LAH 359

Layered Evolution in the Immune System

A Model for the Ontogeny and Development of Multiple Lymphocyte Lineages

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A BIT OF HISTORY

CD5, originally called Ly-1 and for a time called Lyt-1, was first recognized as a surface antigen on a functional subset of murine T cells that currently corresponds to the CD4 (helper) T-cell subset. An extensive series of studies, starting with some in which we participated,[1,2] showed that helper T cells were selectively depleted by cytotoxic treatment with conventional antisera absorbed to be specific for Ly-1. Much of the work that initially distinguished the helper/inducer T-cell subset from the suppressor/cytotoxic subset was based on selective depletion studies using these highly absorbed conventional cytotoxic antibodies. Nevertheless, once we were able to produce a monoclonal antibody reagent that detected Ly-1 and could be used both in FACS and in depletion studies, we realized that Ly-1 was actually expressed on all T cells, rather than just on the helper subset. Furthermore, in collaboration with Lanier and his colleagues, we showed that this pan-T-cell antigen was even expressed on some B-cell tumors.[3]

At about this time, Kyoko Hayakawa and Randy (Richard) Hardy arrived at Stanford. Hayakawa had shown previously that a subset of murine B cell that participated in the regulation of IgG anti-hapten antibody responses could be depleted by cytotoxic anti-Ly-1 antibodies. She wanted to pursue this work in our laboratory. Rather than continuing with the previous methodology, however, she decided to join forces with Hardy and make the search for B cells that express Ly-1 part of an overall analysis of B-cell heterogeneity in normal and immunologically compromised mice. These B-cell studies,[4-7] conducted with the prototype dual-laser FACS instrument built by David Parks and the engineers in our FACS development group, provided the first demonstration of the power of multiparameter FACS analysis. In effect, they defined and partially characterized most of the currently known B-cell subpopulations.

Actually, Chused and colleagues published the first (refereed) demonstration of Ly-1 expression on normal murine B cells.[8] The Hayakawa and Hardy studies, presented initially at a New York Academy of Science meeting (1982)[9] and published shortly thereafter,[3,4,9] provided a more extensive characterization of the surface markers on the "Ly-1 B" cells[1] and, more importantly, implicated these cells in autoimmune processes. Ann Cooke, visiting from the Middlesex Hospital in London, next suggested (personal communication) that a large number of Ly-1 B cells might be found in the murine peritoneal cavity, because cells producing autoantibodies, such as those we

showed were produced by Ly-1-bearing B cells (*e.g.,* to bromelain-treated mouse erythrocytes), had been shown to inhabit this locale. The production of these antibodies connected the Ly-1 B cells to Haughton's work with the CH series of murine B-cell tumors, many of which express Ly-1 and some of which produce autoantibodies to bromelain-treated mouse erythrocytes.

These findings, together with cell transfer and repertoire studies from groups led by John Kearney (Alabama) and Klaus Rajewsky (Cologne) established the foundations of the Ly-1 B story. Hardy and Hayakawa, who left our laboratory in 1985, continued to make outstanding findings in this area, first in T. Kishimoto's laboratory in Japan and now in their own laboratory at the Fox Chase Cancer Center in Philadelphia. Alan Stall, Paul Lalor, Frans Kroese, Tom Kipps, and Sharon Adams from our laboratory also made major contributions to the early work. Studies by Irmgard Forster, Don Holmgren, George Janossy, Anne Cooke, Peter Lydyard, Fred (A.T.) Steinberg, Kenneth Ault, and many others similarly deserve mention; however, a detailed review of the contributions made by these (and other) groups will have to await a more serious account of Ly-1 history than I am willing to attempt here. Suffice it to say, we are glad that this early work opened a productive area of research that drew us together in the first meeting dedicated to Ly-1 B-cell studies.

B-CELL LINEAGES

Collectively, the early work on Ly-1 B cells led to the recognition of physically and functionally distinct B-cell subpopulations/lineages that develop at different times during ontogeny. This view, strange and unpopular at first, has now become central to studies of human and animal lymphocyte biology in a wide variety of laboratories throughout the world (as the attendance at this meeting indicated). Part of the reason for this interest stems from the demonstration that the B cells in one of these lineages (*i.e.,* CD5 B cells, now called B-1 cells[a]) offer a potentially useful model for investigating the properties and responses of the human B cells involved in chronic lymphocytic leukemia (B-CLL)[10,11] and certain autoimmune diseases. The naturally occurring antibodies (and autoantibodies) produced by these B-1 cells have also generated a fair amount of interest, as have the mechanisms that generate diversity and define the repertoire of these self-replenishing B cells. Finally, of course, many laboratories (including our own) are interested in the functional and physical properties that distinguish the murine B-cell lineages and the developmental mechanisms that control the order in which these lineages appear during ontogeny.

We have been particularly concerned with the implications of these findings for the development of the overall immune system. In effect, our findings led us to suggest that the B-cell lineages that we have demonstrated reflect the existence of a stratified immune system in which sequentially developing hematopoietic stem cells give rise to

[a]The participants at this (New York Academy of Sciences) meeting on CD5 B cells adopted workshop designations for the three currently identified populations/lineages of mature B cells: conventional B cells, which constitute the majority of B cells in the adult lymphoid organs; and B-1 cells, which encompass what have previously been referred to as Ly-1 (or CD5) B cells. B-1 cells can be divided into B-1a cells, which express CD5 and constitute the majority of the Ly-1 B population in normal animals; and B-1b cells, which are similar in most ways to B-1a cells but do not express CD5. (These latter cells have been previously referred to as the Ly-1 B-cell "sister" population). For a more complete description of this notation change, please see the article by Kantor *et al.* in this volume.

successive layers of functionally distinct B cells, T cells, and myeloid cells that interact to provide the varied functions of the immune system as we know it.

Our earlier studies subdivided murine B cells into two distinct lineages: conventional B cells, which comprise over 95 percent of the B cells in adult spleen and lymph nodes; and B-1 cells, (formerly called Ly-1 or CD5 B cells), which are a small fraction of the B cells in the spleen but abundant in the peritoneal (and pleural) cavities.[6] We originally took these lineages to be B-cell subsets with distinctive FACS phenotypes and functional properties. Cell transfer studies, however, in lethally irradiated recipients soon revealed major differences in the reconstitution behavior of these two kinds of cells. In essence, B-1 cells are readily reconstituted by transfers of mature, Ig-bearing B-1 cells from the peritoneal cavity but are poorly reconstituted by adult bone marrow. Conventional B cells, by contrast, are fully reconstituted by transfers of progenitors from bone marrow or spleen but are not detectably reconstituted by transfers of peritoneal B cells.[12,13] These findings, which indicated that B-1 cells and conventional B cells develop from different progenitors, provided the first evidence of lineage distinctions among B cells.

Later cell-transfer experiments confirmed this hypothesis by showing that B-1 cells isolated either from the peritoneum or the spleen of adults or neonates are self-replenishing in adoptive recipients and that B220-negative, Ig-negative bone marrow cells, which readily reconstitute conventional B cells, reconstitute B-1a cells very poorly.[13,14] In addition, Kearney and colleagues[15] showed that fetal omental cells selectively reconstitute B-1 cells in *scid* recipients.

FEEDBACK REGULATION OF B-1 CELL DEVELOPMENT

Studies with anti-μ-treated mice further extended these findings.[16,17] In these studies, which follow from our early allotype suppression work,[18] we used antibodies to IgM allotypes to deplete B cells from Ig-allotype heterozygous or homozygous neonates and charted the recovery of conventional B cells and B-1 cells after termination of the treatment. In all cases, we found that conventional B cells recover rapidly when the treatment antibody disappears; however, the development of B-1 cells is seriously compromised and fails entirely under certain conditions. Thus, as in the reconstitution studies, we detect marked differences in the developmental patterns of B-1 and conventional B cells.

The interference with B-1 development traces to the activity of a developmental feedback mechanism through which mature, self-replenishing B-1 cells specifically terminate the *de novo* development of additional B-1 cells in adult animals. Because of this mechanism, B-1 recovery proceeds only when the antibody treatment depletes all B cells. Thus treating allotype heterozygotes with antibodies to one of the parental IgM allotypes depletes half of the B cells while the antibody is present and permanently blocks the recovery of the depleted B-1 cells. By contrast, treating allotype homozygotes with the same antibody depletes all B cells and allows substantial, albeit not complete, B-1 cell recovery. (Conventional B cells recover equivalently in all cases.)

The recovery of the depleted B-1 cells in the antibody-treated allotype homozygotes is prevented when exogenous B-1 cells are introduced by neonatal transfers of (allotype-congenic) peritoneal B-1 cells or relatively benign B-1 tumors—models for human B-CLL[10,11]—from appropriate donors. In these artificial allotype heterozygotes (allotype chimeras), as in their natural counterparts, the introduction/presence of mature B-1 cells at the time that the treatment antibody disappears permanently blocks the recovery of the depleted B-1 cells. Thus, the progenitors that give rise to conventional B cells function normally throughout life in the presence or absence of

B-1 cells; however, the progenitors that give rise to B-1 cells cease to function after a critical number of B-1 cells develop or are introduced into the animal.

This type of developmental feedback mechanism is at odds with the common understanding of mammalian B-cell development; however, it is well-known for avian B cells, which develop in the bursa during the first weeks of life and persist thereafter by self-replenishment (cell division). In fact, the results from the neonatal treatment studies with anti-μ allotypes that we conducted with mice are matched, virtually experiment for experiment, by results from a series of studies conducted in the chicken.[19] Thus, in a developmental sense, B-1 cells appear to be more primitive than the conventional murine B cells.

On the other hand, recent studies have shown that cells that may be homologous to B-1 cells constitute the predominant B-cell subset/lineage in certain mammalian species. Studies with sheep reveal a bursa-like organ that generates most, if not all, of the animal's B cells during the fetal and neonatal period (see J-C. Weill, this volume). Recent studies with rabbits demonstrate that CD5 is expressed on all B cells in that species. Because conventional murine B cells have been reported to express CD5 when appropriately stimulated, the CD5 expression in the rabbit does not necessarily indicate homology to murine B-1 cells (Ramos and Knight, this volume). Nevertheless, the low IgD phenotype of rabbit B cells, and their slow recovery after deletion by neonatal anti-Ig treatment (R. Mage, personal communication), makes these cells likely candidates for B-1 homology.

A THIRD B-CELL LINEAGE

The B-1 cells that recover in anti-μ-treated allotype-homozygous mice have most of the properties of the B-1 cells found in normal (untreated) animals; however, whereas most B-1 cells in normal animals express the CD5 (Ly-1) marker that we used originally to distinguish B-1 cells, relatively few of the B-1 cells that recover in the treated animals express this marker.[16,17] We were already aware that B-1 populations in certain mouse strains tended to be somewhat enriched for these $CD5^-$ B cells; however, we only became convinced that these cells were real when we discovered their striking predominance in the B-1 population that recovers in the anti-μ-treated animals.

Emboldened by these findings, we sorted and transferred peritoneal $CD5^-$ and $CD5^+$ B-1 cells from normal animals and showed that, within experimental limits, each of these B-1 cell populations reconstitutes itself but not the other.[16,17,20] This phenotypic stability, which is maintained despite expansion of the injected population in the reconstituted recipients, suggested that B-1 cells might be subdivided into two lineages; however, taking the more conservative course, we named the $CD5^-$ population "Ly-1 B *sister* cells" and treated them as a subset of the overall Ly-1 B-cell (B-1 cell) population until recent data from progenitor studies defined them as a distinct lineage.[16] These studies, outlined here and discussed in detail in other presentations at this meeting, demonstrate differences in the sources of progenitors for three kinds of mature B cells: conventional B cells, B-1a ($CD5^+$ Ly-1 B) cells, and B-1b ($CD5^-$ Ly-1 B "sister") cells.

THE LAYERED IMMUNE SYSTEM: AN EVOLUTIONARY MODEL

As we previously discussed,[21] the developmental pattern of the B-cell lineages could be interpreted strictly within the framework of B-cell development; however, there is good reason to integrate these observations into a robust model that is consistent with

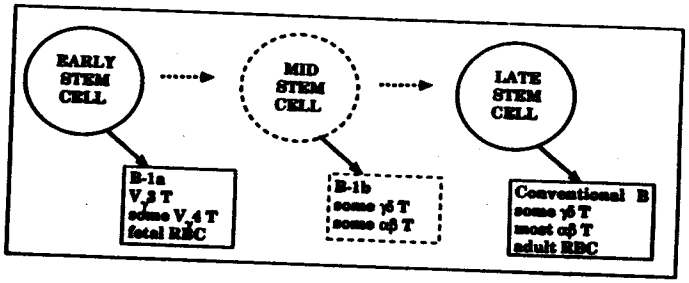

FIGURE 1. Evolutionary layering in the immune system.

the idea that the B-cell progenitors that we have identified arise from distinct pluripotent stem cells that also give rise to progenitors for distinct T-cell lineages (see FIG. 1). Ikuta *et al.* have shown that $V\gamma3^+$ T cells develop from hematopoietic stem cells in the 14-day fetal liver but not from hematopoietic stem cells in adult bone marrow.[22] Havran and Allison and others have presented evidence consistent with these findings demonstrating that Vγ3 T development occurs during late fetal life and does not occur after birth and that Vγ4 and other $\gamma\delta$ T cells develop mainly during late fetal and early neonatal life.[23–25]

The $\alpha\beta$ T cells, like conventional B cells, appear around birth and become predominant as the animal matures. These T- and B-cell populations/lineages, which have a much broader repertoire than their developmentally earlier counterparts, are replenished throughout life by *de novo* differentiation from cells in the bone marrow.

We see these data as evidence that the evolution of the immune system brought a series of stem cells into existence that sequentially give rise to lymphocytes that are similar to their predecessors but have either lost or gained functional capabilities. Inasmuch as the evolutionary success of the latest "layer" in such a system depends on its ability to introduce a selective advantage, each new layer can be expected to increase the sophistication of the immune system with respect to its ability to efficiently protect the animal against challenges within its environment. Thus, whereas B-1 cells tend to create a first line of defense by producing low affinity, broad-specificity antibodies that react with ubiquitous microorganisms, conventional B cells produce high-affinity antibodies that specifically react with particular pathogens. Similarly, the repertoire of $\gamma\delta$ T cells is considerably more restricted than the repertoire of $\alpha\beta$ T cells.[26]

This concept of an evolutionarily layered immune system presents a framework within which the B cell that we have defined here can be organized and related to the T-cell lineages defined in other recent studies. This model makes a number of testable predictions, for example, that the earliest lymphoid stem cells give rise to both B-1 cells and fetal type $\gamma\delta$ cells. Thus, it offers a potentially productive route for unraveling the complexities in the immune system.

Of course, making these kinds of lineage distinctions always poses somewhat of a dilemma. In the broadest sense, all cells in a given animal can be assigned to a single lineage, inasmuch as the zygote is the ultimate progenitor. At the other extreme, the progeny of a single, newly arisen B cell could be treated as a lineage because such B cells are distinguished from each other by unique Ig rearrangements. By and large, however, there appears to be agreement that developmental lineages should be defined as deriving from relatively undifferentiated progenitors that have at least a limited

TABLE 1. Development of the B-1 Cell Repertoire[a]

Pups (<4 weeks)
Progenitors rearrange and give rise to self-replenishing B-1 cells
B-1 repertoire potential is shaped
Adolescents (4–8 weeks)
Feedback blocks new B-1 cell development
Maximal content of the B-1 repertoire is fixed
Adults (<8 weeks)
Individual clones expand or are deleted
B-1 repertoire becomes progressively more restricted

[a] The fixation of the B-1 repertoire early in life makes it distinct from the conventional B-cell repertoire, which is continually replenished from newly arranging bone marrow cells.

capacity for self-renewal and give rise to progeny that are committed to differentiate into cells with particular functional characteristics.

The lineages we propose are consistent with this definition and build upon earlier concepts of the organization of the immune system. In essence, early studies identified three basic types of cells in the hematopoietic system, erythroid, myeloid, and lymphoid, and showed that all three types of cells are reconstituted in irradiated recipients by transfers of either adult bone marrow or fetal liver (*e.g.*, ref. 27). These and related studies led to the idea that these types of cells belong to three distinct lineages (myeloid, lymphoid, and erythroid), but that these lineages derive from a single, pluripotent progenitor (stem cell) that arises during embryonic life and persists as such into adulthood, continually differentiating into self-renewing progenitors for each of the lineages.

Our hypothesis holds to the overall outlines of this concept. As indicated above, however, technological improvements in the methods for distinguishing different types of lymphoid cells now have shown that hematopoietic stem cells in the fetus give rise to subtly, but nonetheless distinctly, different kinds of cells from the stem cells in the adult. Thus we propose that the single stem cell proposed initially is actually a series of stem cells that become functional at progressively later times during development and give rise to lymphoid and other progeny that are progressively better adapted to perform their required functions in higher species.

RAMIFICATIONS OF THE DIFFERENT B-LINEAGE DEVELOPMENTAL PATTERNS

The functional differences already defined for the B-cell lineages are consistent with the evolutionary hypothesis. B-1 developmental mechanisms provide for the ordered development of key components of the B-1 antibody repertoire,[28] which is generated and (initially) selected during the neonatal period.[16,17] The termination of B-1 development from unrearranged progenitors early in life and the persistence of B-1 cells as a self-replenishing population thereafter assures the long-term survival of this neonatally configured B-1 repertoire (TABLE 1).

Analysis of the B-1 repertoire suggests that it consists of a relatively constant core of antibodies whose functions are highly significant to survival. First-line defense against invading pathogens appears to be one of these functions, because B-1 cells respond very rapidly (frequently within 2 days of stimulation) and produce antibodies

that can react with a variety of bacterial and parasitic antigens. More esoteric roles for these cells can also be envisioned, however. For example, autoantibodies produced by B-1 cells could block or facilitate lymphocyte activation by various cytokines; or, such antibodies could block or help antigen-triggered T-cell responses. In addition, because the B-1 repertoire is essentially a reservoir of neonatal immune experience, it provides a vehicle through which maternal antibodies can exert a long-term influence on progeny-immune responses. Thus, as a whole, the B-1 repertoire is conservative in nature and lacking in flexibility.

The conventional B-cell repertoire, by contrast, is highly flexible and can vary substantially from individual to individual because it is largely determined by the nature of the antigens to which a given individual is exposed. These B cells are continually generated *de novo* from unrearranged progenitors in the bone marrow. In effect, the mechanisms that govern their development guarantee a constantly refreshed supply of randomly rearranged cells that can be selected (by antigen) into the long-term surviving B-cell pool, stimulated to develop into memory B cells and mutated to provide the high-affinity antibodies characteristic of the responses produced by these cells. Thus responses produced by conventional B cells tend to be slower, (usually beginning about day 5) but qualitatively better, than responses produced by B-1 cells.

These considerations suggest that evolution has layered the developmental mechanisms that generate conventional B cells over those that generate B-1 cells. B-1 type mechanisms have been shown to exist and to provide (apparently) adequate immune protection for reptiles, birds, and other relatively primitive species in which fetal wastage is common and/or the percentage of animals that survive and reproduce is low. Higher animals apparently require (and have evolved) a more adaptable immune system, however. This requirement apparently stems from a greater need for long-term immune protection; that is, smaller numbers of individuals tend to be conceived and born in such species, and the length of time until reproductive maturity generally tends to be longer.

The immune system in the higher animals could have evolved by jettisoning the primitive mechanisms and replacing them with ones that are better adapted to the life-style of the more advanced organisms. Comparative embryology suggests, however, that evolution tends to proceed by maintaining older structures and building new ones upon them. Thus we believe it likely that evolution has created an immune system in higher animals in which the mechanisms that generate conventional B cells are layered over those that generated self-replenishing B cells in more primitive species, and that the B-1 cells in higher animals represent the retention of useful primitive functions.

REFERENCES

1. HERZENBERG, LEONORE A., KO OKUMURA, HARVEY CANTOR, VICKI L. SATO, FUNG WIN SHEN, E. A. BOYSE & LEONARD A. HERZENBERG. 1976. T cell regulation of antibody responses: Demonstration of allotype specific helper T cells and their specific removal by suppressor T cells. J. Exp. Med. 144: 330-334.
2. CANTOR, HARVEY, ELIZABETH SIMPSON, VICKI SATO, C. GARRISON FATHMAN & LEONARD A. HERZENBERG. 1975. Characterization of subpopulations of T lymphocytes. I. Separation and functional studies of peripheral T cells binding different amounts of fluorescent anti-Thy 1.2 (theta) antibody using a fluorescein activated cell sorter. Cell. Immunol. 15: 180-196.
3. LANIER, LEWIS L., NOEL L. WARNER, JEFFREY A. LEDBETTER & LEONARD A. HERZENBERG. 1981. Expression of Lyt-1 antigen on certain murine B cell lymphomas. J. Exp. Med. 153: 998-1003.

4. HARDY, R. R., K. HAYAKAWA, J. HAAIJMAN & L. A. HERZENBERG. 1982. B-cell subpopulations identifiable by two-color fluorescence analysis using a dual-laser FACS. *In* Immunoglobulin D: Structure and Function. G. Jeanette Thorbecke & Gerrie A. Leslie, Eds.: 399: 112. Annals of the New York Academy of Sciences. New York.
5. HARDY, R. R., K. HAYAKAWA, J. HAAIJMAN & L. A. HERZENBERG. 1982. B-cell subpopulations identified by two-colour fluorescence analysis. Nature 297: 589-591.
6. HAYAKAWA, K., R. R. HARDY, D. R. PARKS & L. A. HERZENBERG. 1983. The "Ly-1 B" cell subpopulation in normal, immunodefective and autoimmune mice. J. Exp. Med. 157: 202-218.
7. HARDY, R. R., K. HAYAKAWA, D. R. PARKS & L. A. HERZENBERG. 1983. Demonstration of B-cell maturation in X-linked immunodeficient mice by simultaneous three-colour immunofluorescence. Nature 306: 270-272.
8. MANOHAR, V., E. BROWN, W. M. LEIBERSON & T. M. CHUSED. 1982. Expression of Lyt-1 by a subset of B lymphocytes. J. Immunol. 129: 532.
9. HAYAKAWA, K., R. R. HARDY, M. HONDA, L. A. HERZENBERG, A. D. STEINBERG & L. HERZENBERG. 1984. Ly-1 B cells: functionally distinct lymphocytes that secrete IgM autoantibodies. Proc. Natl. Acad. Sci. USA 81: 2494-2498.
10. STALL, A. M., M. C. FARINAS, D. M. TARLINTON, P. A. LALOR, L. A. HERZENBERG, S. STROBER & L. A. HERZENBERG. 1988. Ly-1 B-cell clones similar to human chronic lymphocytic leukemias routinely develop in older normal mice and young autoimmune (New Zealand Black-related) animals. Proc. Natl. Acad. Sci. USA 85: 7312-7316.
11. FORSTER, I., H. GU & K. RAJEWSKY. 1988. Germline antibody V regions as determinants of clonal persistence and malignant growth in the B cell compartment. EMBO J. 7: 3693.
12. HAYAKAWA, K., R. R. HARDY, L. A. HERZENBERG & L. A. HERZENBERG. 1985. Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. J. Exp. Med. 161: 1554-1568.
13. HAYAKAWA, K., R. R. HARDY, A. M. STALL, L. A. HERZENBERG & L. A. HERZENBERG. 1986. Immunoglobulin-bearing B cells reconstitute and maintain the murine Ly-1 B cell lineage. Eur. J. Immunol. 16: 1313-1316.
14. KANTOR, AARON B., ALAN M. STALL, SHARON ADAMS, LEONARD A. HERZENBERG & LEONORE A. HERZENBERG. 1992. Differential development of progenitor activity for three B cell lineages. Proc. Natl. Acad. Sci. USA. In press.
15. SOLVASON, N., A. LEHUEN & J. F. KEARNEY. 1991. An embryonic source of Ly1 but not conventional B cells. Int. Immunol. 3: 543-550.
16. LALOR, P. A., L. A. HERZENBERG, S. ADAMS & A. M. STALL. 1989a. Feedback regulation of murine Ly-1 B cell development. Eur. J. Immunol. 19: 507-513.
17. LALOR, P. A., A. M. STALL, S. ADAMS & L. A. HERZENBERG. 1989b. Permanent alteration of the murine Ly-1 B repertoire due to selective depletion of Ly-1 B cells in neonatal animals. Eur. J. Immunol 19: 501-506.
18. BLACK, SAMUEL J. & LEONORE A. HERZENBERG. 1979. B cell influences on the induction of allotype suppressor T cells. J. Exp. Med. 150: 174-183.
19. RATCLIFFE, M. J. H., O. LASSILA, J. R. L. PINE & O. VAINIO. 1986. Avian B cell precursors: surface immunoglobulin expression is an early, possibly bursa-independent event. Eur. J. Immunol. 16: 129-133.
20. HERZENBERG, L. A., A. M. STALL, P. A. LALOR, C. SIDMAN, W. A. MOORE, D. R. PARKS & L. A. HERZENBERG. 1986. The Ly-1 B cell lineage. Immunol. Rev. 93: 81-102.
21. HERZENBERG, L. A. & L. A. HERZENBERG. 1989. Toward a layered immune system. Cell 59: 953-954.
22. IKUTA, K., T. KINA, I. MACNEIL, N. UCHIDA, B. PEAULT, Y. CHIEN & I. L. WEISSMAN. 1990. A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. Cell 62: 863-874.
23. HAVRAN, W. L. & J. P. ALLISON. 1988. Developmentally ordered appearance of thymocytes expressing different T-cell antigen receptors. Nature 335: 443-445.
24. ITO, K., M. BONNEVILLE, Y. TAKAGAKI, N. NAKANISHI, O. KANAGAWA, E. G. KRECKO & S. TONEGAWA. 1989. Different gd T-cell receptors are expressed on thymocytes at different stages of development. Proc. Natl. Acad. Sci. USA 86: 631-635.

25. LAFAILLE, J. J., A. DECLOUX, M. BONNEVILLE, Y. TAKAGAKI & S. TONEGAWA. 1989. Junctional sequences of T cell receptor gd genes: implications for γδ T cell lineages and for a novel intermediate of V-(D)-J joining. Cell 59: 859-870.
26. TONEGAWA, S., A. BERNS, M. BONNEVILLE *et al.* 1989. Diversity, development, liganda, and probable functions of gd T cells. Cold Spring Harbor Symp. Quant. Biol. LIV: 31-44.
27. TILL, J. E. & E. A. MCCULLOCH. 1980. Hemopoietic stem cell differentiation. Biochem. Biophys. Acta 605: 431-459.
28. KEARNEY, J. F., M. VAKIL & N. SOLVASON. 1989. The role of idiotypic interactions and B-cell subsets in development of the B-cell repertoire. Cold Spring Harbor Symp. Quant. Biol. LIV: 203.