

B-cell responses to vaccination at the extremes of age

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Abstract | Infants and the elderly share a high vulnerability to infections and therefore have specific immunization requirements. Inducing potent and sustained B-cell responses is as challenging in infants as it is in older subjects. Several mechanisms to explain the decreased B-cell responses at the extremes of age apply to both infants and the elderly. These include intrinsic B-cell limitations as well as numerous microenvironmental factors in lymphoid organs and the bone marrow. This Review describes the mechanisms that shape B-cell responses at the extremes of age and how they could be taken into account to design more effective immunization strategies for these high-risk age groups.

Infant

A ≤ 12-month-old human baby or a ≤ 3-week-old mouse (experimental definition).

Neonate

A ≤ 28-day-old human infant or a ≤ 7-day-old mouse.

Respiratory and diarrhoeal diseases caused by some pathogens — respiratory syncytial virus (RSV), measles virus, *Bordetella pertussis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), rotavirus or *Salmonella* spp. — claim the lives of more than 1.6 million young children each year¹, and the global infant mortality rate still amounts to 49 deaths per 1,000 live births (see the [World Health Organization \(WHO\) health statistics](#) website). The abrupt transition at birth from a sterile intra-uterine environment to a foreign environment places neonates in a challenging situation. Innate immune responses can be readily elicited at birth, although with specific characteristics (reviewed in REF. 2), but they are transient and insufficient for immune protection. Furthermore, early immune protection initially relies on IgG antibodies of maternal origin³, the levels of which decline after birth with a half-life of 21–30 days. Therefore, infants would become vulnerable to infections within a short time frame without the development of adaptive immunity to confer sustained protection. As adaptive immunity in infants is readily amenable to enhancement through vaccination, great efforts and resources have been dedicated to infant immunization, which has had a notable impact on morbidity and mortality. However, early-life immune responses are weaker and of shorter duration than those that are elicited in immunologically mature hosts. Consequently, vaccine efficacy in this vulnerable population is limited.

This susceptibility to infection at the start of life is mirrored in those who approach the end of the normal life expectancy curve. Indeed, the elderly not only have problems in dealing with new pathogens but also have difficulties in responding to pathogens that they

have previously overcome, from which they can suffer increasingly severe symptoms. Numerous infections have a higher incidence in the elderly and have a higher mortality rate than in younger adults^{4,5}. For example, in the United States, the yearly return of influenza and RSV infections between 1990 and 1999 was responsible for 51,203 and 17,358 deaths, respectively⁶. In an average year, influenza, invasive pneumococcal disease and hepatitis B account for more than 50,000 deaths in the United States, with older individuals being 1,000 times more likely to die of these vaccine-preventable diseases than children living in developed countries⁷.

Current vaccines mediate their protective efficacy through the generation of neutralizing antibodies. Decades of studies have shown that antigen exposure in early life results in blunted, delayed or undetectable antibody responses to infections and immunizations. Historically, the neonatal immune system has been considered to be poorly competent in generating immune responses and instead to be polarized towards the induction of immune tolerance⁸. A conceptual switch occurred in the late 1990s through numerous demonstrations that adult-like B- and T-cell responses could be achieved in early life under specific conditions of stimulation (reviewed in REFS 9, 10). However, as early-life antibody responses markedly differ from those elicited in mature hosts, great challenges remain to be encountered during vaccine development. At the other extreme of age, the capacity to induce protective antibody at a sufficient titre to prevent infection declines significantly with age: in individuals of 65 years or older, influenza¹¹ and hepatitis B¹² vaccines induce protective antibody titres in less than half of their recipients.

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Table 1 | **Human B-cell responses at the extremes of age**

Cell type or site	Characteristics in infants	Characteristics in the elderly
Naive B cells	Decreased expression of cell-surface receptors (CD21, CD40, CD80 and CD86)	Limited generation of naive B cells
Plasma cells	Limited IgG responses to protein antigen under 12 months of age	Poor IgG responses to protein antigen
	Limited IgG responses to most polysaccharide antigens under 18–24 months of age	Poor IgG responses to most polysaccharide antigens
	Limited persistence of IgG antibodies under 12 months of age	Decreased persistence of IgG antibodies
Memory B cells	Effective priming of memory B cells at and before birth	Accumulation of memory B cells of restricted diversity
	Progressive diversified IgG repertoire	Limited diversified IgG repertoire
	Limited affinity maturation under 4–6 months of age	Affinity maturation is not affected by age
Germinal centres	Impaired germinal centre responses*	Impaired germinal centre responses*
	Limited functional FDC network*	Limited functional FDC network*
Bone marrow	Limited access to plasma cell niches*	Limited access to plasma cell niches*

Responses are compared with healthy adults. *Shown in mice. FDC, follicular dendritic cell.

In this Review, we discuss the mechanisms that could account for the limitations of B-cell responses at the extremes of age, which are both interesting biological phenomena and key considerations for the design of more effective immunization strategies.

B-cell responses in early life

Postnatal maturation of antibody responses. The vulnerability of children younger than 18–24 months of age to encapsulated bacteria such as pneumococcus, Hib and meningococcus has long been thought to reflect a general failure to generate T-cell-independent B-cell responses to most bacterial polysaccharides (reviewed in REF. 13). However, age also has a direct effect on the magnitude of antibody responses to T-cell-dependent protein antigens (TABLE 1). As an example, RSV infection only triggers limited T-cell-dependent B-cell responses in young infants, which has implications for the development of live-attenuated intranasal RSV vaccines¹⁴. Infant vaccine studies worldwide have shown that there is an age-dependent stepwise increase in the rates of seroconversion and the magnitude of antibody responses, regardless of whether repeat immunization is administered^{15,16}. In fact, the use of accelerated schedules in which immunizations are initiated at 6–8 weeks of age and repeated at 4-week intervals increases the proportion of infants who fail to respond to certain immunogens^{17,18} and might decrease protective efficacy against many important infant pathogens, such as pertussis^{1,19}. Direct evidence of the postnatal maturation of antibody responses was provided by single-dose studies with measles–mumps–rubella^{20,21} and hepatitis A²² vaccines. The IgG responses that can be eventually elicited in infants by several doses of vaccine wane rapidly, such that most children have low or undetectable antibody concentrations 6–9 months after their primary infant immunization series^{18,23}. This can result in a resurgence of vulnerability to infection unless repeat antigen exposure occurs after the first year of life^{24,25}.

Induction of a primary response. The mechanisms that shape B-cell responses in early life were identified using neonatal mouse immunization models that were developed to reproduce the main limitations of immune responses to vaccines that are administered at an early age (reviewed in REFS 9,26) (FIG. 1). There are numerous differences (TABLE 1) between neonatal and adult mouse splenic B cells (reviewed in REF. 10), although fewer differences have been identified by comparing human peripheral B cells. Specifically, human neonatal B cells express lower levels of the co-stimulatory molecules CD40, CD80 and CD86, which decreases their responses to CD40 ligand (CD40L) and interleukin-10 (IL-10)²⁷ expressed by T cells. Splenic marginal zone infant B cells express lower levels of CD21 (REF. 28), which limits their capacity to respond to polysaccharide–complement complexes²⁹. The expression of TACI (transmembrane activator and calcium-modulating cyclophilin-ligand interactor; also known as TNFRSF13B), an important co-stimulatory receptor, is also decreased on both neonatal mouse³⁰ and neonatal human B cells, particularly those born prematurely²⁷.

In addition, B-cell responses in early life are influenced by numerous extrinsic factors (TABLE 1). Antibodies of maternal origin bind to vaccine antigens in an epitope-specific manner and therefore prevent infant B cells from accessing immunodominant vaccine epitopes³¹. Furthermore, human³² and mouse³³ neonates have low levels of serum complement component C3, which limits their responses to antigen–C3d complexes. The human spleen contains fewer marginal zone macrophages (which have a crucial role in the induction of an antibody response through the trapping of particulate antigens) in neonates than in adults²⁸, and the cells differ in their capacity to produce cytokines³⁴. In infant mice, B-cell responses are limited by a marked delay in the maturation of the follicular dendritic cell (FDC) network, resulting from the failure of FDC precursors

T-cell-independent B-cell response

A B-cell response that is elicited without the requirement for CD4⁺ T-cell co-stimulation, which is a characteristic of responses to most polysaccharide antigens.

T-cell-dependent B-cell response

A B-cell response that requires co-stimulatory signals provided by CD4⁺ T cells, a characteristic of responses to all protein or conjugate vaccines.

Seroconversion

The development of detectable specific antibodies in the serum as a result of infection or immunization.

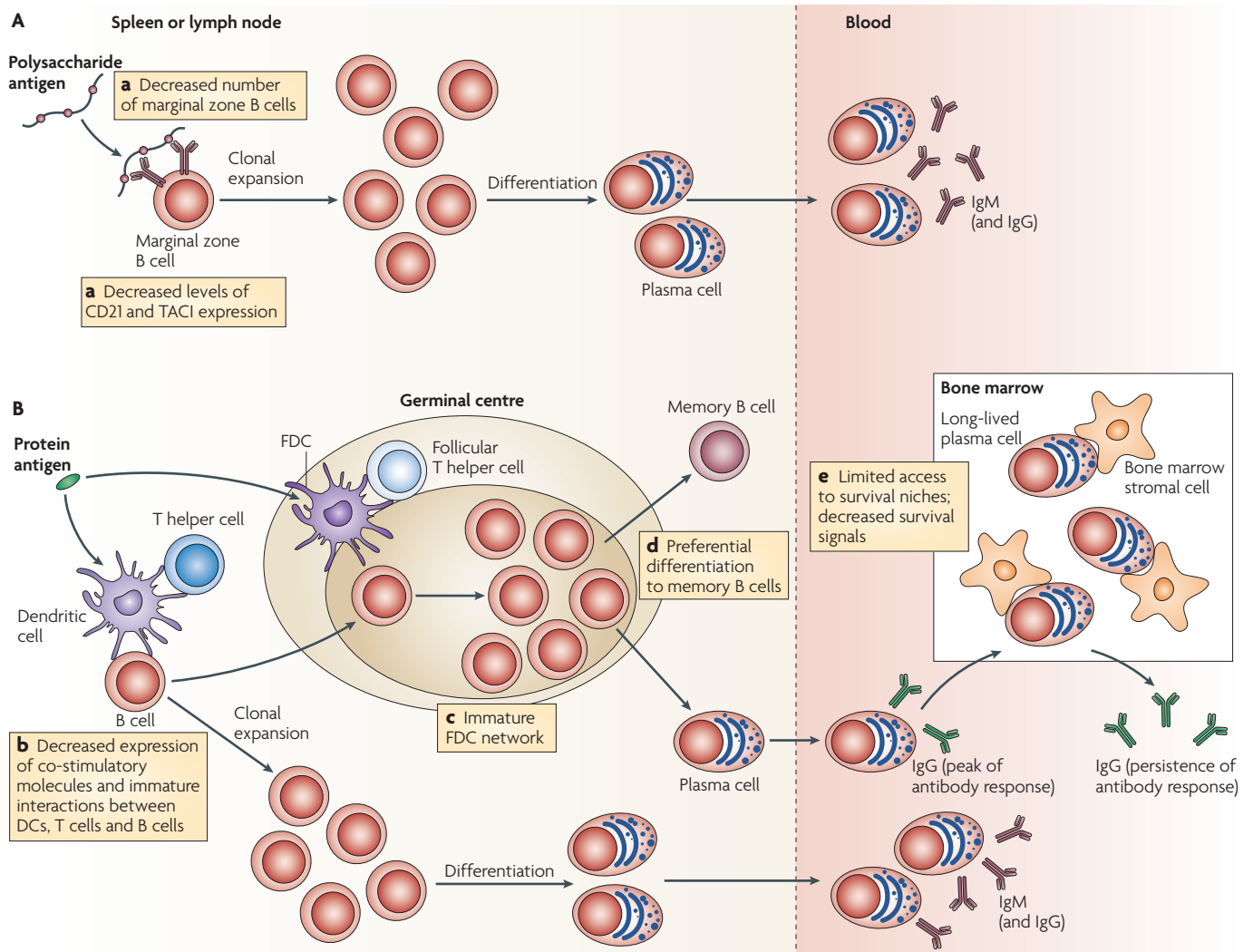


Figure 1 | Early-life limitations of B-cell responses. A | Polysaccharides reach the marginal zone of lymphoid organs, bind to marginal zone B cells and drive their differentiation to short-lived plasma cells. In early life, a decreased number of marginal zone B cells and decreased levels of expression of CD21 and/or TAC1 (transmembrane activator and calcium-modulating cyclophilin-ligand interactor) limit the activation capacity of B cells (a), resulting in the generation of fewer plasma cells. **B** | Proteins reach the B-cell zone in lymphoid organs through free fluid diffusion, triggering T-cell-independent responses, and/or reach the T-cell zone through dendritic cell (DC) transport. Early-life B-cell activation is limited by the decreased expression of co-stimulatory receptors (CD40, CD80 and CD86) and by immature B-cell–DC–T-cell interactions (b). This affects extrafollicular and germinal centre responses. Activated B cells, attracted by antigen-bearing follicular DCs (FDCs) that nucleate germinal centre reactions, receive help from follicular T helper cells, proliferate, undergo somatic hypermutation and affinity maturation, and switch from IgM- to IgG-, IgA- or IgE-producing cells. The immaturity of the FDC network (c), or of other crucial germinal centre factors, limits early-life germinal centre responses. Germinal centre B cells differentiate into memory B cells or plasma cells. In early life, there is a preferential differentiation towards memory B cells (d). By contrast, fewer plasma cells are elicited, resulting in lower peak IgG titres. Short-lived plasma cells must access survival niches that are located in the bone marrow to differentiate into long-lived plasma cells. The decreased persistence of early-life IgG responses might result from limitations of access to bone marrow niches and/or their failure to provide appropriate survival signals (e), such as a proliferation-inducing ligand (APRIL).

to respond to B-cell-mediated lymphotoxin- α signalling³⁵. FDCs nucleate germinal centre reactions by attracting antigen-specific B cells, retain antigens in the form of immune complexes that are highly stimulatory to B cells and provide signals that lead to somatic hypermutation and class-switch recombination. The immaturity of the FDC network therefore delays the induction and limits the magnitude of germinal centre responses, even when potent adjuvants that induce

adult-like B-cell, T-cell and DC activation patterns are used³⁵ (C.-A.S., unpublished observations). It is difficult to establish whether functional FDC maturation is delayed in humans. However, the slow increase in the size and number of lymph node germinal centres with age³⁶ and the induction of fewer plasma cells following infant immunization, compared with adult immunization, indicate that human infants have limited germinal centre reactions³⁷.

Moreover, immature DC–T-cell interactions might also limit infant IgG responses. In addition, neonatal CD4⁺ T-cell responses differ from those elicited later in life, showing preferential T-helper-2-cell polarization (reviewed in REF. 10). This could either support or limit neonatal antibody responses, depending on the antigen-specific B-cell requirements for co-stimulation. It is unclear whether infant T cells express sufficient CD40L to provide optimal CD40-mediated co-stimulation to infant B cells. Potent adjuvants (such as CpG oligonucleotides in mice) can induce adult-like DC and T-cell activation patterns, but they fail to induce adult-like antibody responses^{35,38}. This highlights the control that is exerted by microenvironmental determinants such as FDCs on B-cell responses in early life. All of these factors (TABLE 1) have an age-dependent influence on immune responses by limiting the kinetics and magnitude of antibody responses to both polysaccharide and protein antigens in early life.

Antibody persistence. The long-term maintenance of specific antibodies with a short half-life requires the persistence of antibody-producing B cells, which can either be continually produced from a pool of memory B cells or can persist as long-lived plasma cells. Antibody-mediated depletion of memory B cells, which does not affect plasma cells, has shown that the plasma-cell stage is independent of the memory B-cell pool¹². Indeed, the short persistence of mouse antibody responses in early life (TABLE 1) results from the failure to establish and maintain an optimal plasma-cell pool in the bone marrow³⁹. In infant mice, post-germinal-centre plasmablasts readily home towards the bone marrow compartment⁴⁰ but fail to establish themselves as long-lived plasma cells because of insufficient differentiation and survival signals from bone marrow stromal cells⁴⁰. This was recently ascribed to the insufficient production of a proliferation-inducing ligand (APRIL; also known as TNFSF13), which is a crucial factor for the establishment of the bone marrow plasma-cell pool by early-life bone marrow stromal cells⁴¹. Whether similar limitations affect the establishment and/or persistence of bone marrow plasma cells in early human life is not known. However, studies in human infant twins have shown that although the magnitude of primary vaccine responses is controlled by genetic determinants⁴², their persistence is influenced by environmental factors⁴³. This supports our hypothesis that the limited persistence of antibody responses in early life results from exposure to a large load of environmental antigens, which results in competition for access to a limited set of plasma-cell survival niches in the bone marrow.

Priming and affinity maturation. Although neonatal antigen exposure rarely elicits marked antibody responses, it can effectively prime the immune system for enhanced subsequent responses^{44,45} (TABLE 1). This priming capacity was recently used to accelerate the development of anti-pertussis responses by administering a priming vaccine dose at birth, initially in mice²⁶ and recently in humans^{46,47}. Surprisingly, however, neonatal human immunization can

sometimes fail to enhance subsequent antibody responses (for example, for tetanus or diphtheria toxoids⁴⁸) or even impair such responses (for example, for pertussis antigens⁴⁹). Such vaccine interference could result from B-cell competition for limited resources (such as co-stimulatory signals), from changes in T-cell polarization, from the active induction of regulatory T cells or from as yet unidentified mechanisms⁵⁰.

The acquisition of full antigen responsiveness also requires a progressive diversification of the IgG antibody repertoire. Earlier studies reported a delayed acquisition of somatic mutations in the IgG V_H (variable region of the immunoglobulin heavy chain)⁵¹ and V_L (variable region of the immunoglobulin light chain) regions during infant antibody responses⁵², suggesting that there is limited affinity maturation of antibodies (TABLE 1). However, work in mice has shown that protein-based human vaccines can induce adult-like avidity profiles in infants, in contrast to vaccines that are based on unique epitopes such as haptens⁵³. Furthermore, affinity maturation has also been shown to occur in response to infant immunization with the Hib vaccine⁵⁴, and the infant and adult rotavirus-specific B-cell repertoires are also highly related⁵⁵.

The diversity of IgG transcripts and somatic mutation frequency increase within the first months of life⁵⁶, and adult levels of somatic mutation were reported at 8 months of age in unselected peripheral blood B cells⁵¹. So, age-related differences in the immunoglobulin repertoire and variance of B-cell responses to immunization seem to arise from selection rather than from changes in recombination of the immunoglobulin locus itself⁵⁷. Nevertheless, the avidity of measles-specific IgG is significantly weaker when elicited in 6- or 9-month-old infants than in 12-month-old infants⁵⁸. Therefore, neonatal immunization does prime antigen-specific B and T cells, but this priming can accelerate, leave unchanged or impair subsequent antibody responses and affinity maturation. The determinants of this process are unknown, and identifying them will require further studies.

Determinants of memory responses. The administration of a single dose of vaccine at birth can fail to elicit specific antibodies while priming for subsequent secondary responses, which indicates a preferential neonatal differentiation pathway towards memory B cells rather than plasma cells. Several factors seem to contribute to this B-cell differentiation pattern (BOX 1). The fate of antigen-specific naive B cells and their differentiation to short-lived plasma cells, long-lived plasma cells or memory B cells is controlled by early B-cell activation signals. High-affinity B cells are actively recruited to the plasma-cell pool, whereas moderate-affinity B cells remain as memory B cells in the secondary lymphoid organs⁵⁹. So, decreased initial B-cell receptor affinity and/or delayed affinity maturation of neonatal naive B cells might decrease the strength of the signal and favour memory B-cell differentiation. The limited expression of CD21 by infant B cells also supports the generation of memory B cells and impairs the development of plasma cells⁶⁰, which would be supported

Box 1 | Favoured memory over antibody responses: evolutionary adaptation?**Mechanisms that favour memory B-cell rather than plasma-cell differentiation:**

- Decreased strength of B-cell receptor (BCR) signalling by naive B cells
- Decreased available antigen as a result of binding by maternal antibodies
- Limited expression of B-cell co-stimulatory receptors (CD21, CD40, CD80 and CD86)
- Limited co-stimulatory signals to B cells (through CD40 ligand, serum complement component C3, cytokines, B-cell-activating factor (BAFF) and/or a proliferation-inducing ligand (APRIL))
- Delayed follicular dendritic cell (FDC) maturation, which limits germinal centre reactions
- B-cell competition for simultaneous access to limited germinal centre resources
- Limited support for the establishment of bone marrow plasmablasts
- Intense exposure to environmental antigens, which results in B-cell competition for simultaneous access to limited bone marrow survival niches

Potential evolutionary factors that limit plasma-cell differentiation in early life:

- Antibodies of maternal origin decrease the antigen load and provide protection, so there is no drive for plasma-cell differentiation
- Preventing plasma-cell differentiation until memory B cells have been primed and/or reactivated could limit potentially harmful cross-reactive responses to self polysaccharides and glycosylated proteins during the vulnerable periods of fetal and postnatal development
- As a mechanism to limit responses against prevalent but harmless environmental antigens (resource sparing)
- As a mechanism to prepare the post-weaning period for prompt reactivation in case of repeat exposure

by CD40-mediated signalling, cytokines such as IL-21 (REF. 61) and interactions with ligands such as B-cell-activating factor (BAFF; also known as TNFSF13B) and APRIL⁶². Remarkably, these plasma-cell-supporting factors are all expressed at low levels early in life (BOX 1), unless additional activation signals are provided to enhance DC and T-cell activation.

Furthermore, early-life B cells might have to compete for limited resources within the germinal centre, which impairs antibody responses. Indeed, plasma-cell differentiation can be 'forced' in early life by providing additional DC activation signals. For example, Hib vaccines in which the bacterial polysaccharides are conjugated to the meningococcal outer membrane protein complex (PRP–OMP vaccine), which induces Toll-like-receptor-2-mediated DC activation⁶³, induce higher antibody responses to the first vaccine dose than the other Hib conjugate vaccines⁶⁴. However, this is at the expense of the memory B-cell pool, as PRP–OMP-induced B cells can not be readily reactivated by boosting. Similarly, providing three early infant doses of potent vaccines, such as serogroup C meningococcus–tetanus conjugates, forces primary antibody responses to the detriment of memory B-cell responses⁶⁵.

Therefore, a combination of factors results in the preferential differentiation of early-life B cells towards memory B cells instead of long-lived plasma cells in a pattern that is so strikingly conserved (at least between mice and humans) that it suggests to us an evolutionary adaptation (BOX 1). Importantly, although it has been shown that the memory B-cell pool can be formed early in life, this should not be considered as

evidence that its magnitude or persistence is similar to that elicited in immunologically mature hosts. Recent observations of the failure of booster vaccines to elicit a memory response in adolescents or young adults that had been primed against hepatitis B virus in infancy suggest that infant-triggered memory B cells might not be life long^{66,67}. Whether this reflects a smaller B-cell pool in infants and/or the influence of as yet undefined homeostatic factors remains to be determined.

In summary, a large number of B-cell intrinsic factors and extrinsic determinants cooperate to limit the induction and the persistence of antibody-secreting plasma cells in early life, while supporting the preferential induction of memory B-cell responses.

B-cell responses in later life

Antibody responses in adults and older individuals. Perhaps the greatest difference between B-cell responses at life's extremes regards their serological profiles. Successful recovery from viral and bacterial infections is associated with good humoral immune responses, as shown by the increased levels of specific antibodies following infection. Surviving to old age requires a successful response to a wide range of potential pathogens and the generation of a prodigious 'reservoir' of immunological memory. Measurable titres of serum antibody are stable and have the capacity to be maintained for prolonged periods. Antibody responses to viruses, such as varicella-zoster, measles and mumps, have half-lives of 50 years or more, whereas antibody responses to non-replicating protein antigens, such as tetanus and diphtheria toxins, decline with half-lives of 10–20 years⁶⁸. This suggests that the persistence of antigen contributes to the continued production of antibody, as indicated by studies in mice⁶⁹.

The capacity to respond to new potential pathogens does not increase at an exponential rate throughout life. Indeed, both extremes of life are linked by an inability to respond to new antigens, albeit for mechanistically different reasons (TABLE 1). Evidence for this in older individuals comes from the measurement of changes in specific antibody levels at defined times after vaccination. The ability of influenza vaccine to induce protection is related to age, with an efficacy between 70% and 90% in those under 65 years of age, but of 30% to 40% at best for those over 65 years of age^{11,70}. Similarly, responses to pneumococcal polysaccharides⁷¹ and hepatitis B vaccines are compromised by old age¹², and antibody responses are of shorter duration in healthy older people⁷² (TABLE 1).

Epidemiological studies have shown that populations of older individuals have increasing titres of autoantibody as they age⁷³. These autoantibody titres are not usually associated with autoimmune disease, possibly because of the low affinity of the antibodies. More than half of healthy older individuals have antibodies for non-organ-specific autoantigens (such as nucleoprotein or IgG)⁷⁴, which might result from their generation as a by-product of responses to other antigens. Indeed, an early study showed that shortly after vaccination with tetanus toxoid, there was a significant increase in the frequency of B cells producing rheumatoid factor, accompanied by an increase in plasma levels of IgM rheumatoid factor⁷⁵. There is also

Rheumatoid factor

An autoantibody specific for the Fc portion of IgG, which is most relevant in rheumatoid arthritis.

an age-dependent increase in the prevalence of organ-specific antibodies (such as thyroid-specific antibodies)⁷⁶. The increase does not seem to be exponential, as studies showed that the prevalence of organ-specific autoantibodies in centenarians is similar to that found in individuals under 50 years of age⁷⁶.

Further changes in the serological profile in older individuals were revealed by the presence of a single spike on electrophoresis gels within the region of the gel that is associated with immunoglobulins. These paraproteins are produced by a clone of plasma cells with a defined single specificity — a condition known as benign monoclonal gammopathy. The prevalence of this condition increases with age, with 3.2% of individuals over 50 years of age suffering from the disease, 5.3% of those over 70 years of age and 7.5% of those over 85 years of age⁷⁷.

The differences in the serum antibody profile between these age groups include the increase in the amount of serum immunoglobulin between neonates or infants and elderly individuals⁷⁸, the increased presence of autoantibodies and low affinity antibodies in the elderly and the occurrence of an over-representation of specific classes of antibody from individual B-cell clones in some older individuals (TABLE 1). The following sections discuss how these changes are a reflection of changes in the B-cell pool.

Cellular profile. Comparison between the first and later years of life reveals considerable differences in the peripheral B-cell pool. Sites of naive B-cell production decrease with age: early in life most bones contain marrow that is actively producing B cells, whereas in adults the haematopoietic bone marrow is restricted to the vertebral bones, sternum, ribs, flat bones of the cranium and pelvis, and the end of long bones. These active sites gradually decrease further, and fat deposits accumulate in the marrow cavity as a result of the differentiation of stromal cells to adipocyte-like cells⁷⁹ (FIG. 2). These changes probably alter the specialized stromal-cell microenvironment and result in the potential loss of specialized niches. The age-related decrease in the production of new B cells from their precursors, which has been described by many investigators, has indicated a decrease in the absolute numbers of early B-cell progenitors, including both pro-B and pre-B cells (reviewed in REF. 80). However, antigen encounter continues to induce immune responses and the proliferation of B cells throughout life. Therefore, with time, the average age of B cells in the pool, as measured in terms of the number of cell divisions undertaken, increases because the contribution of new B cells from the bone marrow decreases but the absolute number of B cells remains similar.

The most marked difference between infants and elderly individuals is a progressive shift from a population that contains many naive B cells and few memory B cells to one that contains mostly memory B cells and few naive B cells in later years (FIG. 2). Subsets in the B-cell pool can be defined on the basis of their expression of CD10, CD19, CD23, CD27, CD38, CD44, CD77 and CD95 (REF. 81). Of these, CD27 has been used as a universal marker to define the memory B-cell subset⁸¹. However, the discovery of CD27⁺ memory B cells⁸² indicates that

the memory B-cell pool is more heterogeneous than expected⁸³, which questions the use of this single marker as a method of identifying these cells. Analysis in humans is further hampered because a representative sample of the B-cell pool might not be provided by the peripheral blood. A prime example of this comes from responses to smallpox vaccination, which can be maintained for at least 70 years. Specifically, experiments on vaccinia-virus-specific memory B cells in individuals that had been vaccinated more than 30 years earlier were used to calculate that 10–20 million of these IgG⁺ memory B cells were present in the spleen, whereas less than 10⁵ could be detected in the blood. So, although these memory B cells represent just over 0.2% of all of the IgG⁺ cells in the spleen and therefore make a discernable contribution to the splenic B-cell population, they only correspond to 0.07% of the IgG⁺ cells in the blood⁸⁴. Consequently, the identification and assessment of absolute numbers of specific memory B cells might be difficult to determine from blood samples.

Determinants of the B-cell pool. The progressive and unidirectional nature of B-cell development means that ageing should be associated with an increase in the number of plasma cells, but it is not clear whether this process is accompanied by an increase in the number of survival niches. The number of these niches is thought to be finite^{85–87} but might depend on age; age-related changes in the bone marrow could alter the number of survival niches, and access to these niches is known to regulate the size of the plasma-cell pool⁷⁸. In old mice, an impaired ability of the aged bone marrow to support plasma-cell survival was proposed to account for the decreased number of long-lived bone marrow plasma cells⁸⁸. Whether age-related changes in the bone marrow alter plasma-cell number in older humans is unknown, as is how these changes could account for the increased prevalence of monoclonal gammopathy in older individuals⁸⁹.

Determinants of the B-cell response. The failure of older individuals to produce an antibody response of the same magnitude as that from a young individual led to studies that aimed to determine whether this defect was due to the absence of a specific contributing cell type or a component of the response. Histological analysis of lymph nodes from human subjects has shown that the number and size of germinal centres are significantly decreased in older individuals (TABLE 1), and these changes are accompanied by an increase in the amount of adipose tissue⁹⁰. These alterations suggest that there might be limitations to the proliferative phase of the immune response in the elderly. Indeed, studies examining mitogen-induced proliferation of T cells from older individuals show that these T cells have considerably less proliferative capacity *in vitro* compared with T cells from younger individuals⁹¹. B cells from old mice also show decreased proliferation *in vitro* compared with B cells from younger animals⁹², although this effect does not seem to be as marked as for T cells. A possible explanation for this might come from experiments looking at telomeres and telomerase, which have a central role in the replicative lifespan of cells. Comparison

Benign monoclonal gammopathy

A condition in which a low or non-quantifiable level of a monoclonal paraprotein is detected in the blood by protein electrophoresis.

Telomere

A region of repetitive DNA at the end of chromosomes, which protects the end of the chromosome from destruction.

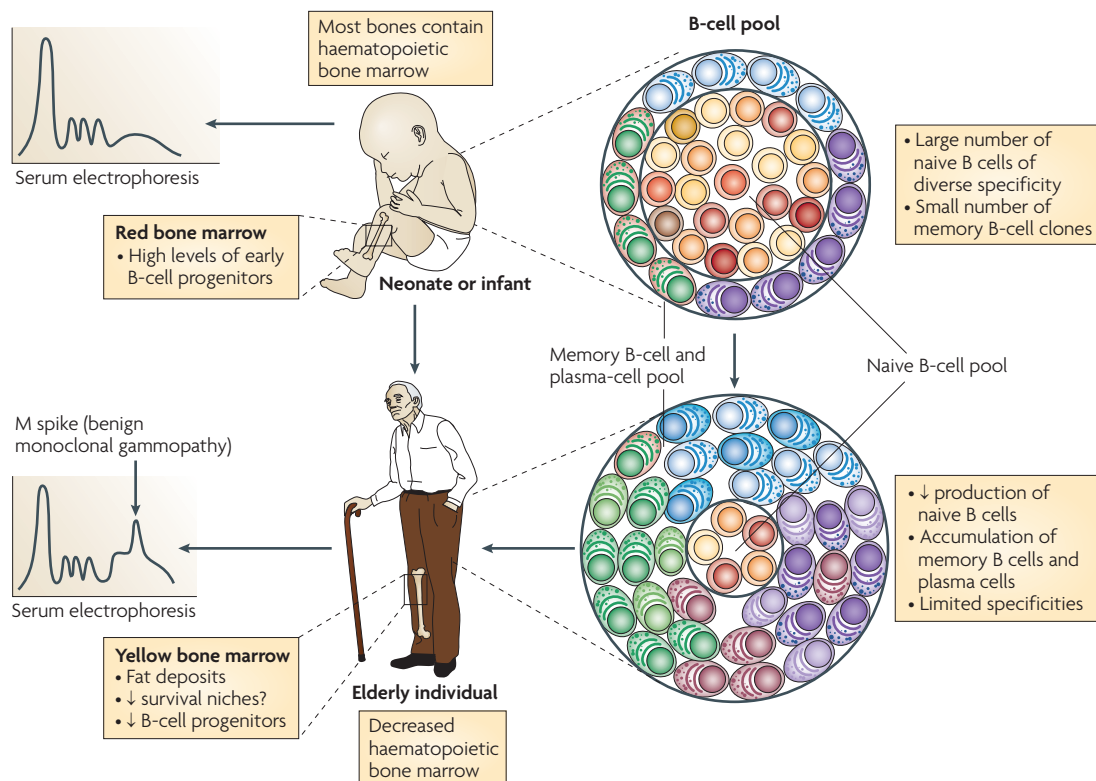


Figure 2 | Comparative changes in the B-cell pool. In early life, the haematopoietic bone marrow generates large numbers of pre-B cells and contains naive B cells of diverse specificities and a small number of memory B-cell clones. With age, the production of naive B cells declines, contributing to the decrease in the capacity to respond to new antigens. Memory B cells and plasma cells of limited specificities accumulate, and occasional dysregulated plasma cells might produce monoclonal IgG (M spike); this might limit the access of new plasma cells to functional bone marrow niches. In addition, differences are observed between the bone marrow of neonates or infants and the elderly. Specifically, in early life, most bones contain marrow that has high levels of B-cell progenitors and is actively producing B cells. Age-related changes in the bone marrow, including the accumulation of fat deposits, are associated with the potential loss of specialized survival niches and a decrease in the number of B-cell progenitors.

of naive and memory CD4⁺ T cells from the blood of healthy adult donors revealed that naive cells consistently have longer telomeres than memory cells (which have undergone extensive proliferation)⁹³. By contrast, no such consistent decline in telomere length was noted when naive and memory B cells were analysed⁹⁴, despite the fact that B-cell differentiation is accompanied by considerable cell division. Lengthening of the telomeres through the induced expression of telomerase in the germinal centre during proliferation and differentiation might account for this observation⁹⁴. Another study has shown that there does seem to be some age-related loss of telomere length in B cells, but that the rate of telomere shortening in B cells is significantly slower than that seen in CD4⁺ T cells⁹⁵.

Others have concentrated their studies on the role of FDCs and immune complexes in the age-related decrease in B-cell responses. Immune complexes on FDCs have been reported to stimulate B-cell proliferation in the germinal centre⁹⁶. In addition, studies in mouse models showed that although the numbers of FDCs in young and old animals are not altered, there is a change in their functional ability. Specifically, FDCs from old mice have been shown to have a marked decrease (>90%) in their

expression of low-affinity Fc receptor for IgG (FcγRII) compared with young animals, which has been proposed to contribute to the decline in the retention of immune complexes in the germinal centre⁹⁷. This might be responsible for the decrease in number and size of germinal centres, and might contribute to the shorter duration of antibody responses that is observed in the elderly (TABLE 1).

An alternative explanation for the decrease in antibody responses with age might be related to the expression level of CD86 on B cells. Young animals that have been injected with a CD86-specific antibody have a decreased ability to form germinal centres and their B cells fail to undergo somatic mutation of immunoglobulins and to produce high-affinity antibody⁷⁴. These results prompted the hypothesis that the decreased antibody responses in older individuals could be due to decreased expression of CD86 on their B cells. Initial experiments failed to support this notion, as germinal centre B cells from old mice have a normal activation phenotype⁹⁸. Further experiments sought to determine whether the defect was upstream of this process. One such early step is the interaction between CD40 on B cells or antigen-presenting cells and CD40L on T cells, which has been linked with the upregulation

of expression of CD86 and, consequently, with successful B-cell responses⁹⁹. Adoptive transfer of T cells from old animals to young hosts resulted in decreased B-cell proliferation and differentiation in response to antigen, whereas no difference in B-cell proliferation and differentiation was noted when young T cells were transferred to young and old hosts. These age-related defects might be due to the decreased level of expression of CD40L on the T cells from old animals^{100,101}.

Several studies indicate that older humans have the capacity to generate a diverse immunoglobulin repertoire and a similar range of immunoglobulin rearrangements as younger individuals^{102,103}. Some studies suggest that although the quantity of antibody that is produced does not change greatly with age, the quality declines markedly, with older individuals producing fewer antigen-specific antibodies and a higher number of antigen-non-specific antibodies¹⁰⁴. This limitation cannot be explained entirely by defects in the B-cell population; problems with other cell populations (such as FDCs and T cells) could also be implicated.

Changes in antibody responses in older individuals are associated with a decline in the production of naive B cells and the accumulation of memory B cells. This increase in memory B cells is not paralleled by a similar increase in the number of plasma cells, which indicates that these two populations are not intimately linked or that there is a decrease in the number of accessible and functional plasma-cell survival niches in the bone marrow. The shift in the ratio of naive to memory B cells, which might be due in part to an intrinsic property of the stem cells of old animals¹⁰⁵, provides a plausible explanation for the age-associated restriction in the B-cell repertoire. Evidence for a decline in the repertoire comes from recent studies of spectratype analysis of the immunoglobulin heavy-chain variable region complementarity-determining region in DNA. These studies revealed a marked age-associated decrease in the B-cell repertoire that could involve clonal B-cell expansion *in vivo*¹⁰⁶.

In summary, the two extremes of age share a similarly high vulnerability to common pathogens such as RSV, influenza virus and *S. pneumoniae*. Remarkably, they share similar difficulties in eliciting sustained protective antibody responses, and this essentially results from the influence of a limited number of non-B-cell determinants, including FDCs and bone marrow survival niches for plasma cells.

Towards improved immunization strategies

Circumventing the limitations of antibody responses to vaccination at the extremes of age is not trivial (TABLE 2). When high levels of neutralizing antibody are required for protection against pathogens in early life (such as RSV and influenza virus), new adjuvants and/or delivery systems that increase B-cell activation and plasma-cell differentiation will be required. When there is sufficient time for the induction of a memory response and its subsequent activation, the preferential differentiation pathway of early-life B cells towards memory B cells could benefit from ‘early prime – later boost’ immunization strategies. This implies defining the earliest age at which specific vaccine antigens can efficiently prime neonatal B and T cells of the appropriate phenotype. It also implies giving the final vaccine dose after a sufficiently long interval (3–4 months) for affinity maturation to occur. Defining strategies that elicit the appropriate balance between plasma cells and memory B cells, result in sustained antibody production and avoid vaccine interference by altered B- and/or T-cell responses⁵⁰ will be the next challenge. Providing passive protection through maternal immunization¹⁰⁷ is an attractive alternative for pathogens to which exposure occurs early, although addressing the issues of low acceptance and fear of litigation will be complicated.

In older individuals, increased doses of antigen or new adjuvants might enhance vaccine immunogenicity¹⁰⁸. However, the main limiting factor is the age-associated atrophy of the haematopoietic tissue and

Table 2 | **Circumventing the limitations of vaccine antibody responses at the extremes of age**

Mechanisms	Potential strategies
Primary activation of naive antigen-specific B cells	
Increasing recruitment of naive B cells	<ul style="list-style-type: none"> • Administering higher doses of vaccine antigen • Increasing antigen retention by improving depot formulation and antigen delivery systems • Improving B-cell targeting with new antigen delivery systems • Administering repeated primary immunization doses
Increasing activation of naive B cells	<ul style="list-style-type: none"> • Developing new adjuvants, delivery systems and immunomodulators
Enhancement of plasma-cell responses	
Increasing B-cell activation	<ul style="list-style-type: none"> • Administering higher doses of vaccine antigen • Developing new adjuvants and immunomodulators
Increasing plasma-cell differentiation	<ul style="list-style-type: none"> • Developing new adjuvants and immunomodulators
Improving plasma-cell survival	<ul style="list-style-type: none"> • Improving access of plasma cells to functional bone marrow niches
Enhancement of memory B-cell responses	
Increasing memory B-cell differentiation	<ul style="list-style-type: none"> • Administering lower doses of vaccine antigen
Recalling memory B-cell responses	<ul style="list-style-type: none"> • Extending vaccine schedules (late boosters) • Administering repeat booster doses

primary lymphoid organs. The accumulation of fat in the thymus and the bone marrow is associated with decreased production and export of new B and T cells, resulting in a peripheral lymphocyte pool that is shaped by previously encountered antigen. Decreased production of lymphocytes creates a system with fewer naive cells and, consequently, with increased vulnerability to pathogens that have not been encountered previously. In addition, the powerful homeostatic forces that maintain lymphocyte numbers through the proliferation of existing clones result in a greater number of cells that have

reached their replicative limit, leaving older individuals vulnerable even to infections that have been successfully dealt with in the past. Reversal of thymic atrophy by castration¹⁰⁹, the use of ligands for growth hormone receptors¹¹⁰ and IL-7 (REF. 111) have shown some success. Although these therapies have concentrated on T cells, they might also have an effect on B-cell development: older mice treated with IL-16 show a 1.6-fold increase in the number of pre-B cells compared with control animals¹¹². How much of this might eventually apply to older humans remains to be determined.

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 APRIL | BAFF | CD27 | CD40 | CD40L | CD80 | CD86

FURTHER INFORMATION

Claire-Anne Siegrist's homepage: <http://pathology.unige.ch/group-siegrist.html>
 World Health Organization (WHO) health statistics: <http://www.who.int/whosis/whostat/2008/en/index.html>

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