

Characteristics of B Cells and B Cell Responses in Aged Individuals

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Abstract Ageing individuals are immunologically characterized by loss of immunological protection and responsiveness, and autoimmunity occurs with increased incidence. Here we review studies on specific age-related changes in B cell generation, activation, and maintenance as well as characteristics of B cell responses and antibody levels during ageing in relation to other cells and factors that are interwoven with B cell biological processes. In the elderly, fewer new B cells are generated, B cell responsiveness to antigens is impaired, and smaller and fewer germinal centers are formed within an immune response with participation of less potent T cells and follicular dendritic cells, leading to the production of reduced, often insufficient plasma cell numbers. Recall responses in the elderly appear to be limited by the cells' proliferative capacity. Hence, vaccine responsiveness is often insufficient and autoantibody production emerges in the elderly. In total, B cells appear to be less affected by age as compared with T cells. Recently, new concepts have been developed to counteract immunosenescence beyond active vaccination, comprising the generation of specific monoclonal antibodies for passive vaccination, immune rejuvenation by immunoablation followed by autologous stem cell transplantation, and modulation of lifestyle.

Keywords Age • Antibody • B cell • Blood • Bone marrow • Frailty • Germinal center • Human • Immunosenescence • Immunity • Immunoglobulin • Memory B cell • Mortality • Mouse • Naïve B cell • Plasma cell • Pre-B cell • Protection • Recombinant antibody • Spleen • Vaccination

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Abbreviations

AID	Activation-induced cytidine deaminase
APRIL	A proliferation-inducing ligand
ASCT	Autologous stem cell transplantation
BCR	B cell receptor
BM	Bone marrow
CDR	Complementarity-determining region
CSR	Class-switch recombination
EBNA	EBV nuclear antigen
EBV	Epstein–Barr virus
FDC	Follicular dendritic cell
HSC	Hematopoietic stem cell
LMP	Latent membrane protein
mRNA	Messenger RNA
NP	Nitrophenol
PC	Phosphorylcholine
Pre-B cell	Precursor B cell
Pro-B cell	Progenitor B cell
RAG	Recombination activating gene
RF	Rheumatoid factor
RSV	Respiratory syncytial virus
SARS	Severe acute respiratory syndrome
SHM	Somatic hypermutation
SLE	Systemic lupus erythematosus

1 Introduction

During the last 100 years, mankind experienced a great increase of life expectancy. Individuals reaching great age are often affected by limited immunocompetence, referred to as “immunosenescence.” In B cell biology, immunosenescence is reflected by altered quantity, quality, and composition of B cells and antibodies, and their spatial organization during an immune response and the memory phase. Germinal centers as key structures for B cell differentiation and selection are impaired in numbers, size, and function. To this end, other cells and processes underlying senescent impairment, such as T cells, cytokine production, and antigen presentation as well as the nonlymphoid B cell microenvironment, regulate and impact age-dependently on B cell function and responses.

B lymphocytes generate specific antibodies that mediate clearance of pathogens in the body and at mucosal surfaces and thus are key players in the immune system. B cells may further act as antigen-presenting cells and modulate immune responses by the secretion of cytokines [1].

Briefly, B cells are generated from hematopoietic stem cells (HSC) in the bone marrow (BM), where they mature and become naïve B cells which are released into the periphery. During their maturation in the BM, B cells rearrange their immunoglobulin genes encoding the B cell receptor and antibody molecules, thus determining the antigen specificity of an individual B cell. Gene rearrangement activity alternates with cell proliferation, so a highly diverse repertoire of B cells is generated, each carrying an individual specificity and unique B cell receptor (BCR). Naïve B cells can be activated by binding an antigen through the BCR. This is followed by proliferation and differentiation into either plasmablasts, which secrete large amounts of antibody of the same specificity as the BCR, or into memory B cells, which remain in the periphery after the acute immune response has ceased. Such memory B cells can be activated by the same antigen to differentiate again into plasmablasts or memory B cells, both being of enhanced potency and responsiveness to recurrent antigen challenge. Somatic hypermutation (SHM), class switch recombination (CSR), and selection for antigen affinity are characteristics of germinal center reactions and memory B cell responses engaging T cell help, which may occur in spleen, lymph nodes, and mucosa-associated lymphoid tissue. Terminally differentiated antibody-secreting plasma cells and memory B cells can persist for years or decades in one's body, with yet not fully defined contributions of self-renewal and longevity of these cells. Whereas memory B cells reside in secondary lymphoid organs and the blood, plasma cells reside in the BM [1, 2]. For almost all of these B cell differentiation stages, properties, and functions, age-related changes are detectable, being mainly associated with reduced immune function and protection. As, e.g., T cells are apparently more greatly affected by age than B cells [3], some works have directly or indirectly questioned the inherent senescence of B cells [4–7], and the contributions of inherent and extrinsic factors to senescent B cell responses remain to be elucidated [8, 9]. It is generally discussed whether age-related immune phenomena reflect or cause the reaching of great age or whether immunosenescence is a process of remodeling the immune system or an indication of immunodeficiency [9, 10]. In this regard, healthy centenarians often show a different pattern of changes as compared with the 70–85-year-old population with a considerable impact of frailty.

Inconsistent results and conclusions have been published in research on age-related changes to B cells and their function, including critical points [8]. Hence, it is difficult to draw a clear picture of aged B cell responses. At least some variation originates from the heterogeneity of human subjects analyzed regarding their genetic background, lifestyle, hidden or overt comorbidities, or general frailty. To this end, the SENIEUR protocol was established, containing donor admission criteria for immunogerontology studies [11].

2 The Contribution of B Cells to Age-Related Illness

2.1 Infectious Diseases

Numerous studies have documented that aged individuals have a highly increased risk of dying from infectious diseases. Most deaths caused by infections are in old individuals. In the USA, 78% of influenza-associated deaths and 90% of respiratory syncytial virus (RSV)-associated deaths occurred among people over 65 years of age [12]. During the 2002–2003 severe acute respiratory syndrome (SARS) outbreak, the patient's age was strongly associated with outcome, i.e., the fatality rate was more than 3 times higher in people over 60 years of age as compared with people under 60 years of age [13].

At the same time, aged individuals respond less frequently and less efficiently to common vaccinations [14], such as against tetanus [15], hepatitis B [16, 17], pneumococcal polysaccharides [18], and influenza [19–21], including mucosal vaccines [22]. Failure to respond appropriately to influenza vaccination appears to be associated with specific B cell properties, such as low blood IgD⁺ B cell numbers and reduced in vitro lymphocyte response to pokeweed mitogen in at least some aged individuals [23], and with higher IgG titers to cytomegalovirus in the young and elderly [24].

2.2 B Cell Malignancies and Autoimmunity

The risk of developing B-cell-derived malignancies and autoimmunity increases with advancing age. Both types of disease and their treatment, such as irradiation, surgical tissue removal, immunosuppression, and cytostatics, may or do affect B cell responses in addition to intrinsic immunosenescence [25].

B cells can be the subject of malignant transformation at all known differentiation stages [26, 27], resulting in a variety of lymphomas, such as B cell non-Hodgkin lymphoma, or Reed–Sternberg cells leading to Hodgkin disease or monoclonal plasma cell expansions, such as multiple myeloma. Besides the life-threatening primary malignant disease, an excess of clonal B cells may additionally impair immune responses by competing with normal B cells for survival and cell migration factors, habitats, and activation signals (e.g., in the germinal center of lymphoid organs). The clonal expansion of B cells also disturbs or destroys the microarchitecture of lymphoid tissue, thus impairing processes involving B cells at the respective sites, e.g., B lymphopoiesis and plasma cell residency in the BM or follicle organization within spleen and lymph nodes. For example, approximately 20% of patients with monoclonal gammopathies of undetermined significance show reductions in uninvolved antibody levels [28].

B cells represent the major target and natural reservoir of Epstein–Barr virus (EBV). More than 90% of all humans are infected with EBV, and in EBV⁺

individuals, virus-containing B cells are rare. Some B cell malignancies originating from germinal center reactions are associated with infections by EBV, i.e., Burkitt lymphoma and Hodgkin lymphoma. Coherently, EBV-associated B cell tumors can express EBV-encoded proteins like EBV nuclear antigen (EBNA)-1, EBNA-2, latent membrane proteins (LMP)-1 and LMP2a, or combinations thereof [29].

Autoimmunity per se is not associated with suppressed responsiveness to antigenic challenge [30]. However, widely used immunosuppressive drugs such as steroids, cyclophosphamide, and TNF- α blocking antibodies target lymphocytes, dampen B cell responses [31], and are associated with increased susceptibility to infections [32]. The success of B cell depletion underlines the key role of B cells in immune responses to autologous antigens [33]. Patients treated with B cell depletion therapy with rituximab (used in both autoimmunity and CD20⁺ lymphoma treatment) do not develop sufficient antibody titers when vaccinated during therapy [34, 35], but remain widely protected during therapy because previously established plasma cells are not affected and respective antibody titers are slightly or not affected by rituximab treatment [36, 37]. Autoimmunity additionally increases the risk for malignant B cell transformation, which is reflected, e.g., by marginal zone lymphoma cells often carrying rheumatoid factor (RF) specificity [38].

3 Age-Related Characteristics of Senescent B Lymphocytes

Immunosenescence can impact on properties of B cells and B cell responses and B cell responses through intrinsic changes of the B lineage cells but is importantly influenced by multiple extrinsic factors: cytokines, interacting and supportive cells, and structures.

3.1 *Impaired B Lymphogenesis During Ageing*

In old age, B cell generation and differentiation in the BM is impaired in both quality and quantity. Tenfold to 20-fold higher frequencies of clonable progenitor B-cells (pro-B cells) and precursor B cells (pre-B cells) were detected in mice shortly after birth as compared with mice aged 6–8 months [39]. Whether these age-related changes are due to an intrinsic defect of HSC [40] or originate from an aged microenvironment of B lymphopoiesis [41] or result from thymic involution is a matter of discussion.

Aged long-term reconstituting HSC display a reduced B cell generative capacity as compared with new, young HSC. This limitation is reflected by alterations of specificities in the B cell repertoire [42] that might be caused by age-related changes in HSC properties [43]. Potential B cell intrinsic defects comprise the

reduced ability to process survival signals [44] and reduced mitotic activity in old versus young animals [45].

A crucial step in initial B cell development is the expression of recombinases that allow V(D)J recombination in pro-B cells, allowing them to become pre-B cells: recombination activating gene (RAG)-1 and RAG-2 proteins [1]. RAG-1 messenger RNA (mRNA) levels in total BM reflecting active early B cell differentiation increase from birth until 2 months of age, remains detectable at 5 months and is severely reduced at the latest by 10 months [46]. Indeed, the BM of old mice contained comparable numbers of pro-B cells, but fewer than the half the number of pre-B cells as compared with young mice after cyclophosphamide-induced lymphopenia. In the same model, in old mice splenic B cells representative of peripheral B cells were present at one third of the numbers of those in young mice and comparatively had a restricted repertoire [31].

It was suggested that increased apoptosis of pre-B cells would account for their reduced numbers in the BM of old mice, involving reduced expression of the anti-apoptotic protein Bcl-xL [47–50]. Although pro-B cell numbers and RAG-1 mRNA amounts within this fraction were normal in aged mice [47, 51], they expressed less RAG-2 and showed less V(D)J recombinase activity [52, 53] and gave rise to fewer pre-B cells in vivo and in vitro [47, 49]. Both D to J and V to DJ recombination were diminished in old mice at the stages of early and late pro-B cells, so defective pre-BCR expression was suggested to contribute to pre-B cells apoptosis [54]. Indeed, surrogate light chain expression was decreased in pre-B cells of senescent mice [55] and was associated with decreased *E2A* expression [56]. When B cells expressing IgH and IgL transgenes were introduced, which do not need to undergo rearrangement, the numbers of transgenic B cells were normal, whereas endogenous B cell numbers with natural rearrangement of IgL and IgH genes were reduced in aged mice [51].

Early B cell development appears to be dependent and regulated by thymic activity. Both athymic nude mice and old euthymic mice show decreased numbers of BM pre-B cells, fewer of these express RAG-1 protein, and the decline of RAG-1 expression during ageing follows similar kinetics as thymic activity and involution [46, 57]. Coherently, thymus engraftment induces B cell development in X-linked immune-deficient mice [58], whereas thymus cells from aged animals were not able to reconstitute B cell maturation in the BM [59]. Injections of IL-16 or supernatants from activated CD8⁺ but not CD4⁺ T cells can restore RAG-1 expression and pre-B cell numbers in athymic mice in vivo [54, 57].

Defects in the aged microenvironment of B lymphopoiesis have been suggested to contribute to reduced B cell generation [49, 52, 60], which therefore might be influenced by age-related changes of bone structure (e.g., osteoporosis) and stromal cell senescence [61, 62]. Indeed, BM stromal cells can rescue young but not old B cell precursors from dexamethasone-induced apoptosis through IL-7 and insulin-like growth factor 1, by counteracting apoptosis through Bax, Bcl-2, and Bcl-x_L.

In human BM, B cell subsets (e.g., pre-B cells and pro-B cells) reflecting maturation of B cells can be detected at comparable frequency throughout ageing (until 88 years), with maintained proliferative capacity [63, 64].

3.2 Lifespan, Survival, and Turnover of B Cells

The capacity of B cells to be maintained as a whole population by cell replication could be limited by the length of telomeres [65]. Human germinal center B cells carry longer telomeres than naïve resting B cells and express telomerase, an enzyme extending telomeric restriction fragments upon in vitro and in vivo activation [66–70]. Hence, the way and strength of B cell activation might influence B cell responses by determining telomere lengthening. During ageing, telomeres of both peripheral blood naïve and CD27⁺ memory B cells shorten, but inducible telomerase expression remains unaffected by age [71, 72].

Survival of individual B cells is further dependent on receptors and signaling molecules expressed by B cells, such as BCR, BR3, and phospholipase C- γ_2 [74–77]. In EBV-infected B cells, virally encoded proteins can substitute the function of survival-promoting B cell surface receptors. Whereas tonic BCR signaling can be replaced by LMP2a expression [78], CD40 can be simulated by LMP1 [79–81]. B cell survival importantly depends on factors produced in the B cell's microenvironment, such as B cell activating factor (BAFF), a proliferation-inducing ligand (APRIL), and interleukins [1]. This allows various senescence-associated phenomena to impact on B cell survival.

Mature B cells appear to be self-sufficient and not intrinsically limited in live organisms, as a joint effect of cell survival and homeostatic proliferation. Consistently, functional B cells could be recovered more than 10 months after their transfer to *scid* mice, and can be utilized in antibody responses [82]. The half-life of peripheral mature B cells from old mice is increased (decreased turnover) and reduced levels of newly generated B cells are detected in the periphery as demonstrated using in vivo 5-bromo-2-deoxyuridine labeling experiments. In old mice, the numbers of immature splenic B cells were found to be reduced fourfold, with a concurrent reduction of the numbers of BM surface IgD⁻/surface IgM^{int/high} B cells, but not least mature surface IgD⁻/surface IgM^{very low} B cells [83]. After 4 weeks of 5-bromo-2-deoxyuridine labeling, 15% of mature and transitional B cells were newly made in old mice compared with 30% in young mice. Immature B cell production rates did not differ between old and young mice in this study, so defective replenishment of peripheral compartments rather than impaired B lymphopoiesis was reasoned to be responsible for reduced peripheral counts of immature B cells in aged mice [84]. In summary, the mature peripheral B cell compartment seems to stably persist in ageing mice, and this has been shown to be independent of age [85, 86].

Relatively little is known about human B cell turnover. Circulating naïve B cells from old individuals were less than those from young individuals susceptible to apoptosis in terms of annexin V binding after 1–2 days of peripheral blood mononuclear cell culture [87]. Circulating B cell turnover rates assessed by incorporation of heavy glucose slightly decreased with age and differed for

CD27⁻ naïve B cells (approximately 0.5% of cells divide each day) and CD27⁺ memory B cells (approximately 2.6% of cells divide each day) [88].

Memory B cells and long-lived plasma cells constitute the immunological B cell memory. For both, long-term persistence in the absence of antigens has been demonstrated [89–91] and directly challenged for memory B cells [92] or questioned as a means to provide humoral memory for plasma cells [93, 94]. There is accumulating evidence for a distinct regulation of the memory B cell compartment and humoral memory provided by plasma cells [36, 37, 95–98], as reflected by persistent antibody production in the absence of memory B cells. They are maintained in the body at different sites and by distinct mechanisms. Memory B cells reside in lymphoid tissues such as spleen [96], tonsils [99], lymph nodes, mucosa-associated lymphoid tissue, and the blood [1] and depend on the presence of the spleen [100], reflected by their gradual disappearance from peripheral blood after splenectomy in humans [95] and concurrent increased susceptibility to microbial infections. Low frequencies of circulating memory B cells proliferate, allowing the maintenance of the whole population [88] and single specificities. By contrast, most mature plasma cells reside mainly in the BM [101, 102], where they can survive and secrete antibody potentially for decades in old mice and humans [91, 97, 103], reflected by persistent antigen-specific antibody titers in human blood serum of adults aged 20–65 years [104]. Long-lived plasma cells are maintained by a yet incompletely explored, hence hypothetical, survival niche which comprises, e. g., stromal cells and their products, such as IL-6 and CXCL12, as well as extracellular matrix components such as fibronectin or hyaluronans [105–108]. The BM microenvironment becomes less potent to support B cell development with age [31] and senescent stromal cells exhibit limited survival and functionality [61], which might implicate a role of the senescent BM microenvironment in reduced plasmablast recruitment and plasma cell survival [109], similarly as shown in 1-week-old mice not yet having adult immune capabilities [110, 111]. At very young age, BM stromal cells largely fail to support plasma cell survival and persistent antibody memory [110, 111]. Thereafter, from 6 to 14 weeks of age, approximately 20% of plasma cells may become long-lived, as demonstrated in lupus-prone New Zealand Black/New Zealand White mice (Hoyer, BF, Voigt, C, Mumtaz, IM, Panne, D, Moser KV, Eilat D, Manz RA, Radbruch A, Hiepe F, manuscript submitted), allowing for filling of survival niches and development of a respective antibody repertoire and increase of blood serum immunoglobulin concentrations to adult plateau values. Plasma cells settling in such niches early in life apparently provide very persistent antibody levels, as illustrated by autoantibodies which arise in patients with systemic lupus erythematosus (SLE) approximately 10 years before manifestation/diagnosis of the disease (e.g., anti-Ro, anti-La) [112] are especially hard to eradicate by therapy [37]. In adulthood, serum immunoglobulin levels remain overall constant and plasma cell niches are saturated. Consistently, only approximately splenic 3% of plasma cells formed within 2 months may become long-lived in old mice as compared with 20% in young mice within the same time period (Hoyer, Voigt, Radbruch, Hiepe et al., unpublished results). Hence, newly generated plasma cells induced within new, systemic immune responses compete

with resident plasma cells for habitat. Newly acquired, long-lasting humoral immunity thus reflects successful competition and is accompanied by the stoichiometric loss of resident, polyclonal plasma cells [97, 113, 114]. In this regard, competition and subsequent mobilization of resident plasma cells might contribute to the slow decrease of antigen-specific titers for some antigens [104] (see later). Other, mutually nonexclusive mechanisms to maintain antigen-specific antibody titers may exist, i.e., homeostatic proliferation of mature plasma cells and continuous de novo generation of plasma cells from memory B cells activated in a bystander mode [94] or by a persistent antigen [93, 115]. More than 95% of BM plasma cells in adult humans do not perform cell division in terms of Ki-67 expression [114]. Therefore, homeostatic proliferation as a means to maintain the plasma cell population over the long term until old age cannot be excluded. Given that homeostatic plasma cell proliferation exists, again changes of the BM microenvironment might impact on antibody titers as a function of age. For the continuous generation of plasma cells from memory B cells, ageing could impact on the B cells' ability to be activated and to proliferate, as discussed in other sections of this chapter.

3.3 Altered Composition of Peripheral B Cell Subsets in Aged Humans

The numbers and frequencies of total CD19⁺ B cells in peripheral blood were found to be reduced with age [87, 116–118], and detection of reduced B cell counts in blood (clustered with low CD4⁺ T cells and in vitro T cells response) predicts a higher mortality and susceptibility to infection [119]. Most of the studies observed changes in the composition of peripheral blood B cells of CD27-expressing memory B cells [120, 121] and CD27-non-expressing naïve B cells dependent on age. From birth until adulthood, the proportion of memory B cells among total circulating B cells increases from approximately 4% in the cord blood to approximately 10% at 1 year of age in peripheral blood, until adult levels of approximately 30% to 50%, and the proportion increases further in aged individuals [71, 122–124]. A generally larger variation of peripheral B cell composition was detected in old humans; however, it did not confirm the increase of the proportion of memory B cells with age [116]. Decreasing proportions of memory B cells with age were also observed [87]. It remains unclear to what degree B lymphopoiesis and naïve B cell output to the periphery is affected by age in humans (see earlier). As most antigen-experienced “memory” B cells do not recirculate but reside in lymphoid tissues such as spleen [96] or mucosal tissues [99], it remains unclear whether or not CD27⁺ B cell counts in peripheral blood reflect B cell memory as a whole. To this end, human tonsils contained constant proportions of memory B cells and plasma cells in 21 samples obtained from donors of different ages, maximum 73 years. At the same time, naïve B cells were relatively enriched with ageing, with a concurrent

reduction of germinal center B cells, which reached their minimum frequency at approximately 45 years, remaining constant afterwards [125].

3.4 Age-Related Changes in the B Cell Repertoire

Analyses of the impact of age on the B cell repertoire are often restricted to specific Ig gene families and cell subsets or are limited to specific experimental conditions. For example, analyses performed with total white blood cells do not allow consideration of the contributions of memory versus naïve versus transitional B cells to total blood B cells that have been analyzed, thus introducing considerable bias. Hence, inconsistent observations have been made, including increase, reduction, and stability of the repertoire.

An age-related decreased repertoire diversity of Ig V_H complementarity-determining region (CDR) 3 of circulating B cells was described, characterized by clonal B cell expansions in vivo in individuals over 86 years of age [126] and splenic B cells of aged mice [127, 128], reflecting a restricted BCR repertoire showing that a smaller, restricted number of sequences give rise to B cell clones in aged individuals [129]. The loss of diversity correlated with a poorer health status and prospective mortality [126]. Peripheral blood CD19⁺ B cell V_H family utilization was biased towards expression of V_H4–34, V_H3–23, V_H1–69, V_H4–59, and V_H1–2 in elderly individuals, whereas V_H3–9 was found solely in young individuals and V_H3–7 was only expressed in the elderly [130]. Tonsillar B cells of the V_H4 family did not show age-dependent changes in repertoire diversity [125], as did the circulating B cell repertoire of adult individuals who were analyzed twice, 9 years apart. In four out of five donors, no apparent changes in the V_H family distribution were detected [131]. Human splenic germinal center accumulate antigen-selected B cells during ageing, as reflected by a higher mutational frequency and R/S ratio detected within CDR. The opposite effects were observed when analyzing germinal center B cells from young versus old Peyer's patches [132].

The overall Ig gene usage exhibits changes with age in mice [133], particularly being influenced by the age of T cells engaged in antigen-specific immune responses (see later).

3.5 Mucosal B Cells During Ageing

Analyses of mucosal B cell responses mainly point to reduced function in aged individuals, and again there are conflicting data that suggest normal mucosal B cell responses in old age.

Oral cholera toxin challenge induced normal plasma cell numbers in spleen and mesenteric lymph nodes with regular humoral immune responses in old mice, whereas IgG⁺ and IgA⁺ plasma cells in the lamina propria and IgA⁺ plasma cells

in Peyer's patches were detected at reduced levels in old animals [134]. When an antigen was applied into peritoneum or stomach, specific plasma cell formation declined with age in spleen and peripheral lymph nodes, but not in mesenteric and bronchial (mediastinal) lymph nodes [135, 136].

It was shown that mucosal B cell responses in normal C57BL/6 mice become strongly impaired as early as 1 year after birth, as reflected by eradication of the humoral and secretory antibody response to cholera toxin with ovalbumin as an adjuvant, including the generation of specific secretory IgA. When the same analyses were performed in 2-year-old mice, only little further impairment was observed [137]. The age-related impairment of secretory antibody responses was demonstrated further in senescent rats that showed reduced IgA⁺ plasma cell numbers in the intestinal lamina propria. Concurrently, IgA⁺ B cells in Peyer's patches accumulated to double the numbers of those in young animals, and fewer $\alpha_4\beta_7$ integrin⁺ IgA⁺ plasmablasts were detected in blood circulation. Old rats additionally showed diminished density of blood-vessel-expressing mucosal addressin cell adhesion molecule 1 (the ligand of $\alpha_4\beta_7$ integrin) in the lamina propria, so impaired IgA⁺ plasmablast migration was concluded as an effect of immunosenescence [138].

Investigations in unimmunized B6D2F1 mice aged 24–26 months showed no age-related differences in the frequency and phenotype of germinal center B cells residing in Peyer's patches. Among these germinal center B cells, IgA⁺ B cells were more frequent, with a concurrently reduced frequency of IgM⁺ B cells, which in turn showed increased mutational frequencies within their sequences. Apparently, CD80 (B7.1) and CD86 (B7.2) were expressed by more germinal center B cells in Peyer's patches in aged than in young mice. Non-germinal-center (peanut agglutinin low) B cells displayed comparable phenotype and frequencies in old and young mice [139].

In humans, nasal immunization with influenza vaccine elicited a local secretory IgA response in approximately 50% of the vaccinated aged approximately 67 years on average [140], showing the mucosal B cell and antibody responses are basically operational in some but not all aged humans.

4 B Cell Responses in the Elderly

4.1 Degenerated Germinal Center Reactions in Old Age

Germinal center reactions are a complex process in which B cells are selected for high-affinity binding to an antigen. Germinal centers thus play a key role in successful humoral immune responses. Besides B cells, other cells and factors are integral parts of germinal centers. In aged mice and humans, germinal centers are impaired and less frequently generated.

Germinal centers are abundantly detected in children's and infants' lymph nodes, fewer are seen in adults, and the numbers of germinal centers are strongly reduced, sometimes undetectable, in ageing individuals aged 40–90 years, especially when mesenteric and cervical lymph nodes which are normally exposed to antigenic stimulation are analyzed. Paracortical and medullar areas within the lymph nodes showed slight but gradual reduction with advancing age. The lymph nodes with rather infrequent antigenic stimulation, i.e., peripheral; cubital, axillary, and popliteal lymph nodes, displayed age-dependent replacement of lymphatic parenchyma by fat (lipomatous atrophy) [141]. Histological analyses of inguinal, cervical, and axillar human lymph nodes showed no differences in the number and size of follicles and relative CD20⁺ B cell counts including IgM⁺ and IgG⁺ B cells between children and adolescents under 20 years of age as compared with individuals aged 67–88 years. However, when clonal T cells expansions were additionally considered, the germinal center area was bigger when related to the mantle zone, and total CD20⁺ and especially IgM-expressing cells were more frequent in tissues containing clonal T cell expansions when compared with samples without clonal T cell expansions in young individuals. In samples from aged donors, these differences were not observed [142].

In the mouse, germinal center numbers and size are clearly reduced with age [5, 143–145], whereas the kinetics of germinal center dissociation was unaffected [143].

4.2 B Cell Proliferation and Activation

B cell proliferation appears to be decreased overall in aged individuals [84, 88, 146], and this characteristic is sustained when activation-induced B cell proliferation is analyzed. Defective B cell proliferation was suggested to contribute to immunosenescence [15, 147]. As an example, anti-CD40 or lipopolysaccharide-stimulated splenic B cells from old mice proliferated less within 48 h as compared with those from young animals, whereas no differences in the CD40 expression and activation-induced expression of CD86 and CD69 were observed [148]. However, impaired CD86–CD28 interactions leading to failure of T cell–B cell interactions are discussed as a source of immunosenescence [8, 147].

4.3 Class-Switch Recombination and Somatic Hypermutation

CSR and SHM are integral processes of affinity maturation of B cells. Both depend on the expression of activation-induced cytidine deaminase (AID) [149]. AID expression can be affected by reduced levels of E47 protein encoded by *E2A*, which is subject to age-dependent regulation. Murine splenic B cells from old mice express less E47 [150, 151] and age-related reduction of E47 mRNA stability

has been described [152]. Impaired CSR was detected in murine B cells stimulated with anti-CD40 and B cell activating factor in vitro [153]. Similarly, human peripheral blood B cells from aged individuals express fewer *E2A* transcripts, and lower AID protein levels and reduced CSR can be observed [150, 154].

SHM is impaired in B cell responses when either B cells or T cells originating from aged donors are transferred to *scid* mice [155]. In an immune response to nitrophenol (NP), Ig V_H hypermutation could not be detected when old mice were immunized, whereas selection for the antigen appeared to be operational in these mice at a level of competition between nonmutated B cell clones [8, 85]. Differing from that, it was also hypothesized that not SHM but selection is impaired in aged individuals [156]. In nonimmunized mice, antibodies originating from plasma cells carrying mutated Ig rearrangements accumulate with age in the blood serum, indicating accumulation of mutations in the plasma cell compartment. This accumulation was partly independent on CSR, but was abrogated when mice were kept under germ-free conditions or in the absence of β/δ T cell receptors [157].

An analysis of human peripheral and cord blood B cell expressing V_H6 donors showed similarly reduced mutational frequency and incidence of mutated V_H6 rearrangements in cord blood and aged donors' blood as compared with normal adult blood, reflecting impaired affinity maturation in early and late life [158]. The type and location of base substitutions did not differ between young and old samples, but the *R/S* ratio was higher in CDR heavy chain regions from old samples, indicative of the accumulation of selected B cells [159]. When IgM⁺ B cells of the V_H5 family were analyzed, no significant alterations were detected in the mutational frequency, the frequency of mutated sequences, and CDR3 lengths, whereas V_H5 IgG sequences showed a significant increase of mutational frequency [160, 161]. The mutational frequency and *R/S* ratios were found to be negatively affected by age in human V_K4 rearrangements from peripheral blood [162].

4.4 Aged T Cells and Antigen-Presenting Cells Contribute to Defective B Cell Responses in Old Age

Age-related changes, mostly defects, have been noted in the germinal center with regard to T helper cells and follicular dendritic cells (FDC) as essential components of a regular germinal center B cell response.

T cells exhibit age-related functional and configurational changes. Importantly, naïve T cells of aged individuals exhibit impaired antigen responsiveness and proliferation in vivo and in vitro, most likely contributing significantly to reduced helper function, thus negatively affecting B cell responses and differentiation [163, 164]. To this end, the role of CD154 (CD40L) expression is discussed [6, 8]. Numbers of B cells expressing CD40 were found to be marginally reduced in aged individuals' blood, reflecting the overall reduction of total CD19⁺ B cells with age [124].

In humans, peripheral CD4⁺ and CD8⁺ T cell composition is skewed towards a memory phenotype with advancing age [165], as similarly observed for B cells.

Adoptive transfer studies of aged versus young T cells identified the old age of CD4⁺ T cells as a major origin of reduced germinal center formation and antigen-specific B cell and humoral IgG response in young hosts, whereas no difference in B cell responsiveness was noted when young CD4⁺ T cells were transferred to aged hosts [6].

In a study analyzing the contribution of aged CD4⁺ T cells versus aged B cells to the immune response to NP in lymphocyte-reconstituted *scid* mice, it was shown that the V_H gene repertoire was dependent mainly on the age of donated CD4⁺ T cells, rather than on the age of B cells. The engagement of aged T cells led to the expression of Ig families not expressed when young T cells were transferred [155].

Successful germinal center reactions further depend on the presence of FDC that retain immune complexes and may present them to B cells [166]. In aged mice, impaired FDC networks were observed as they retained fewer immune complexes and were associated with reduced number and size of germinal centers and reduced memory B cell output [5]. Functional FDC impairment appears to be caused by reduced expression of Fcγ receptor II and slight reduction of complement receptor 1 and complement receptor 2 expression as well as the reduced presence or absence of immune-complex-containing vesicles (icosomes) within FDC. Expression of FDC-M2, indicative of the capability of FDC to trap immune complexes, is also strongly reduced in aged mice [167]. Further, in the lymph nodes of aged but not young mice, tingible body macrophages with a yet unclear role for the germinal center reaction were found to be absent during an immune response to protein antigen [168].

A radiolabeled antigen was used to analyze antigen retention in young versus old mice. The capabilities to trap and retain the antigen in lymphoid tissue became detectable 3 weeks after birth and persisted throughout life. In old animals, the antigen did not translocate to lymphoid follicles but remained at the subcapsular sinus and adjacent superficial cortex [169]. In summary, defective antigen presentation appears to constitute a source of B cell response impairment independent of intrinsic T cell and B cell defects.

4.5 Impaired B Cell Responses in Aged Mice

The vast majority of studies reviewed document impaired B cell responses in aged mice, with less specific B cells and plasma cells generated in fewer germinal centers, resulting in antibodies with a less diverse repertoire, lower avidity, and lower protectivity.

Age-related reductions of both total and immunization-induced antigen-specific B-cell-containing germinal center numbers were observed in aged (19–21 months) versus young (2–3 months) mice [170]. In old mice as compared with young mice, fewer NP-specific IgG⁺ plasma cells were generated in the spleen after

immunization with NP–chicken γ -globulin, and their numbers dropped to lower levels during the maintenance phase [170]. During the primary response in old mice, plasma cells failed to establish as efficiently in the BM as they did in young mice. Upon booster immunization, old mice also generated fewer plasma cells in the spleen and BM than young mice, but with significant increase in antibody-secreting cell numbers as compared with the primary response. Specific serum titers were also generated at tenfold lower levels in aged mice, and remained impaired also in a secondary vaccination with tenfold or greater reduction in old mice as compared with young mice. When V_H186-2^+ B cells indicative of antigen-specific B cells were analyzed, reduced accumulation of somatic mutations in aged mice was observed and old mice failed to select plasma cells with higher mutations into the BM [170]. Consistently, the spleen of aged mice harbors more antigen-specific low-affinity IgM^+ plasma cells and fewer class-switched plasma cells. The BM contained decreased numbers of high- and low-affinity plasma cells in old mice as compared with young mice. Reconstituting old mice with splenocytes from young mice led to restoration of a young plasma cell response pattern in the spleen, but not the BM, reflecting the reduced capacity of the BM to recruit and maintain plasma cells [144]. Aged mice produced less frequently splenic foci containing specific B cells, and within these, fewer specific B cells (less than 50%) were observed after immunization with keyhole limpet hemocyanin–phosphorylcholine (PC), reflecting operational but diminished antigen-specific B cell responses in old animals [171]. Old mice utilize atypical Ig genes in their plasma cells generated in immune responses to PC, *Streptococcus pneumoniae* R36a vaccine, and trinitrophenol [172–174]. Such antibodies appear to be less protective, as demonstrated by loss of immune protection to pneumococcal infection of mice into which a PC-specific antibody generated in old mice was injected [173]. In contrast to results from vaccinations with haptens, the B cell response to influenza virus vaccination does not show an age-related decline in terms of specific B cell generation and repertoire diversity [175].

The primary humoral B cell response to tetanus toxoid is less effective in approximately 1-year-old mice than in young mice, accompanied by diminished germinal center count and size and reduced lymphoid and plasma cell turnover in response to the antigen within popliteal lymph nodes [145]. Aged mice generated reduced numbers of splenic plasma cells which respond to another T-dependent antigen, and especially the numbers of those of IgG isotype and of high avidity were diminished. The transfer of spleen cells from old animals suppressed the generation of a regular plasma cell compartment in young mice upon immunization [176].

The generation of anti-idiotypic antibodies (such as RF) was hypothesized as a regulatory mechanism leading to reduced humoral immune responsiveness and protection [174, 177]. The numbers of these antibodies increase with advancing age in the serum of mice and humans (see later) and appear to negatively influence the responsiveness of splenic B cells from syngeneic, but not genetically different donors transferred to old hosts [178]. The magnitude of anti-idiotypic antibody production induced by a tetanus vaccination was associated with a lower tetanus-specific antibody response [179].

5 Antibody Levels and Specificity in Old Age

5.1 *Effects of Age on Antibody Levels*

Total serum antibody levels are stable in adult individuals and show a modest increase during ageing in mice and humans, affecting IgG and IgA rather than IgM antibodies. The levels of IgG1 and IgG3 are often shown to be increased with age, whereas the level of IgG4 is reduced [15, 180–184]. In old people, the level of soluble IgD has been shown to be significantly reduced to approximately 20% of that in young people [123, 185], whereas IgE levels remain unchanged [185]. Results differ in some details, e.g., the age at which significant differences in antibody levels can be detected and the magnitude of change. Although not determined specifically in old individuals, the physical *in vivo* half-life of both human and murine antibodies has been determined to be 3 weeks or less [186, 187], so age-related effects on antibody levels must occur at the level of plasma cell generation and persistence (see earlier). Stromal cells may fine-tune antibody secretion involving interactions of stromal-cell-derived CCL2 with CCR2 expressed by plasma cells [188]. Hence, senescent stromal cells [61] might impact on plasma cell homeostasis and antibody titers, as demonstrated in old mice being impaired in maintaining plasma cells [144]. The BM environment is further affected by osteoporosis and enlarging fat deposits at older age [189].

Antigen-specific antibody titers remain detectable at protective levels until the age of 65 years for viral and protein antigens [104]. Whereas for replicating antigens, i.e., viral specificities, antibody titer half-lives were virtually unchanged with age, those for protein antigens (tetanus and diphtheria toxoid) declined slowly with a half-life of more than 10 years, despite regular booster immunizations [104]. In line with these findings, anti-RSV IgG titers were undiminished in individuals at 70–80 years of age as compared with young controls at approximately 35 years of age, independent of frailty, whereas among US citizens older than 70 years, fewer than 30% had protective tetanus-specific IgG titers [190]. These specificity-related differences might arise from the different nature of the antigen and respective heterogeneity of immune activation in terms of timing, duration, antigen affinity, localization of the immune response, and replicating, sometimes persistent antigen acquired by natural infection versus nonreplicating, degradable antigen often applied by artificial challenge, i.e., vaccination.

Besides the amounts of antibody, the avidity is also affected by age. Upon annually repeated influenza vaccinations, young vaccinees developed H1N1-specific IgA antibodies of significantly higher avidity than in old subjects. Similar trends were observed for IgG antibodies and antibodies against H3N2 antigen [191]. Besides a reduced humoral response to a 23-valent polysaccharide vaccine, the elderly additionally exhibited reduced avidity of specific IgG as compared with young controls. Sera from old vaccinees less frequently protected mice from death by pneumococcal challenge [192].

In mice, transfer experiments of splenocytes from differently aged animals to hosts revealed maximum antibody avidity when splenocytes came from adult mice, as compared with lower avidity when the same cells were donated by very young or old animals (10 days and 2 years old) [193].

Glycosylation patterns of antibodies can have drastic effects on antibody function and appear to be associated with autoimmunity [194–196]. Total human serum IgG showed an age-related kinetics of the relative incidence of agalctosylated N-linked oligosaccharides, which were suggested to be associated with rheumatoid arthritis [197], with a minimum at 25 years and higher incidence at birth and advancing age [197]. Hence, age-associated glycosylation pattern could also contribute to both the development of autoantibodies and perhaps autoimmunity in aged individuals (see later).

5.2 *Emergence of Autoantibodies in the Elderly*

The incidence of an antibody to autologous antigen (autoantibody) production in humans increases with age (starting at an age of 70–80 years), depending on the type of autoantibody [183]. A similar phenomenon has been described in mice [198–200]. Among centenarians, no further increase but a strong reduction of the prevalence of autoantibody-positive individuals was observed, e.g., for antithyroglobulin and anti-gastric parietal cell antibodies (PCA) antibodies, but not for RF [118, 201]. Differences have been noted between organ-specific and nonspecific autoantibodies with regard to their relation to the frailty of tested individuals. A screening of 148 European individuals at the age of 100 years revealed almost 80% of them expressed at least one autoantibody of 15 organ-specific and nonspecific autoantibodies tested, with the highest prevalence of IgM RF (26.6%). Autoantibodies did not reflect autoimmune disease, but organ-specific in contrast to nonspecific autoantibodies were rarely detected in individuals without comorbidities or disabilities. At least one organ-specific antibody was detected in 32% of the same cohort [118, 202], confirming the findings of earlier studies [181, 203]. Old individuals also display an increased usage of V_H4 gene family members, which are enriched for rearrangements potentially leading to autoreactive antibodies [204], especially V_H4-34 , which has been found associated with cold agglutinin disease [130].

In individuals who have tested positively for autoantibodies, these commonly do not translate into autoimmune disease [205, 206]. Nevertheless autoantibodies can be detected 7–9 years before the onset of SLE, leaving it open to question whether the autoantibody-positive elderly are in the stage of “benign autoimmunity” preceding overt disease [112]. Autoantibodies may arise as by-products of vaccine responses in mice [207] and humans [208, 209]. Transiently increased RF production was observed after tetanus and influenza booster vaccinations of normal healthy individuals [208, 209]. The relative increase in the underlying IgG reactive IgM^+ B cell population correlated with the amounts of tetanus toxoid specific IgG measured in blood serum [208] and was more profound than the vaccination induced IgM-RF production measured in the blood serum. A role for $CD5^+$ B cells within autoantibody production appears to be implicated

as peritoneal cavity CD5⁺ B1 cells have been shown to be a source of IgMRF [210]. The development of chronic lymphocytic leukemia, which occurs at rather old age, often shows a CD5⁺ phenotype [204]. However, the numbers of CD5⁺ B cells become reduced with age in peripheral blood [118, 124].

6 The Role of B Cells and Antibodies in Counteracting Immunosenescence

Although old individuals often show impaired immune responses, they benefit from vaccinations with T-dependent and T-independent antigens, as, e.g., reflected by reduced rates of infections that they were vaccinated against, hospitalization rates, and numbers of outpatient visits [211–213]. In addition, combined parenteral and mucosal (nasal) vaccination [214] provides additional protection in the elderly [215].

Apart from vaccinations, increasing efforts are being made to generate recombinant monoclonal antibodies from antigen-specific B cells isolated *ex vivo* from immune donors to neutralize pathogens without previous vaccination, thus circumventing age-related and other limitations in vaccine development and responsiveness. Clearance of antibody-bound pathogens further depends critically on the complement system and macrophages, which are also affected by age. Various techniques have been used to generate such neutralizing antibodies specific for influenza [216, 217] HIV [218], SARS coronavirus [219], and RSV [220].

A radical attempt to reactivate juvenile immune capabilities is to reconstitute the immune system from autologous stem cells after immunoablation. Autologous stem cell transplantation (ASCT) is currently used to treat severe autoimmunity [221, 222] and lymphoma and indeed leads to strong clinical improvement in SLE and multiple sclerosis [221, 223]. Besides reactivation of thymic activity [221, 223], treatment leads to eradication of peripheral blood B cells and subsequent establishment of a circulating B cell population that mostly (approximately 90%) consists of naïve B cells at 1–5 years after ASCT. Beside proteasome inhibition, e.g. using Bortezomib, immunoablation with rabbit antithymocyte globulin plus ASCT is also the only known treatment to eradicate plasma cells and consequently specific antibody and autoantibody production. Immunoablation/ASCT treatment targets both the pathogenic and protective immune cells and thus patients carry a high risk of suffering from sometimes fatal infections.

The reactive capacity and of the immune system immunoprotection generated earlier are influenced by lifestyle, and B cell parameters have been used to analyze the influence of lifestyle on the immune system. An improvement of antigen-specific serum IgG and splenic B cell function as well as reduced IgG catabolism was demonstrated when mice were allowed to perform voluntary wheel running [224, 225], whereas intense exercise is associated with characteristics of immunodepression [226]. Salivary secretory IgA levels are significantly increased after 1 h of choir singing [227] and are further influenced by emotional state [228]

and mental exercise [229]. The impact of nutrition and drug consumption has been described [230], but not always confirmed [231]. Caloric restriction is associated with longer life span in mouse models [232]. Hence, lifestyle factors, such as nutrition (e.g., vitamins [233, 234]), diet, and physical and mental activity, could represent ways to counteract immunosenescence apart from medical interventions [235].

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