

Competence and competition: the challenge of becoming a long-lived plasma cell

Andreas Radbruch^{*†}, Gwendolin Muehlinghaus^{*}, Elke O. Luger[§], Ayako Inamine^{*}, Kenneth G. C. Smith^{||}, Thomas Dörner^{*¶} and Falk Hiepe^{*‡}

Abstract | Plasma cells provide humoral immunity. They have traditionally been viewed mainly as short-lived end-stage products of B-cell differentiation that deserve little interest. This view is changing, however, because we now know that plasma cells can survive for long periods in the appropriate survival niches and that they are an independent cellular component of immunological memory. Studies of the biology of plasma cells reveal a mechanism of intriguing simplicity and elegance that focuses memory provided by plasma cells on recently encountered pathogens while minimizing the 'fading' of memory for pathogens encountered in the distant past. This mechanism is based on competition for survival niches between newly generated plasmablasts and older plasma cells.

^{*}Deutsches Rheuma-Forschungszentrum Berlin, Charitéplatz 1, D-10117 Berlin, Germany.

[†]Department of Rheumatology and Clinical Immunology, Charité - Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany.

[§]Allergy-Center-Charité, Department of Dermatology and Allergy, Charité - Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany.

^{||}Cambridge Institute for Medical Research and the Department of Medicine, University of Cambridge School of Clinical Medicine, Box 139, Addenbrooke's Hospital, Cambridge CB2 2XY, UK.

[¶]Clinical Hemostaseology and Transfusion Medicine, Charité - Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany.

Correspondence to A.R.
e-mail: radbruch@drfz.de
doi:10.1038/nri1886

Published online
15 September 2006

Secreted antibodies provide specific humoral immunity¹. The biology of plasma cells, which secrete these antibodies², remains enigmatic and is a matter of considerable debate. Of particular interest is whether plasma cells are long-lived and form a pool of memory plasma cells that is separate from the pool of memory B cells^{3–7}. In principle, there are three competing concepts (FIG. 1), which are not mutually exclusive, that might explain humoral memory (that is, the continued presence of antibodies of a given specificity). First, memory might arise by continued generation of short-lived plasma cells from memory B cells, a process that would be driven by persisting antigen⁷. Second, memory might arise by continued generation of long-lived plasma cells with a defined half-life from memory B cells, a process that would be activated by signals from cytokine receptors and Toll-like receptors^{4,8}. And/or, third, memory might arise by unconditional survival of plasma cells in survival niches in the bone marrow^{3,5,9–11}.

The lifespan of plasma cells has been proposed to range from several days to several months^{5,9,12,13}. Here, we discuss the alternative view that plasma-cell survival is conditional. Recent evidence indicates that plasma cells rapidly die as a result of the stress on the endoplasmic reticulum (ER) that is caused by massive production of antibody, unless an unfolded-protein response (which is protective) is induced by external survival signals¹⁴. Such signals contribute to the formation of a molecular

survival niche, which can be located in secondary lymphoid organs, inflamed tissue or the bone marrow. Plasma-cell precursors, which are known as plasmablasts, migrate to these niches, and after they arrive, their ability to respond to survival signals depends on the stimuli that they received during earlier activation. For plasmablasts, the migratory phase lasts only ~1 week, which is a short time within which to find a survival niche. Owing to survival signals that are derived from the niche, plasma-cell survival is not limited, and plasma cells are long-lived. The number of niches is finite, thereby limiting the number of long-lived plasma cells. If a niche is destroyed — for example, by resolution of inflamed tissue — or if a plasma cell is dislocated from its niche, it will die. This concept presents a simple way to maximize antibody production at the peak of an immune response and to maintain a protective concentration of serum antibodies following resolution of the response, providing immunity to subsequent challenges. Plasma-cell survival therefore follows a simple rule of life: success depends on being in the right place at the right time.

From plasmablasts to long-lived plasma cells

It is widely accepted that the switch from expression of membrane-bound antibody, functioning as an antigen receptor, to secretion of antibody marks the terminal differentiation of B cells into plasmablasts and then, ultimately, into plasma cells (which do not divide)¹⁰,

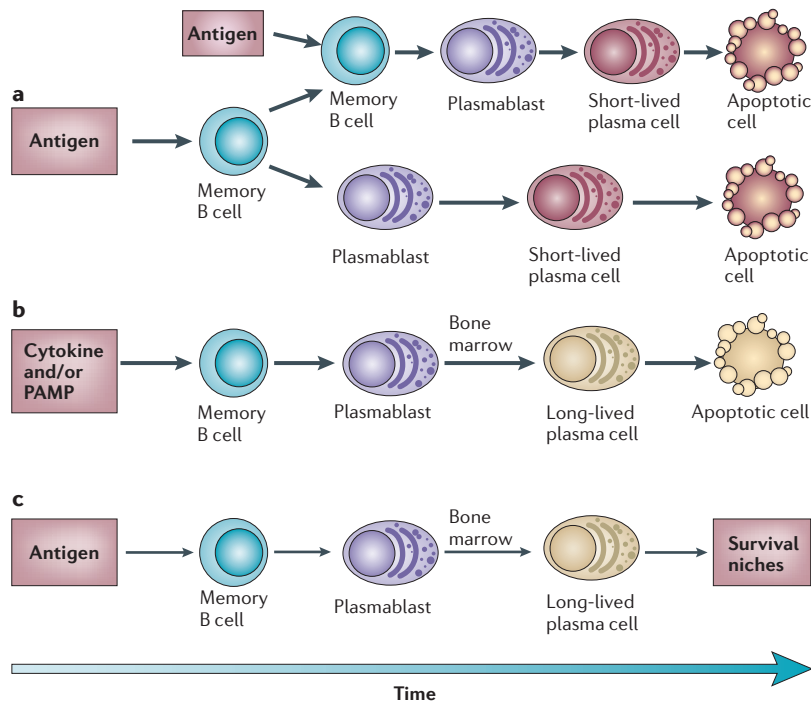


Figure 1 | Three concepts for the maintenance of serum concentrations of specific antibody. **a** | Constant generation of short-lived plasma cells from memory B cells, by a chronic antigen-driven immune reaction. **b** | Replacement of long-lived plasma cells that have a defined half-life of several weeks with bystander-activated plasmablasts. **c** | Generation of plasmablasts that are competent to home to survival niches. Survival depends on habitation in survival niches. PAMP, pathogen-associated molecular pattern.

although it has not been formally excluded that plasma cells might developmentally revert into B cells¹⁵.

B cells develop in the liver of fetuses and later in the bone marrow of adults from haematopoietic stem cells¹⁶. Immature B cells, which are characterized by expression of membrane-bound IgM, leave the bone marrow and differentiate into the B1- and B2-cell subsets. These cells differ in their localization and their activation requirements, with B2 cells being the classical B-cell lineage. B cells continue their development in the periphery, maturing through two transitional stages (T1 and T2), which are still poorly defined in functional terms, into cells that express cell-surface IgM and IgD and are known as mature naive B cells¹⁷. At the T2 stage, some B cells home to the marginal zone of the spleen and remain there as naive non-circulating B cells, which are known as marginal-zone B cells. However, most B cells at the T2 stage mature into naive follicular B cells, which continue circulating to the follicles of the spleen and lymph nodes through the bone marrow until they die or encounter their cognate antigen.

Stimulation of B cells by antigen results in either cell death or proliferation and differentiation into plasmablasts, which secrete antibody, depending on the developmental stage of the B cells and the additional signals that the cells receive. When follicular B cells are activated by antigen and receive T-cell help, they form germinal centres, and their antibody-encoding genes

are modified by class-switch recombination and somatic hypermutation^{16,18,19}. Plasmablasts can develop from any type of activated B cell — including naive, marginal-zone, follicular, germinal-centre and memory B cells — but it is not clear whether plasmablasts of all of these origins are competent to become long-lived plasma cells. When follicular B cells receive co-stimulation from activated T cells, the activated follicular B cells can develop not only into plasmablasts but also into memory B cells, which are long-lived²⁰, express cell-surface antibody but do not secrete antibody, and can react quickly to a subsequent antigen challenge. Both memory B cells and long-lived plasma cells express antibodies that are of switched class and have been affinity matured by somatic hypermutation²¹, both of which are hallmarks of the germinal-centre reaction, although class-switch recombination and somatic hypermutation can also occur in B cells that have been activated outside germinal centres²².

So far, it is not clear whether, following activation, naive B cells can gain competence to become long-lived plasma cells or whether only plasmablasts derived from germinal-centre or memory B cells have this competence. There is evidence, however, that bone-marrow plasma cells are more stringently affinity selected than are memory B cells, indicating that germinal centres — where affinity selection occurs — have a crucial role in the process of bone-marrow plasma-cell generation²³. Maintenance of serum antibody concentrations is a characteristic of secondary immune responses but can also be observed in primary immune responses^{24–27}. It remains to be determined whether the maintenance of antibody concentrations that is observed following a primary immune response is due to long-lived plasma cells generated from activated naive B cells or whether it results from reactivation of specific memory B cells that were generated in the primary immune response^{27,28}.

The competence to become a long-lived plasma cell is distinct from the basic instruction to become a plasma cell. The development of plasma cells depends on several factors, including the following: the presence of interleukin-21 (IL-21)²⁹; activation of the gene *PRDM1* (positive-regulatory-domain containing 1; which encodes the transcription factor **BLIMP1**, B-lymphocyte-induced maturation protein 1)³⁰; subsequent suppression of the master transcription factor of B-cell development, **PAX5** (paired box protein 5); and expression of a stable isoform of the transcription factor **XBPI** (X-box-binding protein 1), which is generated by differential splicing³¹. So far, there are no known molecular correlates of competence to become a long-lived plasma cell. There is, however, evidence that long-lived plasma cells and short-lived plasma cells constitute distinct populations and that all plasmablasts do not have the competence to become long-lived plasma cells³². In the spleens of (NZB × NZW)F₁ mice, there are two populations of plasma cells: a population of long-lived plasma cells, and a rapidly renewing population of short-lived plasma cells^{32,33}. Such stable coexistence of long-lived plasma cells and

Plasma cell

A non-dividing, terminally differentiated, immobile antibody-secreting cell of the B-cell lineage.

Unfolded-protein response

A response that increases the ability of the endoplasmic reticulum to fold and translocate proteins, decreases the synthesis of proteins, and causes the arrest of the cell cycle and apoptosis.

Plasmablast

A dividing antibody-secreting cell of the B-cell lineage that has migratory potential. These cells can further mature into plasma cells, which do not divide.

Marginal-zone B cell

A mature B cell that is enriched mainly in the marginal zone of the spleen, which is located at the border of the white pulp.

Germinal centre

A highly specialized and dynamic microenvironment that gives rise to secondary B-cell follicles during an immune response. It is the main site of B-cell maturation, leading to the generation of memory B cells and plasma cells that produce high-affinity antibody.

Class-switch recombination

A molecular alteration of the constant-region gene of the antibody heavy chain (C_H) that leads to a switch in expression from the C_{μ} (or C_{δ}) region to one of the other C_H genes. This leads to a switch in the class of the antibody that is displayed on the cell surface of the B cell (and that plasma cells subsequently produce) — from IgM (or IgD) to IgG, IgA or IgE — without altering the specificity of the antibody. This imparts flexibility to the humoral immune response and allows it to exploit the different capacities of the antibody classes to activate the appropriate downstream effector mechanisms.

Somatic hypermutation

A unique mutation mechanism that is targeted to the variable regions of rearranged immunoglobulin gene segments. Combined with selection for B cells that produce high-affinity antibody, somatic hypermutation leads to affinity maturation of B cells in germinal centres.

(NZB $\times$ NZW)F₁ mice

The F₁ generation of the cross between NZB mice and NZW mice. (NZB $\times$ NZW)F₁ mice have a disease that closely resembles the human disease systemic lupus erythematosus.

Systemic lupus erythematosus

(SLE). An autoimmune disease in which autoantibodies specific for DNA, RNA or proteins associated with nucleic acids form immune complexes that damage small blood vessels, especially in the kidneys. Patients with SLE generally have abnormal B- and T-cell function.

short-lived plasma cells in the same organ implies that competence to enter the pool of long-lived plasma cells, which is lost in the rapidly renewing population, can be based on specific co-stimulatory signals that enable a plasma cell to respond to survival signals (discussed later), on the capability to home to sites where such signals are provided, or on both of these factors.

The migration of plasmablasts to survival niches — for example, in the bone marrow — is a crucial differentiation step for the generation of long-lived plasma cells. Accumulation of plasma cells in the bone marrow is a T-cell-dependent process³⁴, indicating that there must be a specific co-stimulatory instruction at some stage of plasma-cell development. Although a small proportion of long-lived plasma cells persist in the spleen of both humans and mice^{5,32,35,36}, most long-lived plasma cells are found in the bone marrow^{37,38}. However, under chronic inflammatory conditions, plasma cells are found in inflamed tissues: for example, in the kidneys of (NZB $\times$ NZW)F₁ mice³⁹ and patients with systemic lupus erythematosus (SLE)⁴⁰, and in the synovia of patients with rheumatoid arthritis^{41,42}. The lifespan of these plasma cells is probably limited by the inflammatory condition of the organ (discussed later). The generation of plasmablasts in secondary lymphoid organs and the persistence of these cells as plasma cells in the bone marrow indicates that detachment from secondary lymphoid organs and migration to, and attachment to, the bone marrow are crucial steps in selecting plasmablasts to become long-lived plasma cells.

The molecules endothelial-cell selectin (E-selectin) ligand and platelet selectin (P-selectin) ligand^{43,44}, CD11a, CD18 (REFS 45,46) and CD44 (REFS 47,48) have been identified as relevant adhesion molecules expressed by plasma cells. The chemokine receptors CXCR5⁴⁹ and CC-chemokine receptor 7 (CCR7)^{45,50}, which control germinal-centre traffic, are downregulated at the cell surface of plasmablasts. So plasmablasts no longer respond to the corresponding chemokines^{51,52}, disabling them from returning to the sites of immune reactions. **CXCR4** is expressed by plasmablasts, and its ligand, CXCR4-chemokine ligand 12 (**CXCL12**), is expressed in the red pulp of the spleen, as well as the medullary cords of the lymph nodes and the stroma of the bone marrow. Plasmablasts deficient in CXCR4 are greatly impaired in their ability to home to the bone marrow, although there are probably alternative mechanisms of attraction to the bone marrow^{51,53,54}. Although many B cells express CXCR4, the upregulation of CXCR4 by B cells that are CXCR4^{-low} is induced by B-cell activation and is independent of T-cell help⁵⁵. Therefore, CXCR4 is also expressed by plasmablasts that cannot become long-lived plasma cells, and additional signals are required for an activated B cell to become a plasmablast that is competent to enter the pool of long-lived plasma cells.

The presence of interferon- γ (IFN γ), a hallmark of inflamed tissue, increases both the expression of the chemokine receptor **CXCR3** by B cells differentiating into plasmablasts and the expression of CXCR3 ligands by cells

present in inflamed tissue, indicating that IFN γ might be particularly important for the attraction of plasmablasts to inflamed tissue^{55,56}. In mucosal immune responses, expression of the chemokine receptors CCR9 and CCR10 can be induced, allowing IgA-secreting plasma cells to home to mucosal sites. The CCR9 ligand, CC-chemokine ligand 25 (CCL25), is mainly restricted to the small intestine. By contrast, the CCR10 ligand, CCL28, is produced by epithelial cells in various mucosal tissues, indicating that this chemokine has a broad role in mucosal plasma-cell trafficking^{57–60}. One challenge of future studies is to determine whether antibody-secreting cells, either those generated⁶¹ or resident⁶² at mucosal sites, are long-lived plasma cells.

Following a systemic secondary immune response, the plasmablasts that are generated have been shown to migrate towards CXCR3 and CXCR4 ligands in transwell assays^{52,58,63,64}. Then, within 1 to 2 weeks of their generation, plasmablasts from mice lose the ability to migrate towards gradients of CXCR3 and CXCR4 ligands (that is, CXCL9, CXCL10, CXCL11 and CXCL12)⁵², consistent with the finding that resident antibody-secreting cells of the spleen and the lymph nodes show little or no migratory potential^{50,58}. This switch from plasmablasts, which have migratory capacity, to resident long-lived plasma cells, which do not, presents intriguing options for the regulation of the humoral immune response and humoral memory.

Survival of plasma cells

Antibody-secreting cells that have been generated *in vitro* or isolated *ex vivo* die within a few days^{47,65}. *In vivo*, most plasma cells that are generated in a given immune reaction disappear from the secondary lymphoid organs within a few days^{9,11,36,66}. In a secondary immune response in mice, ~10–20% of the plasma cells that are generated survive in the bone marrow for more than 2 weeks⁶⁷. In humans who have been vaccinated against tetanus, the proportion of specific antibodies that persists in the memory phase of the immune response is 10% of that of the peak response, probably reflecting a corresponding elimination of 90% of the antibody-secreting cells that were initially generated⁵⁸. The persisting antibody-secreting cells can survive *in vivo* for long periods^{12,32,68}. For example, Slifka and colleagues¹³ showed that plasma cells persisted in mice for more than 1 year in the absence of any detectable precursors. And Manz and colleagues^{5,69} showed that, 60 days after the onset of a secondary immune response to ovalbumin, more than 90% of ovalbumin-specific plasma cells in the bone marrow were long-lived and survived without proliferating for at least the next 60 days.

Taken together, the short lifespan of plasma cells *in vitro* (and, for many of them, *in vivo*) and the extended survival of plasma cells in the bone marrow implies that, to survive, plasma cells require both specific signals from the bone marrow and a molecular competence to respond to these signals. This competence is based on heritage: for example, whether the cells were derived from memory B cells and if the cells were appropriately instructed during activation (although

we do not yet fully understand the molecular nature of the instructive signals). This competence depends on expression of the transcription factor BLIMP1, because after conditional inactivation of the *Prdm1* gene (which encodes BLIMP1), long-lived plasma cells disappear from the bone marrow, and a corresponding decrease in antigen-specific serum antibody concentrations is observed⁷⁰. Whether conditional inactivation of *Prdm1* leads to the apoptosis of plasma cells or to the dedifferentiation of plasma cells into non-antibody-secreting cells that no longer express the plasma-cell marker **CD138** (also known as syndecan-1) is not clear, although it seems probable that plasma cells undergo apoptosis, owing to ER stress. BLIMP1 increases the expression of the transcription factor XBP1, which in its activated form (generated by differential splicing) induces the unfolded-protein response in plasma cells and therefore controls the ER stress that is caused by the massive production of antibody^{71–73}. The inhibition of XBP1 in plasmacytoma cells leads to ER-stress-induced apoptosis⁷⁴.

As well as BLIMP1 and XBP1, the competence of plasma cells to survive also depends on the nuclear factor Aiolos, because long-lived plasma cells cannot be derived from **Aiolos**-deficient B cells⁷⁵. The precise role of Aiolos in plasma-cell differentiation is not yet clear. As a member of the Ikaros family, it might be involved in the rearrangement of chromatin, and perhaps it interacts with BLIMP1, which has previously been described to interact with, and to be functionally dependent on, chromatin modifiers^{76–78}. It is less probable that the continued presence of Aiolos is required for the survival of plasma cells in the bone marrow, because these cells no longer divide and therefore might be less prone to epigenetic restoration (that is, to the reversion of epigenetic imprinting of 'plasma-cell genes').

Several signals that both alone and synergistically support the survival of bone-marrow plasma cells have been reported. Two research groups have isolated plasma cells from the bone marrow and identified signals that extend the survival of plasma cells *in vitro*. Among these signals are CXCL12 (the ligand for CXCR4), tumour-necrosis factor (**TNF**), IL-5, **IL-6** and ligands for CD44 (such as hyaluronic acid)^{47,79}. Other candidate factors were inefficient at promoting plasma-cell survival, at least when tested alone; these include IL-2, IL-4, IL-7, IL-10, IL-18, transforming growth factor- β , stem-cell leukaemia factor (also known as TAL1), leukaemia inhibitory factor, and antibodies that crosslink CD19, CD38, CD40 or CD138.

In vitro, IL-6 is the most efficient survival factor for bone-marrow plasma cells, and there is a marked synergy between CD44 and IL-6 in supporting survival⁴⁷. Whether this synergy is based on the ability of CD44 to induce expression of IL-6 (REF. 80) has not yet been determined. As an inducer of the expression of *PRDM1*, the role of IL-6 might be to maintain expression of BLIMP1. Induction of *PRDM1* might also explain the survival effect of members of the TNF family¹⁵.

As well as TNF itself, survival factors could include B-cell-activating factor (**BAFF**) or a proliferation-inducing ligand (**APRIL**), both of which are ligands for the B-cell maturation antigen (BCMA) receptor, which is expressed by plasma cells⁸¹. Although both ligands have been reported to prolong the survival of bone-marrow plasma cells in bone-marrow cultures *ex vivo*⁸¹, other evidence indicates that BAFF does not exert its function on plasma cells but, instead, on plasmablasts⁸². Blocking BAFF- and APRIL-mediated signalling with a **TACI** (transmembrane activator and calcium-modulating cyclophilin-ligand interactor)–immunoglobulin fusion protein reduces the number of resident plasma cells in the bone marrow by two-thirds, and **BCMA**-deficient mice have less than 20% of the resident bone-marrow plasma cells of wild-type mice⁸¹. Although effects on plasma-cell precursors might contribute to the observed reduction in bone-marrow plasma-cell numbers, the available data strongly indicate that BAFF and APRIL influence the survival of plasma cells in the bone marrow, either directly or indirectly. The data also indicate that BAFF and/or APRIL do not function by inducing the expression of IL-6, because IL-6-deficient mice have no defect in the maintenance of humoral memory responses^{47,79}, despite the important role of IL-6 in the survival of bone-marrow plasma cells *ex vivo*. Recently, the plasma-cell marker CD138 was identified as a receptor for APRIL that is involved in the homing of plasma cells to the bone marrow. Inhibition of BAFF and APRIL, but not BAFF alone, was found to prevent the survival and/or the migration of newly formed plasma cells to the bone marrow⁸³. So, despite having made considerable progress, we still do not completely understand all of the signals that allow plasma cells to survive in the bone marrow.

Termination of the longevity of plasma cells

Ex vivo, CXCL12 is a potent survival factor for resident bone-marrow plasma cells⁴⁷, although neither these cells nor resident lymph-node plasma cells can migrate towards gradients of CXCL12 (REFS 50,52), unlike their immediate precursors, plasmablasts^{52,63,64}. Mice with B cells deficient in the CXCL12 receptor, CXCR4, have a markedly reduced number of bone-marrow plasma cells, although this could result mainly from the disturbed homing of plasmablasts to the bone marrow⁶³. Later, in the memory phase of an immune response, the number of plasma cells in the bone marrow of CXCL12-deficient mice stabilizes⁵³. The relevance of CXCL12 for survival of bone-marrow plasma cells *in vivo* has yet to be shown directly, but in the bone marrow, plasma cells are located close to stromal cells that express CXCL12 (REF. 54). This localization offers an intriguing explanation for how plasmablasts, migrating towards CXCL12, could directly compete for this location with plasma cells (which are resident and do not migrate) and replace these plasma cells (FIG. 2).

The mode of action of such replacement is not clear, although it is tempting to speculate that, in a direct physical competition, a mobile plasmablast might dislodge an immobile plasma cell. Plasma cells that have been dislocated from their survival niche would be

Rheumatoid arthritis

An immune disorder that is characterized by symmetrical polyarthritis, often progressing to crippling deformation after years of synovitis. It is associated with systemic immune activation, with acute-phase reactants — as well as rheumatoid factor (antibodies specific for IgG), which forms immune complexes that are deposited in many tissues — being present in the peripheral blood.

Antibody-secreting cells

A term that encompasses both plasmablasts, which proliferate, and plasma cells, which do not proliferate. It is used when both cell types might be present.

Aiolos

A lymphoid-regulatory protein that is a member of the Ikaros family. It is a haematopoietic-cell-specific transcription factor that is involved in B-cell development and function.

Ikaros family

A family of zinc-finger-containing transcription factors. These factors are pleiotropic regulators of haematopoiesis and are required for the generation of lymphocyte and dendritic-cell lineages, as well as lymph nodes and Peyer's patches.

Stromal cells

Cells of non-lymphoid origin that form the framework of each organ. By expressing various molecules, these cells can support adhesion, proliferation and survival of distinct cell subsets.

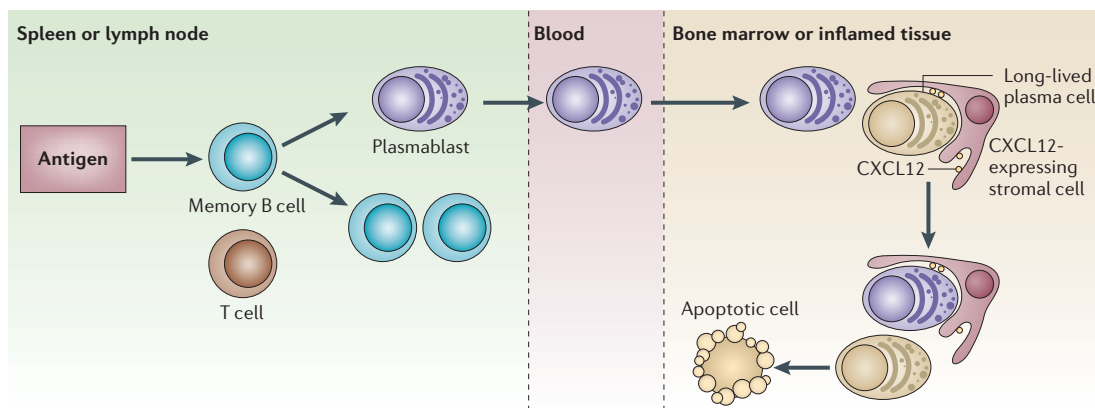


Figure 2 | Competition between plasmablasts and plasma cells for survival niches, which is driven by CXCL12, both as an attractant of plasmablasts and as a survival factor for long-lived plasma cells. When memory B cells are activated by antigen in a T-cell-dependent manner, either they proliferate or they differentiate into plasmablasts, which secrete antibody, are migratory, and also proliferate. Plasmablasts then leave the secondary lymphoid organ (the spleen or the lymph node) and express the chemokine receptors CXCR3 and CXCR4. The chemokine that is bound by CXCR4 is CXCL12, which is probably also an integral component of plasma-cell survival niches: so plasmablasts are attracted by plasma-cell survival niches. Plasma cells that reside in these survival niches are not migratory. Mobile plasmablasts that dislocate a resident plasma cell therefore have a competitive advantage. After an immobile plasma cell has been dislocated from its niche, it cannot return to a survival niche, and it dies. The successful plasmablast differentiates into a plasma cell and loses its migratory potential. This direct competition ensures that only as many plasma cells of 'old' specificities (that is, for antigens that were previously encountered) are removed from the bone marrow as plasmablasts with the 'new' specificity are recruited to the pool of memory plasma cells.

prone to die rapidly, presumably as a result of ER stress. Supporting evidence for this competitive dislocation of resident memory plasma cells comes from a recent flow-cytometric analysis of T-cell-dependent secondary immune responses in humans⁶⁴. About 1 week after immunization against tetanus, a wave of migrating plasmablasts are found in the blood. Most (if not all) of these cells are specific for the tetanus-toxoid antigen and express large amounts of HLA-DR. At the same time, plasma cells are mobilized into the blood, and they express small amounts of HLA-DR and are not specific for tetanus toxoid. These plasma cells probably reflect the repertoire of memory plasma cells in the bone marrow and are specific for antigens that were encountered previously: for example, antigens from the causative agents of diphtheria or measles^{4,64}. Direct competition between migrating plasmablasts and resident plasma cells of the bone marrow for CXCL12-containing survival niches would be the most efficient way to eliminate only as many plasma cells from the memory plasma-cell pool as is required to include cells of a new specificity (FIGS 2,3).

Survival factors for long-lived plasma cells — such as CXCL12, IL-6, BAFF and/or APRIL, and TNF — are also expressed in inflamed tissues^{52,81,84–86} and in activated secondary lymphoid tissues. In the spleens of (NZB × NZW)F₁ mice, ~30% of the CD138-expressing antibody-secreting cells are long-lived plasma cells³³. In the spleen and the lymph nodes of normal mice, cells with the phenotype of long-lived plasma cells are much less abundant but are still detectable^{32,50,69}. In successful immune responses, the presence of plasma-cell survival niches in inflamed tissues and activated secondary

lymphoid tissues is an efficient way of providing maximal amounts of specific antibodies at the site of inflammation. Such survival niches also provide an elegant way to terminate the peak response, by resolution of the inflammation as a consequence of a successful immune reaction. Resolution of inflammation will lead to degeneration of plasma-cell survival niches, and plasma cells in these tissues will die, because they are deprived of survival signals and cannot migrate to other, functional, survival niches, such as those in the bone marrow (FIG. 4).

Size, repertoire and fading of humoral memory

The number of bone-marrow survival niches for long-lived plasma cells seems to be limited to encompass 0.1–1% of all bone-marrow cells in healthy individuals⁸⁷. The bone marrow therefore has a capacity for 10⁶ memory plasma cells, at most, in mice⁸⁸ and can be estimated to have a capacity for in the order of 10⁹ memory plasma cells in humans⁸⁹. The repertoire of humoral memory also needs to be limited to maintain protective titres of serum antibodies in an individual. An estimate of the memory-cell repertoire can be obtained by comparing the number of plasma cells and the concentration of antibodies specific for a given antigen, after the immune response has reached a steady state^{5,47}. In mice, 40 days after secondary immunization, the total number of bone-marrow plasma cells specific for tetanus toxoid and ovalbumin is ~2 × 10⁴ and 7.5 × 10⁴, respectively^{5,90}. Ovalbumin-specific plasma cells provide steady-state serum concentrations of ovalbumin-specific antibodies of 2–4 mg per ml: that is, 4–8 mg per mouse^{5,47}. Therefore, one mouse

Flow-cytometric analysis
A method that allows simultaneous detection of cell-surface and intracellular molecules on single cells, through the use of fluorescently labelled antibodies.

memory plasma cell maintains 56 ± 22 ng of ovalbumin-specific serum IgG. In humans, serum concentrations of IgG specific for tetanus toxoid and for the capsular polysaccharide antigen of *Streptococcus pneumoniae* are similar, whereas the concentration of antibodies specific for the capsular polysaccharide antigen of *Haemophilus influenzae* is ~ 20 -fold lower⁹¹. In vaccinated human adults, the concentration of tetanus-toxoid-specific serum IgG ranges from 10 to 20 μg per ml ^{4,91}, implying corresponding memory plasma-cell numbers between 0.8×10^6 and 1.6×10^6 (provided that human and mouse plasma cells secrete similar amounts of antibody and that the half-life of human IgG *in vivo* is similar to that of mouse IgG)⁹². The discrepancy between the serum concentration of specific antibodies in mice and humans might reflect the potential of the bone marrow of young mice to accommodate more plasmablasts. This larger capacity might be due to a lack of competition

resulting from an incompletely developed bone-marrow plasma-cell repertoire, which in turn might result from the youth of the mice and/or from their housing in specific-pathogen-free conditions⁶⁹. In human adolescents, the concentration of tetanus-toxoid-specific serum IgG is about twofold higher than in human adults, reaching 40 µg per ml⁹¹.

The recruitment of $\sim 10^6$ new plasmablasts to a resident memory plasma-cell pool with a capacity of $\sim 10^9$ cells implies that the repertoire of human humoral memory is in the order of 1,000 main specificities. This is also reflected in the frequency of specificities of plasma cells that are detectable in the blood after vaccination and have presumably been mobilized from the bone marrow by migrating plasmablasts. The frequency of such plasma cells secreting antibodies specific for any of the tested bystander antigens has been shown to be $\sim 0.1\%$ for each antigen⁴.

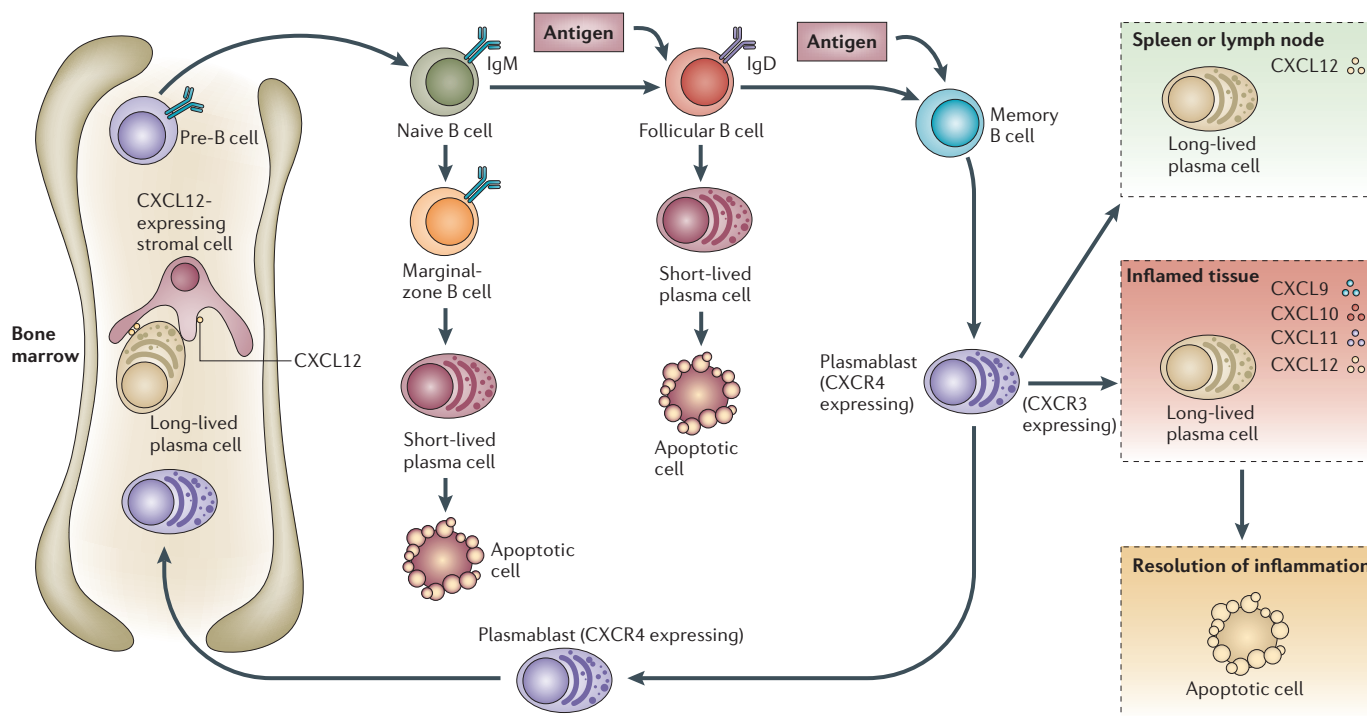


Figure 3 | Ontogeny of plasma cells. The heritage and the dynamics of plasma-cell life in humoral immune responses are shown. B cells that are generated in the bone marrow exit as precursor B cells (pre-B cells), which are immature and express IgM. These cells further mature into naive B cells and then into either marginal-zone B cells or follicular B cells. When activated, these marginal-zone and follicular B cells can differentiate into plasmablasts (not shown) and short-lived plasma cells, both of which can secrete antibody. Alternatively, with the help of T helper cells, follicular B cells can also differentiate into memory B cells, which are long-lived, and express antibodies of switched class and high affinity for antigen. When reactivated by antigen, memory B cells can differentiate into plasmablasts, which are competent to become long-lived plasma cells. A small proportion of these plasmablasts stay in the secondary lymphoid organ (the spleen or the lymph node) where they were generated. Most of the plasmablasts migrate either to inflamed tissue, under the control of interferon- γ -induced expression of CXC-chemokine receptor 3 (CXCR3; which binds CXC-chemokine ligand 9 (CXCL9), CXCL10 and CXCL11), or to the bone marrow, under the control of chemotaxis towards CXCL12 (which binds CXCR4). All three tissues have finite numbers of plasma-cell survival niches. Plasmablasts that succeed in the acquisition of such a niche differentiate into plasma cells and become immobile. Resolution of inflamed tissue after a successful immune response terminates the survival niches in the tissue and therefore eliminates the resident plasma cells, and this is the peak of the immune response. In the bone marrow, and to a lesser degree in secondary lymphoid organs, long-lived plasma cells survive and provide humoral memory.

Peak response — inflamed tissue

- Plasmablasts migrate to inflamed tissue
- Plasma cells survive in niches in inflamed tissue and in the spleen
- Successful immune reaction terminates survival niches in inflamed tissue
- Dislocated plasma cells cannot relocate and therefore die

Humoral memory — bone marrow

- Plasmablasts invade the bone marrow and compete with resident plasma cells for occupancy of survival niches
- Plasmablasts become long-lived plasma cells
- Dislocated plasma cells cannot relocate and therefore die
- Memory slowly fades owing to competition

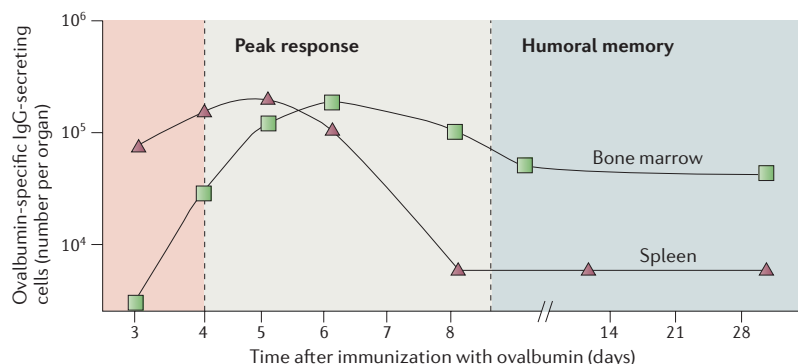


Figure 4 | The kinetics of the humoral immune response in BALB/c mice following secondary immunization with ovalbumin. There is a peak in the number of antibody-secreting cells detected in the spleen, on day 5 after immunization. Between day 6 and day 8 after immunization, the number of antibody-secreting cells in the spleen then decreases to less than 5% of the number present at the peak of the response. In the bone marrow, the number of antibody-secreting cells detected peaks on day 6 after immunization. From day 10 after immunization, a constant number of antibody-secreting cells (~70,000) persists. The kinetics reflect the migration of antigen-specific plasmablasts from secondary lymphoid organs to the bone marrow and the successful competition of 10–20% of migrating plasmablasts for bone-marrow survival niches.

How can humoral memory for antigens that were encountered in the distant past be maintained without losing the ability to generate memory of new specificities? Clearly, the limited overall capacity of humoral memory requires the elimination of plasma cells specific for 'old' antigens to allow cells of each new specificity to be included. The concept of inclusion of new specificities through competition between migrating plasmablasts and resident plasma cells — that is, the elimination of as many 'old' plasma cells as new ones that are adopted — provides an optimal solution for maximizing the plasticity of humoral memory.

From the estimate of the repertoire of humoral memory that is stated here and detailed elsewhere⁶⁷, it can be predicted that humoral memory for an old antigen would wane at a frequency of 0.1% for each generation of cells with a new specificity. After 692 such challenges, the serum concentration of antibody specific for the old antigen would still be 50% of the originally established concentration. How many years would it take for this decrease in antibody concentration? In a cohort of 50 apparently healthy blood donors, 2 showed spontaneously increased frequencies of migrating plasmablasts, indicating an antigen challenge had occurred 6–7 days before the donation, although it is unclear whether these migrating plasmablasts were

competent to home to the bone marrow (H. Mei, A.R. and T.D., unpublished observations). Because such peripheral-blood plasmablasts can be detected only for 2 days after challenge⁶⁴, the frequency of antigenic adoptions to humoral memory can be extrapolated to be a maximum of 30 per year. Under these conditions, the serum concentration of an average antibody specificity would decrease to 50% of the original steady-state concentration within 23 years. This corresponds well to the observed stability of humoral memory of particular specificities. It has been estimated that protection against a standardized environmental challenge with the causative agent of tetanus would still be provided by serum concentrations of 3 µg per ml^{91,93}, which is less than 20% of the original steady-state concentration of 10–20 µg per ml. Therefore, after protective humoral memory specific for a given pathogen has been established, it could last for a lifetime.

Long-lived plasma cells and immunopathology

Because they are the product of systemic T-cell-dependent activation of B cells, long-lived plasma cells can be an essential component of immunopathology. This is particularly evident for IgE-mediated allergy, because the half-life of serum IgE is only ~12 hours⁹². Persistent production of specific IgE in the apparent absence of the corresponding antigen is a hallmark of type 1 allergies in humans⁹⁴. To a certain extent, allergen-specific IgE production can be transferred by the transplantation of bone marrow from allergic individuals to non-allergic individuals⁹⁵, although the transfer of this property has not been unambiguously shown to be due to long-lived plasma cells. Moreover, a study by Holt and colleagues¹² showed that IgE-secreting cells (and IgG-secreting cells) in allergic rats survive X-ray irradiation, which is sufficient to eliminate memory B cells but not long-lived plasma cells; this study is an early example of long-lived plasma cells secreting pathogenic IgE.

Long-lived plasma cells, however, might not be the only source of allergen-specific IgE. Mucosal immune responses might have an important role in IgE responses to antigen challenges in the lungs and gut. At present, it is unclear whether the mucosa-associated lymphoid tissue generates long-lived plasma cells. IgA-secreting plasmablasts that are generated in mucosal immune responses have been shown to express CCR9 and CCR10 (REF. 58) (in addition to CXCR4), which would allow them to home to the mucosae (with an unknown life expectancy) rather than to the bone marrow. Whether CXCR3-expressing plasmablasts, which are 'pro-inflammatory', are generated in immune responses to allergens is also unclear. The generation of such cells would require the simultaneous presence of IL-4 (which induces class-switch recombination of antibody genes to yield IgE⁹⁶) and IFNγ (which induces expression of CXCR3 (REF. 55)) during the activation of B cells to become plasmablasts. A genetic polymorphism in the CXCR3 gene has recently been shown to be associated with the risk of developing asthma⁹⁷. The local production of allergen-specific IgE in inflamed tissues and the presence of allergen-specific

Type 1 allergy

An allergy or hypersensitivity of type 1 is an abnormal immune response that involves IgE specific for otherwise harmless proteins.

B cells in inflamed tissues have been described by several research groups. However, the lifespan and the persistence of these cells have not been determined, and their origin — which could be from a local immune reaction or an immune reaction in adjacent lymphoid organs — is unclear^{98–100}. The resistance of allergies to immunosuppression, and the persistence of allergen-specific IgE despite immunosuppressive therapy^{101,102}, indicates that long-lived plasma cells make a substantial contribution to IgE-mediated allergy, because long-lived plasma cells are resistant to immunosuppression^{13,33}.

The generation of autoreactive antibodies is a hallmark of autoimmune diseases. For several such diseases, autoantibodies have been identified as the main agents of immunopathology, as well as valuable diagnostic markers^{103–105}. Examples of these diseases are Hashimoto's thyroiditis¹⁰⁶, antiphospholipid syndrome¹⁰⁷, myasthenia gravis¹⁰⁸ and bullous skin diseases¹⁰⁹. This list is incomplete, and more diseases might be included in the future, after more detailed analyses of their immunopathology. After the pathogenic potential of autoantibodies of the IgE class has been further elucidated (for example, in Churg–Strauss syndrome), a close link between IgE-mediated autoimmunity and IgE-mediated allergies might emerge¹¹⁰.

In patients with SLE, autoantibodies specific for intracellular antigens are prevalent. These autoantibodies contribute to immunopathology by forming immune complexes, binding complement and binding Fc receptors¹⁰³, and they might be involved in the defective clearance of apoptotic material¹¹¹. Such autoantibodies also show hallmarks of T-cell-dependent activation of B cells: that is, somatic hypermutation and class-switch recombination. Cells that secrete autoantibodies can be generated from cells that express non-autoreactive antibodies by somatic hypermutation^{112,113}. Interestingly, the serum concentrations of autoantibodies specific for particular autoantigens are stable, whereas the concentrations of autoantibodies specific for other autoantigens fluctuate according to flares of the disease¹¹⁴. This indicates that the former might be secreted by long-lived plasma cells, whereas the latter might be secreted by plasmablasts and short-lived plasma cells. Accordingly, large numbers of plasmablasts are detectable in the blood of patients with SLE during disease flares, and these cells express highly mutated antibodies, presumably of mainly autoreactive specificities^{115,116}.

In (NZB × NZW)F₁ mice, long-lived plasma cells are generated early in ontogeny. These long-lived plasma cells include cells that secrete autoantibodies. They survive in the bone marrow and, as a defined subpopulation of a remarkable size, in the spleen. Interestingly, at later stages of pathogenesis, a separate subpopulation of plasmablasts and short-lived plasma cells is detectable in the spleens of (NZB × NZW)F₁ mice^{33,117}. These plasmablasts and short-lived plasma cells lack the competence to compete with the long-lived plasma cells for survival niches, indicating that the short-lived plasma cells are formed by a different type of immune reaction and providing strong evidence that an activated B cell can develop into an antibody-secreting cell in several

distinct ways. The persistence of an expanded stable population of long-lived plasma cells in the spleens of (NZB × NZW)F₁ mice indicates that the survival niches to support these cells are formed early in disease pathogenesis. Such cells are no longer formed later in disease pathogenesis, when constant generation of short-lived plasma cells is a hallmark of pathology. In patients with rheumatoid arthritis, the persistent expression of characteristic serum autoantibodies — class-switched rheumatoid factor specific for autologous IgG and antibodies specific for citrullinated peptides — is observed years before onset of clinical symptoms of the disease, indicating again that the generation of autoreactive long-lived plasma cells is an early step in pathogenesis in ~50% of patients¹¹⁸. Similarly, the presence of characteristic serum autoantibodies before the onset of disease has been observed in patients with type 1 diabetes, SLE or coeliac disease^{119–121}.

As well as the bone marrow and the spleen, the inflamed kidneys of (NZB × NZW)F₁ mice provide survival niches for plasma cells. Independent of their specificity, migrating plasmablasts that are competent to become long-lived plasma cells can home to inflamed kidneys but not to non-inflamed kidneys³⁹. The number of plasma-cell survival niches in the inflamed kidneys of (NZB × NZW)F₁ mice is remarkably high. In an ovalbumin-specific immune response in these mice, as many long-lived plasma cells survived in the kidneys as in the bone marrow³⁹. Extensive chronic inflammation might therefore contribute to systemic hypergammaglobulinaemia (that is, an increased concentration of serum IgG) and the immunopathology that is associated with this³⁵. However, when the inflammation is successfully resolved by treatment with anti-inflammatory agents the survival niches in the tissue are eliminated.

Concluding remarks

In their survival niches, resident long-lived plasma cells are resistant to therapies that target the activation and/or proliferation of lymphocytes: for example, irradiation with X-rays¹² or γ-rays¹³, or treatment with mitomycin C¹³, cyclophosphamide³³ or steroids (such as prednisolone)⁶⁸. Long-lived plasma cells and the allergen- or autoantigen-specific antibodies that they secrete might therefore be important components in the perpetuation of allergy and autoimmune disease that are beyond the reach of available therapies. At present, most (if not all) of these therapies efficiently control the symptoms of disease but do not provide a cure. Curative therapy might require elimination of long-lived plasma cells that secrete pathogenic antibodies. Although the resolution of inflammation that is achieved by current therapies might reduce the number of long-lived plasma cells that reside in inflamed tissues and secondary lymphoid organs, the elimination of long-lived plasma cells that reside in the bone marrow will require a different approach. So far, the elimination of these cells has been achieved only by complete immunoablation using antithymocyte globulin (a depleting antibody that targets cells of the thymus, including plasma cells)^{122–124}. More specific targeting of long-lived plasma cells remains a challenge of the future.

Autoreactive antibody
An antibody that is specific for a self-antigen.

Citrullinated peptide
A peptide that incorporates the amino acid citrulline. These peptides are generated post-translationally by peptidylarginine deiminases. The citrulline moiety is the essential part of the antigenic determinant towards which characteristic autoantibodies in patients with rheumatoid arthritis are generated.

1. Behring, E. V. & Kitasato, S. Ueber das Zustandekommen der Diphtherie-Immunität und der Tetanus-Immunität bei Thieren. *Dtsch. Med. Wochenschr.* **16**, 1113–1114 (1890) (in German).
2. Fagraeus, A. Antibody production in relation to the development of plasma cells. *In vivo* and *in vitro* experiments. *Acta Med. Scand.* **130**, 7–122 (1948).
3. Ahmed, R. & Gray, D. Immunological memory and protective immunity: understanding their relation. *Science* **272**, 54–60 (1996).
4. Bernasconi, N. L., Traggiai, E. & Lanzavecchia, A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science* **298**, 2199–2202 (2002).
Introduces the concept that polyclonal bystander activation of memory B cells maintains serum antibody concentrations (that is, humoral memory).
5. Manz, R. A., Thiel, A. & Radbruch, A. Lifetime of plasma cells in the bone marrow. *Nature* **388**, 133–134 (1997).
Directly shows longevity of bone-marrow plasma cells.
6. Szakal, A. K., Kosco, M. H. & Tew, J. G. Microanatomy of lymphoid tissue during humoral immune responses: structure function relationships. *Annu. Rev. Immunol.* **7**, 91–109 (1989).
7. Zinkernagel, R. M. *et al.* On immunological memory. *Annu. Rev. Immunol.* **14**, 333–367 (1996).
8. Traggiai, E., Puzone, R. & Lanzavecchia, A. Antigen dependent and independent mechanisms that sustain serum antibody levels. *Vaccine* **21**, S35–S37 (2003).
9. Cooper, E. H. Production of lymphocytes and plasma cells in the rat following immunization with human serum albumin. *Immunology* **4**, 219–231 (1961).
10. Nossal, G. J. Genetic control of lymphopoiesis, plasma cell formation, and antibody production. *Int. Rev. Exp. Pathol.* **1**, 1–72 (1962).
11. Schooley, J. C. Autoradiographic observations of plasma cell formation. *J. Immunol.* **86**, 331–337 (1961).
12. Holt, P. G., Sedgwick, J. D., O'Leary, C., Krska, K. & Leivers, S. Long-lived IgE- and IgG-secreting cells in rodents manifesting persistent antibody responses. *Cell. Immunol.* **89**, 281–289 (1984).
13. Slifka, M. K., Antia, R., Whitmire, J. K. & Ahmed, R. Humoral immunity due to long-lived plasma cells. *Immunology* **8**, 363–372 (1998).
14. Iwakoshi, N. N. *et al.* Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. *Nature Immunol.* **4**, 321–329 (2003).
15. Shapiro-Shelef, M. & Calame, K. Regulation of plasma-cell development. *Nature Rev. Immunol.* **5**, 230–242 (2005).
16. Rajewsky, K. Clonal selection and learning in the antibody system. *Nature* **381**, 751–758 (1996).
17. Carsetti, R., Rosado, M. M. & Wardmann, H. Peripheral development of B cells in mouse and man. *Immunol. Rev.* **197**, 179–191 (2004).
18. MacLennan, I. C. Germinal centers. *Annu. Rev. Immunol.* **12**, 117–139 (1994).
19. Liu, Y. J. & Arpin, C. Germinal center development. *Immunol. Rev.* **156**, 111–126 (1997).
20. Schitteck, B. & Rajewsky, K. Maintenance of B-cell memory by long-lived cells generated from proliferating precursors. *Nature* **346**, 749–751 (1990).
21. Tarlinton, D. M. & Smith, K. G. Apoptosis and the B cell response to antigen. *Int. Rev. Immunol.* **15**, 53–71 (1997).
22. William, J., Euler, C., Christensen, S. & Shlomchik, M. J. Evolution of autoantibody responses via somatic hypermutation outside of germinal centers. *Science* **297**, 2066–2070 (2002).
23. Smith, K. G. *et al.* *bcl-2* transgene expression inhibits apoptosis in the germinal center and reveals differences in the selection of memory B cells and bone marrow antibody-forming cells. *J. Exp. Med.* **191**, 475–484 (2000).
24. Ochsenbein, A. F. *et al.* Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proc. Natl Acad. Sci. USA* **97**, 13263–13268 (2000).
25. Smith, K. G., Light, A., Nossal, G. J. & Tarlinton, D. M. The extent of affinity maturation differs between the memory and antibody-forming cell compartments in the primary immune response. *EMBO J.* **16**, 2996–3006 (1997).
26. Driver, D. J., McHeyzer-Williams, L. J., Cool, M., Stetson, D. B. & McHeyzer-Williams, M. G. Development and maintenance of a B220⁺ memory B cell compartment. *J. Immunol.* **167**, 1393–1405 (2001).
27. Blink, E. J. *et al.* Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. *J. Exp. Med.* **201**, 545–554 (2005).
28. Dörner, T. & Radbruch, A. Selecting B cells and plasma cells to memory. *J. Exp. Med.* **201**, 497–499 (2005).
29. Ozaki, K. *et al.* Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. *J. Immunol.* **173**, 5361–5371 (2004).
30. Turner, C. A. Jr., Mack, D. H. & Davis, M. M. Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. *Cell* **77**, 297–306 (1994).
31. Lin, K. I., Tunyaplin, C. & Calame, K. Transcriptional regulatory cascades controlling plasma cell differentiation. *Immunol. Rev.* **194**, 19–28 (2003).
32. Ho, F., Lorton, J. E., MacLennan, I. C. & Khan, M. Distinct short-lived and long-lived antibody-producing cell populations. *Eur. J. Immunol.* **16**, 1297–1301 (1986).
33. Hoyer, B. F. *et al.* Short-lived plasmablasts and long-lived plasma cells contribute to chronic humoral autoimmunity in NZB/W mice. *J. Exp. Med.* **199**, 1577–1584 (2004).
Shows that both long-lived and short-lived plasma cells produce autoantibodies.
34. Koch, G. & Benner, R. Differential requirement for B-memory and T-memory cells in adoptive antibody formation in mouse bone marrow. *Immunology* **45**, 697–704 (1982).
35. Ellyard, J. I. *et al.* Antigen-selected, immunoglobulin-secreting cells persist in human spleen and bone marrow. *Blood* **103**, 3805–3812 (2004).
36. Smith, K. G., Hewitson, T. D., Nossal, G. J. & Tarlinton, D. M. The phenotype and fate of the antibody-forming cells of the splenic foci. *Eur. J. Immunol.* **26**, 444–448 (1996).
37. McMillan, R. *et al.* Immunoglobulin synthesis by human lymphoid tissues: normal bone marrow as a major site of IgG production. *J. Immunol.* **109**, 1386–1394 (1972).
38. Medina, F., Segundo, C., Campos-Caro, A., Gonzalez-Garcia, I. & Brieva, J. A. The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. *Blood* **99**, 2154–2161 (2002).
39. Cassese, G. *et al.* Inflamed kidneys of NZB/W mice are a major site for the homeostasis of plasma cells. *Eur. J. Immunol.* **31**, 2726–2732 (2001).
40. Hutloff, A. *et al.* Involvement of inducible costimulator in the exaggerated memory B cell and plasma cell generation in systemic lupus erythematosus. *Arthritis Rheum.* **50**, 3211–3220 (2004).
41. Tsubaki, T. *et al.* Accumulation of plasma cells expressing CXCR3 in the synovial sublining regions of early rheumatoid arthritis in association with production of Mig/CXCL9 by synovial fibroblasts. *Clin. Exp. Immunol.* **141**, 363–371 (2005).
42. Schroder, A. E., Greiner, A., Seyfert, C. & Berek, C. Differentiation of B cells in the nonlymphoid tissue of the synovial membrane of patients with rheumatoid arthritis. *Proc. Natl Acad. Sci. USA* **93**, 221–225 (1996).
43. Bullard, D. C. *et al.* Infectious susceptibility and severe deficiency of leukocyte rolling and recruitment in E-selectin and P-selectin double mutant mice. *J. Exp. Med.* **183**, 2329–2336 (1996).
44. Underhill, G. H., Minges Wols, H. A., Fornek, J. L., Witte, P. L. & Kansas, G. S. IgG plasma cells display a unique spectrum of leukocyte adhesion and homing molecules. *Blood* **99**, 2905–2912 (2002).
45. Ellyard, J. I., Avery, D. T., Mackay, C. R. & Tangye, S. G. Contribution of stromal cells to the migration, function and retention of plasma cells in human spleen: potential roles of CXCL12, IL-6 and CD54. *Eur. J. Immunol.* **35**, 699–708 (2005).
46. Pabst, O. *et al.* Egress of newly generated plasma cells from peripheral lymph nodes depends on β_2 integrin. *J. Immunol.* **174**, 7492–7495 (2005).
Provides evidence that β_2 -integrins are required for the exit of plasma cells from peripheral lymph nodes.
47. Cassese, G. *et al.* Plasma cell survival is mediated by synergistic effects of cytokines and adhesion-dependent signals. *J. Immunol.* **171**, 1684–1690 (2003).
48. Barker, H. F., Hamilton, M. S., Ball, J., Drew, M. & Franklin, I. M. Expression of adhesion molecules LFA-3 and N-CAM on normal and malignant human plasma cells. *Br. J. Haematol.* **81**, 331–335 (1992).
49. Allen, C. D. *et al.* Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5. *Nature Immunol.* **5**, 943–952 (2004).
50. Wehrli, N. *et al.* Changing responsiveness to chemokines allows medullary plasmablasts to leave lymph nodes. *Eur. J. Immunol.* **31**, 609–616 (2001).
Describes the role of CXCR4 in plasmablast migration.
51. Hargreaves, D. C. *et al.* A coordinated change in chemokine responsiveness guides plasma cell movements. *J. Exp. Med.* **194**, 45–56 (2001).
52. Hauser, A. E. *et al.* Chemotactic responsiveness toward ligands for CXCR3 and CXCR4 is regulated on plasma blasts during the time course of a memory immune response. *J. Immunol.* **169**, 1277–1282 (2002).
53. Nie, Y. *et al.* The role of CXCR4 in maintaining peripheral B cell compartments and humoral immunity. *J. Exp. Med.* **200**, 1145–1156 (2004).
Shows that plasma-cell accumulation in the bone marrow is impaired in CXCR4-deficient mice. However, following immunization, normal numbers of bone-marrow plasma cells are generated, indicating that CXCR4 is important, but not essential, for this process.
54. Tokoyoda, K., Egawa, T., Sugiyama, T., Choi, B. I. & Nagasawa, T. Cellular niches controlling B lymphocyte behavior within bone marrow during development. *Immunity* **20**, 707–718 (2004).
Shows that plasma cells and CXCL12-expressing stromal cells colocalize in the bone marrow.
55. Muehlinghaus, G. *et al.* Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into plasma cells. *Blood* **105**, 3965–3971 (2005).
56. Baggiolini, M. Chemokines and leukocyte traffic. *Nature* **392**, 565–568 (1998).
57. Bowman, E. P. *et al.* The intestinal chemokine thymus-expressed chemokine (CCL25) attracts IgA antibody-secreting cells. *J. Exp. Med.* **195**, 269–275 (2002).
58. Kunkel, E. J. & Butcher, E. C. Plasma-cell homing. *Nature Rev. Immunol.* **3**, 822–829 (2003).
59. Kunkel, E. J. *et al.* CCR10 expression is a common feature of circulating and mucosal epithelial tissue IgA Ab-secreting cells. *J. Clin. Invest.* **111**, 1001–1010 (2003).
60. Lazarus, N. H. *et al.* A common mucosal chemokine (mucosae-associated epithelial chemokine/CCL28) selectively attracts IgA plasmablasts. *J. Immunol.* **170**, 3799–3805 (2003).
61. Etchart, N. *et al.* Intranasal immunisation with inactivated RSV and bacterial adjuvants induces mucosal protection and abrogates eosinophilia upon challenge. *Eur. J. Immunol.* **36**, 1136–1144 (2006).
62. Hiepe, F. & Radbruch, A. Is long-term humoral immunity in the mucosa provided by long-lived plasma cells? A question still open. *Eur. J. Immunol.* **36**, 1068–1069 (2006).
63. Cyster, J. G. Homing of antibody secreting cells. *Immunol. Rev.* **194**, 48–60 (2003).
64. Odendahl, M. *et al.* Generation of migratory antigen-specific plasma blasts and mobilization of resident plasma cells in a secondary immune response. *Blood* **105**, 1614–1621 (2005).
Reports that the mobilization of plasmablasts into the blood occurs together with the mobilization of resident bone-marrow plasma cells. Provides evidence for the concept of competition between plasmablasts and plasma cells.
65. Nossal, G. J., Szenberg, A., Ada, G. L. & Austin, C. M. Single cell studies on 19S antibody production. *J. Exp. Med.* **119**, 485–502 (1964).
66. Nossal, G. J. & Makela, O. Kinetic studies on the incidence of cells appearing to form two antibodies. *J. Immunol.* **88**, 604–612 (1962).
67. Höfer, T. *et al.* Adaptation of humoral memory. *Immunol. Rev.* **211**, 295–302 (2006).
68. Miller, J. J. 3rd. An autoradiographic study of plasma cell and lymphocyte survival in rat popliteal lymph nodes. *J. Immunol.* **92**, 673–681 (1964).
69. Manz, R. A., Lohning, M., Cassese, G., Thiel, A. & Radbruch, A. Survival of long-lived plasma cells is independent of antigen. *Int. Immunol.* **10**, 1703–1711 (1998).

70. Shapiro-Shelef, M., Lin, K. I., Savitsky, D., Liao, J. & Calame, K. Blimp-1 is required for maintenance of long-lived plasma cells in the bone marrow. *J. Exp. Med.* **202**, 1471–1476 (2005).
71. Shapiro-Shelef, M. *et al.* Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity* **19**, 607–620 (2003).
72. Iwakoshi, N. N., Lee, A. H. & Glimcher, L. H. The X-box binding protein-1 transcription factor is required for plasma cell differentiation and the unfolded protein response. *Immunol. Rev.* **194**, 29–38 (2003).
73. Shaffer, A. L. *et al.* XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* **21**, 81–93 (2004).
74. Lee, A. H., Iwakoshi, N. N., Anderson, K. C. & Glimcher, L. H. Proteasome inhibitors disrupt the unfolded protein response in myeloma cells. *Proc. Natl Acad. Sci. USA* **100**, 9946–9951 (2003).
75. Cortes, M. & Georgopoulos, K. Aiolos is required for the generation of high affinity bone marrow plasma cells responsible for long-term immunity. *J. Exp. Med.* **199**, 209–219 (2004).
76. Ren, B., Chee, K. J., Kim, T. H. & Maniatis, T. PRDI-BF1/Blimp-1 repression is mediated by corepressors of the Groucho family of proteins. *Genes Dev.* **13**, 125–137 (1999).
77. Yu, J., Angelin-Duclos, C., Greenwood, J., Liao, J. & Calame, K. Transcriptional repression by Blimp-1 (PRDI-BF1) involves recruitment of histone deacetylase. *Mol. Cell. Biol.* **20**, 2592–2603 (2000).
78. Gyory, I., Wu, J., Fejer, G., Seto, E. & Wright, K. L. PRDI-BF1 recruits the histone H3 methyltransferase G9a in transcriptional silencing. *Nature Immunol.* **5**, 299–308 (2004).
79. Minges Wols, H. A., Underhill, G. H., Kansas, G. S. & Witte, P. L. The role of bone marrow-derived stromal cells in the maintenance of plasma cell longevity. *J. Immunol.* **169**, 4213–4221 (2002).
80. Uchiyama, H., Barut, B. A., Mohrbacher, A. F., Chauhan, D. & Anderson, K. C. Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion. *Blood* **82**, 3712–3720 (1993).
81. O'Connor, B. P. *et al.* BCMA is essential for the survival of long-lived bone marrow plasma cells. *J. Exp. Med.* **199**, 91–98 (2004).
Defines a role for BCMA in the establishment or maintenance of humoral memory.
82. Avery, D. T. *et al.* BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J. Clin. Invest.* **112**, 286–297 (2003).
83. Ingold, K. *et al.* Identification of proteoglycans as the APRIL-specific binding partners. *J. Exp. Med.* **201**, 1375–1383 (2005).
84. Hirano, T. & Kishimoto, T. Interleukin 6 and plasma cell neoplasias. *Prog. Growth Factor Res.* **1**, 133–142 (1989).
85. MacLennan, I. & Vinuesa, C. Dendritic cells, BAFF, and APRIL: innate players in adaptive antibody responses. *Immunity* **17**, 235–238 (2002).
86. Schneider, P. The role of APRIL and BAFF in lymphocyte activation. *Curr. Opin. Immunol.* **17**, 282–289 (2005).
87. Terstappen, L. W., Johnsen, S., Segers-Nolten, I. M. & Loken, M. R. Identification and characterization of plasma cells in normal human bone marrow by high-resolution flow cytometry. *Blood* **76**, 1739–1747 (1990).
88. Haaijman, J. J., Schuit, H. R. & Hijmans, W. Immunoglobulin-containing cells in different lymphoid organs of the CBA mouse during its life-span. *Immunology* **32**, 427–434 (1977).
89. Trepel, F. Number and distribution of lymphocytes in man. A critical analysis. *Klin. Wochenschr.* **52**, 511–515 (1974).
90. Pihlgren, M. *et al.* Delayed and deficient establishment of the long-term bone marrow plasma cell pool during early life. *Eur. J. Immunol.* **31**, 939–946 (2001).
91. Schauer, U. *et al.* Levels of antibodies specific to tetanus toxoid, *Haemophilus influenzae* type b, and pneumococcal capsular polysaccharide in healthy children and adults. *Clin. Diagn. Lab. Immunol.* **10**, 202–207 (2003).
92. Vieira, P. & Rajewsky, K. The half-lives of serum immunoglobulins in adult mice. *Eur. J. Immunol.* **18**, 313–316 (1988).
93. Simonsen, O. *et al.* Revaccination of adults against diphtheria. II: combined diphtheria and tetanus revaccination with different doses of diphtheria toxoid 20 years after primary vaccination. *Acta Pathol. Microbiol. Immunol. Scand. C* **94**, 219–225 (1986).
94. Lichtenstein, L. M., Ishizaka, K., Norman, P. S., Sobotka, A. K. & Hill, B. M. IgE antibody measurements in ragweed hay fever. Relationship to clinical severity and the results of immunotherapy. *J. Clin. Invest.* **52**, 472–482 (1973).
95. Hallstrand, T. S. *et al.* Long-term acquisition of allergen-specific IgE and asthma following allogeneic bone marrow transplantation from allergic donors. *Blood* **104**, 3086–3090 (2004).
96. Bergstedt-Lindqvist, S. *et al.* Interleukin 4 instructs uncommitted B lymphocytes to switch to IgG1 and IgE. *Eur. J. Immunol.* **18**, 1073–1077 (1988).
97. Cheong, H. S. *et al.* CXCR3 polymorphisms associated with risk of asthma. *Biochem. Biophys. Res. Commun.* **334**, 1219–1225 (2005).
98. Chvatchko, Y., Kosco-Vilbois, M. H., Herren, S., Lefort, J. & Bonnefoy, J. Y. Germinal center formation and local immunoglobulin E (IgE) production in the lung after an airway antigenic challenge. *J. Exp. Med.* **184**, 2353–2360 (1996).
99. Astrom, K. *et al.* Analysis of germ line transcripts and IL-4 mRNA expression in the adenoids suggests local IgE switching. *Allergy* **55**, 1049–1055 (2000).
100. Coker, H. A., Durham, S. R. & Gould, H. J. Local somatic hypermutation and class switch recombination in the nasal mucosa of allergic rhinitis patients. *J. Immunol.* **171**, 5602–5610 (2003).
101. Wyczolkowska, J., Brzezinska-Blaszczyk, E. & Maslinski, C. Kinetics of specific IgE antibody and total IgE responses in mice: the effect of immunosuppressive treatment. *Int. Arch. Allergy Appl. Immunol.* **72**, 16–21 (1983).
102. Brunette, M. G., Bonny, Y., Spiegelblatt, L. & Barrette, G. Long-term immunosuppressive treatment of a child with Takayasu's arteritis and high IgE immunoglobulins. *Pediatr. Nephrol.* **10**, 67–69 (1996).
103. Martin, F. & Chan, A. C. Pathogenic roles of B cells in human autoimmunity: insights from the clinic. *Immunology* **20**, 517–527 (2004).
104. Lipsky, P. E. Systemic lupus erythematosus: an autoimmune disease of B cell hyperactivity. *Nature Immunol.* **2**, 764–766 (2001).
105. von Muhlen, C. A. & Tan, E. M. Autoantibodies in the diagnosis of systemic rheumatic diseases. *Semin. Arthritis Rheum.* **24**, 323–358 (1995).
106. Chiovato, L. *et al.* Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. *Ann. Intern. Med.* **139**, 346–351 (2003).
107. de Groot, P. G. & Derksen, R. H. Pathophysiology of the antiphospholipid syndrome. *J. Thromb. Haemost.* **3**, 1854–1860 (2005).
108. Hughes, B. W., Moro De Casillas, M. L. & Kaminski, H. J. Pathophysiology of myasthenia gravis. *Semin. Neurol.* **24**, 21–30 (2004).
109. Amagai, M. Autoimmunity against desmosomal cadherins in pemphigus. *J. Dermatol. Sci.* **20**, 92–102 (1999).
110. Gross, W. L. Churg–Strauss syndrome: update on recent developments. *Curr. Opin. Rheumatol.* **14**, 11–14 (2002).
111. Carroll, M. C. A protective role for innate immunity in systemic lupus erythematosus. *Nature Rev. Immunol.* **4**, 825–831 (2004).
112. Brard, F., Shannon, M., Prak, E. L., Litwin, S. & Weigert, M. Somatic mutation and light chain rearrangement generate autoimmunity in anti-single-stranded DNA transgenic MRL/lpr mice. *J. Exp. Med.* **190**, 691–704 (1999).
113. Shlomchik, M. *et al.* Anti-DNA antibodies from autoimmune mice arise by clonal expansion and somatic mutation. *J. Exp. Med.* **171**, 265–292 (1990).
114. Ishii, Y., Nagasawa, K., Mightumi, T. & Niho, Y. Clinical importance of persistence of anticardiolipin antibodies in systemic lupus erythematosus. *Ann. Rheum. Dis.* **49**, 387–390 (1990).
115. Jacobi, A. M. *et al.* Correlation between circulating CD27^{high} plasma cells and disease activity in patients with systemic lupus erythematosus. *Arthritis Rheum.* **48**, 1332–1342 (2003).
116. Odendahl, M. *et al.* Disturbed peripheral B lymphocyte homeostasis in systemic lupus erythematosus. *J. Immunol.* **165**, 5970–5979 (2000).
117. Hiepe, F. *et al.* Long-lived CD20^{neg} plasma cells contribute significantly to autoantibody production in SLE and represent a novel and essential target for cellular therapies. *Arthritis Rheum.* **50**, S260 (2004).
118. Nielen, M. M. *et al.* Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum.* **50**, 380–386 (2004).
119. Scofield, R. H. Autoantibodies as predictors of disease. *Lancet* **363**, 1544–1546 (2004).
120. Arbuckle, M. R. *et al.* Development of autoantibodies before the clinical onset of systemic lupus erythematosus. *N. Engl. J. Med.* **349**, 1526–1533 (2003).
121. McClain, M. T. *et al.* The prevalence, onset, and clinical significance of antiphospholipid antibodies prior to diagnosis of systemic lupus erythematosus. *Arthritis Rheum.* **50**, 1226–1232 (2004).
122. Zand, M. S. *et al.* Polyclonal rabbit antithymocyte globulin triggers B-cell and plasma cell apoptosis by multiple pathways. *Transplantation* **79**, 1507–1515 (2005).
123. Jayne, D. & Tyndall, A. Autologous stem cell transplantation for systemic lupus erythematosus. *Lupus* **13**, 359–365 (2004).
124. Statkute, L. *et al.* Antiphospholipid syndrome in patients with systemic lupus erythematosus treated by autologous hematopoietic stem cell transplantation. *Blood* **106**, 2700–2709 (2005).

Acknowledgements

We thank C. Raulfs for critical reading of the manuscript and the members of the Berlin B Cell Club for helpful discussions. We are especially grateful to R. A. Manz for helpful comments. This work was supported by the Deutsche Forschungsgemeinschaft (Germany).

Competing interests statement

The authors declare no competing financial interests.

DATABASES

The following terms in this article are linked online to:

Entrez Gene:
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 Aiolos | APRIL | BAFF | BCMA | BLIMP1 | CD138 | CXCL12 | CXCR3 | CXCR4 | IL-6 | PAX5 | TAC1 | TNF | XBP1

FURTHER INFORMATION

Deutsches Rheuma-Forschungszentrum Berlin:

<http://www.drzf.de/indexp-7.htm>

Access to this links box is available online.