

Selecting B cells and plasma cells to memory

Thomas Dörner and Andreas Radbruch

Humoral immunity appears to be based on immunological memory provided by memory plasma cells, which secrete protective antibodies, and memory B cells, which react to antigen challenge by differentiating into plasma cells. How these differentiation pathways relate to each other, how cells are selected into these memory populations, and how these populations are maintained remains enigmatic.

More than 100 years after the original discovery of humoral immunity by Kitasato and Behring in 1891 (1), we have finally reached an understanding of antibodies, which are the molecular basis of humoral immunity. Understanding the cells that make these antibodies, however, remains a challenge. The work of Blink and collaborators in this issue (2) provides evidence that in systemic immune responses that are dependent on help by T lymphocytes both memory B cells and memory plasma cells are generated in secondary lymphoid organs; the two cell types then exit these organs independently and compete for survival niches with preexisting memory cells. In the primary immune response of mice to the experimental antigen 4(hydroxy-3)-nitrophenyl, coupled to keyhole limpet hemocyanin (NP-KLH), both memory B cells and antibody-secreting cells with specificity for the antigen appear in the blood within 1 week after immunization. This is surprisingly early, and as fast as in secondary immune reactions in humans. Odendahl and collaborators have recently shown the appearance of a wave of antibody-secreting cells on days 6 and 7 after secondary immunization of humans with tetanus antigen, whereas antigen-binding memory B cells appeared only from day 8 onwards and remained constant in numbers at least until day 34 (3). The classification of cells in the present study by Blink et al.

(2) is based on several surrogate markers of B cell differentiation (B220, PNA-binding, CD38, and CD138), the expression of surface antibodies that bind antigen versus the secretion of antigen-specific antibodies, and expression of the transcription factor Blimp-1, a hallmark of plasma cell differentiation. Both memory B cells and memory plasma cells express affinity-matured, class-switched antibodies, and thus are probably the output of germinal center reactions, although recent work suggests that somatic mutation can also occur in B cells activated outside of germinal centers (4, 5).

Selection of memory B cells

Blink et al. (2) showed that although the numbers of circulating memory B cells remained constant from early on in the immune response, the affinity of their antibodies increased. Also, the circulating antigen-specific B cells were attracted by the chemokine CXCL13, a ligand of CXCR5, which allows cells to navigate back into the secondary lymphoid organs. Surprisingly, interruption of the immune response on day 7 by ablation of T cell help eliminated at least 50% of the circulating antigen-binding B cells with memory phenotype, and there is no evidence that the other 50% are really long-lived. This raises the question whether the antigen-specific B cells circulating early in the response are bona fide memory B cells. With respect to CD38 and PNA staining, they have a memory phenotype, but their lifespan in the absence of restimulation is rather limited. Apparently, recruitment of circulating B cells to the memory pool requires additional

instruction. Alternatively, circulating B cells might only become memory B cells if they find appropriate survival conditions outside of restimulating secondary lymphoid organs. Thus, similar to plasma cells, there might be short- and long-lived memory B cells likely determined by their affinity for the respective antigen and their survival potency, which is determined by the expression of genes that mediate survival signals and prevent apoptosis (Fig. 1). Inside and outside of “niches” providing the essential survival signals, the phenotype of such B cells may be the same, but their functions and lifespan could be very different.

Although it is not clear whether recirculating antigen-binding B cells are true memory B cells, in any case these cells do not have an impressive potential to develop into antibody-secreting cells upon adoptive transfer in the absence of antigen. Blink et al. (2) performed this experiment on day 14 of the primary immune response, and confirmed that there are essentially no cells circulating in blood that can develop into antibody-secreting cells within 4 days in the spleen or bone marrow in the absence of antigen. It remains unclear whether the few antibody-secreting cells (<100) detected in spleen and bone marrow after transfer of 10^7 blood leukocytes have been derived from the transferred memory B cells or from antibody-secreting cells. Although the authors do not explicitly comment on it, the 75 antibody-secreting cells recovered 4 days after transfer of cells from spleen and bone marrow nicely correspond to the 50–75 antibody-secreting cells contained in the 10^7 blood leukocytes transferred on day 14 after immunization, as can be estimated from the data in Fig. 5 of the paper (2). Unfortunately, the adoptive transfer of antibody-secreting cells from blood has not been successful so far, so it is difficult to confirm this assumption directly.

T.D. and A.R. are at University Hospital Berlin and Deutsches Rheumaforschungszentrum Berlin, 10098 Berlin, Germany.

CORRESPONDENCE

T.D.: thomas.doerner@charite.de

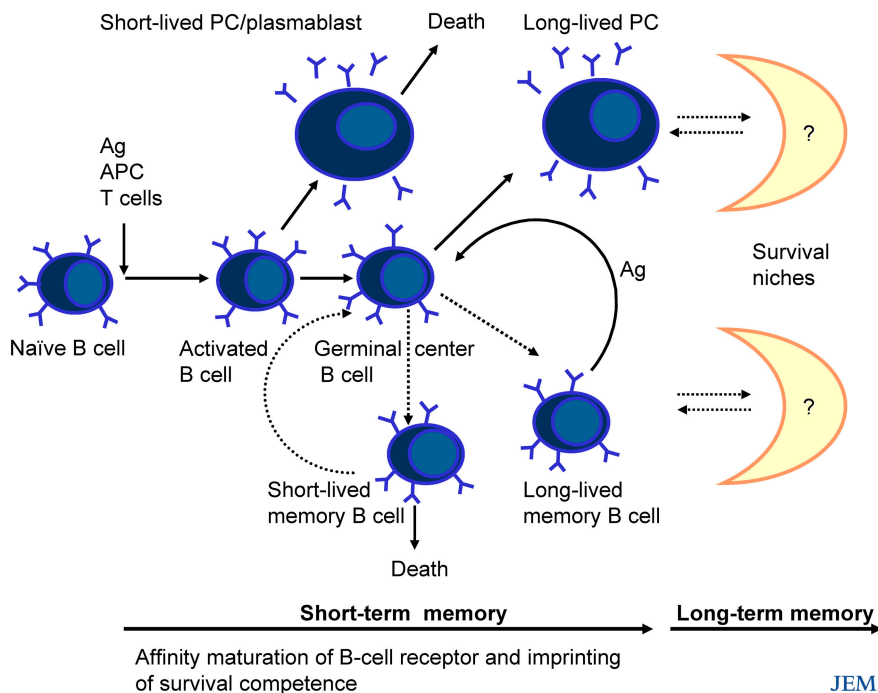


Figure 1. Hypothetical model for the generation of short- and long-lived memory B cells and antibody-secreting cells in primary immune reactions. Short- and long-lived memory B cells and plasma cells are the product of not only affinity maturation but also of an acquired ability to survive, which likely is related to successful competition for survival niches and factors.

Selection of memory plasma cells

In the adoptive transfer experiments of Blink et al. (2), it is remarkable that on day 14 of a primary immune response only 25 antibody-secreting cells can be transferred with 10^7 blood leukocytes to the bone marrow of a recipient, whereas 25,000 antigen-specific antibody-secreting cells are present in the bone marrow of the donor at this time (again calculated from Fig. 5, B and C [reference 2]). When did these cells arrive in the bone marrow from the blood? In human secondary immune reactions, a wave of specific antibody-secreting cells emerges in blood on days 6–8, and very few are found thereafter (3, 6). Admittedly, this cannot directly be compared with primary murine immune responses, but the question remains, how long would a similar wave of antibody-secreting cells be detectable in the small volume of murine blood? In the data shown in Fig. 5 A of the paper (2), there is no difference between day 0 and day 7 with respect to the blood frequencies of $CD138^+$ and *Blimp-1*-expressing cells. One could argue that for primary immune responses the wave of blood-antibody secreting

cells could be delayed or spread out over a longer period of time. The present data do not rule out this possibility, but there was no indication of an increase in blood-borne $CD138^+$, *Blimp-1*-expressing cells on day 9 either.

In the same analysis (2), blood taken on day 7 of the mouse primary immune response lacked not only a significant population of antigen-specific antibody-secreting cells, it also lacked a population of cells expressing high levels of *Blimp-1*. Such cells are found in spleen and bone marrow on day 7, and they may represent more mature antibody-secreting cells, probably plasma cells. In the human secondary response to tetanus vaccine, such cells are mobilized into the blood and are characterized as cells expressing little MHC class II (3). Interestingly, these human plasma cells are apparently not specific for the vaccine. Their numbers are increased markedly, at the same time when vaccine-specific antibody-secreting cells are found in the blood. The latter express MHC class II highly, a hallmark of plasmablasts, but little *CXCR3* and *CXCR4*. Nevertheless, these plasmablasts can migrate in

response to the corresponding chemokines. Studies on the migration potential of murine plasmablasts and plasma cells suggest that by 12 days after their generation, in the course of their differentiation from plasmablasts into plasma cells, antibody-secreting cells have switched their response to *CXCL12*, the ligand of *CXCR4*, from migration to survival (7). This observation has prompted us to speculate that plasmablasts, when competing with resident plasma cells of the bone marrow, may have a competitive advantage, because once dislodged from their survival niches plasma cells cannot relocate efficiently (3). The lack of migration of plasma cells towards chemoattractants may also explain why adoptive transfers of bone marrow-resident plasma cells poorly reconstitute the host (8). In the murine primary immune response, Blink et al. (2) found no evidence for mobilization of resident plasma cells from the bone marrow into the blood, but the analysis does not focus on whether plasmablasts of the primary immune response compete with resident plasma cells of the bone marrow for survival niches, and provides no clue on the long-term stability of antigen-specific serum antibody titers and numbers of antibody-secreting cells in the bone marrow.

Instead, the analysis focuses on the question of whether there is an antigen-binding immediate precursor of plasma cells not expressing B220 in spleen or bone marrow, as had been described previously. Such cells should express *Blimp-1*, since they have not been found in *Blimp-1*-deficient mice. Apart from the question of whether such cells do exist at all (9), they do definitely not exist in the primary immune response analyzed here, among the cells binding the antigen. It should be noted that in previous experiments (10) adoptively transferred, B220-negative bone marrow cells did not react to antigen by differentiation into antibody-secreting cells in a secondary murine immune reaction either, leaving little room for such memory cells in immunity.

Selection of high affinity

Affinity maturation is a hallmark of immunological memory, but how are cells

expressing antibodies of high affinity for the antigen selectively recruited to the memory compartment? In the primary immune response analyzed by Blink et al. (2), B cells with memory phenotype leave the secondary lymphoid organs for the blood ~2–3 days after somatic hypermutation of their antibody genes, that is, at any time point of the immune reaction the mutation frequency of blood B cells equals that of splenic B cells 2–3 days previously. However, B cells expressing antibodies with the high affinity mutation 33Trp/Leu were released into the blood as soon as they were generated. Since the numbers of specific memory B cells in blood remain constant over the first 28 days of the immune reaction, the increasing mutation frequency in this population must be based on turnover of the cells. This turnover could be due to a limited lifespan of the B cells released early on in the immune response, or to recirculation of blood B cells into the secondary lymphoid organs and their further affinity maturation there, as discussed by Blink et al. (2). In conjunction with the preferential recruitment of B cells with high affinity antibodies to the blood, and irrespective of the underlying mechanism, this turnover will result in a population of the most affinity-matured blood-borne antigen-specific B cells at the end of the immune response. Whether all or just some of these B cells are then recruited to the compartment of long-lived memory B cells remains to be shown (Fig. 1).

The preferential recruitment of cells secreting high affinity antibodies into the bone marrow is another interesting observation of Blink et al. (2). Such cells probably can no longer signal through an antigen receptor, and any selective advantage would have to be implemented before their differentiation into antibody-secreting cells. Indeed, the affinity maturation observed between day 7 and day 14 for cells secreting specific antibodies, in bone marrow and blood, could simply reflect a lack of survival potency of antibody-secreting cells generated in the early phase of a primary immune response. It will be interesting to learn whether antibody-secreting cells of primary immune responses have up-regulated expression of antiapoptotic genes and receptors for survival signals, such as CXCR4 (7). One could imagine that antibody-secreting cells will persist in the bone marrow if they are generated from memory B cells expressing high affinity antibodies, and have already been programmed for survival, for example, by up-regulating antiapoptotic genes. Such a mechanism would ensure that protective humoral immunity by serum antibodies is provided by plasma cells secreting antibodies with an affinity as high as the affinity of the antibodies of the corresponding long-lived memory B cell population, that is, the reactive humoral memory.

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