Mucosal
Immunology
Update

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The cover depicts a collage of photos of mucosal inductive sites. Counterclockwise from the top, a view of a lymphoid aggregate in the airway (courtesy of John Bienenstock, in the “Handbook of Mucosal Immunology” with permission); a line drawing of the Peyer’s patch from Peyer’s original manuscript, 1677; a scanning electronmicrograph of M-cells overlying a patch (courtesy of Jacques Pappo). The background is another scanning electronmicrograph of M-cells.

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Obituary—In Memory of Professor Anne Ferguson, President of SMI

Anne Ferguson Collee passed away peacefully on 21 December 1998, after fighting courageously against the spread of a carcinoma for a couple of months. Most of this time she was able to stay at home surrounded by her devoted husband Gerry and two children, Kathleen and Douglas. She bore her illness with great fortitude and retained her spirit and good humour till the end.

Anne Glen was born in Glasgow, Scotland, in 1941. She married the sociologist John Ferguson in 1966 but was widowed in 1989. She was later on joined by Gerald (Gerry) Collee, Professor of Medical Microbiology at the University of Edinburgh and Chief Bacteriologist to the Edinburgh Royal Infirmary, and they were married in 1995. Anne graduated from the University of Glasgow in 1964 as the most distinguished student. She continued at the same university as a Pre-registration House Officer in Medicine (1964-65), Lecturer in Medicine (1965-69), and Lecturer in Bacteriology and Immunology (1969-74). During this period she carried out her first substantial scientific work under direction of Professor Delphine Parrot, and received her PhD in immunology in 1974. Thereafter Anne moved to the University of Edinburgh and became a Lecturer in Medicine (Gastroenterology) and Consultant Physician at Western General Hospital, Edinburgh. In 1987 she became Professor of Gastroenterology and during the period 1991-94 she was chairman of the Department of Medicine. She became the President of SMI in 1996 and thus died at the end of her 3-year period which she served with great enthusiasm and skill. She was the first woman to hold this important office since the Society was established in 1986.

Anne was a highly esteemed colleague, being quite unique in combining clinical work and administrative duties with science at an impressive international level for more than two decades. She was one of the pioneers in the field of mucosal immunology. She used to say that: If you are well organized, the family need not suffer if a woman continues to work full time while her children are small. Anne's vitality and appetite for life were a source of inspiration, and we will miss her a lot. We are all saddened and shaken by this tragic loss, and our deep-felt sympathy goes to her beloved husband Gerry and to the rest of her dear family. We will remember Anne with great respect and cherish the good memories she gave us.

Per Brandtzaeg  
Past-President, SMI

Obituary—Professor Anne Ferguson  
(Anne Collee) 1941-98

Everyone is shocked and saddened by the death, aged 57, of Anne Ferguson, President of SMI, on December 21. Anne had been a major figure in world gastroenterology and mucosal immunology for the last 25 years and will be sadly missed by family and friends alike.

Anne was brought up in Glasgow and attended Notre Dame School. A brilliant student, she gained a first class honours degree in physiology and then M.B., Ch.B. with honours at Glasgow University. She was awarded the Brunton Medal as the outstanding medical graduate of her year. After her clinical training at the Royal Infirmary in Glasgow she was appointed lecturer in the new department of Bacteriology and Immunology at the Western Infirmary in Glasgow in 1969 where she did a Ph.D. with Delphine Parrot. She was appointed senior lecturer in gastroenterology at the Western General Hospital in Edinburgh in 1975 and received a personal chair in gastroenterology from the University of Edinburgh in 1987. She was head of the Department of Medicine at the University of Edinburgh from 1991-94. She was a fellow of the Royal College of Physicians of Glasgow, Edinburgh and London, a fellow of the Royal College of Pathologists and a fellow of the Royal Society of Edinburgh. At a national level she served on the Committee on Safety of Medicines, The MRC Gene Therapy Advisory Board, and the Spongiform Encephalopathy Advisory committee.

Anne worked in many areas of mucosal immunology and was one of the few UK gastroenterologists who felt comfortable with immunology. Her Ph.D. with Delphine Parrot was continued on page 4
the first detailed study of intraepithelial lymphocytes. With Delphine she also noticed that when allografts of mouse fetal small intestine were implanted under the kidney capsule of adult mice, the mucosa took on an appearance similar to celiac disease during the rejection process. This formed the basis for a successful grant application to the charity Action Research for the Crippled Child and analysis of this system formed the basis of my PhD studentship. Anne and Delphine very quickly whipped me into shape after a rocky start. Implanting these grafts was a very tedious business and I have happy memories of Anne and I sitting down at a bench and doing 30-40 mice in an afternoon. She was the first to propose in the mid 1970’s that celiac disease was due to a lamina propria T cell response to gluten. In 1975 she published a landmark paper in the Lancet where she showed that biopsies from celiac patients but not controls produced a macrophage migration inhibitory factor when challenged in organ culture with gliadin. It has taken another 20 years for the Oslo groups’ of Ludwig Sollid and Per Brandtzæg to show formally that Anne was correct. She also had the prescience to suggest that Crohn’s disease might also involve inappropriate gut T cell activation, again formally shown by many groups in the 1990’s. She did landmark work on oral tolerance in the late 70’s and 80’s. More recently she moved away from animal experiments into more clinical studies and felt strongly that the difficulty of doing this kind of work was more than compensated by the fact that it was in patients with real diseases.

This empathy with patients, both adult and children, was very strong. She was a superb clinician and there was a very moving oration at her funeral from a patient with Crohn’s disease who had been under her care for many years. This empathy extended to the community of students and research fellows whom she trained over the years. I first met Allan Mowat when he did an intercalated BSc on IEL with Anne while a medical student in Glasgow and after graduation, he went on to do a Ph.D. with Anne in Edinburgh. Stefan Strobel, Maureen Bruce and Allan Lamont all did Ph.D.’s on oral tolerance in Edinburgh with Anne and later Eduardo Arranz from Spain and Riccardo Troncone from Naples trained with her. Over the years her circle of friends and colleagues throughout the world increased. She was in great demand as a speaker and chairperson at international meetings where her common sense shone. One of the last meetings she was able to attend took place at Bart’s on October 2 where as president of SMI she opened the first European Mucosal Immunology meeting. She appeared somewhat under the weather and shortly afterwards became ill. She had particular friends in the world of pediatric gastroenterology and was one of the few adult gastroenterologists who was also a member of the European Society for Paediatric Gastroenterology and Nutrition.

Anne was also a talented athlete at university. She represented Glasgow University in athletics and was a competitive middle-distance runner. She was also a member of the Scottish women’s basketball team.

Anne was very typically Scottish. She loved mountaineering and the highlands of Scotland. It was through mountaineering that she met her first husband John whom she married in 1966. She helped him through a long illness in the 1980’s before his death from cancer in 1989. Anne and John had a cottage in a remote location on the west coast of Scotland, lacking all amenities except running water. Many of us had the pleasures and rigors of holidays there; the views were wonderful when it stopped raining. They lived in a large Georgian house on Newhaven Rd. in Edinburgh where visitors were always welcome for a chat and a dram. They had two adopted children, Kathleen and Douglas and I had the pleasure of being in the Ferguson household the day Kathleen arrived.

One of the great pleasures in recent years was seeing how happy Anne was in the company of her second husband, Professor Gerry Collee, Emeritus Professor of Medical Microbiology at the University of Edinburgh and they married in 1995. A wonderful man of great intelligence and humour, he brought peace and stability into Anne’s life, making the last few years amongst the happiest she had experienced. Anne will be missed by all who knew her and mucosal immunology has lost a deeply caring person to whom many of us turned for advice in times of personal and professional difficulty.

Thomas T. MacDonald
St. Bartholomews Hospital, London
Two distinct populations of B cells (B-1 and B-2) were first identified in the early 1980’s. B-1 cells have been shown to make a substantial contribution to IgA plasma cells, particularly in the intestine. In this issue four original reviews examine B-1 cell ontogeny, differentiation, function and role in the common mucosal immune system. Berland and Wortis discuss the hypothesis that commitment to the B-1 phenotype occurs as a result of activation of B cells by surface IgM cross-linking in the absence of T cell help, rather than lineage commitment at the pre-B cell stage (the lineage hypothesis). Beagley and colleagues go on to demonstrate that IgA+ mucosal B cells of B-1 and B-2 lineages have different cytokine requirements for terminal differentiation to IgA plasma cells and that IgA+ B-1 and B-2 cells have different antigenic repertoires with B-1 cells mediating responses predominantly to T-independent microbial antigens while B-2 cells respond mainly to T-dependent antigens. All four reviews highlight the IL-5 dependence of B-1 cell differentiation. Erickson and Waldschmidt discuss the requirements for T cell dependent (and independent) activation of B-1 cells. They suggest that rapid induction of B-1 cells to produce antibody may also occur through non-classical helper cells such as CD4-CD8-TCRγδ T cells, TCRγδT cells and mast cells all of which secrete IL-5 and are found at mucosal surfaces. This alternate mechanism has the advantage of a rapid response to microbial invasion through the mucosal lining prior to the activation of more sophisticated CD40-CD40L driven B-2 cell responses. Finally Hiroi and colleagues suggest that sIgA+ B-1 cells may populate mucosal surfaces independently of B-2 cell migration from Peyer’s patches.

The ontogeny and function of B-1 cells

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Abstract
B cells with different histories of antigenic exposure exhibit different phenotypes. For example naive B cells, germinal center B cells, and memory B cells can readily be distinguished from one another by surface staining. There also exist, in mice and humans, phenotypically distinct populations of B cells, termed B-1 and B-2, that have been proposed to represent entirely separate B cell lineages. According to this view, the unique properties of these cells are considered to be determined in their development prior to the pre-B stage. We and others have suggested an alternative model; B-1 cells represent the outcome of particular signaling events in mature B cells and the B-1 phenotype is therefore potentially open to any B cell. Specifically, we propose that B-1 cells arise as a consequence of activation by surface IgM crosslinking in the absence of cognate T cell help, in conjunction with other signals.

In this review we will consider the evidence for the “lineage” and “induced differentiation” models of B-1 development and the implications of each for understanding B-1 cell function. But first we will turn to some of the properties of B-1 cells that make the question of their origin interesting.

Properties of B-1 Cells
B1 cells are IgMint, IgDint, CD23, CD43+, and CD45 (B220)+. They are further divided into B-1a which express CD5 and B1b which do not. Because most work has dealt with B-1a cells we will restrict our discussion to these cells. In addition, unless explicitly noted, this review pertains to murine B-1 cells. Human B-1 cells, although very similar to those in mice, both in phenotype and apparent function, have not been as much studied, in part because of the difficulties of doing experimentation.
In addition to differences in surface phenotype, B-1a cells exhibit a number of intriguing properties that suggest an important role for them in innate immunity and defense against bacterial pathogens, particularly in the gut, as well as possible contributions of 1a cell dysfunction to autoimmunity.

**Anatomical Localization**

In the neonate, B-1a cells make up a significant fraction (up to 30%) of both splenic and peritoneal B cells. By eight weeks of age, in most strains, only 2-5% of splenic B cells are CD5+ while peritoneal B cells remain about 30% CD5+. B-1a B cells are largely absent from lymph nodes and Peyer’s Patches.

**Antibody Repertoire**

Fetal/neonatal B-1a cells frequently produce antibodies reactive with bacterial antigens as well as with self antigens such as ssDNA and nuclear proteins. This is a consequence of the biased use of D-proximal V_H regions which results from non-random VDJ recombination. Because fetal cells do not express terminal deoxynucleotidyl transferase (TdT) the repertoire lacks N insertions.

A bias towards 3'-V_H region usage is not seen in the adult, peritoneal B-1a population. Still, among these cells there is an enrichment for B cell receptors (BCR’s) specific for bacterial polysaccharides and glycolipids. For example, the vast majority of B cells specific for Phosphatidylcholine (PtC) and the O/core of bacterial LPS are contained in the B-1a pool. This has generally been attributed to the use of a restricted subset of V regions; however, a recent study of V_H region usage single cell PCR of FACS sorted cells indicates otherwise (1). Although some V_H families were found to be used in B-1a cells and not in conventional B cells, in general B-1a cells used V_H regions from multiple families and the relative frequencies of use correlated with the sizes of the families. The peritoneal B-1a repertoire lacks somatic mutations, contains a few antibodies with N insertions, and these tend to be shorter than in antibodies produced by conventional B cells. Thus the B-1a repertoire is skewed toward germ-line sequences. This, together with the diversity of B-1a V region usage and the high frequency of B-1a reactivities to bacterial antigens, suggests that many germ line V regions have evolved to react with common bacterial antigens.

**B-1a Self-Renewal and Feedback Inhibition**

Unlike conventional B cells, the B-1a compartment can be fully reconstituted in irradiated mice by injection of mature, fully differentiated B-1a cells. Thus, these cells are capable of self-renewal in the absence of a new generation of B-1a cells from precursors. Interestingly, B-1a cell numbers are maintained by a feedback inhibition mechanism that regulates entry of new cells into the B-1a compartment.

**Origins of B-1 Cells**

B1 cells have been proposed to represent a distinct B cell lineage based on the following experimental results. 1) Fetal omentum cells give rise exclusively to B-1 cells when transferred into SCID recipients. 2) Fetal liver cells give rise to both conventional and B-1 cells when transferred into SCID or irradiated recipients. 3) Adult bone marrow only inefficiently replenishes the B-1 compartment of irradiated mice.

The simplest interpretation of these results is that committed B-1 cell progenitors are present in the fetal omentum and liver but largely absent from adult bone marrow. However the existence of such committed precursors cannot alone account for B-1 development since in immunoglobulin transgenic mice receptor specificity profoundly influences the B cell phenotype. Thus, in transgenic mice expressing an immunoglobulin specificity found on B-2 cells, transgene expressing B cells are found only in the B-2 compartment. Conversely, in mice transgenic for a B-1 cell derived immunoglobulin, transgene expression is found predominantly on B-1 cells (Table 1). This indicates that BCR-mediated selection events are critical in B-1 and B-2 cell development. Consistent with this, genetic alterations that affect BCR signaling have consequences for the relative numbers of B-1 and B-2 cells. Mice unable to signal at all through their BCR (as a result, for example, of loss of surface IgM [sIgM] by m <sub>m</sub> deletion) have no B cells. However, mutations that affect the quality or threshold of antigen evoked signaling affect B-1 cell numbers while, in most cases, having little affect on the B-2 compartment (Table 2). Mutations that decrease the threshold for BCR signaling generally result in increased numbers of B-1a cells. Those that increase the threshold or impair activation cause decreases in B-1a cell numbers.
One way of accommodating these results within the committed precursor model is to posit the following: IgM+ cells arise that are committed either to be B-1 or B-2 B-1 lineage cells require BCR signaling for survival, self-renewal and expansion. B-2 lineage cells apoptose in response to BCR signaling. BCR signaling would be triggered by self-antigens or frequently encountered foreign antigens and thus expression of receptors specific for such antigens would kill B-2 lineage cells and promote survival, self-renewal and expansion of B-1 lineage cells. Expression of a receptor with no reactivity with these antigens would allow survival (or generation) of B-2 but not B-1 cells. Recent results of Clarke and Arnold cast doubt on this model (2).

Previous work from this group had shown that in VH12/Vk4 transgenic mice, which express an immunoglobulin specific for PtC, PtC reactive B cells are found predominantly in the B-1a compartment (see Table 1). In their more recent study they crossed these mice onto x-linked immunodeficiency disease (xid) mice. Xid mice have few B-1a cells (along with other immune system defects) due to defective BCR signaling (see Table 2). In the resulting VH12/Vk4/xid mice there was a reduction in PtC-reactive B-1a cells, as might be expected from the xid phenotype. In addition, there was an increase in PtC-specific B-2 cells (B0 in their nomenclature). An easy way to explain this increase in PtC-specific B-2 cells within the committed precursor model is to say that the xid mutation not only inhibits BCR signals necessary for positive selection of B-1 cells, but also inhibits signals necessary for the negative selection of B-2 cells. However the xid mutation is not known to hinder BCR mediated apoptosis. If anything it enhances it.

We have proposed an alternative model of B-1 cell development that we believe accounts more readily for the results summarized above. According to this model, B-1a cells are generated as a consequence of activation of a B cell by surface IgM crosslinking in the absence of T cell help. The original impetus behind this model was the observation that treatment of conventional splenic B cells with F(ab’), anti-IgM induced expression of CD5 as well as the ability to be activated by PMA in the absence of ionomycin, a property of B-1a cells. Treatment with LPS, CD40L or fixed activated T cells resulted in proliferation but no CD5 induction.

Although it induces CD5, anti-IgM treatment fails to cause down-regulation of IgD or CD23. Thus, additional signals are necessary for B-1a development. Mice transgenic for IL-5 have increased numbers of B-1a cells while IL-5 and IL-5R knockout mice have reduced B-1a cell numbers early in life until six weeks of age. Depletion of IL-10 by anti-IL-10 injection into adult mice also reduces B-1a cell numbers (although eight week old IL-10 knockout mice have normal numbers of B-1a cells) suggesting that this cytokine plays a role in B-1a development as well. It is possible that IL-5 and/or IL-10, in conjunction with BCR crosslinking, promote differentiation to or survival of B-1a cells.

The major strength of the induced differentiation model of B-1a origins is how readily it accounts for the effects of BCR antigen specificity and signaling function on B-1 vs B-2 development. Cells bearing a BCR specific for crosslinking self-antigens or exogenous thymus independent type 2 (TI-2) antigens will be driven into the B-1a pool. If continued BCR signaling is required for longevity or self-renewal, the repertoire of this pool will be skewed towards

<table>
<thead>
<tr>
<th>Transgene (Source)</th>
<th>Specificity</th>
<th>Phenotype of Transgene+ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ 17.2.25 (normal spleen)</td>
<td>4-Hydroxy-3-nitrophenyl (NP)</td>
<td>B2</td>
</tr>
<tr>
<td>V(_{H})11 (B1a)</td>
<td>Phosphatidylcholine (PtC) (depending on light chain)</td>
<td>B1a if PtC binding; B2 if not PtC binding</td>
</tr>
<tr>
<td>V(<em>{H})12V(</em>{k})4 (B1a)</td>
<td>PtC</td>
<td>B1a</td>
</tr>
<tr>
<td>HyHEL10 (normal spleen)</td>
<td>Hen Egg Lysozyme</td>
<td>B2</td>
</tr>
<tr>
<td>4C8 (NZB mouse)</td>
<td>Mouse RBC (pathogenic)</td>
<td>B1a (in peritoneum only)</td>
</tr>
<tr>
<td>3-83 (normal spleen)</td>
<td>H-2(^{a}) and H-2(^{b})</td>
<td>B2</td>
</tr>
</tbody>
</table>
self-antigens and foreign antigens to which the animal is repeatedly exposed. This would explain why in neonates the B-1a repertoire tends to react to self-antigens while in the adult peritoneum the repertoire is skewed toward reactivity with bacterial TI-2 antigens as well as autoantigen.

One fact that this model as presented thus far does not explain is that adult bone marrow is a less efficient source of B-1a cells than fetal liver. Since each tissue retains its characteristic ability (or inability) to reconstitute B-1a cells even when they are coinjected into irradiated mice, there must be an intrinsic difference in the potential of each to generate B-1a cells. One well established difference between lymphopoiesis in these two tissues is that in bone marrow N insertions are introduced at the joints of VDJ recombination whereas in fetal liver this does not happen. This is due to the absence of TdT in fetal liver. Thus, the fetal repertoire is essentially that encoded in the germ line. Much of this repertoire may have been selected evolutionarily to encode reactivity with self and common bacterial antigens and thus B cells derived from fetal liver precursors would be expected to be selected into the B-1a pool at a higher frequency than bone marrow derived B cells since in the latter case N insertions will have driven the specificity away from that encoded in the germ line.

It is important to emphasize that there are two different lineages of B cell development, fetal/neonatal and adult. B cells from these two lineages have different phenotypes. The presence or absence of TdT and N insertions is one such difference. Another recently described difference is in MHC class II expression. B cells derived from adult bone marrow express class II molecules at the pre-B cell stage while those derived from fetal/neonatal precursors do not express class II until several days after slgM expression. Using the presence of IgM+, classII- immature bone marrow B cells as a marker for fetal/neonatal B cell lymphopoiesis, Stall et. al. demonstrated that this developmental system persists until somewhere between one and three months of age. Thus it is possible that the B-1a cells that do arise after bone marrow transfer are derived from fetal/neonatal precursors. This has led Stall et. al. to propose that the induced differentiation model of B-1 development applies to fetal/neonatal development but that adult precursors are unable to give rise to B-1 cells. Establishing whether or not this is true will require isolating pure populations pro-B cells from fetal and adult sources and testing their potential in transfer experiments. If there is a difference, it will be necessary to determine if this is due to signaling or repertoire differences between these populations.

In several species (see Table 3) the entire B cell population displays many of the phenotypic traits characteristic of the B-1 cells of mice and humans. This suggests the possibility that the B-2 lineage arose as a late evolutionary modification. While this is an attractive model, there is no evidence that directly supports this model as opposed to the alternative, that some of the features of B-1 cells are made manifest by antigen-triggered signal induction of mature B cells.

In summary we believe that the induced differentiation model described here more satisfactorily accounts for the origin of B-1a cells. In particular, it more easily accounts for the phenotype of VH12/Vk4/xid mice as described above.

**B-1 Cell Functions**

**Role in Natural Antibody Production.**

Cell transfer studies indicate that B-1a cells are responsible for the bulk of natural antibody in the mouse. Natural antibody arises

---

**Table 2**

<table>
<thead>
<tr>
<th>B cell receptor signalling mutants exhibit altered B-1a cell development</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mutations that prevent B-1 cell formation</strong></td>
</tr>
<tr>
<td>Knockout of proto-oncogene vav</td>
</tr>
<tr>
<td>Knockout of PKCβ</td>
</tr>
<tr>
<td>Mutation of Igα</td>
</tr>
<tr>
<td>Knockout of CD19</td>
</tr>
<tr>
<td>Knockout of CD21</td>
</tr>
<tr>
<td>Mutation of Bruton’s tyrosine kinase</td>
</tr>
<tr>
<td>X-linked immunodeficiency (point mutation)</td>
</tr>
<tr>
<td>Knockout</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Mutations that increase B-1 cell numbers</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation of SHP-1</td>
</tr>
<tr>
<td>Motheaten and motheaten viable</td>
</tr>
<tr>
<td>Knockout of CD22 (two of four studies)</td>
</tr>
<tr>
<td>Knockout of Lyn (young mice only)</td>
</tr>
<tr>
<td>Transgenic overexpression of CD19</td>
</tr>
</tbody>
</table>
Table 3
Comparison of B cell Ontogeny and Phenotype

<table>
<thead>
<tr>
<th>TRAIT</th>
<th>Rabbit</th>
<th>Cattle</th>
<th>Chicken</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of B-cell</td>
<td>GALT</td>
<td>GALT</td>
<td>GALT</td>
<td>GALT</td>
</tr>
<tr>
<td>IPP</td>
<td>IPP</td>
<td>IPP</td>
<td>IPP</td>
<td>IPP</td>
</tr>
<tr>
<td>Rearrangement mechanism</td>
<td>gene conversion</td>
<td>likely to be gene conversion</td>
<td>gene conversion</td>
<td>conversion</td>
</tr>
<tr>
<td>N regions (TdT)</td>
<td>Yes</td>
<td>No</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>IgD</td>
<td>No</td>
<td>No</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>CD5⁺</td>
<td>All</td>
<td>?</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>Somatic mutation</td>
<td>Yes</td>
<td>?Yes</td>
<td>?</td>
<td>Yes in IPP?</td>
</tr>
</tbody>
</table>

GALT = Gut associated lymphoid tissue
IPP = Ileal Peyer’s patch
TdT = terminal deoxynucleotidyl transferase

in the absence of experimentally introduced antigen, is IgM (and hence fixes complement extremely efficiently), and reacts broadly with bacterial and self antigens. It functions as a first line of defense against pathogens. In addition it appears to enhance responses to TD antigens by promoting complement-mediated localization of antigen to follicular dendritic cells (FDC’s) (5,6). Thus, in mice engineered to be unable to produce the secreted form of IgM (while retaining the ability to produce all other Ig isotypes) TD responses are impaired (6,7). The response is restored if immunization is accompanied by treatment with normal mouse serum (7). Interestingly, secretory IgM-deficient mice have elevated numbers of B-1a cells, indicating that serum IgM levels regulate B-1a cell numbers (6,7).

Role in TI-2 Responses.
As discussed above, we believe that activation of a naive B cell by TI-2 antigen, possibly in conjunction with other signals, drives it to differentiate into a B-1a cell. Peritoneal B-1a cells have been shown to continuously secrete low levels of antibody. Thus, the B-1a compartment may have evolved to provide a constant level of antibodies directed against certain, frequently encountered TI-2 antigens. This does not necessarily implicate these cells in acute responses to immunization with TI-2 antigens. In fact, it has been shown that transferred peritoneal B-1a cells fail to respond to immunization with the TI-2 antigen NP-Ficoll. In the same study, the transferred cells did respond to immunization with the TI-2 antigen α(1-3)dextran. This difference could be due to an absence of B-1a cells specific for NP-Ficoll in the peritoneum. Alternatively, B-1a cells may, for some unknown reason, be unable to respond to this antigen.

The induced differentiation model would predict that any conventional B cells responding to a TI-2 antigen should be induced to become B1a. Consistent with this, in mixed peritoneal cell (PerC)/bone marrow (BM) chimeras immunized with the TI-2 antigen polyvinyl pyrrolidine (PVP), responding cells are derived exclusively from the BM yet have the phenotype of B-1 cells (7). This could be due to BM derived B-1a cells present before immunization. More likely, given the inefficiency of B-1a generation from bone marrow, it is due to the induction of the B-1a phenotype on responding (conventional) cells.

Role in TD Responses.
B-1a cells do not play a determining role in TD responses since xid mice, which have few B-1a cells, exhibit quite good responses to TD antigens. Nonetheless, cell transfer studies into SCID mice indicate that B-1a cells are capable of responding to T cell help. Other studies have indicated that a significant fraction of plasma cells in spleen secreting IgG₁, a prototypical TD isotype, as well as those secreting IgM and IgG₂a are derived from B-1a cells. In the gut lamina propria up to 50% of IgA secreting plasma cells are B-1a derived. Since the hallmark of TD responses is affinity maturation, which occurs in germinal centers (GC’s), it is important to know if these B1a-derived plasma cells contain somatic mutations. In one study, PerC/BM radiation chimeras were constructed and it was determined that the B cells in spontaneously occurring splenic germinal centers are bone marrow and not PerC derived (3). It is possible that this is due to the repertoire of peritoneal B-1a cells and not the result of an intrinsic inability of B-1a cells to be recruited into GC’s. In humans, peripheral blood CD5⁺ B cells, like mouse peritoneal B cells, lack somatic mutations. However, IgA secreting plasma cells of the lamina propria are extensively somatically mutated. If, as in mice, about...
half of these cells are B-1a derived, this would suggest that B-1a cells, or their derivatives, can in fact enter GALT-associated GC’s. Alternatively, in humans CD5+ B cells may not give rise to IgA plasma cells in the lamina propria.

Role in Autoimmunity

In several instances increased numbers of B-1a cells correlate with autoimmune disease. Thus, NZB and motheaten mice, which are prone to autoimmunity, have elevated numbers of B-1a cells. Crossing either strain of mice with xid eliminates B-1a cells but not conventional B cells and suppresses autoimmunity. Autoimmunity and increased CD5+ cells are also seen in mice transgenic for the Fli-1 gene and in mice with a targeted disruption of the lyn gene, both of which contain elevated CD5+ B cells.

The strongest evidence for a role of B-1a cells in autoimmunity is from a transgenic model of hemolytic autoanemia (9). In mice transgenic for a pathological anti-RBC immunoglobulin cloned from an NZB mouse, transgene expressing B cells have the B-1a phenotype and are found exclusively in the peritoneum where they are sequestered from RBCs. Over time, these mice begin to display autoimmune disease. Activation of the B-1a cells by administration of LPS accelerates disease onset. Elimination of the B-1a cells by intraperitoneal injection of mouse RBC or ddH2O protects from disease.

Conclusions

In mice and humans there are two B cell lineages, fetal and adult, differing at least in the expression of TdT and MHC class II. There also exist two populations of mature B cells, B-1 and B2, differing in phenotype and function. Evidence from immunoglobulin transgenic mice demonstrates that receptor specificity determines whether a B cell will be B-1 or B-2. B-1 cells unlike B-2 cells are not generated when BCR-mediated signaling is compromised. We argue that BCR cross-linking together with additional signals can drive B-2 cells to differentiate into B-1 cells.

Acknowledgments

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References


T helper cell activation of B-1 cells

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Abstract

During T cell dependent B cell activation, CD40 ligation has been shown to be a key step for progression of B cells into their proliferation and differentiation programs. Although most studies demonstrate CD40 engagement to be critical for B cell responses, others suggest the presence of a CD40 independent means by which T helper (Th) cells drive B cells. In studying this phenomenon in more detail with Th2 clones derived from CD40 ligand (C40L) deficient mice, our laboratory found a complete absence of conventional B cell activation upon culture with CD40l- clones under cognate conditions. Curiously, B-1 cells were still observed to undergo proliferation and differentiation when cultured with CD40l- Th2 clones. Further studies were performed with recombinant CD40L and cytokines to better understand how B-1 cells respond in a Th2 environ-
ment. These experiments demonstrated B-1 cells to respond strongly to CD40 engagement, but only in the presence of IL-5. Additionally, B-1 cells were still found to proliferate and differentiate in the absence of a CD40 agonist when both IL-4 and IL-5 were present. This observation thus clarifies how CD40L+/− Th cells can activate B-1 cells, and may explain the residual T cell dependent antibody response observed in CD40 and CD40L deficient mice, as well as in X-linked hyper-IgM patients. These findings also suggest a more primitive means by which B-1 cells can rapidly produce antibodies.

Introduction

Recent investigation has revealed a central role for CD40 and its ligand in T cell-dependent B cell activation (reviewed in 1). Subsequent to binding and endocytosing protein antigens, B cells process and present peptides from the proteins in their class II molecules. These class II:peptide complexes attract CD4+ Th cells, via engagement of T cell receptors (TCR), and initiate the first phases of contact between B and Th cells. Signals delivered to T cells as a result of TCR clustering induce a transient upregulation of CD40L, designated as CD154, and interaction with CD40 on B cells. In vitro studies either with murine splenic B cells or human peripheral and tonsillar B cells have demonstrated the CD40-CD40L interaction as a crucial step in T cell dependent B cell activation, as this event renders the B cell fully competent to respond to further cytokine signals provided by the Th cells. Cytokines such as IL-4, IL-5, IL-6, and IL-10 promote proliferation, differentiation, and isotype switching after CD40 stimulation. Collectively, these events lead to the germinal center reaction and the production of memory cells.

Although this model appears to explain the basis for most Th cell-dependent B cell responses, a number of reports suggest the presence of T cell driven responses which are CD40-CD40L independent. Immunization of X-linked hyper-IgM (X-HIM or CD40L mutated) patients with protein antigens results in a modest but definite antibody response (2). In addition, several groups have demonstrated T cell clones derived from X-HIM patients to promote proliferation, IgM production, and even switching of normal human B cells upon co-culture (1). CD40 or CD40L deficient mice also exhibit residual IgM antibody responses upon challenge with protein antigens (1). Together, these observations pose a challenge to the notion that CD40-CD40L interactions are essential for T cell dependent antibody responses.

In order to resolve this apparent discrepancy, one could propose that Th cells normally utilize a number of pathways to stimulate B cells. In addition to CD40-CD40L, this scenario would include stimulation through other Th cell surface ligands or factors. Alternatively, one could suggest that dependence on CD40-CD40L is B cell subset specific. The B cell compartment is now understood to consist of a number of B cell populations (reviewed in 3).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Stimulation of conventional B cells* with wild-type or CD40−/− Th2 clones†</th>
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<tbody>
<tr>
<td></td>
<td>Wild-type</td>
</tr>
<tr>
<td>CD23/class II upregulation³</td>
<td>++</td>
</tr>
<tr>
<td>Proliferation⁴</td>
<td>++++</td>
</tr>
<tr>
<td>IgM secretion⁵</td>
<td>++++</td>
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<tr>
<td>IgG1 secretion⁵</td>
<td>++</td>
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1 Percoll sedimented (70-75% interface) T cell-depleted Balb/c spleen cells.
2 Rested T cell clones were stimulated for 24 hours with plate-bound anti-CD3 (15-2C11).
3 600,000 B cells were incubated with 600,000 anti-CD3 stimulated and 0.8% paraformaldehyde fixed T cell clones in 48 well plates for 48 hours in the presence of anti-IL-4 and anti-IL-5 antibodies. Harvested cells were analyzed by flow cytometry for levels of CD23 and class II expression on B-220+ cells.
4 40,000 B cells and 40,000 anti-CD3 stimulated and mitomycin C treated T cell clones were incubated in 96 well plates for 72 hours in the presence of 100 units/ml of IL4. Exogenous IL-4 was added to normalize for potentially different levels of secretion by the Th cells. Cells were pulsed with H³-Thymidine for the last 4 hours of culture. Maximal responses are equivalent to approximately 80,000 CPM.
5 40,000 B cells and 40,000 anti-CD3 stimulated and mitomycin C treated T cell clones were incubated in 96 well plates for 6 days in the presence of 1000 units/ml of IL4. Exogenous IL-4 was added to normalize for potentially different levels of secretion by the Th cells. Secretion of Ig was measured by isotype-specific ELISA. Maximal IgM and IgG1 responses are equivalent to approximately 30,000 ng/ml and 1,000 ng/ml respectively.
These include follicular and marginal zone B cells in the spleen, and B-1 and B-2 B cells in the peritoneal cavity. Experiments testing in the in vitro response of resting murine splenic B cells or human peripheral blood and tonsillar B cells, are most likely assessing the activity of conventional (follicular or B-2) B cells. When measuring humoral responses in vivo, however, it is possible that antibodies are derived from some or all of the B cell subsets. Thus, depending upon the type of B cells responding, the characteristics of a T cell dependent response may vary.

**Capacity of Murine CD40L Deficient Th Clones to Support Activation of Conventional B Cells**

In order to better understand the role of CD40-CD40L in the activation of murine B cell subsets, our laboratory derived a number of Th2 clones from CD40L deficient (CD40L−/−) mice. These clones were obtained by allostimulation (H-2b anti-H-2d) and directed towards the Th2 pathway by culture with IL-4 and IL-10. Wild-type (CD40L+/+) clones were similarly derived and used for comparison. Initial experiments examined the capacity of wild-type and CD40L−/− Th2 clones to stimulate resting B cells isolated from spleen. B cells were tested for the induction of activation antigens, proliferation, differentiation, and isotype switching. In order to assess the helper activity of the Th cells in a polyclonal manner, rested clones were stimulated with plate-bound anti-CD3. As expected, wild-type Th2 clones induced increased levels of CD23 and MHC class II antigens, strong proliferation, and production of IgM and IgG1 from splenic B cells, as summarized in Table 1. Regardless of the assay however, CD40L−/− Th2 clones were unable to invoke any form of activation in this population. Neither induction of activation antigens, proliferation, nor IgM and IgG1 secretion were evident in co-cultures of CD40L−/− Th2 and B cells (Table 1). Since the Th2-B cell co-cultures consisted of H-2b alloreactive Th cells and BALB/c (H-2b) B cells, it is possible that recognition of B cell class II molecules in the absence of CD40 ligation triggered a negative response (4). This was controlled for by examining the activity of H-2k and H-2b splenic B cells when cultured with the CD40L−/− Th clones. These B cells likewise failed to register any response in the various assays, indicating that the CD40L−/− Th2 clones are fundamentally unable to promote proliferation and differentiation of resting conventional B cells. These results are consistent with studies of Noelle and co-workers demonstrating the ability of an anti-CD40L antibody to completely block proliferation of splenic B cells induced by activated CD40L+ Th cell membranes (4). They also agree with the findings of Parker and colleagues in which splenic B cells from CD40−/− mice were unable to proliferate and differentiate in response to anti-CD3 activated wild-type Th clones (6).

**Capacity of Murine CD40L Deficient Th Clones to Support Activation of B-1 Cells**

Using the CD23 marker in conjunction with B-220 (CD45R) one can delineate two major B cell subsets in the peritoneal cavity of mice (3). B-220+CD23− cells comprise the B-1 (both B-1a and B-1b) cell population. Using electronic cell sorting, we therefore sort purified the B-220+CD23− B cell subset and examined their capacity to respond to both wild-type and CD40L−/− anti-CD3 stimulated Th2 clones. Similar to results obtained with conventional B cells, the purified B-1 population proliferated vigorously and produced both IgM and IgG1 when stimulated with wild-type Th2 cells (Table 2). Of interest however, the B-1 cells exhibited a definite proliferative response when co-cultured with activated CD40−/− Th2 clones. Although the response was approximately 50% of that obtained with wild-type clones, it was still significant. In addition, CD40−/− Th2 clones stimulated B-1 cells to secrete levels of IgM that were 50-70% of that observed with wild-type Th2 cells. Wild-type and CD40−/− clones induced equivalent amounts of IgG1. Taken together, these data suggest that conventional resting B cells are strictly dependent upon CD40 ligation for expansion and differentiation when interacting with Th cells. Although B-1 cells respond optimally to CD40-CD40L dependent cognate help, these results further point to a CD40-CD40L independent means of T cell directed B-1 cell stimulation.

**Role of IL-5 in Driving CD40-CD40L Independent B-1 Cell Stimulation**

Given the finding that CD40−/− Th2 clones induce expansion, differentiation and switching in B-1 cells, the question arises as to whether this alternative (non-CD40L) helper activity is derived from surface molecules or soluble factors. Since previous reports had suggested B-1 cells to respond to IL-5 in an au-
tonomous fashion and to express IL-5 receptors (7, 8), we tested the effect of a blocking anti-IL-5 antibody in CD40-/- Th2-B-1 cell co-cultures. As shown in Table 3, neutralization of IL-5 completely abrogated the proliferative response of B-1 cells to the CD40-/- Th2 clones, suggesting T cell derived IL-5 to play a key role in CD40-CD40L independent stimulation. Further experiments examined the capacity of anti-IL-5 and anti-CD40L antibodies to block the response of B-1 cells cultured with wild-type Th2 clones. Whereas anti-IL-5 and anti-CD40L antibodies by themselves reduced the response by only a modest level, as listed in Table 3, the addition of both completely eliminated B-1 cell stimulation. Collectively, these results suggest two independent means by which Th cells can activate B-1 cells. One is centered through CD40, and the other through the IL-5 receptor. Elimination of either of these pathways allows for rescue through the other, and the opportunity for B-1 cells to still proliferate and differentiate. Neutralization of both pathways results in a complete blockade of B-1 cell activation.

**Respective Roles of IL-4 and IL-5 in Conventional and B-1 Cell Activation**

Whereas Th2 clones stimulate conventional B cells in a CD40-CD40L dependent manner, the data summaries in Tables 2 and 3 clearly indicate that helper T cells can activate B-1 cells using at least two different pathways. In order to better resolve these pathways and the relative contributions of IL-4 and IL-5, conventional and B-1 cells were stimulated with combinations of soluble recombinant CD40L trimer (CD40L), IL-4 and IL-5, and were examined for proliferation. From the results in Table 4, it is evident that conventional B cells responded optimally when cultured with CD40L + IL-5 and suboptimally with CD40L + IL-5. These data are consistent with results obtained with Th2-B cell co-cultures and demonstrate three signal combinations capable of stimulating B-1B cells. The most efficient is clearly CD40 ligation in the presence of IL-5, with either CD40 ligation + IL-4 or IL-4 + IL-5 providing a weaker but sufficient set of stimuli. Based on these observations, similar experiments were performed with B-1 cells to assess their capacity to differentiate and isotype switch in the presence of CD40L, IL-4, and IL-5. The results are summarized in Table 5, and demonstrate a similar pattern. Maximal levels of IgM resulted when B-1 cells were cultured with CD40L + IL-5, with lower amounts induced by either CD40L + IL-4 and CD40L + IL-5, with lower amounts resulting from treatment of B-1 cells with IL-4 + IL-5. Tables 4 and 5 also show that IL-5 by itself could induce a modest level of B-1 cell expansion and differentiation.

**Discussion**

The results presented herein suggest resting conventional B cells (follicular or B-2 B cells) respond to Th cell dependent help in a CD40-CD40L restricted manner. Since most B cell biologists utilize conventional B cells for in vitro studies, it is not surprising that experiments with Th clones, membranes from activated Th cells, or recombinant CD40L have suggested CD40-CD40L interactions to be central in Th cell mediated activation. The present results also provide evidence that B-1 cells can respond to Th cells in a CD40-CD40L independent manner. Although B-1 cells are optimally stimulated when CD40 engagement occurs (in the presence of IL-5), these cells can also expand and differentiate when exposed to IL-4 and IL-5. These findings may thus explain the residual antibody response to protein antigens observed in both CD40 and CD40L deficient mice, as well as in X-HIM patients.
The finding that IL-5 is a key cytokine in Th2 driven B-1 cell activation is consistent with previous reports demonstrating this subset to constitutively express IL-5 receptors, and to exhibit modest levels of proliferation and differentiation when treated with this factor (7, 8). Further studies with IL-5 transgenic and knockout mice have demonstrated a prominent role for this cytokine in the development and maintenance of the B-1 cell population. Together with the present results, the question arises as to the meaning of IL-5 driven B-1 cell responses in both systemic and mucosal immunity. Perhaps the answer rests in the proposed phylogenetic layering of the B cell compartment (reviewed in 9). Although still controversial, a strong body of evidence suggests that B-1 and B-2 cells are products of distinct development branches or lineages (9). In addition, B-1 cells are thought to represent a more primitive or phylogenetically older B cell population (9). Should this be the case, one could speculate that early in vertebrate development, primitive B cells and helper cells collaborated in a less restricted, or CD40-CD40L independent manner. This collaboration could have relied upon the use of cytokines, such as IL-4 and IL-5, a mechanism still available to the B-1 cell population. It is established that B-1 cells can contribute to primary responses against bacteria (such as the T15 restricted anti-phosphorycholine response (9)) and are a significant source of precursors for IgA plasma cells in the gut lamina propria (9). How much of this response is cytokine driven and CD40-CD40L independent is unknown, but potentially testable. Moreover, given the option of B-1 cells to respond in the absence of CD40 ligation, it is possible that any cell which produces IL-4 and IL-5 can serve as a helper cell to this subset. In
addition to classical CD4+ Th cells, CD4CD8TCRαβ T cells, TCRγδ T cells, and even mast cells may provide this function. It is of interest that the latter three cell types are enriched in mucosal sites, and TCRγδ T cell deficient mice have reduced mucosal IgA responses (10). This presents the possibility that non-classical helper cells and B-1 cells may collaborate to produce a rapid, albeit primitive response upon invasion through the mucosal lining. Similar to T cell independent anti-carbohydrate responses, this primitive B-1 cell mechanism may serve to hold the invading agents in check until more sophisticated CD40-CD40L driven responses are fully operational.

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References

Mucosal IgA responses in cytokine gene knockout mice: Differential cytokine requirements for IgA secretion by B-1 and B-2 cells

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Abstract

Mucosal lymphoid tissues are the only tissues where large numbers of IgA-secreting plasma cells are found under normal physiological conditions and the numbers are maintained by a combination of recruitment of precursor cells and local proliferation. Two tissue sources of IgA plasma cell precursors have been identified in the mouse, Peyer’s patches and the peritoneal cavity. Peyer’s patch IgA+ B cells can be induced to secrete IgA in vitro by both IL-5 and particularly IL-6. In IL-6−/− mice IgA B cell numbers in lamina propria are greatly reduced (>60%). In these mice mucosal immune responses to T-dependent antigens such as ovalbumin (OVA) are greatly diminished whereas responses to microbial antigens such as phosphorylcholine (PC) and LPS are actually increased, compared to wild type mice, after challenge with attenuated Salmonella. Residual intestinal IgA+ cells in IL-6−/− mice are enriched in B cells coexpressing CD5, suggesting that these cells are of B-1 origin. Peritoneal cavity B-1 cells are
induced to secrete IgA in vitro by IL-5 but not IL-6. IL-5−/− mice produce considerably less mucosal IgA against PC or LPS following mucosal challenge with Salmonella compared to normals while responses to intestinal OVA challenge remain intact. There was a small but significant decrease in total IgA cell numbers in IL-5−/− mice compared to controls. These findings indicate that IgA+ mucosal B cells are derived from both B-1 and B-2 precursors, that these lineages of B cells have different cytokine requirements for differentiation to IgA plasma cells and also have different antigenic repertoires with B-1 cells mediating responses to predominantly microbial (T-independent) antigens while B-2 cells respond to predominantly T-dependent antigens.

**B cell lineages/subsets in the mouse**

Expression of CD5 (Ly-1, Leu-1), a marker at first thought to be restricted to helper T lymphocytes, on a number of mouse and human B cell tumors and then some normal B cells was first reported in the early 1980’s. Since that time other phenotypic differences have been noted between these B-1 cells (Ly-1, CD5 B cells) and conventional B-2 cells. Thus B-1 cells in the mouse peritoneal cavity were shown to be IgMHi/IgDLo, CD11b (Mac 1)+, B-220Lo, to spontaneously express the IL-5R and to be negative for CD23 (FcγR). B-1 cells can be further divided into B-1 a cells which express CD5 and B-1 b cells that are CD5−. In this review, however, we will consider only B-1 a cells. B-1 cells were shown to be most abundant in peritoneal and pleural cavities, constituted a minor fraction of splenic B cells and were rarely detected in other lymphoid tissues such as lymph nodes and Peyer’s patches. B-1 cells are found in small numbers in the thymus and in the salivary glands and female reproductive tract (personal observations). In humans, B-1 cells show less tissue specificity making up 10-25% of B cells found in adult peripheral blood and lymphoid organs. Conventional B-2 cells are IgM+IgD−, CD5+, CD11b, B-220+, CD23− and usually only express IL-5R following activation. B-1 cells produce the majority of serum IgM and also make significant contributions to serum IgG3 and IgA. The B-1 cell antigenic repertoire appears skewed toward T-independent antigens found on microorganisms (eg phosphorylcholine, α1-3 dextran and LPS) and some auto antigens. B-1 cells are also thought to use a restricted number of available V regions and V-(D)-J rearrangement in B-1 cells is characterised by absent or reduced levels of N region addition. Modification of B-1 antibody responses by somatic mutation is also reduced in B-1 cells compared to B-2 cells. Considerable, and often heated debate has occurred in recent years as to whether B-1 and B-2 cells represent distinct lineages or arise as a result of stimulation by different types of antigen. This debate will not be continued here but is addressed in the accompanying paper by Dr. Wortis.

**Cytokine regulation of in vitro IgA secretion**

Mucosal immunologists became interested in peritoneal cavity B-1 cells when it was shown that these cells reconstituted almost 50% of intestinal IgA plasma cells in allotype congenic chimeras (1). Following adoptive transfer to SCID mice B-1 cells also gave rise to large numbers of IgA plasma cells in intestinal lamina propria and reconstituted both intestinal and serum IgA in recipients (2). These results showed that mucosal IgA+ B cells in mice arose from two sources: Peyer’s patches (PP), as had been demonstrated in earlier studies, and peritoneal cavity (PerC) B-1 cells. Many human IgA plasma cells in intestinal LP were also shown to express CD5 again suggesting that these cells may be derived from B-1 cells (3). In vitro studies had shown that PP B-2 cells were induced to secrete IgA by the cytokines IL-5 and most importantly IL-6 (4). Both of these cytokines acted on cycling, IgA-committed cells and the effect was seen in the absence of mitogen costimulation. Culture of PerC B cells (in the presence or absence of the B cell mitogen STM) with these cytokines showed that these cells may be derived from B-1 cells (3). In vitro studies had shown that PP B-2 cells were induced to secrete IgA by the cytokines IL-5 and most importantly IL-6 (4). Both of these cytokines acted on cycling, IgA-committed cells and the effect was seen in the absence of mitogen costimulation. Culture of PerC B cells (in the presence or absence of the B cell mitogen STM) with these cytokines showed that IL-5, either alone or in combination with IL-2, induced IgA secretion by these cells, however, IL-6 was without affect (5). Earlier studies by others had also shown that IL-5 enhanced PerC B cell proliferation, spontaneous IgM secretion and trinitrophenyl-conjugated lipopolysaccharide (TNP-LPS) plaque-forming cell (PFC) responses. IL-5 acted on IgA+ cells in PerC as removal of IgA-expressing B cells by cell sorting abrogated the affect of IL-5(2). In older (>20 weeks) BALB/c mice 8-10% of PerC CD5+ B cells coexpressed IgA. Thus, it appeared that the cytokine requirements for in vitro IgA secretion differed for B-1 and B-2 cells.
IgA production in cytokine gene knockout mice

The availability of mice with targeted disruption of particular cytokine genes (cytokine gene knockout mice) provided valuable tools for evaluating the importance of individual cytokines for production of mucosal IgA. IL-6-/- mice were shown to be deficient in IgA-producing cells in the intestinal lamina propria with numbers reduced by >60% compared to wild type control mice(IL-6+/+) (6). Furthermore IL-6-/- mice mounted poor mucosal IgA responses in the intestine and lungs following mucosal immunisation with ovalbumin (OVA), a defect that could be overcome by vector-directed IL-6 gene therapy. Additional experiments with normal mice and rats has shown that direct co-injection of IL-6 plus OVA into Thiry-Vella loops at the time of intestinal boosting increases the levels of OVA-specific IgA secreting cells compared to animals boosted with OVA alone (Bao et. al., Immunology and Cell Biology, in press). Examination of residual IgA+ B cells in the lamina propria of IL-6-/- mice demonstrated that these cells were enriched for cells that coexpressed CD5 (5) suggesting that these cells were derived from B-1 cell precursors.

Studies in IL-5-/- mice had shown that these mice had a defect/delay in the development of CD5+ B-1 cells (7), particularly during the first 6 weeks of life in this study. This was further demonstrated by a significant reduction in the number of CD5+IgM+ cells in both the peritoneal cavity and lamina propria of IL-5-/- mice compared to wildtype controls (8). Similarly IgA+ cells coexpressing CD5 were also reduced 3-4 fold in the PerC and LP of IL-5-/- mice compared to normal IL-5+/+ mice. In these mice there was also a small but statistically significant decrease in total numbers of IgA plasma cells in the gut. Knockout and control mice were then immunized with OVA by intra-Peyer’s patch (IPP) injection, followed 14 days later by an intraduodenal OVA boost. Fecal pellets were collected from all animals on a daily basis, the antibody extracted and assayed by ELISA for OVA-specific antibody. Prior to immunisation no OVA-specific antibody in fecal extracts and intestinal washes collected at sacrifice did not differ between IL-5+/+ and IL-5-/- mice (Figure 1) nor was there any difference in intestinal anti-ovalbumin containing cells of any subclass (IgA/ IgG/IgM/IgE) between IL-5-deficient and normal mice. Thus mucosal immune responses to the T-dependent antigen OVA were greatly reduced in IL-6-/- animals (deficient in B-2 cells) but were unaffected in IL-5-/- animals despite significantly reduced numbers of B-1 cells in both PerC and LP.

Reconstitution of B-1 cell-deficient CBA/N Xid mice with PerC B (enriched in B-1 cells) cells but not B cells from other lymphoid tissues (spleen, Peyer’s patch, bone marrow) restored normal immune responses to Salmonella LPS or the hapten phosphorylcholine (PC) following challenge with live Staphylococcus (9), confirming that B-1 cells are responsible for immune responses against these antigens. In reconstituted animals serum IgM levels also returned to normal levels. We have used an attenuated aroA/aroD mutant of Straphylococcus to orally challenge IL-5-/- and IL-6-/- mice in order to determine if either cytokine deficiency affects the immune response to PC and LPS. Knockout and normal mice were immunized orally on weeks 0 and 5 with 10⁸ viable bacteria per mouse, and anti-PC and anti LPS levels in fecal extracts determined at weekly intervals. Total, IgA and IgG PC-specific antibody levels in fecal extracts were significantly (p<0.001) reduced in IL-5-/- mice compared to controls (8) after the first immunization (weeks 1 and 2) and after the booster immunization (weeks 6, 7 and 8) and at the termination of the experiment at week 10 anti-PC levels in gut wash of knockout animals was reduced by >50% compared to controls (Figure 1). This reduction in IL-5-/- mice was also apparent when PC-specific B cell numbers were determined in sections of intestinal lamina propria (data not shown). Intestinal antibody responses to Salmonella LPS followed a similar pattern to that seen for PC, being significantly reduced in IL-5-deficient mice. Thus the lack of IL-5 in these mice results in an impaired intestinal antibody response to the antigens PC and LPS, despite these mice having only a minor decrease in total intestinal IgA cell numbers. This can be attributed to the reduced numbers of B cells of the B-1 cell lineage.
We next infected IL-6−/− mice with attenuated S. typhimurium using the same protocol as used in IL-5−/− mice. In IL-6−/− mice PerC B-1 cell numbers were significantly increased compared to control animals as shown by increased numbers of IgM+/CD23- and CD5+/IgM+ cells, despite a reduction in total B cell numbers in most lymphoid tissues (Beagley et. al., submitted). Consistent with earlier findings of reduced B cell numbers in intestinal lamina propria (6) the levels of antibody (total Ig, IgM, IgG and IgA) in fecal pellet extracts and gut wash were reduced by 60-80% in IL-6−/− mice (Figure 2) compared to controls. Despite this decrease in total intestinal antibody, pre-immunisation levels of PC-specific antibody in fecal pellet extracts was actually increased (data not shown) and LPS-specific antibody of all isotypes (Figure 2) increased significantly in IL-6−/− mice while antibody levels only increased marginally in wildtype IL-6+/+ mice over pre-immunisation levels. The increase in antibody of the IgG subclass was mainly due to IgG1, consistent with B-1 cells being a major source of this antibody.

**Discussion**

The studies reported here demonstrated a greater than 50% reduction in B-1 cells in the PerC of IL-5−/− mice and a similar decrease in B-1-derived IgA plasma cells. Anti-PC and Staphylococcus LPS mucosal IgA responses following oral challenge with attenuated S. typhimurium were also decreased by 30-60% in knockout mice compared to controls, whereas, intestinal antibody responses following mucosal challenge with OVA (an antigen not recognized by B-1 cells) were not significantly different to those of control mice. Mice deficient in IL-6 (IL-6−/−) showed a >60% reduction in lamina propria B cells and a 60-80% reduction in antibody levels in intestinal wash. Following oral challenge with attenuated S. typhimurium, however, anti-PC and anti-LPS antibody levels in gut wash of IL-6−/− mice were significantly increased compared to wild type controls. IL-6−/− mice were unable to mount a mucosal response to OVA however. These data support the concept of a layered evolution of the mucosal immune system (10) with primitive B-1 cells being overlayed with evolutionarily more advanced B-2 cells. Both B cell lineages are committed to IgA production at mucosal surfaces but have both differing cytokine requirements for IgA secretion and different antigenic repertoires. The fact that both lineages preferentially isotype-switch to IgA in a mucosal setting reinforces the importance of this.
isotype for mucosal protection. Interestingly CD4-CD8 TCRαβT cells and TCRγδT cells, both of which are considered to be evolutionarily primitive T cells and which are found in the intestinal mucosa, spontaneously secrete IL-5 and could thus support mucosal IgA production by B-1 cells. IL-6 is produced by multiple cell types in the gut mucosa including epithelial cells, macrophages, Th2 helper cells and some B cells, all of which may support B-2 cell IgA secretion.

Both IL-5−/− and IL-6−/− mice, however, suffer no apparent adverse health problems and while antibody responses against PC/LPS and OVA respectively are greatly reduced in these knockouts they are not totally ablated by the cytokine deficits. This is likely due to the built-in redundancy of cytokine networks. The IL-5 receptor (IL-5R) shares common receptor chains with the IL3 and GM-CSF receptors and it may be possible that these cytokines support the residual B-1 cell functions in IL-5−/− mice. Similarly cytokines such as IL-11, LIF or CNTF may support some B-2 cell development in IL-6−/− mice due to the sharing of common receptor elements. The affect of cytokine gene inactivation on total B cell numbers was greatest in the IL-6−/− mice. This suggests that the more advanced B-2 cells may be able to expand to compensate for a deficiency in B-1 cell development seen in IL-5−/− mice but that the more primitive B-1 cell lineage cannot compensate for the failure of B-2 cell development in the IL-6−/− mice.

Acknowledgments
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Figure 2
Intestinal immunoglobulin production in IL-6−/− and wildtype control mice. Following oral immunisation with S. typhimurium fecal extracts were collected from IL-6−/− and control mice and assayed for total and LPS-specific immunoglobulin by ELISA.

References
IL-5R+, B-1 cells: Are they CMIS independent B cells for mucosal IgA plasma cells?

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Abstract

IL-5R is a critically important cytokine-specific receptor for the differentiation of sIgA+B-1 cells but not B-2 cells into plasma cells and subsequent IgA production in mucosal effector tissues. Furthermore, it was suggested that sIgA+B-1 cells arise from the CMIS-independent pathway while sIgA+B-2 cells are where they arose from IgA inductive tissues (e.g., Peyer’s patch) (Fig. 1). Since the distribution of B-1a, B-1b and B-2 cells differed in several mucosa-associated tissues, it would be interesting and important to examine in a future study the contribution of these different sources of sIgA+B cells for the induction of antigen-specific mucosal immune responses against TD, TI-1 and TI-2 antigens and their specific requirements of Th1 and Th2 cytokines.

Introduction

A unique feature of the mucosal immune system is represented by the presence of IgA inductive and effector compartments for the induction of antigen-specific IgA responses. For example, IgA inductive tissues such as gut-associated lymphoreticular tissue (GALT) or Peyer’s patches (PP) containing a high frequency of IgA-committed B cells [surface IgA+ (sIgA+) B cells] and Th1/Th2 cells are interconnected with IgA effector sites including the intestinal lamina propria (LP) via the common mucosal immune system (CMIS) (5). As reviewed in the accompanying manuscript by Wortis, B cells have been shown to be separated into at least two subsets, B-1 and B-2, based on the expression of CD5 and CD45/B-220, the intensity of surface IgM (sIgM) and sIgD, as well as the differences in anatomical localization and functional characteristics. An interesting observation associated with the mucosal immune system is that up to 40% of IgA-producing cells in the murine intestinal lamina propria arise from a pool of B-1 precursors derived from the peritoneal cavity which may represent a lineage separate from that of conventional PP B cells (1). Another study using transgenic mice has provided additional supportive evidence that intestinal IgA plasma cells are derived from B-1 cells (2). In addition, up to 50% of intestinal B cells are CD5+, a large number of which secrete IgA in humans (3). Taken together, these findings suggest that B-1 cells could be an important source for IgA-producing cells in mucosal tissues. Inasmuch as the reduction of B-1 cells was a common feature to both IL-5+ and IL-5R+ mice (6, 7), we focused our study on exploring the role of IL-5R and B-1 cells for the development of IgA-producing cells in mucosa-associated tissues using the latter gene-disrupted murine model.

Deletion of IL-5R signaling cascade resulted in reduction of IgA levels

The IgA levels in mucosal secretions including fecal extracts (feces) and saliva were reduced in IL-5Rα-/- mice (~50%) when compared with wild type mice (9). However, the levels of IgA and IgG were not changed in serum, while the IgM levels were reduced by ~60% (8).

Furthermore, analysis of IL-5+ mice revealed a similar finding that the levels of mucosal IgA were also reduced (8). Taken together, these results suggested that the removal of the IL-5Rα or IL-5 specific gene led to the selective impairment of IgA production at mucosal compartments rather than at the systemic site.

Deficiency of IgA-producing cells in mucosal tissues of IL-5Rα+/- mice

In order to directly demonstrate specific reduction of IgA-producing cells in IgA effector tissues of IL-5Rα mice, mononuclear cells were isolated from different mucosal-associated tissues. The isotype-specific ELISPOT assay was used to determine and com-
pare the frequency of IgA-producing cells in IL5Ra-/- and wild type mice. Interestingly, the number of IgA-producing cells was decreased in mucosal effector tissues including intestinal lamina propria (i-LP), submandibular gland (SMG) and nasal passage (NP) but not in inductive sites such as PP and NALT (9). The frequency of IgA-containing cells was also evaluated in the tissue sections of intestine and SMG by confocal imaging immunohistological analysis. Enumeration of the IgA-producing cells in SMG and i-LP (e.g., duodenum, jejunum and ileum) revealed that the numbers of IgA plasma cells were more reduced in IL5Ra-/- mice in comparison to wild type mice. This immunohistological analysis further confirmed the result obtained by IgA isotype specific ELISA and ELISPOT assay where partial impairment of the IgA induction pathway was always seen in the mucosal effector tissues (e.g. i-LP, SMG and NP) of IL5Ra-/- mice.

**Deficiency of B-1 cells in mucosal effector sites of IL5Ra-/- mice**

To determine the exact contribution of B-1 and B-2 cells in the reduction of mucosal IgA in IL5Ra chain deficiency, our experiment sought to elucidate the exact frequency of these different subsets of B cells in mucosal inductive and effector tissues of IL5Ra-/- and normal background mice. We found B-1 cells to be much more frequent in mucosal effector tissues (e.g., i-LP, NP and SMG) than in mucosal inductive tissues (e.g., PP and NALT), the latter showing instead a predominance of B-2 cells. Mucosal B-1 cells can be further classified into B-1a (IgM<sup>high</sup>, IgD<sup>low</sup>, CD5<sup>+</sup>) cells and B-1b (IgM<sup>high</sup>, IgD<sup>low</sup>, CD5<sup>+</sup>) cells based on the expression of CD5 (9). A high frequency of B-1a cells was more typical of SMG, while a predominance of B-1b cells was characteristic of the i-LP and NP of wild type mice (9). For IL5Ra-/-, both B-1 cells in the effector tissues were eroded while B-2 cells in the inductive site were not (9). These results suggest that IL5R is essential for the development of localized B-1a and B-1b cells in mucosal effector sites, while GALT- and NALT-derived B-2 cells are exempted from IL5R dependency.

**Reduction of sIgA+ B-1 cells but not B-2 cells in IgA effector site of IL5Ra-/- mice**

To further specify IL-5-dependent IgA B cells, the frequency of sIgA+ B cells in B-1a, B-1b and B-2 cell subsets was examined in mononuclear cells isolated from mucosal effector tissues of IL5Ra-/- and control background mice. The numbers of sIgA+ B-2 cells were similar in both groups of mice (Table 1), while the frequency of sIgA+ B-1a and B-1b cell fractions was significantly reduced in IL5Ra-/- mice (Table 1) (9). This reduction predominantly affected sIgA+ B-1a in SMG and sIgA+ B-1b cells in i-LP (Table 1), suggesting that the IL-5/IL-5R signalling pathway is essential for the development of sIgA+ B-1a and B-2 cells at these effector sites.

**Analysis of cytokine receptor (IL-2Ra, IL-5Ra and IL-6R) expressions on sIgA+ B cells**

When the expression of cytokine receptor for the known IgA-enhancing factors (e.g., IL-2, IL-5 and IL-6) was examined in the different subsets of sIgA+ B cells in wild type mice, the levels of IL-5Ra (36%) were found to be much higher than those of IL-6R (8%) in sIgA+ B-1 cells (9). On the other hand, IL-6R (~56%) was more prevalent rather than IL-5Ra (~31%) on sIgA+ B-2 cells. In IL5Ra-/- mice, the preferential expression of IL-6R on sIgA+ B-2 cells was comparable to that of the wild type group. According to these results, we hypothesized that the development of sIgA+ B-1 cells requires a cytokine signal provided via the IL-5/IL-5R pathway since they preferentially express IL-5R. In contrast, sIgA+ B-2 cells can be developed via a signal provided via IL-6/IL-6R even in the absence of the IL-5/IL-5R signalling pathway since B-2 cells express both IL-5Ra and IL-6R.
IL-5-induced IgA production from i-LP B-1 cells in vitro

To prove our hypotheses described above, sIgA+ B-1 or B-2 cells were isolated from i-LP of IL-5Rα-/- and wild type mice and then co-cultured with or without IL-5 and/or IL-6 to induce IgA production in vitro B cell culture system. When sIgA+ B-1 cells from wild type mice were incubated with IL-5 but not IL-6, high levels of IgA synthesis were induced. In contrast, B-2 cells produced IgA in the presence of IL-5 and/or IL-6 (9). In the case of IL-5Rα-/- mice, the level of IgA production was not changed when B-1 cells were cultured with IL-5 and/or IL-6 in comparison to the control wells (without the cytokine) (9). On the other hand, B-2 cells from IL-5Rα-/- mice produced high levels of IgA in the presence of IL-6 (9, 10). These findings indicate that the IL-5/IL-5R signaling pathway is essential for the differentiation of sIgA+ B-1 cells into IgA-producing plasma cells, but may be compensated for sIgA+B-2 cell differentiation by the signal provided by the IL-6/IL-6R pathway in the situation of IL-5R gene deletion.

References
Announcements

Society Business

• The notice for membership renewal for 1998 contained a window in which members were able to donate money to the Graham Jackson Fund as well as the Emerging Countries Fund. The membership should be congratulated on their generosity to both funds. Approximately $400 was raised for the Jackson Fund and $500 for the Emerging Countries Fund. These funds will be made available for their respective goals.

• Recently, the Governing Council approved the suggestion by Drs. Kagnoff and Kiyono to create an educational fund in support of mucosal immunology in developing countries. The goals of this fund are to facilitate the development of educational efforts in these countries. Currently, the priorities are to increase the cash available to support these efforts, hence, a request for contributions from members was included in your renewal notice. Interested individuals are asked to seek contributions from industry, government or private foundations to augment the fund as well as provide suggestions for their use. Consideration of requests for projects will begin in 1999. Please contact the Secretary-Treasurer, Peter Ernst, at the Society Business Office with your suggestions and requests.

• Cards announcing the 10th International Congress and including an application for membership in the Society are available to anyone who would like to distribute them to interested groups. Please contact the Society Business Office if you require these cards.

• Recently, members should have received their new SMI Membership Directory. If you did not, please contact Mr. Tom Peno by email at smi@paimgmt.com to reach the SMI offices in Washington. Also, please carefully read your description in the directory. If any information is incorrect or missing, forward the corrections on to Mr. Peno.

• Members are also requested to provide Nominations for Councillors in Europe and North America. Pending the receipt of these nominations, a ballot will be mailed to all members.

Meeting Announcements

• Eighth International Symposium on Coeliac Disease in Naples, Italy, from April 22-24, 1999. For more information, visit their website at http://www.socmucimm.org/meetings-celiac.htm or contact Jean Gilder, Congress Secretariat, by fax 39 81 546 3781 or by email: jgcon@tin.it.

• The 10th International Congress of Mucosal Immunology will be held in Amsterdam, The Netherlands, from June 27-July 1, 1999. For more information, visit their website at http://www.socmucimm.org/tenth.htm or contact Dr. E. van Rees by email: e.van_rees.cell@med.vu.nl.


• The Banff Inflammation Workshop will be held from March 4-6, 1999 at Buffalo Mountain Lodge, Alberta, Canada. For more information, please contact John Wallace by email at WallaceJ@acs.ucalgary.ca.

• Second Annual Conference on Vaccine Research: Basic Science-Product Development-Clinical & Field Studies, 28-30 March 1999, Hyatt Regency Bethesda, Bethesda, Maryland, USA. To be placed on the mailing list to receive conference announcements and the Call for Abstracts (available November 1998), please forward your name, affiliation, postal address, phone & fax numbers, and email address to: Kip Kantelo/NFID. For more information, send an email at: e-mail: kkantelo@aol.com or visit the website at (http://www.nfid.org/conferences/vaccine99).

Society for Mucosal Immunology — Application for Membership

Membership in the Society for Mucosal Immunology is open to all immunologists, physicians, dentists, veterinarians, biochemists, or other scientists who do research in or who have an active interest in mucosal immunology, and who have published at least one first-authored paper in a peer-reviewed journal. Society membership includes a subscription to Mucosal Immunology Update. (Please Type or Print)

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Please mail completed application form with $10 application fee and $60 annual dues (total $70) payable by check in U.S. dollars or by VISA or MasterCard account number (please include expiration date and MasterCard 4-digit interbank number) to Dr. Peter Ernst, Secretary-Treasurer, The Society for Mucosal Immunology, 4340 East West Highway, Suite 401, Bethesda, MD 20814-4411, U.S.A. Or e-mail to smi@paimgmt.com.