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The development and repertoire of B-1 cells (CD5 B cells)

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A small subset of mouse and human B cells produces much of the serum immunoglobulin, including many common autoreactive antibodies, and accounts for most cases of B-cell chronic lymphocytic leukemia. An exciting recent conference focused on the development, repertoire and lineage classification of these cells. The meeting was convened for a discussion of 'CD5 B cells' but ended with a discussion of 'B-1 cells' (see Ref. 1).*

Hayakawa and co-workers[2] originally distinguished a subset of mouse B cells that bear low levels of the pan-T-cell glycoprotein Ly-1 (CD5). These Ly-1$^+$ B cells (now called B-1a cells[1]) have received increasing attention in the subsequent eight years, mainly because of the existence of a homologous population in humans, their high representation in chronic lymphocyte leukemia in humans and mice, and their potential importance in autoimmune disease. This report focuses on two major themes: (1) the characterization of differences among B-1a, B-1b and conventional B (also known as B-2) cells and (2) evidence that classifies these populations as separate lineages in the mouse.

Phenotype

B-1 cells can be distinguished from conventional B cells by anatomical localization, functional characteristics and gene expression. The expression of a series of cell surface molecules detectable by flow cytometry was discussed (for reviews, see Refs 3–5). All B-1 cells in the mouse are bright for immunoglobulin M (IgM) and dull-to-moderate for IgD and B220. In contrast, conventional B cells are dull for IgM, and bright for IgD and B220. In the peritoneal cavity, B-1 cells are Mac1(CD11b)$^+$ (A. Stall, Columbia Univ.) and FcεR(CD23)$^-$ (T. Waldschmidt, Univ. of Iowa), while conventional B cells are Mac1$^-$ and FcεR$^+$. However, these two markers are less useful in the spleen as Mac1 is not expressed on splenic B-1 cells, and both splenic B-1 cells and marginal zone B cells lack FcεR.

Mouse B-1 cells can be divided into two very similar populations, B-1a cells, which express detectable levels of surface CD5, and B-1b cells, which do not. Within experimental limits, each of these B-1-cell populations replenishes itself, but not the other, when transferred into irradiated recipients with congenic bone marrow (Stall).

H. Wortis (Tufts Univ., Boston) showed that cultured conventional B cells from spleen (Ig-D^{br} CD5$^-$CD23$^+$) can be induced to gain CD5, lose CD23 and express low levels of IgD following treatment with anti-μ and interleukin 6 (IL-6), but not lipopolysaccharide (LPS)[6]. These cells do not express Mac1 in culture. It is not known whether or not they self replenish when transferred into irradiated recipients, and whether or not they express Mac-1 in the peritoneal cavity.

CD72 is the ligand for CD5

K. Thielemans (Vrije Universiteit, Brussels) and J. Parnes (Stanford) identified the B-cell surface protein CD72 (Lyb-2 in mouse) as the ligand for CD5 (Ly-1)[7]. CD72 is present on pre-B cells and all mature B cells but is absent from plasma cells. Investigation of the CD5–CD72 interaction may provide insights into the differences between B-1 and conventional B cells and into T-cell–B-cell communication. Thus far, there are no known functional differences between B-1a and B-1b cells; however, the presence of both Lyb-2 and Ly-1 on B-1a cells, but only Lyb-2 on B-1b cells, suggests that such differences will be found.

Self replenishment, feedback regulation and IL-10 help define B-1 cells

What physiological characteristics can be used to distinguish between B-1 and conventional B cells? First, B-1 cells maintain their numbers by self replenishment; conventional B cells do not. Second, B-1 cells exert a feedback regulation that limits *de novo* production of B-1 cells from progenitors, starting at about the time mice are weaned[8]. Third, as described by A. O'Garra (DNAX, Palo Alto), IL-10 is produced by LPS-stimulated B-1, but not conventional B, cells. Moreover, M. Howard and H. Ishida (DNAX, Palo Alto) suggested that IL-10 may be an autocrine factor that is essential for B-1-cell development. They showed that animals treated from birth with anti-IL-10 antibodies have normal levels of cells in the spleen and thymus, but are B-1 cell deficient, with essentially no B cells in the peritoneal cavity. Consistently, these mice have reduced levels of serum IgM.

Repertoire

B-1 cells are disproportionately represented in the production of autoreactive antibodies and use a restricted set of *V* genes. H. Gu and K. Rajewsky (Institut für Genetik, Köln) presented two important

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results based on analyses of gene expression from polymerase chain reaction (PCR)-amplified cDNA libraries of sorted B-cell populations. First, nontemplated N-region sequences are rarely inserted in the B-1a cells that arise earliest in ontogeny[9]. Some of these B-1a cells maintain themselves by self replenishment throughout the life of the animal. B-1a cells arising later (>1 month) have more N-region diversity and in this sense are more similar to conventional B cells. Second, positive selection is important in determining the repertoire. Pre-B cells from neonatal liver or adult bone marrow appear to utilize a wide range of V_H genes within the large J558 family. In contrast, peripheral B cells, both B-1 and conventional B, show dominant expression of particular V_H genes in J558. The Köln group postulates that positive recruitment between the pre-B and B-cell transition brings all peripheral B cells into a long-lived pool[10,11].

Since B-1 cells develop early in ontogeny, repertoire differences may, at least in part, reflect selection by different endogenous antigens, including anti-idiotypic B cells (M. Vakil, Univ. of Alabama, Birmingham), present in the fetus and not in the adult. K. Hayakawa (Fox Chase Cancer Center, Philadelphia) and others presented evidence that positive selection by endogenous antigens is a major mechanism for the germ-line-encoded antibody specificities prevalent in the B-1-cell population. This results in an increased level of anti-thymocyte autoantibodies and anti-phosphatidylcholine (PtC, also known as anti-bromelain-treated mouse red blood cells (BrMRBC)) antibodies in the B-1-cell population. Anti-PtC hybridomas show multiple, independent rearrangements using V_H11 (Hayakawa), as has also been observed in lymphomas and with V_H12 (S. Clarke, Univ. of North Carolina). PCR amplification of sorted pre-B and B cells from the adult bone marrow indicates that conventional B cells also rearrange V_H11, although subsequent selection does not occur[12].

Finally, Hayakawa presented preliminary data from V_H11 transgenic (Tg^+) mice, which do not have B cells with endogenous rearrangements. There is an over-representation of B-1a cells and the anti-PtC specificity is restricted to the Tg^+CD5^+ B cells and absent in the Tg^+CD5^- B cells. No increases in B-1a cell frequency are observed when the transgene encodes for a specificity normally attributed to conventional B cells[13]. Hayakawa and Hardy conclude that the use of a 'preferred specificity' can result in the expansion of the B-1a population.

λ5 knock out

Perturbations in B-cell development are also observed when the λ5 gene is knocked out by homologous recombination (D. Kitamura, Rajewsky; A. Kudo, F. Melchers, Basel). λ5 is part of the pseudo-light-chain complex, μ–ψL, which consists of μ chain in association with λ5 and V pre-B proteins and is present on some pre-B cells; it is presumably involved in V_H-positive selection prior to light chain synthesis. Loss of λ5 does not affect B-1-cell development in the peritoneum or spleen; however, there is a much delayed development of B-2 cells. This may reflect the decreased susceptibility of B-1 cells to perturbations in B-cell development and/or their characteristic ability to self replenish from surviving cells.

Demonstration of distinct B-cell lineages

Three independent groups, with different experimental systems, introduced evidence for distinct B-1- and conventional B-cell lineages. The question of separate lineages is best addressed early in the differentiation pathway, that is at the progenitor (stem cell) level. R. Hardy (Fox Chase Cancer Center, Philadelphia) demonstrated that hematopoietic stem cells (HSC), enriched by sorting Thy-1^{lo}/Lin^- (including $B220^-$) cells from fetal or neonatal liver, readily repopulate B-1 cells in irradiated severe combined immunodeficient (SCID) recipients; however, similarly sorted and transferred HSC from adult bone marrow do not. In separate experiments, Hardy showed that adult bone marrow pro-B cells ($B220^+CD43^+HSA^+$) reconstitute mostly conventional B cells[14], while similar fetal liver pro-B cells yield only B-1 cells. Thus pro-B cells, which have undergone D_H–J_H but not V_H–D_HJ_H rearrangement are committed to particular lineages when isolated from fetal versus adult sources.

N. Solvason and J. Kearney (Univ. of Alabama, Birmingham) demonstrated the independent development of B-1- and conventional B-cell lineages in a cell transfer system. They showed that progenitors that give rise to B-1 cells, but not those that give rise to conventional B cells, are present in the 13-day fetal omentum. Thus, B-1 cells, but not conventional B cells, develop at a distinct site associated with mesodermally-derived peritoneal lining. Fetal liver, which may also include mesodermal tissue, yields both B-1 and conventional B cells[15]. The human fetal omentum is also a site for B-cell generation with a bias toward the production of B-1 cells (Solvason).

Finally, the existence of independent B-cell progenitors was demonstrated by the co-transfer of 14-day fetal liver (BAB strain, IgH b allotype) and adult bone marrow (Balb strain, a allotype) into irradiated recipients. Although fetal liver is capable of reconstituting both B-1 cells and conventional B cells, the development of these B-cell lineages from progenitors was shown to proceed independently. In some co-transfer recipients, all conventional B cells were derived from the bone marrow source. The fetal liver reconstituted B-1 cells in these recipients, but failed to reconstitute conventional B cells (A. Kantor).

Progenitors for B-1a and B-1b cells

Kantor and Stall distinguish progenitors ($B220^-$) for B-1b cells from progenitors for B-1a cells by their ability to persist into adulthood. Progenitors for B-1a cells, although abundant in fetal liver, diminish with age and are rare in adult bone marrow. Progenitors for B-1b cells are also active in fetal liver (and omentum, Solvason); however, in contrast to the progenitors for B-1a cells, they readily persist into adulthood, as evidenced by bone marrow reconstitution of peritoneal $IgM^{br}IgD^{lo}Mac1^+CD23^-CD5^-$ cells. Pro-B cells, isolated from fetal liver and adult bone marrow by Hardy also reconstitute peritoneal cells with the B-1b FACS-phenotype ($IgM^{br}IgD^{lo}CD5^-$).

Several questions were raised regarding the equivalence of B-1b cells in normal animals (or fetal liver recipients) and B-1b cells that arise from adult bone marrow transfers. It is not yet clear whether the bone-marrow-reconstituted B-1b cells can self replenish and produce IL-10. The repertoire of bone-marrow-derived B-1b cells and fetal-derived B-1 cells also needs to be compared. The extent to which B-1b cells are repopulated from progenitors in the normal adult bone marrow is also unclear. A feedback mechanism limits the emergence of both B-1a and B-1b cells from their progenitors starting at two-to-three weeks of age, forcing these cells to persist subsequently by self replenishment[8].

Questions regarding lineage

The issue of lineage assignment was the most actively debated topic at the meeting. L. Herzenberg (Stanford) stated that there was compelling evidence for three independent B-cell progenitors and hence three distinct B-cell lineages (described above). She further proposed a global model of lymphocyte development, based on the existence of three stem cells (lymphoid progenitors), each capable of yielding specific sets of T and B cells[16]. She suggested that B-1a cells and early γδ T cells represent the most primitive layer of the immune system, whereas conventional B cells and αβ T cells represent the most developed layer. K. Ikuta (Stanford) supported the connection between the fetal-derived B-1a cells and the early γδ cells. He showed that Vγ3 T cells can arise from fetal, but not adult, HSC.

Most of the lineage discussion, however, focused on the validity of the B-cell assignments. Hardy and Hayakawa agreed with the B-1a- versus conventional B-cell lineage distinction; however, they withheld judgement on the designation of B-1b cells as a separate lineage, awaiting further demonstration of the equivalence of fetal-derived B-1b cells and adult bone-marrow-derived B-1b cells.

Wortis, who had previously questioned the existence of two B-cell lineages[6], concurred that fetal and adult-derived B cells represent separate lineages. At present, he questions the relationship between fetal progenitors and mature CD5$^+$ B-1a cells. Wortis argues that the functional phenotype of B-1a cells *in vivo* is a consequence of surface IgM (sIgM) crosslinking by antigens with repetitive epitopes, that is thymus-independent, type-2 antigenic stimulation. He presumes that fetal-derived B cells are much more likely than adult-derived B cells to enter the B-1a pathway solely by virtue of their distinct repertoire, which includes specificities with a high chance of encountering endogenous or common exogenous sIgM-crosslinking antigens. As discussed at the meeting, this mechanism has not yet been demonstrated *in vivo* and is inconsistent with several types of evidence. With respect to repertoire, Rajewsky's group has shown that the V_H gene usage of adult and fetal pre-B cells are similar within the J558 family and that N-region insertions are observed in B-1a cells arising at four weeks. With respect to stimulation, Hayakawa has shown that, three-to-four days following trinitrophenol (TNP)-Ficoll immunization, all anti-TNP plaque-forming cells in the spleen are CD5$^-$ (Ref. 17), and Rajewsky presented data showing that B-1a cells can give a T-cell-dependent response to PC.

On another issue, Howard raised the question as to whether micro-environmental factors (for example, local cytokines and different stimulatory pathways), rather than restrictions in the developmental potential of donor cells, could control progenitor development into B-1 or conventional B cells. Although not discussed in the plenary sessions, data from the co-transfers rule out this possibility. That is, all sites in a recipient should be equally accessible to injected progenitors. If the progenitors from bone marrow and fetal liver are functionally equivalent, and if the site at which a given progenitor lands controls its development, then the progeny of the co-injected progenitors from bone marrow and fetal liver should be proportionately represented in all B-cell populations. Kantor and Stall showed this is not the case; the distribution of B cells derived from adult bone marrow (few B-1a cells) is clearly different from that derived from fetal liver (many B-1a cells) in co-transfer recipients. Thus, the B-cell developmental potential is inherent in the progenitors, rather than solely determined by micro-environmental factors in the recipient.

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