Do developing B cells need antigen?

Jean-Claude Weill and Claude-Agnès Reynaud

Just as potentially useful T cells are positively selected by MHC–peptide complexes in the thymus, it has been proposed that self or commensal bacterial epitopes might select B cell populations with the capacity to recognize polysaccharide or protein structures on pathogens. Recent studies indicate that the repertoire of B cells entering the periphery is not shaped by specific stimuli, but that mature B cell subsets may be under different selective pressures.

The genome of vertebrates contains a large set of genes that are involved in the function of the immune system. Among them are the V genes that code for the variable part of the B cell and T cell receptors. For T cells, the stringent selection imposed in the thymus by the host MHC–peptide complexes is known to drive the specificity of the variable part of the TCR. For B cells, the question of what selects V genes during evolution, and also during their somatic lifespan, is highly debated. What makes the B cell story more complicated is the absence of an organ equivalent to the thymus in which negative and positive selection through the B cell antigen receptor (BCR) would take place. The existence of such an organ, referred to as a "mutant breeding organ" was speculated by N.K. Jerne in 1971 (1). Jerne's proposal was in line with the idea that development of the preimmune B cell repertoire would occur by somatic mutation and selection acting on a small number of germline genes (2). At the same time, an opposing model was proposed in which antibody diversity was predicted to be the product of a large pool of V genes in the genome, already selected against specific antigens (3).

We know now that a solution in between these two propositions has evolved for the human and mouse B cell system. Functional antibody receptors are formed continuously in the bone marrow by joining 50 to 100 functional V heavy chain (V_H) and V light chain (V_L) genes to diversity (D), joining (J), and constant (C) region gene segments. The imprecision of this site-specific recombination reaction generates a large amount of antibody diversity. It is accepted that immature B cells recognizing self-epitopes with strong affinity arise at a high rate and are purged from the system during the early steps of the antibody repertoire formation in the bone marrow (4), but a role for positive selection is unclear.

Although mice and humans lack an obvious mutant breeding organ in which to study positive selection, in many species B cell development occurs in gut-associated lymphoid tissues (GALT), which may fulfill this role. In this issue, Rhee et al. (5) show that the positive selection operating on the preimmune B cell repertoire of a GALT species, the rabbit, is not specific for the antigen binding sites, but selects B cells on the basis of a few amino acid residues in the framework region of the heavy chain variable genes. We would like to discuss these results in the context of the two different modes of B cell formation: the ongoing differentiation in bone marrow or the diversification in GALT.

The GALT species

The GALT model of B cell development has been described in many species, including chicken, rabbit, and sheep, and differs completely from the bone marrow mouse model, both in terms of V gene organization and of mechanisms of B cell repertoire formation.

B cell development in GALT was first described in the chicken, in which there is one functional V_H–V_L gene pair that rearranges in each B cell during embryonic development. This unique antibody, which harbors some junctional (and heavy chain combinatorial) CDR3 variability, is diversified before and after hatching in the bursa of Fabricius, a primary lymphoid organ that differentiates during embryonic development as a dorsal diverticulum of the cloaca. This diversification takes place by gene conversion, a nonreciprocal homologous recombination process, using a large pool of pseudogenes as donors (6). An almost infinite number of antibody specificities can be generated by this process, which excludes that a selective pressure could act on the pseudogenes to retain any specificity among this vast repertoire stored in bits and pieces in the pseudogene pool. In this model, the bursa is a perfect mutant-breeding organ, where positive selection could be exerted on the BCR of the newly produced B cells by the gut-associated food and bacterial antigens that have direct access to proliferating B cells through the bursal duct. B cell development proceeds similarly in the sheep, taking place in ileal Peyer's patches before and after birth (7). In this species, postrearrangement diversification proceeds through hypermutation, using a somewhat larger gene pool than chickens (8). In the rabbit, despite the fact that there are many functional V_H genes, only one is rearranged in most B cells. This V_H gene is further diversified by gene conversion and hypermutation in the appendix and ileal Peyer's patches during development (9). The main difference with chicken and sheep is that B cell development starts after birth in rabbits and requires the colonization of the gut flora.

J.-C.W. and C.-A.R. are at INSERM U373, Faculté de Médecine René Descartes, 75730 Paris Cedex 15, France.

CORRESPONDENCE
J.-C.W.: weill@necker.fr
OR
C.-A.R.: reynaud@necker.fr

Common features of B cell development in the GALT species, which include also cattle, pigs, horses, and dogs, are the use of a restricted set of *V* genes for rearrangement and an almost unlimited amount of diversity generated by gene conversion and/or hypermutation after rearrangement (10). The development and diversification of B cells occur in lymphoid follicles along the gut during the first months of life, with the primary B cell organ involuting or behaving as a secondary lymphoid organ after that stage. In addition, there is no de novo production of B cells throughout the life of the animal, implying that the naive B cell clones that have reached the periphery during ontogeny will remain and/or self-renew for the life of the animal.

Transitional B cells

In these somatic models of B cell repertoire formation, it seems plausible that positive selection triggered by recognition of epitopes on commensal bacteria could send an army of B cells to the periphery selected to recognize similar structures on pathogens. However, in the chicken ligation of the bursal duct slowed down B cell proliferation but did not affect diversity (11). In sheep, isolating the B cells from the influence of gut constituents, either surgically in ileal loops or by rearing the animals in germ-free conditions, similarly slowed B cell proliferation but did not affect the rate or targeting of hypermutation (12). Both cases suggested that bacteria were acting as mitotic stimuli and not as selective agents through the antibody receptor. The elegant experiments by Rhee et al. (5) now indicate, as proposed previously by Rose Mage and colleagues (13), that only some gut bacteria are mitogenic during the formation of the rabbit preimmune repertoire through a superantigen-like effect. B cell superantigens stimulate proliferation through the BCR by binding to framework regions which are conserved in *V* gene families. Such a selection process fits well with the structure of the V_H repertoire of rabbit, sheep, or chicken, which comprises a single gene family.

These findings indicate that positive selection is not happening through the BCR antigen binding site. Since the chicken bursa, the sheep ileal Peyer's patches, and rabbit appendix are the primary B cell–producing organs in these animals, the B cells emanating from these organs on their way to the peripheral lymphoid tissues may be considered the equivalent of transitional B cells exiting from the bone marrow in mouse and man. In the latter, a constitutive tonic BCR signal that is independent of antigen binding seems necessary to check the health of the BCR (14, 15) (Fig. 1). A mitotic signal independent of the BCR combining site seems necessary in GALT species to ensure an intense B cell proliferation, allowing the ongoing diversification of the antibody receptor expressed by these cells. In both models, negative selection must actively delete autoreactive clones, and even more so in the mutant breeding organs: only 5% of B cells produced in the sheep GALT are allowed to emigrate to the periphery (16).

B1 and marginal zone B cells

The signal for entry into the mature B cell populations mediating T-independent responses against carbohydrate antigens may also differ between species. In rodents, these responses are mediated largely by B1 and marginal zone B cells that carry germline-encoded antibodies. B cells are most probably selected to the B1 and marginal zone B cell lineages on the basis of their binding specificity as they are generated (17, 18). In GALT species, however, there are few B cells in the periphery displaying a germline encoded antibody receptor, since most B cells leaving the primary B cell organ are highly mutated. T-independent antibody responses against bacterial polysaccharides will therefore involve antibodies with a large range of specificities and affinities. Given the absence of specific selection exerted by the gut bacterial constituents as these B cells develop in the GALT (5), it is difficult to imagine that such selection will occur once they have left this site.

In humans, there is no clear B1 cell subset, and T-independent antipolysaccharide responses are mediated by marginal zone B cells, which harbor mutated antibody genes (19). Moreover, recent work from our laboratory suggests that human marginal zone B cells, similar to B cells in GALT species, diversify their antibody receptors by hypermutation during development be-
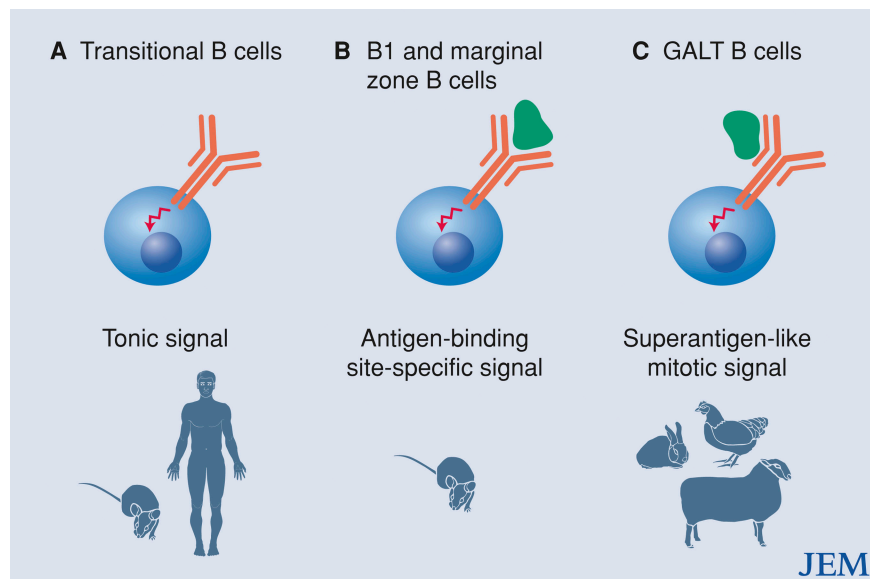
Figure 1. Different modes of BCR signaling are referred to as positive selection. (A) A constitutive tonic signal through the BCR and the Igα–Igβ complex. (B) An authentic antigen binding site–specific signal. (C) A superantigen-like signal that acts through binding of framework residues in the *V* regions. Examples of B cell subsets in which such type of positive selection takes place are shown.

fore external antigen stimulation (20). The intrinsic diversification taking place in this subpopulation may thus preclude a positive selection step on the antigen binding site of B cells involved in T-independent responses in humans and rather supports a nonspecific mitotic stimulation.

Long-lived recirculating B cells

There remains the question of whether there is an antigen-specific positive selection for B2 cells at the stage of entry in the long-lived recirculating pool. In mouse and man, naive B cells recirculating in the periphery carry germline-encoded receptors. The essential property of this germline-encoded BCR will be to fold and to signal correctly. This function is probably assayed constantly throughout the B cells lifetime with the requirement for BCR–dependent survival signals (21). On antigen encounter, the antigen binding site of B2 cells shifts from a loose poly-specific structure to a tighter conformation created by hypermutation and selection in germinal centers that is highly specific for that particular antigen (22). In GALT species, the naive repertoire already displays mutated antibodies, the specificity of which will be improved by further steps of diversification in responding germinal centers. In both models, it remains a matter of debate whether a tonic, antigen-independent signal will be sufficient for entry in the long-lived recirculating naïve B cell pool, or whether this step will also involve, as suggested from several experimental settings, a more specific BCR signaling event (23, 24).

Concluding remarks

In conclusion, although there may be a pathogen-driven selective force for some specific genomic *V* genes, for most of them evolution has simply ensured that they can be efficiently targeted by somatic diversification processes (12, 25, 26). It is clear that positive selection through the BCR specificity does not occur at the transitional stage in GALT species as shown by Rhee et al. (5), and the same may be true for the equivalent B cell stage in species where B cell development occurs in the bone marrow. In rodents, an antigen binding site–dependent positive selection step may select the B1 and marginal zone B cells, which are mainly responsible for responses against bacterial polysaccharides, but such a specific selection may not occur in the functionally equivalent population of either GALT species or humans.

REFERENCES

1. Jerne, N.K. 1971. The somatic generation of immune recognition. *Eur. J. Immunol.* 1:1–9.
2. Cohn, M. 1970. Selection under a somatic model. *Cell. Immunol.* 1:461–467.
3. Hood, L., and D. Talmage. 1970. Mechanisms of antibody diversity: germ line basis for variability. *Science.* 168:325–334.
4. Wardemann, H., S. Yurasov, A. Schaefer, J.W. Young, E. Meffre, and M.C. Nussenzweig. 2003. Predominant autoantibody production by early human B cell precursors. *Science.* 301:1374–1377.
5. Rhee, K.-J., P.J. Jasper, P. Sethupathi, M. Shanmugam, S.-K. Zhai, and K.L. Knight. 2005. Positive selection of the peripheral B cell repertoire in gut-associated lymphoid tissues. *J. Exp. Med.* 200:55–62.
6. Reynaud, C.-A., B. Bertocci, A. Dahan, and J.-C. Weill. 1994. Formation of the chicken B cell repertoire: ontogenesis, regulation of Ig gene rearrangement and diversification by gene conversion. *Adv. Immunol.* 57:353–378.
7. Reynolds, J.D., and B. Morris. 1983. The evolution and involution of Peyer's patches in fetal and postnatal sheep. *Eur. J. Immunol.* 13:627–635.
8. Reynaud, C.A., C. Mackay, R.G. Mueller, and J.-C. Weill. 1991. Somatic generation of diversity in a mammalian primary lymphoid organ: the sheep ileal Peyer's patches. *Cell.* 64:995–1005.
9. Becker, R.S., and K.L. Knight. 1990. Somatic diversification of immunoglobulin heavy chain VDJ genes: evidence for somatic gene conversion in rabbits. *Cell.* 63:987–997.
10. Lanning, D., B. Osborne, and K.L. Knight. 2004. Immunoglobulin genes and generation of antibody repertoires in higher vertebrates: a key role for GALT. *In* Molecular Biology of B Cells. F.W. Alt, T. Honjo, and M.S. Neuberger, editors. Elsevier Science Co., Inc., New York, NY. pp 433–448.
11. Lassila, O., I. Lefkovits, and A. Alanen. 1989. Immunoglobulin diversification in bursal duct-ligated chickens. *Eur. J. Immunol.* 19:1343–1345.
12. Reynaud, C.-A., C. Garcia, W.R. Hein, and J.-C. Weill. 1995. Hypermutation generating the sheep antibody repertoire is an antigen-independent process. *Cell.* 80:115–125.
13. Pospisil, R., G.O. Young-Cooper, and R.G. Mage. 1995. Preferential expansion and survival of B lymphocytes based on V_H framework 1 and framework 3 expression: "positive" selection in appendix of normal and V_H–mutant rabbits. *Proc. Natl. Acad. Sci. USA.* 92:6961–6965.
14. Bannish, G., E.M. Fuentes-Panana, J.C. Cambier, W.S. Pear, and J.G. Monroe. 2001. Ligand-independent signaling functions for the B lymphocyte antigen receptor and their role in positive selection during B lymphopoiesis. *J. Exp. Med.* 194:1583–1596.
15. Wienands, J., O. Larbolette, and M. Reth. 1996. Evidence for a preformed transducer complex organized by the B cell antigen receptor. *Proc. Natl. Acad. Sci. USA.* 93:7865–7870.
16. Reynolds, J.D. 1986. Evidence of extensive lymphocyte death in sheep Peyer's patches. I. A comparison of lymphocyte production and export. *J. Immunol.* 136:2005–2010.
17. Hayakawa, K., M. Asano, S.A. Shinton, M. Gui, D. Allman, C.L. Stewart, J. Silver, and R.R. Hardy. 1999. Positive selection of natural autoreactive B cells. *Science.* 285:113–116.
18. Lopez-Carvalho, T., and J.F. Kearney. 2004. Development and selection of marginal zone B cells. *Immunol. Rev.* 197:192–205.
19. Lucas, A.H., and D.C. Reason. 1999. Polysaccharide vaccines as probes of antibody repertoires in man. *Immunol. Rev.* 171: 89–104.
20. Weller, S., M.C. Braun, B.K. Tan, A. Rosenwald, C. Cordier, M.E. Conley, A. Plebani, D.S. Kumararatne, D. Bonnet, O. Tournilhac, et al. 2004. Human blood IgM "memory" B cells are circulating splenic marginal zone B cells harboring a pre-diversified immunoglobulin repertoire. *Blood.* 104: 3647–3654.
21. Kraus, M., M.B. Alimzhanov, N. Rajewsky, and K. Rajewsky. 2004. Survival of resting mature B lymphocytes depends on BCR signaling via the Igalpha/beta heterodimer. *Cell.* 117:787–800.
22. Wedemayer, G.J., P.A. Patten, L.H. Wang, P.G. Schultz, and R.C. Stevens. 1997. Structural insights into the evolution of an antibody combining site. *Science.* 276:1665–1669.
23. Gaudin, E., M. Rosado, F. Agenes, A. McLean, and A.A. Freitas. 2004. B-cell homeostasis, competition, resources, and positive selection by self-antigens. *Immunol. Rev.* 197:102–115.
24. Pillai, S., A. Cariappa, and S.T. Moran. 2005. Marginal zone B cells. *Annu. Rev. Immunol.* 23:161–196.
25. Wagner, S.D., C. Milstein, and M.S. Neuberger. 1995. Codon bias targets mutation. *Nature.* 376:732.
26. Xu, J.L., and M.M. Davis. 2000. Diversity in the CDR3 region of V(H) is sufficient for most antibody specificities. *Immunity.* 13:37–45.