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Title
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Permalink
https://escholarship.org/uc/item/98r69907

Journal
Springer Seminars in Immunopathology, 26(4)

ISSN
0344-4325

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Publication Date
2005-03-01

Peer reviewed

Surveillance B lymphocytes and mucosal immunoregulation

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Abstract:

Mucosal lymphocyte homeostasis involves the dynamic interaction of enteric microbiota, the intestinalflora and host epithelium, and the mucosal immune system. Multiple host components play critical roles in mediating this homeostasis. Dyisregulation of mucosal lymphocyte homeostasis results in a variety of instestinalintestinal disorders, notably inflammatory bowel diseases like ulcerative colitis and Crohn's disease. that strike at over one-million people annually in the United states. One key cellular compartment incomponent regulating homeostasis areis comprised of the B-lymphocytes (B-cells) that reside in gut associated lymphoid tissue (GALT). This tissue compartment includes Ppeyer's Ppatch, isolated lymphoid follicles,tissue lamina propria, and mesenteric lymph nodes. Recent data has pointed to two new and exciting aspects of B-cells in the gut. First, there has been progress on identification and functional analysis of abundant isolated lymphoid follicle B-cell that are key mediators of IgA genesis. Second, the several groups have now clarified the functional identification and characterization of immunoregulatory B-cells in the gut. This review examines the novel aspects of these B-cells and examines how each play a role in mediating mucosal homeostasis in this bacterial laden compartment.

Introduction

The gut mucosa is the immunologic center of the body, harboring 80% of the body's leukocyte population. These leukocytes are absolutely required for host homeostasis with an estimated 100 trillion bacteria that reside in the intestine. Dysregulation of homeostasis results in a class of diseases collectively termedcalled inflammatory bowel disease.

The intestinal mucosa is comprised of several highly specialized cellular and anatomic components, each of which is required in order to maintain homeostasis. First, only a single epithelial cell layer separates enteric flora and environmental antigen from the sterile host. This cell layer actively contributes to barrier modes of defense via mucus production (1****refs), anti-microbial peptides (notably the Paneth cell subpopulation; 2-4 ****refs from Ganz, Oulette), and microbial sensing functions which permit the epithelium to modify its production of barrier products, and recruitment of leukcocytes (5-7****refs from Madara group). Throughout the intestine, subepithelial lymphoid tissue is segmentally deployed beneath specialized, Second, associated with the epithial layer at the host-antigen interface are readily identifiable follicles called, the follicular- associated epithelium (FAE). FAE differentiation is induced by the underlying lymphoid cells, and is specialized for the traits permitingpermitting antigen sampling from the lumenal environment for delivery to the subjacent lymphoid compartment (8-10****refs). Underlying theThese lymphoid sites are comprised of either P FAE are lymphoid aggregates, peyer's Ppatches (PP) and or isolated lymphoid follicles (ILF),

each of which are critical for maintenance of homeostasis. Underlying theIn a more diffuse

distribution, the intestinal epithelial cell layer are overlays a lamina propria compartment,

particularly enriched for plasma cells, macrophages, and dendritic cells (11,12refs****), and the

developmentally significant the intensely studied lamina propria and cryptopatch structures (13-

15****Refs). AdditionallyFinally, , mesenteric lymph nodes provide a draining secondary

lymphoid compartment for the intestinal environment. the gut associated mesenteric lymph

nodes are yet another critical mediator in mucosal homeostasis. Together, these compartments

comprise anatomic sites for organization and, induction and effector phases of immunity, which

is absolutely required for fecundity and survival of the host, Figure 1.   (***Peter- a diagram here

would be very nice)..

The leukocytes of these compartments play a key role in maintaining homeostasis to the

enteric flora and food antigens, as well as maintaining immunity to infectious agents. One key

leukocyte in maintaining homeostasis and immunity is the B lymphocyte, B-cell. The most

intensely studied function of B-cells in the mucosa is in the genesis of IgA producing plasma

cells and is reviewed elsewhere (12{Fagarasan, 2003 366 /id}1). While B-cells of the PP and LP

have been intensely studied, two unique populations of B-cells have only recently been

appreciated:. These B-cell populations are those of the isolated lymphoid follicles (ILF), and

immunoregulatory B lymphocytes that reside in the mesenteric lymph node. These B cell

subpopulations, which are components of the innate-like population of surveillance B cells

(****refs- 16-18Bendelac, Kearney reviews), are the subject of this review.

In order to properly form these B-cell compartments, several steps must take place. First,

B-cells must develop and successfully mature at progenitor sites. The in order to enter into the

periphery from either the bone marrow or peritoneum. Such requirements for these initial phases of marrow B-cell lymphopoiesis, and the possibility of serosal or mucosal B cell precursor populations, has been the subject of many excellent reviewsdevelopment have been carefully studied, in the context of other B-cells subsets and will not be discussed here (***refs: 19-23marrow reviews, B-1 self renewal review, PP review). Second, once in the periphery, ILF and PP B-cell precursors must successfully migrate into the gut mucosa and localize into their follicular sites. In the case of the PP, successful clones undergo antigen-dependent activation at that site, and proceed from there to the MLN, and finally to the LP where they take up residence as plasma cells (12****refs). This sequential process has not yet been delineated for ILF B cells. However, there has been substantial progress on the shared and divergent le or MLN. Once in the follicle, each B-cell must have the capability to recognize antigen and respond. Each of these steps represents a unique stage at which the B-cells can be regulated by genetic and molecular factors for development of these two subjacent B cell compartments.

ILF B-cells

Immunoregulatory B-cells of the Gut Associated Lymphoid Tissue

A surprising finding in recent years has been the immunoregulatory role of B-cells in immune responses, observed in models of respiratory inflammation, multiple sclerosis, and inflammatory bowel disease (2-5). In this regard, the detailed study of B-cells in the gut may

provide new insights to mucosal homeostasis. Several groups have reported the immunoregulatory role of B-cells. Tsitoura et al. has demonstrated that antigen specific B-cells are highly effective in inducing tolerance to respiratory antigen (2). In EAE, it has been demonstrated that B-cells play a role in immune modulation in the acute disease process (3). Similarly, mice deficient in B-cells have a defect in the induction of oral tolerance (4). Work completed by the Bhan group has also demonstrated that a CD1 positive B-cell subset induced in the gut under inflammatory conditions performs an immunoregulatory function via effects mediated by IL-10 production (5). Studies in our lab also demonstrate a critical role of B-cells in immune regulation of T-cells from colitic mice (*Wei et al., submitted*).

Each study examining immunoregulatory B-cells of gut was because these studies examined immunoregulatory B-cells in multiple models of chronic intestinal inflammation, including TCR$\alpha^{-/-}$ (5) as well as the transfer models of Gαi2$^{-/-}$ and CDRB^{hi} (*Wei et al., submitted*). Therefore, B-cell protection from colitis is not an artifact of an experimental system. Importantly, CD1d is required to induce IL-10 production in B-cells in the TCR$\alpha^{-/-}$ model of colitis. IL-10 produced by CD1d^{hi} B-cells down-modulates inflammation mediated by IL-1 and STAT3 (5). In both Gαi2$^{-/-}$ and CDRB^{hi} transfer models of colitis, B-cells from mesenteric lymph node (MLN) of wild type mice can protect from colitic T-cells (*Wei et al., submitted*). Protection is associated with expansion of NKT-cell in the MLN and CD4^{+}CD8^{+} T-cells in the gut. B-cells in these model systems home preferentially to MLN and are not detected in the intestine, either IEL, ILF or LPL (*Wei et al., submitted*).

ILF B-cells

Isolated lymphoid follicle B-cells are a unique compartment of B lymphocytes that are found at the host bacterial interface of the bacterial laden intestine. This unique structure has only recently been discovered but significant strides haves been made in order to yield a moderate understanding of how ILF B-cells develop and mediate homeostasis. The focus of this section is to review what is currently known about ILF B-cells.

Discovery / Identification

Isolated lymphoid follicle B-cells were first carefully studied in a comparative fashion in humans (24{Moghaddami, 1998 392 /id}6.) Using the mouse as a model system because of its ready availability and, potential genetic and biochemical manipulation, Hamada et al. was the first to identify isolated lymphoid follicles (ILF's) in the intestine of these animals.

ILF B-cells ILFs were identified in mice as small follicles (~ 0.1 mm), numbering 100-200 sites dispersed along anti-mesenteric aspect of the small intestine. ILFs are comprised primarily of $B220^+$ $CD19^+$ $CD23^+$ IgM^{lo} IgD^{hi} $CD5^-$ $Mac-1^-$ cells. A small but significant IgA+ B-cell population is also present, and ILFs typically display germinal center morphology, both reflecting antigenic stimulation and differentiation of ILF B-cells. In aggregate, the cellularity of this compartment is substantial, and is equivalent or larger than the PP compartment. (25{Hamada, 2002 380 /id}; ***Velazquez et al., submitted).

(***Peter- insert a picture or cartoon here. If a picture, you can use a published one; write the author, and ask if you can use it for the review, and if so, could they send you a TIFF file of the image; also, you'll need to get permission from the journal publisher, using the form from our review journal; you can get guidance on this from the editorial assistant at our review journal). Curiously, unlike PP and lymph nodes, containing mostly B220$^+$CD5$^-$ cellsILFs contain only minimal numbers of T cells, dendritic cells, and macrophages. Thus, the local factors that would normally facilitate T-dependent B cell activation are uncertain. Indeed, genetic and environmental parameters suggest a novel mode of B cell activation at this site (see below). that had in fact formed germinal suggesting an active role for ILF in mucosal homeostasis (7). Other studies have further characterized the structure and phenotype of ILF B-cells (8, *Velázquez et al., submitted*). Additionally, several groups have reported ILF distribution abundance of ILF B-cells suggesting that ILF B-cells are at least as abundant as B-cells of the PP (7, 8, *Velázquez et al. submitted*).

Structure

Structure. Several groups have reported on the structure of ILFs and it is depicted in figure 1. ILF'sILFs are predominated by B-cells that are organized into a central follicle that may contain a germinal center (24{Moghaddami, 1998 392 /id}6, 25{Hamada, 2002 380 /id}7, 26{Lorenz, 2003 393 /id}8) . These organized B-cell structures clearly underlie athe follicular associated epithelium resembling, M-cells (25, {Hamada, 2002 380 /id}7. 26{Lorenz, 2003 393 /id}8). M-cells a, which are known to play a major role in antigen uptake and transport from the lumen to the organized lymphoid structures, such as in the a peyer's patch (PP)PP. Additionally, ILF'sILFs are vascularizedvascularized with vessels containing high endothelial venules, which

implicate that the source of at least some of the leukocytes of the ILF are derived from cell migrating in from blood. Dendritic cells are also found inter-dispersed in ILF'sILFs like that found in PP (25{Hamada, 2002 380 /id}7, 27{Fagarasan, 2002 381 /id}9). Interestingly, some groups have reported that ILF'sILFs lack the interfollicular T-cell regions that are typically found in PP while some B-cell clusters contain interdispersed T-cells (25{Hamada, 2002 380 /id}7, 26{Lorenz, 2003 393 /id}8). This likely represents different stages of antigenic responses of ILF'sILFs. Together, these findings indicate that ILF's are actively involved as a lymphoid organizing center whose state of responsiveness is dynamically regulated.

Phenotype

Phenotype. B and T-lymphocytes of ILF'sILFs have been characterized by phenotype by several groups. In human ILF B-cells are express $CD19^+$, MHC Class II^+, CD20+ an, and $CD45RA^+$. In the human, the T-cells found in the ILF were $CD3^+CD4^+$ (24{Moghaddami, 1998 392 /id}6). A more closerDetails of reported phenotypes of and comparative analysis of ILF T and B-cell phenotypes are tabulated has been conducted iin murine ILF'sILFs (Table 1). ILF B-cells are $B220^+CD5^-$ and are dominated by $IgM^{low}IgD^{hi}$ type of B-cells that are $CD21^{low}CD23+$(25{Hamada, 2002 380 /id}7, *Velázquez et al. submitted*). Similar to PP, ILF IgA^+ plasmablasts are found in 5-6 fold less abundance (25{Hamada, 2002 380 /id}7). Interestingly, ILF B-cells lack Mac-1 expression, a B-1 B-cell marker, as do PP B-cells. Taken together, the phenotype of ILF B-cells suggests that these cells are B2-like cells. However, it is not clear if the markers used to identify B-1 versus B-2 cells from sites of genesis (bone marrow and peritoneum) and induction (spleen and mesenteric lymphoid node) are identical at such a unique inductive/effector sites like ILF and PP.

Distribution

Distribution. Interestingly, the distribution of ILF is similar in both human and mouse, pointing to the conserved importance of such as structure during the course of evolution. In human, ILF'sILFs are found throughout the small intestine, in both jejunum and ileun but are most abundant in ileum. Quantitative analysis shows that jejunal ILF are nine-times more abundant in ileial ILF'sILFs (24{Moghaddami, 1998 392 /id}6.). Similarly, in theIn mouse , ILFs are most abundantly found in the distal small intestine (8, *Velázquez et al. submitted*) and cecum (26{Lorenz, 2003 393 /id}, *Velázquez et al. submitted*).(*Velázquez et al*).

Since enteric bacteria are most abundantly found in the distal small intestine and in the large bowel, in particular cecum, the physical distribution of ILFs is provided the first evidence that the presence ofpoint to the idea that ILF B-cellsthey is modulated,are induced at least in part, by enteric antigenic microbial antigen stimulus, likely enteric bacteria, in the intestinestimulation. However, ILF (or PP) formation of the small intestine is largely independent of commensal bacteria 26, {Lorenz, 2003 8978 /id}25,28{Hamada, 2002 8980 /id}{Casola, 2004 9039 /id}. B cell antigen recognition also does not appear to be required, since good cellularity of PP (28) {Casola, 2004 9039 /id} or ILF (****VelazquezVelázquez et al. submitted)*) compartments occurs in LMPa and MD4 (HEL-specific Ig) transgenic mice. However, germinal center formation and numerical expansion occurs with bacterial stimulation (26,28{Lorenz, 2003 8978 /i)d}{Casola, 2004 9039 /id}. Analysis of these transgenic mice on the RAG2-/- mice background suggest that T cell-dependent bacterial sensing is the key component of the germinal center response (28){Casola, 2004 9039 /id}, an idea also reflected in the failure of germfree mice to support formation of B cell-activating invariant MR-1 restricted T cells (29{Treiner, 2003 8615 /id}).

Studies by several groups have demonstrated that this is likely the case. However, the molecular mechanisms underlying ILF B-cell formation have only begun to be examined. Therefore, of critical importance is to understand the antigenic sensing systems involved in driving ILF B-cell formation and presence.

Function

Antibody formation. In addition to distribution, markers indicate that ILF B-cell actively respond to antigenic stimulus. ILF B-cells are reactive for the germinal center markers GR-7 and PNA (25-27{Hamada, 2002 380 /id}7, {Fagarasan, 2002 381 /id}9, {Lorenz, 2003 393 /id}8). Also, BRDU incorporation into the blasting germinal center ILF is approximately equal to that in PP, indicating the importance of ILF B-cells in antibody maturation in the intestine.

Activation induced cytodine deaminase (AID) is required for the Ig class switch recombination and somatic hypermutation. Deficiency of AID provided an important insight into the role of ILF B-cells in mediating homeostasis in the intestine. AID deficiency resulted in B-cell hyperplasia in the ILF (27{Fagarasan, 2002 381 /id}9). It is hypothesized that because B-cells are unable to mature into antibody producing cells, they assume a hyperactivated state. Most striking was an altered distribution of enteric flora, including a 100- fold increase in the number of anaerobic bacteria, in AID deficient animals. Antibiotic treatment resulted in abrogation of the ILF B-cell hypertrophy (27{Fagarasan, 2002 381 /id}9). Lastly, in hypertropic ILF'sILFs there was a limited, oligoclonal, B-cells receptor specificity (27{Fagarasan, 2002 381 /id}9). These findings highlight the role of mucosal B-cell response in control of the commensal bacterial population, in, suggesting some degree due to of B-cell receptor (BCR) mediated antigenic stimulus. Indeed, clonal expansion of antigen-specific B cells can be guided by

antigenic specificity towards commensal bacteria (30,31){Lanning, 2000 7994 /id}{Bos, 1989 7893 /id}.

Another critical piece of data that highlights the function of ILF B-cells examined class switch recombination of from IgM to IgA in ILF B- –cells. A close examination of inductive sites including mesenteric lymph nodes, PP and ILF demonstrated that only inductive sites contained IgM$^+$ B-cells while effector sites such as the lamina propria contained only IgA$^+$ plasmablasts (32{Shikina, 2004 394 /id}10). Upon examination of markers of recent recombination, only inductive sites such as ILF, did contain markers such as αCT (circular segments of DNA that are looped out as a result of class switching from IgM to IgA) and Iμ-Cα mRNA (germline DNA that recombines as a result of looping out of αCT) during class switch recombination and, AID (32{Shikina, 2004 394 /id}10).

Requirements

Antigen recognition. In order to understand factors that drive the development of ILF B-cells, both BCR and non-BCR requirements must be examined. It is well known that T-cells provide critical factors, such as CD40, required for B-cell maturation to antibody producing plasma cells. ,S secretory IgA is most critical for the maintaining homeostasis in the bacteria laden intestine.

Therefore, as one would predict, ILF B-cells are deficient in genetically deficient TCRβ animals (25{Hamada, 2002 380 /id}7). Athymic nude mice deficient in T-cells are also deficient in germinal center ILF B-cells. Also, ILF B-cells are also deficient in RAG and muMT deficient animals as recombination and IgM-BCR expression is required for development, respectively, (Table II2) table 2 (25{Hamada, 2002 380 /id}7).

Importantly, by examining aA series of genetically altered animals mice have been studied for ILF formation with selective deficiencies in PP, T-cells, cryptopatches (known to contain local T-cell precursors), or a combination of these deficiencies. This analysis showed that or deficient in a combination of these structure, Hamada et al demonstrated that ILF are unique from both PP and cryptopatches in histogenesis and lymphocyte composition (25{Hamada, 2002 380 /id}7).

The Newberry group has further segregated ILF'sILFs based on level of maturity and carefully identified pointed to a critical requirement for ILF'sILFs. ILF require lLymphotoxin (LT) β-receptor from the stromal cell compartment, and LT sufficient B-cells, (Ttable II2). Unlike PP and MLN this interaction is not required during gestation. Therefore, ILF formation can be induced de novo in adult with LTβ-receptor, a striking and unique feature of ILF B-cells (26{Lorenz, 2003 393 /id}8).

A well- characterized and critical function of B-cells in the intestine is the production of IgA. The paradigm is that B-cells of the intestine in the GI differentiate to produce abundant amounts of IgA that can be transported across the epithelium (12{Fagarasan, 2003 366 /id}1). IgA in the lumen of the GI can then bind to and inhibit potentially deleterious bacterial flora. However, it is not clear what is the importance of signals mediated by BCR for the presence of ILF in the intestine. Studies have demonstrated the importance of the BCR signal strength in ILF B-cell homeostasis and defined two key molecular mediators (*Velázquez et al. submitted*).. Deficiency in BCR signaling pathway mediated by Btk (the xid mutation in CBA/N mice) results in an abrogated tonic BCR signal strength and deficiency in specific B-cell subsets (33,34-36), including ILF B-cells (*Velázquez et al. submitted*).   Conversely, if BCR mediated signal is too strong, as in the case of the loss of the negative regulator Gαi2, there is a similar loss of ILF B-

cells (Table 2). Therefore, the proper tonic biochemical signal must be delivered in order to drive ILF B-cell homeostasis while antigen specificity is not an absolute requirement (*Velázquez et al. submitted*).

strength and deficiency in specific B-cell subsets ({Cariappa, 2002 24 /id;Cariappa, 2001 33 /id}11,12, {Martin, 2000 38 /id}13, {Dalwadi, 2003 387 /id}14), including ILF B-cells (*Velázquez et al. submitted*). Conversely, if BCR mediated signal is too strong, as in the case of the loss of the negative regulator Gαi2, there is a similar loss of ILF B-cells, table 2 (Table 2) (*Velázquez et al. submitted*). Therefore, the proper tonic biochemical signal must be delivered in order to drive ILF B-cell homeostasis while antigen specificity is not an absolute requirement (*Velázquez et al. submitted*).

Immunoregulatory B-cells

A surprising finding in recent years has been the immunoregulatory role of B-cells in immune responses, observed in models of respiratory inflammation, multiple sclerosis, and inflammatory bowel disease (37-40{Tsitoura, 2002 367 /id;Wolf, 1996 218 /id;Gonnella, 2001 368 /id;Mizoguchi, 2002 130 /id}). In this regard, the detailed study of B-cells in the gut may provide new insights to mucosal homeostasis. Several groups have reported the immunoregulatory role of B-cells. Tsitoura et al. has demonstrated that antigen specific B-cells are highly effective in inducing tolerance to respiratory antigen (37{Tsitoura, 2002 367 /id}). In the mouse model of multiple sclerosis, EAE, it has been demonstrated that B-cells play a role in immune modulation in the acute disease process (38{Wolf, 1996 218 /id}). Similarly, mice deficient in B-cells have a defect in the induction of oral tolerance (39{Gonnella, 2001 368 /id}). Work completed by the Bhan, Mizoguchi, and their colleagues has also demonstrated that a CD1

positive B-cell subset induced in the gut under inflammatory conditions performs an immunoregulatory function via effects mediated by IL-10 production (40{Mizoguchi, 2002 130 /id}). Studies in our lab also demonstrate a critical role of B-cells in immune regulation of T-cells from colitic mice (*Wei et al., submitted*).

Each study examining immunoregulatory B-cells of gut was unique because these studies examined immunoregulatory B-cells in multiple models of chronic intestinal inflammation, including TCRα$^{-/-}$ (40{Mizoguchi, 2002 130 /id}) as well as the transfer models of Gαi2$^{-/-}$ and CD45RB^{hi} (*Wei et al., submitted*). Therefore, B-cell protection from colitis is not an artifact of an experimental system. Importantly, CD1d is required to induce IL-10 production in B-cells in the TCRα$^{-/-}$ model of colitis. IL-10 produced by CD1d^{hi} B-cells down-modulates inflammation mediated by IL-1 and STAT3 (40{Mizoguchi, 2002 130 /id}). In both Gαi2$^{-/-}$ and CDRB45^{hi} transfer models of colitis, B-cells from mesenteric lymph node (MLN) of wild type mice can protect from colitic T-cells (*Wei et al., submitted*). Protection is associated with expansion of NKT-cell in the MLN and CD4^{+}CD8^{+} T-cells in the gut. B-cells in these model systems home preferentially to MLN, and are not detected in the intestine (ILF, PP, or LPL). An interesting genetic requirement for the formation of these cells is the Gαai2, since null mice lack B cells with this immunoregulatory activity. Since this null mutation results in a selective deficiency of surveillance B cells, the finding suggests that cells with this immunoregulatory activity are included within the surveillance B cell subsets. However, the location of immunoregulatory B cell development, and the nature of their antigen specificity, remains to be defined.

Conclusion: ILF and Immunoregulatory B-cells in Mucosal Homeostasis

Taken together, we have formulated the following model of ILF and MLN B-cells contribute to mucosal lymphocyte homeostasis. In the gut mucosa, there are three major lymphoid organizing centers, two of which have been intensely studied, PP and MLN. The third lymphoid organizing center, ILF'sILFs, have only recently been carefully studied. ILF B-cells, like those of PP, reside at the host bacteria interface and are capable of responding to antigenic challenges.

One major function of ILF B-cells is as an organizing center for IgM+ B-cell maturation into IgA producing plasmablasts. Since ILF cells reside at the host bacteria interface, they provide a first line of antibody responsiveness and protection of the host from both enteric and infectious organisms. A key characteristic of ILF'sILFs that make it unique from PP and MLN is that they are inducible after birth, by LTβ-R. Therefore, ILF'sILFs are dynamically regulated. We hypothesize that ILF respond to enteric flora or infectious pathogens that are able to surpass innate lines of defense, such as mucosa, defensins or galectins. Upon induction, ILF'sILFs drive the maturation of IgA producing cells. The resulting antibody can then bind to and inhibit potentially harmful flora. Based on distribution and abundance, ILF B-cells are at least as abundant as PP B-cells. Therefore, while PP and MLN are also capable of driving B-cell differentiation into IgA plasmablasts, ILF, in a normal individual, may represents the major source of IgA genesis. This fact becomes particularly important upon compromising of mechanical and innate barriers by flora and infectious agents.

In this regard, failure of the appropriate maturation and responsiveness of ILF B-cells may lead to dysregulation of homeostasis. Failure of ILF formation may result in an insufficient

antibody responsiveness that allows for opportunistic pathogensinfectious or enteric flora to establish successful infection. After, some period PP and MLN may be capable of compensating for IgA genesis while a robust CD4 T-cell response here would also contribute to preventing disease progression.

An interesting facet of ILF formation is that there is not an absolute requirement of antigen specificity. This suggetssuggests that other, perhaps innate, sensing systems are responsible for the formation of ILF B-cells. Supporting this hypothesis is the unique absolute requirement of LT/LTβ-R interaction for ILF B-cell formation. An exciting possibility is the evolutionary selection of germline VDJ segments that preferentially recognize enteric and pathogen associated molecular patterns, EAMP and PAMP, respectively. In such an event, EAMP and PAMP engagement would be sufficient to drive a protective antibody response from multiple flora without specific antigenic engagement, an evolutionary favorable characteristic.

B-cells of the mesenteric lymph node formulate a new an exciting component of mucosal homeostasis. Immunoregulatory B-cells of the MLN can act in two unique aspects of homeostasis. They can be induced either during a chronic inflammatory condition or can act to prevent colitis onset. Importantly, CD1d is required to mediate immunoregulation. Therefore, we hypothesize that immunoregulatory B-cells act on NK cells in the MLN. This interaction can drive the selective outgrowth of regulatory CD4+CD8+ T-cells that act as the regulatory cell at the effector site. It appears important the IL-10 is important for this immunoregulation. However, it is not clear what the role of NKT or CD4+8+ T-cells in IL-10 production. Additionally, it is not clear how other immunoregulatory cytokines, such as TGF-beta, or other immunoregulatory CD4 T-cells can also inhibit inflammation in these model systems. It is

possible that these other cytokines and T-regulatory cells represent an additional, redundant, level of regulation to prevent a chronic or hyper-responsiveness in the gut.

In conclusion, recent studies on ILF and immunoregulatory B-cells of the gut highlight the importance of B-cell subsets in mediating homeostasis in intestine. Further studies examining the molecular mechanisms of homing, retention and activation of these cellular subsets to the mucosa will allow us to further understand how to therapeutically modulate the presence and activity of these cell subsets. Such modulation may include activity of immune regulation during chronic inflammation, such as the case in inflammatory bowel disease, or to drive an immune response to an infectious disease in an otherwise nonresponsive host, such as an infant or immunocompromised individual.

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Table 1. Mucosal ILF and PP Markers

	ILF	PP	Technique
Human			
MogHaddami et al.	CD19		IHC
	CD20		IHC
	MHC Class II		IHC
	CD45RA		IHC
Murine			
Hamada et al.	B220+CD5-	B220+CD5-	IHC
	IgM+IgD+	IgM+IgD+	IHC
	CD5+B220-	CD5+B220-	IHC
	CD3-	CD3+	IHC
	CD11c-		IHC, FC
	PNA+	PNA+	FC
	Overlying M-cells	Overlying M-cells	Microscopy
Lorenz et al.	CD19+CD11b-		Follice Isolation
	CD19+IgA-		FC
Velázquez et al	IgM+IgD+CD21lowCD23+	IgM+IgD+CD21lowCD23+	Isolation (DTT)
	IgM-IgD+CD21lowCD23+		Isolation (DTT)
	All B-cells CD19+ and CD5-		Isolation (DTT)

IHC: Immunohostochemistry
FC: Flow Cytometry

Table 2. Mucosal Non-B-cell Receptor Specific Mediated ILF Requirements

	Small Intestine	Large Intestine
Genetic		
Hamada et al.		
Nu/nu (Balb/c)	+++	
RAG2-/- (Balb/c)	+++	
TCRβ-/-	+++	
μm-/-	+	
IL7Rα-/-	+	
LTα-/-	---	
aly/aly	---	
CRγ$^{-/Y}$	---	
Lorenz et al.		
LTβR-/-	---	
LTα-/-	---	
Velazquez et al.		
Btkxid	+	+++
Gαi2	+	+++
Biochemical		
Hamada et al.		
Anti-IL7R	+++	
LTβR-Ig	+++	
LTβR-/- with LTβR-Ig treatment	+++	
LTα-/- with LTβR-Ig treatment	---	
Environmental		
Hamada et al.		
Germ Free	+++	
Lorenz et al.		
Germ Free	---	
Germ Free with SPF cecal content	+++	
Strain		
Velazquez et al.		
C57BL/6	+++	+++
129SvEv	+++	+++
CBA/J	+++	+++
C3H/HeSnJ	++	+++
Hamada et al.		
C57BL/6	+++	
Balb/c	+++	

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Figure 1. Section of Small Intestinal Villus.

IEL

Plasma Cell

Dendritic Cell
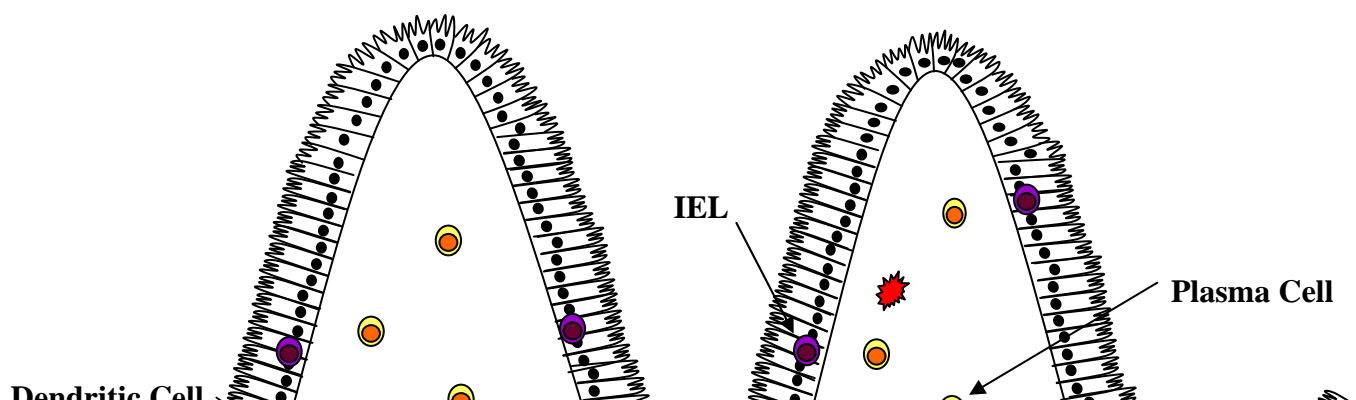
Table I. Mucosal ILF and PP Markers

	ILF	PP	Technique
Human			
Moghaddami et al.	CD19		IHC
	CD20		IHC
	MHC Class II		IHC
	CD45RA		IHC

Murine

Hamada et al.	B220+CD5-	B220+CD5-	IHC
	IgM+IgD+	IgM+IgD+	IHC
	CD5+B220-	CD5+B220-	IHC
	CD3-	CD3+	IHC
	CD11c-		IHC, FC
	PNA+	PNA+	FC
	Overlying M-cells	Overlying M-cells	Microscopy
Lorenz et al.	CD19+CD11b-		Follicle Isolation
	CD19+IgA-		FC
Velázquez et al	IgM+IgD+CD21lowCD23+	IgM+IgD+CD21lowCD23+	Isolation (DTT)
	IgM-IgD+CD21lowCD23+		Isolation (DTT)
	All B-cells CD19+ and CD5-		Isolation (DTT)

IHC: Immunohistochemistry
FC: Flow Cytometry

Table II. Mucosal Non-B-cell Receptor Specific Mediated ILF Requirements

	Small Intestine	Large Intestine
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Nu/nu (Balb/c)	+++	
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TCRβ-/-	+++	
μm-/-	+	

	IL7Rα-/-	+	
	LTα-/-	---	
	aly/aly	---	
	CRγ$^{-/Y}$	---	
Lorenz et al.			
	LTβR-/-	---	
	LTα-/-	---	
Velázquez et al.			
	Btk^{xid}	+	+++
	Gαi2-/-	+	+++

Biochemical

Hamada et al.

	Anti-IL7R	+++	
	LTβR-Ig	+++	
	LTβR-/- with LTβR-Ig treatment	+++	
	LTα-/- with LTβR-Ig treatment	---	

Environmental

Hamada et al.

	Germ Free	+++

Lorenz et al.

	Germ Free	---
	Germ Free with SPF cecal content	+++

Strain

Velázquez et al.

	C57BL/6	+++	+++
	129SvEv	+++	+++
	CBA/J	+++	+++
	C3H/HeSnJ	++	+++

Hamada et al.

	C57BL/6	+++
	Balb/c	+++