CCL25 mRNA (Ericsson et al., 2006), indicating that this endothelial CCL25 may derive from the epithelium.

Adoptive transfer studies using neutralizing antibodies to CCL25 or CCR9^{-/-} T cells have demonstrated a role for CCL25/CCR9 in mediating effector T cell localization to the murine small intestinal epithelium (Johansson-Lindbom et al., 2003; Svensson et al., 2002). Moreover, CCL25 has been demonstrated to enhance α_4 dependent adhesion of murine lymphocytes to MAdCAM-1 in an *in vitro* cell adhesion assay (Hieshima et al., 2004) and desensitization with CCL25/CCR9 antibodies inhibit the adherence of LPL and IEL to small intestinal microvessels (Hosoe et al., 2004).

Despite these findings, CCR9^{-/-} mice have relatively normal numbers of small intestinal CD8 $\alpha\beta$ ⁺ and CD4⁺ T cells (Pabst et al., 2004a; Uehara et al., 2002; Wurbel et al., 2001), suggesting that T cell entry to and maintenance within the small intestinal mucosa can be mediated by CCR9 independent mechanisms..

Together these results demonstrate an important and selective role for CCR9/CCL25 in effector T cell localization to the small intestinal epithelium.

EFFECTOR T CELL HOMING TO THE INFLAMED INTESTINAL MUCOSA

Inflammatory bowel disease

IBD occurs most frequently in people ages 15 to 30 years, but it can also affect younger children and older people. There are significantly more reported cases in western Europe and North America than in other parts of the world. IBD can with by and large be divided into two main types of diseases, namely Crohn's disease (CD) and ulcerative colitis (UC). CD can develop throughout the intestine but with a predominance for the small intestinal terminal ileum and ascending colon while UC is exclusively present in colon (Podolsky, 2002). CD is characterized by transmural inflammation that causes fissuring ulcers and increased fibrous connective tissue. Thus in contrast to CD, that penetrates deep into the intestinal wall, UC demonstrates shallow ulcers that usually are limited to the mucosa and submucosa.

The underlying mechanisms causing IBD is yet to be determined. However in studies using murine models of IBD, development of experimental colitis appears to require the presence of luminal flora. Thus in these mice disease does not occur when the animals are maintained in germfree conditions (Sadlack et al., 1993), but develops rapidly when mice are colonized by commensal bacteria. Thus IBD is thought to result from a dysfunctional and ongoing activation of the mucosal immune system driven by the presence of normal intestinal bacteria flora (Cahill et al., 1997).

The accumulation of lymphocytes within the intestinal mucosa appears to be a central feature of intestinal inflammatory responses occurring in sites of microbial infections or in regions with disrupted epithelial barrier functions. The current section will provide a brief outline of molecules implicated in mediating lymphocyte recruitment to the inflamed intestine.

Role for MAdCAM-1/ $\alpha_4\beta_7$

MAdCAM-1 expression is increased during inflammation in the gut (Briskin et al., 1997) and the importance of $\alpha_4\beta_7$ for intestinal lymphocyte homing appears, at least in part, to be retained during human intestinal inflammation.

Podolsky et al demonstrated an important role for the α_4 integrin in chronic colitis in the cotton-top tamarin, a New World primate that is susceptible to a spontaneous form of IBD that resembles that of human UC (Podolsky et al., 1993). Animals treated with an anti- α_4 antibody showed attenuation of disease compared to placebo controls (Podolsky et al., 1993). Furthermore a neutralizing antibody to $\alpha_4\beta_7$ or MAdCAM-1 has been shown to ameliorate disease in various IBD animal models (Hesterberg et al., 1996; Kato et al., 2000; Picarella et al., 1997). One of these studies used a murine CD45RB^{hi} colitis transfer model (Picarella et al., 1997). Here specific

antibodies to MAdCAM-1 or $\alpha_4\beta_7$, and MAdCAM-1 blocked the recruitment of lymphocytes to the inflamed colonic mucosa and thereby reduced the severity of disease (Picarella et al., 1997).

Treatment with humanized anti- α_4 antibody (natalizumab) in human trials showed benefits for patients with CD in short-term studies (Ghosh et al., 2003). Since the integrin α_4 subunit pairs not only with the β_7 subunit but also with the β_1 subunits, anti- α_4 treatment also inhibit $\alpha_4\beta_1$ mediated adhesion and lymphocyte entry into CNS (Kent et al., 1995). The FDA approved natalizumab in 2004 for treatment of multiple sclerosis (MS), but was 2005 withdrawn after three cases of severe viral infection of the brain during ongoing clinical trials. Since 2006 natalizumab is again available as a treatment for restricted use in MS.

Finally a humanized antibody to $\alpha_4\beta_7$ (MLN02) has successfully been proven in phase II clinical trials in patients with UC and CD (Feagan et al., 2005). Moreover the severe infection complications, seen in patients treated with the less selective anti- α_4 antibody, were not seen in the early phase of the trials with the more selective MLN02 (Feagan et al., 2005).

Taken together, $\alpha_4\beta_7$ and MAdCAM-1 are thought to be involved in the recruitment of lymphocytes to both human and murine inflamed intestinal mucosa.

Role for CCL25/CCR9

The selective expression of CCL25 in the small intestine is maintained in patients with small bowel CD (Papadakis et al., 2001) and patients with small intestinal (but not colonic) CD have increased numbers of circulating CCR9⁺ CD4⁺ T cells (Papadakis et al., 2001). Together these results indicating a potential role for CCR9/CCL25 in T cell recruitment to the inflamed ileum.

In a recent study, Rivera-Nieves et al investigated the effect of neutralizing antibodies to CCR9 and CCL25 in a spontaneous murine model of chronic ileitis (Rivera-Nieves et al., 2006b). In this model CCR9 expressing lymphocytes increased in the initial phase of disease but decreased during later stages (Rivera-Nieves et al., 2006b). CCL25 levels increased in the inflamed compared to non-inflamed ileum (Rivera-Nieves et al., 2006b) and administration of antibodies to CCR9 and CCL25 was demonstrated to only partially ameliorate early but not late chronic murine ileitis (Rivera-Nieves et al., 2006b).

Chemocentryx inc (Mountain View, CA) has recently developed a low molecular weight antagonist to CCR9, Traficet-ENTM, which has demonstrated beneficial effects in two murine models of IBD (Wei Z., 2005). The effect on inflammation in the small intestine was determined in the TNFΔARE murine model developing ileal CD while the effect on colonic inflammation was examined in a murine model of UC where disease is induced by a mutation in the MDR1a gene. Blockade of CCR9 with Traficet-ENTM significantly ameliorated the severity of disease in

both the murine model of ileitis and colitis (Wei Z., 2005). In human phase I trials, the oral administered Traficet-ENTM was well tolerated and sustained levels of the drug was demonstrated (Keshav S.). There are now ongoing phase II trials in CD patients with Traficet-ENTM (Keshav S.).

Together these results suggest that targeting CCL25/CCR9 pathway may provide a potential means for treatment of small bowel CD. Nevertheless the role of CCR9 dependent versus independent lymphocyte entry to the inflamed mucosa under different stages of disease process certainly needs further study.

Since both $\alpha_4\beta_7$ and CCR9 are suggested to play a role in the recruitment of cells to the inflamed small intestinal mucosa, combinatorial therapeutic regimes using both chemokine receptor and integrin antagonist would potentially increase both the specificity and efficiency of the treatment.

Role for additional homing molecules

In addition to $\alpha_4\beta_7$ and CCR9, several adhesion molecules have been implicated in regulating severity in animal models of IBD. For example in a murine model of terminal ileitis, PSGL-1 was found to be expressed on infiltrating intestinal CD4⁺ T cells as well as on small intestinal endothelial cells during disease (Rivera-Nieves et al., 2006a). Treatment with a monoclonal antibody to PSGL-1 ameliorate disease (Rivera-Nieves et al., 2006a), demonstrating a role for PSGL-1 in lymphocyte recruitment to chronically inflamed small intestine. In another set of experiments, blocking antibodies to ICAM-1 and α_4 or ICAM-1 and VCAM-1 demonstrated resolution of active but not chronic murine small intestinal inflammation (Burns et al., 2001). Furthermore antibody blockade of L-selectin together with α_4 integrins improved the outcome of disease by depleting CD4⁺ LPL co-expressing $\alpha_4\beta_7$ and $\alpha_4\beta_1$ from the small intestine (Rivera-Nieves et al., 2005). Together these results define multiple adhesion molecule combinations that mediate the recruitment of CD4⁺ T cells to the inflamed small intestinal lamina propria.

Apart from CCL25 and CCR9, several other chemokines and chemokine receptors have been suggested to be involved in intestinal inflammatory pathogenesis. For example, an increased expression of CCR2 by human SI CD4⁺ LPL has been demonstrated in small bowel CD compared to control ileum (Connor et al., 2004). Upregulated expression of the CCR2 ligands CCL2, CCL7 and CCL8 has been shown in human CD (Banks et al., 2003; Grimm et al., 1996; Mazzucchelli et al., 1996; Wedemeyer et al., 1999). Together these results suggest a preferential recruitment of CCR2⁺ lymphocytes to the inflamed intestinal site. Increased expression of CX3CL1 has been detected on human intestinal microvascular endothelial cells during intestinal

inflammation (Muehlhoefer et al., 2000; Sans et al., 2007). Moreover IBD patients show higher numbers of circulating CX3CR1⁺ cells compared to healthy controls (Sans et al., 2007). This suggests that CX3CL1/CX3CR1 chemokine-receptor pair may also play a role in human intestinal inflammation.

In summary, several chemokine receptors and adhesion molecules have together with their respective ligands been demonstrated to be involved in the recruitment of lymphocytes to the inflamed intestinal mucosa. However their contribution to direct specific lymphocyte populations to the intestinal mucosa remains to be determined.

AIMS OF THIS THESIS

The intestinal mucosa, including the intestinal epithelium and underlying LP, contains a large number of effector/memory T cells that are thought to play a central role in the generation and maintenance of mucosal immune responses and in maintaining mucosal integrity. The current thesis investigates the mechanisms regulating effector T cell recruitment to the small intestinal mucosa focusing on the role of chemokine receptor CCR9 in this process.

The specific aims of this thesis were:

- To describe chemokine receptors involved in CD4⁺ effector Tcell homing to the small intestinal lamina propria.
- To determine the importance of the CCR9/CCL25 chemokine receptor-ligand pair in regional localization of CD8 α β ⁺ effector T cells to the small intestinal mucosa.

PAPER I: GUT-ASSOCIATED LYMPHOID TISSUE PRIMED CD4⁺ T CELLS DISPLAY CCR9-DEPENDENT AND -INDEPENDENT HOMING TO THE SMALL INTESTINE

Background

To examine the role for CCR9 in the *in vivo* recruitment of CD8 $\alpha\beta$ ⁺ to the small intestinal epithelium, an adoptive transfer model was used in which TCR transgenic CD8 $\alpha\beta$ ⁺ OT-I cells were injected into recipient WT mice. Using this model CCR9 was demonstrated to be selectively induced on activated CD8 $\alpha\beta$ ⁺ OT-I cells in MLN but not in PLN and spleen (Svensson et al., 2002). In the same model, treatment with antibodies to the CCR9 ligand CCL25 reduce the localization of effector CD8 $\alpha\beta$ ⁺ OT-I cells to the small intestinal epithelium (Svensson et al., 2002). Accordingly, by using a competitive adoptive transfer system, where CCR9^{-/-} and WT CD8 $\alpha\beta$ ⁺ OT-I cells were co-injected into recipient mice, CCR9 was demonstrated to be critical for the recruitment of CD8 $\alpha\beta$ ⁺ T cell to the small intestinal epithelium (Johansson-Lindbom et al., 2003). Together these results demonstrated (I) intestinal LNs as crucial for the generation of effector CCR9⁺ CD8 $\alpha\beta$ ⁺ T cell and (II) CCR9/CCL25 receptor-ligand pair as critical for effector CD8 $\alpha\beta$ ⁺ T cell recruitment into the small intestinal epithelium.

The intestinal mucosa contains a large number of CD4⁺ T cells spread throughout the LP. Distinct pairs of adhesion molecules present on the T cell and the intestinal vascular endothelial surface mediate the localization CD4⁺ T cells into the LP. Effector CD4⁺ T cells entry into the small intestinal LP is mediated by interactions between $\alpha_4\beta_7$, expressed on the T cell surface and MAdCAM-1 on the endothelial wall (Campbell and Butcher, 2002a; Haddad et al., 2003). In a TCR transgenic adoptive transfer model administration of ovalbumin (OVA) induced PSGL-1 and $\alpha_4\beta_7$ expression on OVA specific CD4⁺ T cells following their activation in MLN (Haddad et al., 2003). Treatment of these mice with neutralizing antibodies to P-selectin (a ligand for PSGL-1) reduced the recruitment of activated CD4⁺ T cells to the LP (Haddad et al., 2003). Thus $\alpha_4\beta_7$ and PSGL-1 expressed on the CD4⁺ T cell surface have been demonstrated to be important for the localization of CD4⁺ T cells to the small intestinal LP.

To gain further insight into intestinal homing mechanisms, the aim was to examine chemokine receptors involved in the recruitment of effector CD4⁺ T cells to the small intestinal LP.

Brief summary of paper I

Endogenous CD4⁺ LPLs express a heterogeneous array of chemokine receptors including CCR9, CCR6, CCR5, CXCR6 and CXCR3. Since CCR9 is selectively induced on CD4⁺ T cell (OT-II cells) activated in MLN and expressed on the majority of OT-II cells entering into the small intestinal LP, CCR9 was suggested as a candidate receptors involved in migration to the intestinal LP. The importance of CCR9 was examined in competitive adoptive transfers with CCR9^{-/-} and WT OT-II cells demonstrating both CCR9 dependent and independent CD4⁺ T cell entry in to the small intestinal LP.

In vivo adoptive transfer model

We have used an adoptive transfer model where OVA specific CD4⁺ (OT-II) cells were transferred into WT recipient mice for examination of chemokine receptor regulation on activated OT-II cells *in vivo*. We administered OVA and the adjuvant LPS i.p. rather than orally since oral administration induces priming in gut draining LNs (Worbs et al., 2006) and we wished to compare chemokine receptor regulation in PLN and MLN. The adjuvant LPS was used since it has been demonstrated to be required for *in vivo* differentiation of CCR9⁺ $\alpha_4\beta_7$ ⁺ gut homing T cells after OVA administration i.p. (Johansson-Lindbom et al., 2003). To exclude that OT-II cells had been activated in another LNs before examination, we performed analysis 2 days after activation a time point when activated cell have been demonstrated to still be sequestered in LNs (Arnold et al., 2004; Campbell and Butcher, 2002a).

To determine the role for CCR9 in the migration of CD4⁺ T cells into the LP, CCR9^{-/-} mice were crossed with OT-II mice for the generation of CCR9^{-/-} OT-II cells. CCR9^{-/-} and WT OT-II cells were adoptively cotransferred into recipient mice and the CCR9^{-/-}:WT OT-II cells ratios determined in peripheral tissues three days after activation, the time point when activated T cells start to enter into peripheral tissues (Figure 5A of Paper I).

In this study adoptive transferred cells were systemically activated by i.p. injections of antigen. However activation of cells with orally administered antigen may be more physiological relevant since intestinal immune responses primarily are initiated by antigens from the intestine. DC migration from the intestinal LP to the MLN is enhanced after oral administration of antigen (Anjuere et al., 2004; Johansson-Lindbom et al., 2005). Administrate antigen orally will

thus potentially target another set of DCs and thus generate effector T cells with a different chemokine receptor profile.

Murine adoptive transfer models are good for the purpose of following immune responses *in vivo*. However the use of abnormally high precursor frequencies in adoptive transfers have been shown to skew the development of effector memory T cells versus central memory T cell (Marzo et al., 2005). Accordingly, the use of high numbers of OT-II cells ($2-6 \times 10^6$) in our adoptive transfers may not generate normal immune responses and thus give non-physiological tissue homing responses.

Results

To identify potential candidate chemokine receptors involved in the localization of CD4⁺ LPL, we isolated CD4⁺ T cells from the small intestinal LP and stained them with a panel of anti-chemokine receptor antibodies. The majority of previously activated/memory CD4⁺ LP T cells expressed CCR9 and CXCR6, 50% CCR5, CCR6 and CXCR3 and a minor population expressed CCR2. Thus CD4⁺ LPL was demonstrated to express a heterogeneous chemokine receptor profile.

Effector T cells entering the intestinal mucosa is thought to derive primarily from intestinal inductive sites such as the MLN and PP. To identify potential receptors involved in the recruitment of CD4⁺ T cell to the intestinal LP, we used an OVA specific TCR transgenic OT-II cell adoptive transfer model. OVA and the adjuvant LPS were administered i.p. as we wished to compare chemokine receptor induction on OT-II cells activated in PLN and MLN. OT-II cells were injected into WT recipient mice and 2 days after activation chemokine receptors and $\alpha_4\beta_7$ were examined on activated cells in MLN and PLN. Activated OT-II cells in MLN and PLN expressed CCR6 and CXCR3 while CCR2, CCR5 and CXCR6 were induced on a minority of the cells. Chemokine receptor CCR9 and integrin $\alpha_4\beta_7$ were selectively induced on activated OT-II cells in the MLN consistent with previous results in the CD8 β^+ OT-I system (Svensson et al., 2002). In contrast CCR8 was induced on activated OT-II cells in PLN and not on activated cells in MLN. This is an interesting finding based on data demonstrating that human skin memory T cells express CCR8 and that CCR8 ligand CCL1 is constitutively produced in non-inflamed skin (Schaerli et al., 2004). Thus the selective expression of CCR9 and CCR8 in gut and skin draining LNs respectively, may suggest a potential role for these chemokine receptors in selective homing to peripheral tissues. Moreover, activated CCR9^{-/-} OT-II cells were induced to express higher levels of CCR5 compared to their WT counterparts after activation in MLN. CCR5 was the only examined chemokine receptor that was differently expressed on CCR9^{-/-} and WT OT-II cells.

MLN DCs have been shown to be important for induction of CCR9 on CD8⁺ T cells (Johansson-Lindbom et al., 2003), we therefore aimed to determine whether these subset of DCs could induce CCR9 and other chemokine receptors on CD4⁺ T cells. OT-II cells activated *in vitro* by OVA pulsed MLN DCs were induced to express CCR9, CXCR3 and $\alpha_4\beta_7$, similar to *in vivo* activated cells. Although CCR6 was induced on *in vivo* activated OT-II cells both in the MLN and PLN this could not be repeated in DC/OT-II co-cultures *in vitro*. Of note, stimulating OT-II cells together with preparations from whole MLN tissues was not sufficient for inducing CCR6 on responding OT-II cells. Taken together, the chemokine receptor profile induced on *in vivo* activated OT-II cells in MLN can largely be reproduced with antigen presenting MLN DCs *in vitro*. However the induction of CCR6 could not be repeated *in vitro* and thus seems to require additional non-DC associated signals in combination with the lymphoid tissue network present within LNs.

Since the majority of endogenous CD4⁺ LPL expressed CCR9 and the receptor was selectively induced on activated OT-II cells in MLN, we continued to examine whether the localization of CD4⁺ T cells to the small intestinal LP was CCR9 dependent. CCR9^{-/-} and WT OT-II cells were cotransferred into recipient mice (activated by i.p. injection with OVA and LPS) and 3 days after activation CCR9^{-/-}:WT OT-II cell ratios were examine in MLN, liver and LP tissues. These data demonstrated that CCR9^{-/-} OT-II cells had a significant disadvantage in localizing to the small intestinal LP compared to the other tissues. However, CCR9^{-/-} OT-II cells were detected within the site, demonstrating CCR9 dependent and independent CD4⁺ T cell homing to the small intestinal LP. The majority of the CCR9^{-/-} OT-II cells entering the intestinal LP failed to express other chemokine receptors examined, suggesting that entry into the LP take place independently of these receptors. Thus CD4⁺ T cell demonstrate both CCR9 dependent and independent localization to the small intestinal LP.

Future directions of interest

Chemokine receptor association with functional or positioning characteristics

CD4⁺ LPL was demonstrated to express a heterogeneous chemokine receptor profile.

This heterogeneity may reflect (I) the presence of different subsets of CD4⁺ LPL in the site (II) that different intestinal region of the intestine are populated by different subsets of chemokine receptor expressing cells. CD4⁺ T cells can be divided into different subsets with different functional properties according to their cytokine producing profile such as IFN γ and IL-4 producing Th1 cells and Th2 cells respectively (Coffman, 2006) and IL-17 producing Th17 cells (Ivanov et al., 2006). Thus chemokine receptor positive subsets of CD4⁺ LPL can be sorted and

stimulated *in vitro* for examination of functional characteristics. Homogenous functional characteristics after *in vitro* culture may suggest that CD4⁺ LPL can be functionally grouped based on their chemokine receptor profile. These results can be used to control the access of subset of cells in to the intestinal mucosa, whose function is for example to increase protection against intestinal pathogens or down regulate intestinal inflammation and thus be involved in protective immune surveillance.

In this study we identified chemokine receptors expressed on CD4⁺ LPL distributed throughout the whole small intestinal LP. Thus the demonstrated heterogeneity in chemokine receptor expression may be due to different subsets of chemokine receptor expressing cells within different subintestinal regions. Moreover the demonstrated CCR9 dependent and independent CD4⁺ T cell entry into the small intestinal LP suggest different localization mechanisms to different regions within the lamina propria. Using immunohistochemistry will visualize the localization of subsets of chemokine receptor positive cells within different regions on the intestinal mucosa.

Functional discrepancies due to differences in CCR5 induction

Activated CCR9^{-/-} OT-II cells were induced to express higher levels of CCR5 compared to their WT counterparts after activation in MLN. We currently cannot explain the reason for this or whether differential regulation of CCR5 expression on CCR9^{-/-} and WT CD4⁺ T cells have any functional consequences for these cells subsequent to their activation. Nevertheless, CCR5 has been shown to be upregulated on a subpopulation of late apoptotic T cells as a tool for chemokine clearance during resolution of tissue inflammation (Ariel et al., 2006). Thus the connection between CCR5 and cell death suggests a potential decreased survival capacity for CCR9^{-/-} compared to WT T cells. This can be further explored by comparing CCR9^{-/-} and WT T cells apoptosis *in vitro* or in competitive adoptive transfers.

In adoptive transfers of CD8 $\alpha\beta$ ⁺ OT-I cells, CCR5 is upregulated on naïve OT-I cells after immunization but prior to antigen recognition in LNs of immunized recipient mice (Castellino et al., 2006). Active recruitment of naïve CCR5⁺ OT-I cells to antigen loaded DC/OT-II cell complexes (where CCL3 and CCL4 are produced) was demonstrated to favor the generation of OT-I memory T cells (Castellino et al., 2006). Whether an early induction of CCR5 (prior to antigen exposure) is differently regulated on CCR9^{-/-} and WT OT-I cells is not known. However it would be interesting to determine in the context of the role for CCR9 in the generation of CD8⁺ memory T cells.

CCR9 in T cell interactions with B cells

After adoptive transfers of CCR9^{-/-} and WT OT-II cells into recipient mice a decreased CCR9^{-/-}:WT OT-II cells ratio was detected for activated cells in MLN compared to the input ratio (see Figure 4C of Paper I). Further analysis of CCR9^{-/-}:WT OT-II cells ratios gating on small and large cells in MLN (Figure 4A) demonstrated a decreased proportion of CCR9^{-/-} OT-II cells within the large compared to the small lymphocyte gate (Figure 4B-C).

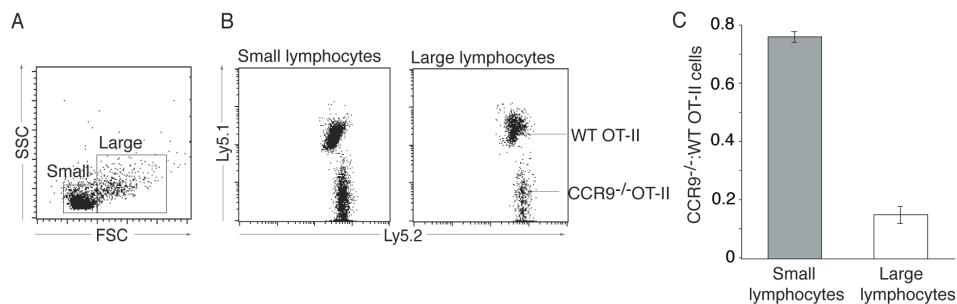


Figure 4. (A) Representative flow cytometry analysis of small and large lymphocytes among transferred OT-II cells in MLN. (B) CCR9^{-/-} (Ly5.2⁻) and WT OT-II (Ly5.1⁺Ly5.2⁺) cells within small and large lymphocyte gate respectively and (C) CCR9^{-/-} to WT OT-II cell ratios within small and large lymphocyte gates. Data represents mean (SEM) from 4 individual mice.

While we currently do not understand the reason for this, we have detected B cell/T cell complexes in the large lymphocyte gate when analyzing MLN cell from animals given OVA and LPS (B Johansson-Lindbom unpublished observation). Thus one potential explanation is that CCR9^{-/-}OT-II cells have an impaired ability to colocalize with B cells. This is supported by the fact that human CCR9⁺ PB CD4⁺ T cells, in contrast to CCR9^{-/-}PB CD4⁺ T cells, are potent inducers of IgA, IgG and IgM producing B cells *in vitro* (Papadakis et al., 2003) and that CCR9 deficient mice show reduced levels of antigen specific serum IgA after immunizations with T cell dependent antigen (Pabst et al., 2004b). Accordingly to these findings, it will thus be interesting to investigate whether CCR9 deficient CD4⁺ T cells demonstrate impaired colocalization or contact with B cells in the induction of humoral immune responses. Thus immunization with T cell dependent and independent antigens will further explore the importance of CCR9 in the generation of proper T cell help for driving B cell responses. Moreover detection of effector molecules (like CD40L on the T cell surface) necessary for the establishment of a functional B

cell help will possibly determined whether T cell/B cell complexes can be formed in the absence of CCR9 expressing T cells.

CD69 and T cell retention within intestinal tissues

As discussed in the introduction, S1P₁ is expressed on T cell and thought to control cell egress from LNs and thymus (Matloubian et al., 2004; Lo et al., 2005a). Moreover CD69 has been shown to form complexes with S1P₁ that lead to inhibition and down modulation of the receptor (Shiow Rosen et al. 2006). Consequently, S1P₁ agonists have *in vivo* been shown to induce down regulation of CD69 on thymocytes and thereby inhibit thymic cell egress (Rosen et al., 2003; Shiow et al., 2006). Thus CD69 expression has been demonstrated to be associated with T cell retention in tissues. Since the majority of CD4⁺ LPL express CD69 (Figure 1A of Paper I), the importance of CD69 in T cell retention within the intestinal LP is highly relevant. Thus a future direction would be to determine the relationship between S1P₁ and CD69 on intestinal T cells and analyze the effect of CD69 for T cell retention within this site.

PAPER II: DIFFERENTIAL HOMING MECHANISMS REGULATE REGIONALIZED EFFECTOR CD8 $\alpha\beta$ ⁺ T CELL ACCUMULATION WITHIN THE SMALL INTESTINE

Background

Effector CD8 $\alpha\beta$ ⁺ T cell migration to the small intestinal epithelium has been demonstrated to depend on CCR9 (Johansson-Lindbom et al., 2003; Svensson et al., 2002). However, when we explored the involvement of CCR9 in the localization of CD4⁺ T cell to the small intestinal LP, both CCR9 dependent and independent localization was observed (Paper I). From this data it was not clear whether the ability to enter the intestinal LP via a CCR9 independent mechanism was selective for CD4⁺ T cells, or whether this discrepancy between CD4⁺ and CD8⁺ T cell homing was due to differences in the localization mechanism of effector T cells to the LP versus the epithelium.

In addition, whether the mechanism of effector T cell migration to the intestinal LP and epithelium was similar throughout the entire length of the small intestine had not been determined. Therefore we wanted to see if there are any regionalized differences in the mechanisms controlling T cell entry to the proximal versus distal small intestine and between the LP and epithelium.

Brief summary of paper II

Here we demonstrate the differences in CCR9 dependent CD8 β ⁺ T cell localization both within and to the separate sites along the length of the small intestine. The CCR9 dependent migration was shown to be more efficient to the proximal compared to the distal small intestinal mucosa, corresponding to higher epithelial expression levels of the CCR9 ligand CCL25 at this site. Migration of cells to the LP was less dependent on CCR9 than migration to the epithelium and the CCR9 independent migration to the LP was found to be PTX sensitive.

In vitro adoptive transfer model

Activation of adoptive transferred TCR transgenic T cells *in vivo* generates different subsets of effector T cells with different homing profiles. Thus examine distinct homing mechanisms is complicated in an adoptive transfer model where transferred cells are activated *in vivo*. We developed a new adoptive transfer model to be able to examine the homing pattern of a homogenous population of CCR9⁺ $\alpha_4\beta_7$ ⁺ OT-I cells. Large numbers of gut homing T cells were generated *in vitro* for subsequent adoptive transfers into recipient mice. Initially OT-I cells were activated by anti-CD3 and anti-CD28 *in vitro* in the presence of RA, since these culture

conditions had been demonstrated to generate $\alpha_4\beta_7$ and CCR9 expressing T cells (Iwata et al., 2004). However this *in vitro* culture condition was not sufficient for down regulation of CD62L. In contrast OT-I cells stimulated with OVA peptide pulsed spleen DCs in the presence of RA generated CD62L^{low} CCR9⁺ $\alpha_4\beta_7$ ⁺ WT OT-I cells and CD62L^{low} $\alpha_4\beta_7$ ⁺ CCR9^{-/-} OT-I cells. The OT-I cells remained their phenotype after expansion in culture media supplemented with IL-7, IL-15 and RA (Figure 2A of Paper II). Thus large numbers of $\alpha_4\beta_7$ ⁺ WT and $\alpha_4\beta_7$ ⁺ CCR9^{-/-} effector OT-I cells could be generated for competitive cotransfers into recipient mice for the comparison of CCR9 dependent entry into different regions of the small intestinal mucosa.

Since both T cell entry and exit from LNs are dependent on G α i signaling (Lo et al., 2005a), PTX can not be used in our *in vivo* adoptive transfer models for examining G α i sensitive homing mechanisms into the intestinal mucosa. *In vitro* generated $\alpha_4\beta_7$ ⁺ CCR9^{-/-} OT-I cells were instead pretreated with PTX and used in short term homing experiments for the examination of whether G α i protein-coupled receptors could mediate the CCR9 independent localization of effector T cells to the intestinal mucosa.

In summary, generation of a homogenous population of cells for transfer into recipient mice is useful for dissection of specific homing mechanisms. The possibility to pre-incubate *in vitro* generated effector T cells with various neutralizing antibodies or signaling inhibitors before assessing their homing potential is a further advantage with this model.

Results

Epithelial cells have been demonstrated to constitutively express CCL25 mRNA in the small intestine (Ericsson et al., 2006; Kunkel et al., 2000; Papadakis et al., 2000; Wurbel et al., 2000).

To determine whether there were any regional variations in CCL25 expression within the small intestine, epithelial CCL25 mRNA levels were assessed by real-time RT-PCR from the duodenum, jejunum, ileum and colon. Duodenum was defined as the first 3 cm after pylorus, jejunum as the following proximal part of the small intestine and ileum as the distal 5 cm of the small intestine before cecum and colon (Figure 5).

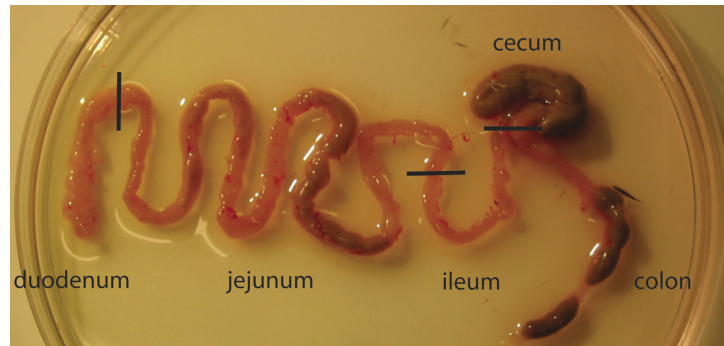


Figure 5. Murine intestine divided into the duodenum, jejunum, ileum, cecum and colon.

The mRNA levels of CCL25 in the distal small intestinal epithelium were significantly reduced in comparison to the proximal small intestine and the reduction in CCL25 mRNA levels in ileum compared to jejunum correlated with reduced levels of CCL25 protein. Together these results demonstrate that the epithelial production of CCL25 decreases from the proximal to the distal small intestine.

To study the involvement of CCR9 in the accumulation of CD8 β ⁺ T cells in the proximal, middle and distal small intestinal epithelium, homogenous populations of CCR9⁺ $\alpha_4\beta_7$ ⁺WT and CCR9^{-/-} $\alpha_4\beta_7$ ⁺ OT-I cells were generated *in vitro* in the presence of OVA peptide-pulsed splenic DCs and RA. Following co-injections of WT and CCR9^{-/-} OT-I cells into recipient mice, CCR9^{-/-} OT-I cells were dramatically disadvantaged in their ability to enter the epithelium in all intestinal regions. The proportion of *in vitro* generated WT OT-I cells among endogenous CD8 $\alpha\beta$ ⁺ IEL was lower in the ileum compared to that of the duodenum and jejunum. Thus the reduced expression of CCL25 in the ileum correlated with less CCR9 dependent T cell entry into this site.

To examine whether there were any differences in the CCR9 dependent migration to the lamina propria versus the epithelium CCR9^{-/-}:WT OT-I cell ratios were determined and compared for the small intestinal LP and epithelium. These results showed a reduced CCR9 dependent localization into the LP compared to the epithelium.

To determine whether the CCR9 independent migration to the LP was mediated by other G α_i protein-coupled receptors, *in vitro* generated CCR9^{-/-} OT-I cells were pretreated with PTX before injection into recipient mice. These cells were significantly disadvantage in their ability to localize to the small intestinal LP compared to untreated cells, thus demonstrating an

active involvement of G α i coupled receptors in the CCR9 independent migration of CD8 $\alpha\beta$ ⁺ T cells to the intestinal LP.

To examine the *in vivo* relevance of CCR9 independent CD8 $\alpha\beta$ ⁺ T cell localization to the intestinal epithelium, CCR9^{-/-} and WT OT-I cells were injected at an equal ratio into recipient mice. Three days after immunization with OVA and LPS i.p. cells were isolated from small intestinal epithelial regions and the CCR9^{-/-}:WT OT-I cell ratios were determined. These results demonstrated OT-I cells to be less dependent on CCR9 in their localization to the distal compared to proximal regions of the intestinal epithelium. Interestingly, in contrast to *in vitro* generated CCR9⁺ $\alpha_4\beta_7$ ⁺ WT OT-I cells (that preferentially localized to the proximal epithelium), *in vivo* generated WT OT-I cells migrated equally well into the duodenal, jejunal and ileal epithelium. Thus CCR9 independent entry mechanisms play an important role in directing *in vivo* activated OT-I cells to the distal small intestinal epithelium.

Future directions of interest

Use of in vitro assay to dissect CCR9 dependent and independent T cell entry into the intestinal mucosa

In Paper II we developed an *in vitro* assay to generate large numbers of CCR9⁺ $\alpha_4\beta_7$ ⁺ T cells that could be used in adoptive transfers. This assay can now be used to explore the mechanisms of effector T cell homing to the intestinal mucosa specifically.

Mechanisms of CCR9 dependent migration

We have demonstrated that the majority of recently entered CD4⁺ T cells within the small intestinal LP express CCR9 and $\alpha_4\beta_7$ (Figure 6 of Paper I). However the role of $\alpha_4\beta_7$ in CCR9⁺ T cell entry into the small intestinal LP remains to be elucidated. Thus pretreatment of *in vitro* generated CCR9⁺ $\alpha_4\beta_7$ ⁺ OT-II cells with neutralizing antibodies to $\alpha_4\beta_7$ before transfer into recipient mice will determine whether $\alpha_4\beta_7$ is critical for T cell entry into the small intestinal LP.

Chemokine CCL25 and its receptor CCR9 are critical for CD8⁺ T cell entry into the small intestinal epithelium (Johansson-Lindbom et al., 2003; Svensson et al., 2002). Chemokine receptor signaling involves a cascade of intracellular signaling pathways, each mediated by several effector proteins. However signaling pathways involved in CCR9 mediated T cell migration is yet to be determined. Thus *in vitro* generated CCR9⁺ $\alpha_4\beta_7$ ⁺ OT-I cells can be pretreated with inhibitors to various effector signaling proteins and injected into recipient mice. Determining the ratio between inhibitor-treated and untreated cells in the intestinal epithelium will thus role out essential CCR9 signaling pathways in T cell entry into the intestine.

The tissue origin of DCs is suggested to play an important role for the induction of T cell homing phenotype. The *in vitro* homing assay can be used to explore the homing profile of effector T cells generated *in vitro* by different DC populations. Using effector T cells activated by peptide pulsed MLN, SI or colonic DCs *in vitro* will gain further insight into their respective imprinting of *in vivo* T cell homing properties to the intestinal mucosa.

Our work has so far been performed in healthy animals to get an understanding of how effector T cell trafficking is regulated into the non-inflamed intestine. To examine differences in CCR9 dependent T cell migration during intestinal inflammation, the localization profile of *in vitro* generated CCR9⁺ $\alpha_4\beta_7$ ⁺WT and CCR9^{-/-} $\alpha_4\beta_7$ ⁺T cells into the intestinal mucosa of mice with ongoing IBD can be compared to that of healthy controls.

Mechanisms of CCR9 independent migration

We have demonstrated both CCR9 dependent and independent localization of effector CD4⁺ T (OT-II) cells and CD8⁺ T (OT-I) cells into the small intestinal LP (Paper I, Paper II).

The integrin $\alpha_4\beta_7$ is induced on activated CCR9^{-/-} OT-II cells in MLN, and expressed on the majority of CCR9^{-/-} OT-II cells that enter the small intestinal LP in our *in vivo* transfer model. Thus we, and others have suggested $\alpha_4\beta_7$ to mediate homing to the small intestinal LP (Paper I, (Campbell and Butcher, 2002a; Haddad et al., 2003)). Using donor cells from animals deficient for $\alpha_4\beta_7$ will determine whether the presence of $\alpha_4\beta_7$ alone is sufficient to inhibit T cell entry into the small intestinal LP. All our *in vitro* generated CCR9 deficient OT-I cells in Paper II express $\alpha_4\beta_7$ (unpublished observation). Thus another way of addressing the importance of $\alpha_4\beta_7$ in the migration to the intestine would be to treat these cells with neutralizing antibodies to the $\alpha_4\beta_7$ integrin prior to use in homing transfers.

The CCR9 independent migration of CD8⁺ T cells into the small intestinal LP was determined to be PTX sensitive and thus mediated by other G α_i coupled receptors (Paper II). Furthermore all *in vitro* generated CCR9 deficient OT-I cells used in our *in vitro* assays expressed the chemokine receptor CXCR3 (unpublished observations). Thus pretreatment of these cells with neutralizing antibodies to CXCR3 before using those in homing assays will rule out the involvement of the G α_i coupled receptor CXCR3 in the localization of CCR9 deficient T cells to the intestinal mucosa. Since the levels of CXCR3 ligands are increased in inflamed intestinal tissues (Grimm and Doe, 1996; Grimm et al., 1996; Mazzucchelli et al., 1996; Ugucioni et al., 1999), CXCR3 may also be an interesting target for T cell entry into the inflamed intestinal mucosa.

Role of antigen administration route in regulating CCR9 dependent and independent localization

In the present studies we have examined OVA specific CD4⁺ and CD8⁺ T cell recruitment to the intestinal mucosa after administration of OVA and adjuvant i.p. Adjuvant was used since antigen administration i.p. has in the presence of LPS been demonstrated to generate large numbers of CCR9⁺α₄β₇⁺ OT-I cells in gut draining LN compared to antigen alone (Johansson-Lindbom et al., 2003). However activation of cells in intestinal LN with orally administered antigen will be more physiological relevant since intestinal immune responses primarily are initiated by antigens from the intestine. Orally administered of OVA with or without adjuvant induces local intestinal mucosal immune responses with induction of larger proportion of CCR9⁺ effector T cells in gut associated LNs (Johansson-Lindbom et al., 2005). This is likely to be due, at least in part, to enhanced targeting of LP DC via the oral route, that are efficient at generating gut tropic effector T cells *in vitro* (Anjuere et al., 2004; Johansson-Lindbom et al., 2005). An important additional line of study is thus to determine the degree of CCR9 dependent and independent T cell recruitment to the intestinal regions following different routes of antigen/adjuvant immunization. Here oral administration of antigen will be of particular interest because of its physiological relevance.

POPULÄRVETENSKAPLIG SAMMANFATTNING PÅ SVENSKA

Ett väl fungerande immunförsvar har kapaciteten att särskilja och oskadliggöra en ofantlig mängd sjukdomsframkallande ämnen och organismer. Immunförsvarets förmåga att skilja strukturer på främmande ämnen från våra egna strukturer i kroppen är viktig, eftersom försvaret annars kan angripa kroppens egna vävnader. Det är denna del av immunförsvaret som inte fungerar då autoimmuna sjukdomar uppstår, såsom när insulinproducerande cellerna skadliggörs vid diabetes eller delar av nervceller bryts ned i hjärnan och MS uppkommer.

Immunförsvaret är mycket grovt uppdelat i det medfödda (innata) försvaret och det adaptiva försvaret. Den medfödda delen består av fysiska barriärer, såsom hud och slem, antimikrobiella substanser samt cytotoxiska och fagocytotiska immunceller. Den innata delen fungerar främst som en första linjens försvar och känner igen ett begränsat antal främmande ämnen, ofta representerade på mikroorganismer. Det adaptiva immunförsvaret består av T och B celler med förmåga att unikt känna igen ett visst ämne (antigen) via receptorer på sin yta. Vid ett immunsvaret har det adaptiva försvaret ett minne som medför ett snabbare och mer specifikt försvar vid en upprepad infektion av samma typ.

Att lymfocyter kommer i kontakt med de antigener de är lämpade att agera mot är avgörande för ett fungerande immunförsvar. Det är bland annat för detta ändamål lymfkörtlar finns. Lymfkörtlar fungerar som mötesplatser för cirkulerande lymfocyter och antigen som transporteras från den angripna vävnaden. Då oerfarna T celler möter de främmande ämnena i körteln aktiveras de och utvecklas till bekämpande effektor T celler. En mängd aktiverade effektor T celler bildar en armé som ges förutsättningar att lämna lymfnoden och ta sig ut till den angripna kroppsvävnaden. Förmågan att ta sig till en viss vävnad styrs av i vilken lymfkörtel T cellen aktiverats. En T cell aktiverad i en lymfkörtel i mesenteriet rekryteras främst till tarmvävnad medan en T cell aktiverad i en perifer lymfkörtel, i exempelvis armhålan, hellre får de förutsättningar som behövs för att ta sig in i hudvävnad. Den selektiva rekryteringen av immunceller till olika delar av kroppen är en förutsättning för att immunförsvaret skall kunna generera ett funktionellt försvar i olika delar av kroppen.

Tarmen är kroppens största immunologiska säte där vi finner majoriteten av kroppens immunceller. Detta försvar måste dels vara specialicerat på att se skillnad mellan den nyttiga bakteriefloran, som exempelvis hjälper oss att bryta ned näringsämnen i tarmen, och dels de patogena bakterier som vi utsätts för via mag-tarmkanalen. Fel fungerande immunresponser i tarmen, med en okontrollerad T cells rekrytering som följd, har visat sig vara inblandade vid uppkomst liksom upprätthållande av inflammatoriska tarmsjukdomar såsom ulcerös colit och

Crohns sjukdom. Det är därför viktigt att vi får en ökad förståelse för de mekanismer som styr rekryteringen av T celler till tarmen.

Vi vet idag att T celler behöver attraherande ämnen (kemokiner) i vävnaden i kombination med kemokinreceptorer på cellytan för att ta sig från blodet ut i kroppsvävnaden. Vi har undersökt vilka kemokinreceptorer som är involverade i migrationen av T celler till tunntarmens slemhinna. Resultat från dessa studier visar att T celler som aktiveras i mesenteriska lymfkörtlar selektivt uttrycker kemokinreceptorn CCR9 och att T celler som inte har förmågan att uttrycka CCR9 missgynnas i sin migration till tarmslemhinnan. Vi har visat att kemokinen CCL25, liganden för CCR9, är gradvis mindre förekommande från tunntarmens främre del, duodenum, till bakre ileum. Detta har en funktionell betydelse då den CCR9-beroende migreringen av T celler är mest förekommande till duodenum medan migrationen av T celler till ileum sker mer oberoende av CCR9. Denna upptäckt visar att T celler kan rekryteras till olika delar av tarmslemhinnan beroende på deras uttryck av kemokinreceptorer.

Sammanfattningsvis ämnar de studier som beskrivs i denna avhandling att ge en ökad kunskap om de grundläggande mekanismer som verkar vid rekryteringen av T celler till tarmslemhinnan. Denna vetenskap kan ligga till grund för framtagandet av metoder som selektivt kan förhindra rekryteringen av oönskade T celler till tarmen. Detta är en attraktiv terapeutisk möjlighet för inflammatoriska sjukdomstillstånd i tarmen där den sjuka i dagsläget främst erbjuds kirurgisk behandling och inflammationsdämpande läkemedel som verkar ospecifikt på hela kroppen.

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“Retar du upp dig på ting som är små,
så är du väl inte större än så.”

Alf Henrikson