Maintenance of immunological memory: a role for CD5+ B cells?

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How memory is retained is an immunological mystery. One possibility, argued here by Fons UytdeHaag and colleagues, is that memory is imprinted in the somatically-mutated Ig expressed by certain CD5+ B cells. The theory proposes that the Ig expressed by this self-renewing population acts as surrogate antigen, selecting and stimulating emerging antigen-specific lymphocytes.

Basic elements in life-long immunity against a pathogen include the generation and maintenance of immunological memory, that is the state of the immune system that results in a secondary immune response upon re-encounter with the antigen. The pathways of memory cell generation are well defined[1–4] but the mechanism(s) responsible for the maintenance of immunological memory is poorly understood. In rodents, memory has been variously ascribed to long-lived cells, to the continuous proliferation of mature antigen-specific cells and to the continuous input of newly formed short-lived cells from the bone marrow[5–9]. Persistence of antigen may be fundamental to B-cell longevity, although there is no definitive proof[10–14].

Here, an alternative hypothesis is proposed for the maintenance of memory, based on the integration of B cells bearing somatically-mutated Ig, into the B-cell network.

Development of 'memory' B cells

After encountering T-cell-dependent or T-cell-independent antigen, a virgin, resting B cell either develops into an antibody-secreting plasma cell or a 'memory' B cell that can respond upon re-encounter with antigen[1,3]. Primary B cells and 'memory' B cells differ in many respects[3,15]. Most importantly, in a primary response, plasma cells usually produce unmutated, multireactive antibodies with low affinity for the antigen, whereas activation of 'memory' B cells results in the production of specific antibody with high affinity for the antigen. Somatic hypermutation, acting selectively on rearranged antibody variable region genes, and antigen-driven selection of mutations are the basis of affinity maturation of the specific antibody molecules expressed by 'memory' B cells[1–3,16–18].

CD5+ B cells

The investigation of B-cell development in mice has revealed two B-cell 'lineages' that appear to develop along separate differentiation pathways (see Refs 19 and 20 for review). B cells emerging continuously from the bone marrow enter the periphery and may be selected by antigen into conventional immune responses (*vide supra*). A functionally distinct 'lineage' of B cells, expressing the CD5 (Ly-1) molecule and apparently not participating in conventional immune responses, arises early in ontogeny, possibly from a source of stem cells independent of the bone marrow[19–22]. These B cells, which predominate in early life but represent only a minor fraction of the B cells in the adult[23], appear to have the capacity for self renewal[24]. They make up a proportion of the B cells that express immunoglobulin with inherent low affinities for self antigens[25]. They produce the so-called natural IgM antibodies that display multireactivity and harbor specificities for certain bacterial antigens[19,20,24,26–29]. In humans, CD5+ B cells (Leu-1+) with similar functional characteristics exist[30–32] (see Refs 19 and 20 for review).

CD5+ B cells in both humans and mice frequently express V_H and V_L germ-line genes in the absence of somatic mutation[33–36]. However, recently we[37] and others[38] have identified somatic mutations in expressed V_H genes that lead to amino acid replacements, which accumulate in the complementarity-determining regions, CDR-I and CDR-II, in human CD5+ Epstein–Barr virus (EBV)-transformed peripheral blood B cells of healthy adults or patients with rheumatoid arthritis[38]. These observations indicate that somatic mutants of CD5+ B cells may be selected by antigen. In the mouse the possibility that *V* genes of CD5+ B cells can be subject to antigen-driven somatic hypermutation has not been directly addressed.

Immune networks

In the mouse there is a high frequency of mutual V_H region interactions, known as connectivity, within the set of antibodies that arises during early B-cell ontogeny[22,39,40]. A large part of this early repertoire is constituted by B cells belonging to the CD5+ B-cell 'lineage' (see Refs 19 and 20 for review). Because of the autonomous activities of CD5+ B cells and the self reactivity and high connectivity of their IgM antibodies, it has been proposed that these cells and their secreted antibodies establish immune networks by interacting with self constituents, including elements of the immune system itself[41]. These immune networks may be essential in the initial development of B-cell and T-cell repertoires, in self–nonself discrimination and in the maintenance of tolerance and memory[41,42]. Immune networks may have little to do with conventional immune responses, which

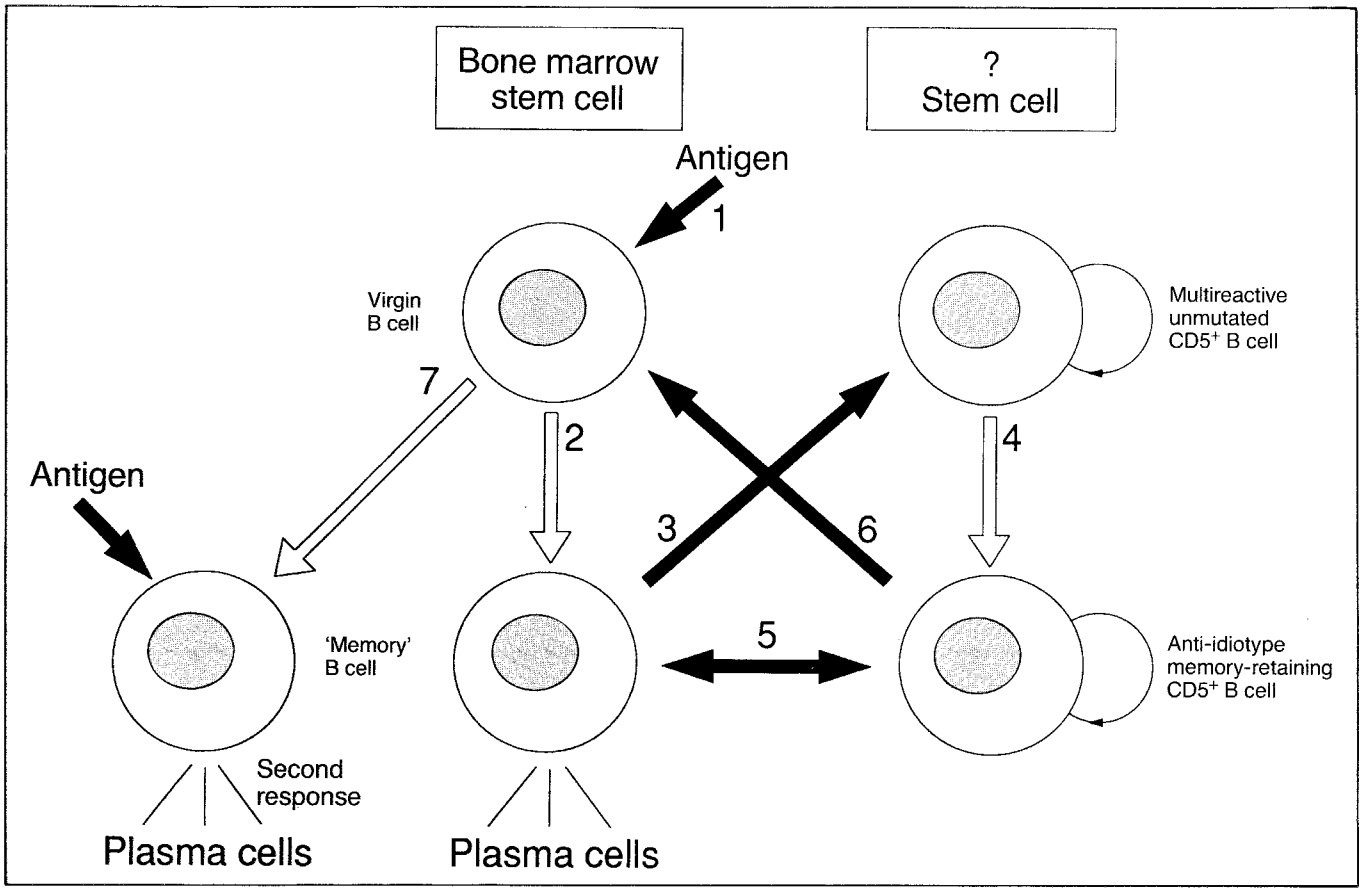

Fig. 1. *A possible scheme for the generation and maintenance of B-cell memory is shown. Solid arrows indicate selection/stimulation by antigen/idiotype; open arrows indicate affinity maturation of antibody V regions; circles with arrows indicate capacity for self renewal.*

are proposed to be discrete, nonnetworked responses that are selected by external antigen[41,42].

How can memory be a property of an immune network if memory B cells originate in the 'unconnected set'? It has been proposed that, although disconnected from immune networks when functioning as effector cells, 'memory' B cells do make connections with immune networks and these interactions determine their survival as 'memory' cells in the resting state. From this viewpoint, survival of lymphocytes in the resting state and the activation of effector functions or proliferation, as well as their decline, are determined by the affinities and concentrations of the receptors involved in the clonal interactions[41,42]. It is our contention that the influence of immune networks on the lifespan of 'memory' B cells is due to regulation or control of clonal responses to antigen by networks, rather than 'imprinting the experience of the immune system with antigen in immune networks'[41], which would make memory a real systemic property.

How does the immune system 'learn about antigens'? Perhaps this occurs via idiotype-driven somatic mutation and selection of antibody V regions of CD5$^+$ B cells.

Memory: a systemic property

Repeated stimulation with the same external antigen results in clonal proliferation and selection of somatic B-cell mutants that express high-affinity receptors for the antigen (see Fig. 1, arrows 1 and 2).

Somatic mutation may alter the *V* regions of antigen-specific 'memory' B-cell clones such that their *V* regions interact with *V* regions of one or more members of the interconnected B-cell network. This does not necessarily imply an integration of these antigen-specific B cells into a network. Because of the temporarily increased clone sizes of antigen-specific 'memory' B cells during the response to antigen, and the greatly increased concentrations of their somatically-mutated *V* regions, the anti-idiotypic CD5$^+$ B-cell clones to which they fit will be stimulated to clonal expansion (see Fig. 1, arrow 3). We propose that *V* region genes encoding anti-idiotype antibodies of CD5$^+$ B cells of immune networks are subject to a process of somatic hypermutation and are then selected by somatically-mutated idiotypes of 'memory' B-cell clones (see Fig. 1, arrow 4). In this way anti-idiotypic CD5$^+$ B cells, which really carry memory in the form of the imprint of the experience of the immune system with antigen, are expanded. Consequently, after antigen clearance, the somatically mutated antibody V regions of these anti-idiotypic memory B cells may stimulate the clonal expansion of mature antigen-specific 'memory' B cells or simply keep these cells alive as 'long-lived' resting cells (see Fig. 1, arrow 5). In either case, immunological memory is maintained in the absence of antigen by a population of self-renewing B cells, the somatically-mutated V regions of which mimic antigen.

In addition, the memory-retaining CD5$^+$ B cells could

select virgin resting B cells or pre-B cells, which daily emerge in adult bone marrow (see Fig. 1, arrow 6). In contrast to anti-idiotypic CD5$^+$ B cells, which are operative in the development of repertoires during ontogeny[41,42], anti-idiotypic memory-retaining CD5$^+$ B cells will select for specificity rather than connectivity. In other words, anti-idiotypic memory-retaining CD5$^+$ B cells expressing somatically-mutated antibody V regions that mimic antigen will select B cells with those combinations of germ-line *V* genes that are most likely to give rise to high-affinity antibody upon re-encounter with antigen (see Fig. 1, arrows 6 and 7).

This proposition raises questions related to the basis of connectivity in immune networks: if antibody *V* regions of CD5$^+$ B cells are subject to somatic hypermutation and now select antigen-specific *V* regions, how, if at all, is their connectivity in immune networks maintained? Perhaps the nucleotide sequences in framework I (amino acid residues 6–24) and in framework III (amino acid residues 67–85) that are conserved between human and murine V_H genes of related families[43] will help to provide an answer. The conservation of these V_H-family-specific coding sequences is especially striking among V_H genes that are preferentially expressed in the early B-cell repertoire. Kearney and co-workers[22] postulated a role for these conserved sites on natural IgM antibodies in establishing connectivity in the developing immune networks. We recently described a human EBV-transformed, CD5$^+$, anti-idiotypic B-cell clone from peripheral blood lymphocytes of a healthy adult deliberately immunized with rabies virus vaccine[37]. This clone showed monoreactivity and high affinity ($K_d = 8.67 \times 10^{-9}$ mol l^{-1}) for its idiotype, which is located in or near the combining site of a monoclonal antibody mapping a linear epitope of the rabies virus glycoprotein[44]. Remarkably, the V_H gene encoding this antibody showed, apart from amino acid replacements in CDR-I and CDR-II, five amino acid replacements in framework III, three of which were located in the conserved site spanning residues 67–85 (Ref. 37). It is, therefore, conceivable that an anti-idiotypic memory-retaining CD5$^+$ B-cell clone selects for specificity when its V region mimics antigen and it can no longer establish connectivity, that is when the antibody framework III region is modified by somatic mutations.

A corollary of this theory is that, although memory is retained exclusively within anti-idiotypic CD5$^+$ B-cell clones and is thus a property of B cells that have originated from immune networks, memory is not necessarily distributed in immune networks, because anti-idiotypic memory-retaining CD5$^+$ B cells have become disconnected.

We have refrained from discussing a possible role for T cells in the generation and maintenance of immunological memory. Melchers[45] recently proposed a model in which two B cells expressing complementary somatically-mutated antibody V regions act in concert with T cells to maintain immunological memory. In his model, processed idiotypic fragments presented via major histocompatibility complex (MHC) class I or class II molecules may activate helper or cytotoxic (suppressor) T cells, respectively, thereby regulating the response of the respective B-cell clones.

In conclusion, we propose that somatically-mutated antibody *V* regions of CD5$^+$ anti-idiotypic memory B cells, which have the capacity for self renewal, may maintain B-cell memory in the absence of antigen. In addition, it is conceivable that these structures also act as 'crossreactive peptides'[14] in the maintenance of T-cell memory[46].

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