Older Human B Cells and Antibodies

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Abstract

B cells have a number of different roles in the immune response. Their excellent antigen presentation potential can contribute to the activation of other cells of the immune system, and evidence is emerging that specialized subsets of these cells, that may be increased with age, can influence the cell-mediated immune system in antitumor responses. They can also regulate immune responses, to avoid autoreactivity and excessive inflammation. Deficiencies in regulatory B cells may be beneficial in cancer but will only exacerbate the inflammatory environment that is a hallmark of aging. The B cell role as antibody producers is particularly important, since antibodies perform numerous different functions in different environments. Although studying tissue responses in humans is not as easy as in mice, we do know that certain classes of antibodies are more suited to protecting the mucosal tissues (IgA) or responding to T-independent bacterial polysaccharide antigens (IgG_2) so we can make some inference with respect to tissue-specific immunity from a study of peripheral blood. We can also make inferences about changes in B cell development with age by looking at the repertoire of different B cell populations to see how age affects the selection events that would normally occur to avoid autoreactivity, or increase specificity, to antigen.

Keywords

Tolerance · B cell repertoire · Autoantibodies · Immunoglobulin genes · Age-related B cells · Aging · Antibody class switching · Affinity maturation · Regulatory B cells · Humoral immunity

Abbreviations

ABCs	Age-related B cells
AID	Activation-induced cytidine deaminase
ANA	Antinuclear antibodies
ANCA	Antineutrophil cytoplasmic antibodies
BCR	B cell receptor
CAP	Community-acquired pneumonia
CDR	Complementarity-determining region
CLL	Chronic lymphocytic leukemia
CMV	Cytomegalovirus
CRP	C-reactive protein
D	Diversity region of immunoglobulin gene

EBV	Epstein-Barr virus
FDC	Follicular dendritic cell
Fw	Framework region
GC	Germinal center
HI	Haemagglutinin inhibition
HIV	Human immunodeficiency virus
HTS	High-throughput sequencing
J	Joining region of immunoglobulin gene
MBL	Monoclonal B cell lymphocytosis
MGUS	Monoclonal gammopathy of undetermined significance
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RSV	Respiratory syncytial virus
TD	T-dependent
T_{FH}	T follicular helper cell
TI T	independent
V	Variable region of immunoglobulin gene

Introduction

It has been well established that the efficiency of the immune system declines with increasing age. Immunosenescence causes increased susceptibility to infectious diseases, and infection is, in fact, the third leading cause of mortality in people aged 65 and over (Albright and Albright 2003). Even in cases where mortality is not an issue, reducing morbidity of infection in older people is an urgent need in order to improve the health of the older generation. As is clearly apparent from the other chapters of this book, there are many components of the immune system that can change with age and are crucial to maintaining an effective immune system. In this chapter, we address current knowledge on age-related changes in the humoral immune system and how this contributes to the immune frailty of the older person.

Role of B Cells in Age-Associated Susceptibility to Infection

The humoral immune system interacts with the other immune components in a number of different ways. The terminal differentiation point of B cell development is the plasma cell, which produces large quantities of antibody. In more recent years, it has become apparent that there are other critical functions of B cells in activating or suppressing immune responses. They can be important as modulators of inflammation (Arnaboldi et al. 2005; Maglione et al. 2007), as regulators of the immune response (Mauri and Menon 2015), and as antigen-presenting cells and activators of T cells (Linton et al. 2000; Crawford et al. 2006; Lund et al. 2006). An overview of B cell development is shown in Fig. 1.

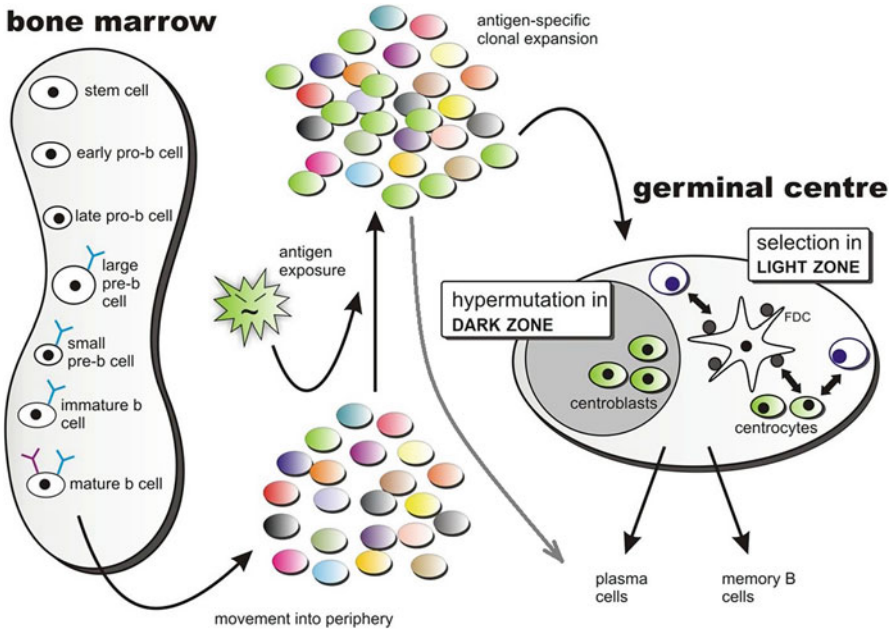

Fig. 1 B cell development. The humoral immune response is mediated by antibodies produced from plasma cells. These plasma cells are the end point in B cell development, which is characterized by (**a**) generation of a huge diversity of different B cells, each carrying a different antibody gene in the bone marrow and (**b**) selection processes using the affinity of the membrane-bound form of the antibody (the B cell receptor) for its antigen as the selection criteria. Diversity is generated by a process of gene rearrangement early on in the development of the cell, in the bone marrow prior to antigen encounter. T-dependent activation of B cells occurs via the germinal center. Innate B cell activation by T-independent antigens can also occur. Selection processes shape the repertoire. In the bone marrow, B cells are selected for survival, or not, on the basis of their antibody recognition – to eliminate inappropriate self-reactivity and encourage reactivity with foreign pathogens. In the germinal center, there is a mutation step, and the resultant B cells carrying improved antibodies are selected; this serves to increase the affinity of the antibody for the relevant antigen. Both generation of diversity and selection of antibody are complex processes that are crucial for an effective humoral immune system. A clear understanding of these processes, and how they are affected with age, is needed in order to comprehend the etiology of age-related inflammatory and infectious disease

B Cell Role in Protection from Infection

In the primary B cell response, antibodies that recognize pathogen, although not necessarily with high affinity, are rapidly produced. They may include the so-called "polyspecific" antibodies, which have the ability to bind to multiple antigens (Meffre and Wardemann 2008; Mouquet et al. 2010; Scheid et al. 2011). The first antibodies are of the IgM class and are crucial for opsonizing pathogens, inducing phagocytosis, and activating the complement cascade. These antibody functions, and the rapidity of this primary response, have been shown to play a

Table 1 Pathogens found frequently in elderly subjects with respiratory, gastrointestinal, and urinary tract infections

Organ system	Pathogen found frequently	B cell role in immune response to pathogen
Respiratory tract	*S. pneumoniae*	B cells crucial for TI response (Barrett and Ayoub 1986)
	H. influenza	Mucosal IgA protects (Pichichero et al. 1981)
	L. pneumophila	B cells required for opsonization (Brieland et al. 1996)
	C. pneumonia	Neutralization by antibody (Peterson et al. 1998)
	Rhinoviruses	Antibody-mediated neutralization (Smith et al. 1996; Zhong et al. 2005)
	Coronaviruses	
	Influenza	
	Respiratory syncytial Virus	
Urinary tract	*E. coli*	IgA secretion inhibits bacterial attachment (Trinchieri et al. 1990; Kantele et al. 2003)
	P. klebsiella	IgM and IgA aids protection (Lepper et al. 2003; Deo et al. 2004)
	P. aeruginosa	Opsonization (Mueller-Ortiz et al. 2004)
	Enterococci	Antibody effective in the presence of complement (Harvey et al. 1992)
Gastrointestinal	*E. coli*	Mucosal IgA protects
	C. difficile	Strong antibody and memory B cells required to protect (Monaghan et al. 2013)

vital role in protection from extracellular bacterial pathogens (Brown et al. 2002). T-independent responses, in general, are particularly important in protection against bacterial pathogens, where the external antigens of the pathogen are nonprotein in nature. Antibodies afford protection against viral infection by neutralizing virus particles, binding and blocking key molecules involved in cellular infection. Similarly, they can also neutralize toxins. Later T-dependent affinity maturation of B cells in an immune response is slower but results in the generation of more highly specific antibodies, often of a different class.

The elderly are susceptible to infections by a wide variety of pathogens, all of which involve B cells and antibodies in the normal course of the immune response (Table 1). The lungs are, in common with other mucosal surfaces of the gastrointestinal and genitourinary tracts, particularly vulnerable to infection by virtue of their exposure to the environment. As is illustrated in Table 1, pulmonary infections are common in older people. The elderly are usually the first to be affected by annual epidemics of respiratory infections and frequently suffer the worst clinically. Mortality figures attributable to influenza and pneumonia are confused by the fact that influenza is very often followed by a secondary infection – most notably by *Streptococcus pneumoniae* (Brinkhof et al. 2006; van der Sluijs et al. 2004; Seki et al. 2004). Resolution of a first infection can leave a person susceptible to a secondary bacterial infection, and this may be of particular concern in older people (Dunn-Walters and Ademokun 2010; Ademokun et al. 2011). It has been reported

that 90% of all pneumonia and influenza deaths and 88% of respiratory syncytial virus (RSV)-associated deaths occur in those aged over 65 years (Thompson et al. 2003; Matias et al. 2014). In the oldest old (85 years and over), there was a 32-fold increased chance of mortality from influenza or influenza-associated pneumonia compared with those aged 65–69 years (Thompson et al. 2003). A prospective study, following 608 healthy elderly, showed that approximately twice as many people had RSV infection than did influenza A infection, and in a period of 2 years, 13% of these patients contracted either influenza or RSV (Falsey et al. 2005). The predicted probability for Older patients with community-acquired pneumonia (CAP) to enter hospital within 28 days of Contracting CAP is 86% (Millett et al. 2015). There is also an increased incidence of pneumococcal septicemia in old people associated with *S. pneumoniae* infection (McIntosh et al. 2005; Weil-Olivier et al. 2012).

It is known that specific antibodies generated during a T-dependent B cell response are crucial for protection against influenza. Ineffective influenza-specific antibody, as assessed by the hemagglutination inhibition (HI) test, is associated with lowered protection from the disease (Goodeve et al. 1983). It has been said that an age-related decrease in influenza protection can be solely accounted for by the reduced T cell help available in the diminished elderly T cell repertoire. However, this does not take into account the fact that the CD4+ T cells themselves may rely on fully functioning B cells for their activation (Rivera et al. 2001; Crawford et al. 2006).

In other areas of humoral immunity, the B cells are even less reliant on T cells for help. Pneumonia is a bacterial infection, caused by a number of different organisms (e.g., *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*) although *S. pneumoniae* is the major cause (AlonsoDeVelasco et al. 1995; Birch and Gowardman 2000; Hageman et al. 2006). Immunity against *S. pneumoniae* is particularly reliant on a healthy B cell population, since the antigenic portion of *S. pneumoniae* is a capsular polysaccharide and a T-independent type II (TI-II) antigen. Unlike a T-dependent B cell response, where the maturation of the B cell antibody relies on T cell help and therefore any failure to respond could be attributed to a failure of T cells, this response is independent of direct T cell help. Therefore a failure to protect against *S. pneumoniae* is more likely to be a failure ascribable to deficits in the B cells themselves.

In children a reduced pneumococcal response can be explained by a lack of marginal zone B cells in the spleen, where the main TI-II responding B cells are thought to reside. However, older people appear to have a fully functioning splenic marginal zone (Banerjee et al. 2000). CD27+IgM+ B cells are thought to be the recirculating equivalent of the splenic marginal zone, provide protection from *S. pneumoniae* (Kruetzmann et al. 2003), and show age-related changes (see below) which may account for a reduced response to TI-II antigens. Removal of IgM from human serum diminishes phagocytosis of *S. pneumoniae* in *in vitro* assays (Park and Nahm 2011), also indicating that IgM has a crucial role in protection from pneumococcal disease. Complementary to this we discovered that, although

polysaccharide-specific IgG was equivalent in old and young patients after vaccination, the production of specific IgM and IgA antibodies was significantly diminished (Ademokun et al. 2011).

Although pulmonary infections of the elderly are the most notable, by virtue of the fact that they cause the most mortality, there are also significant increases in morbidity and mortality from other infections. Bacterial infections of the skin, urinary tract, soft tissue and gastrointestinal tract are all increased with age (Albright and Albright 2003) and infections from organisms such as *Clostridium difficile* and *Candida albicans* seem almost exclusive to the older age groups (Fig. 2). Sepsis is a particular problem in older people, and the severity of disease has been shown to correlate with serum levels of IgM in the elderly (Suzuki et al. 2016).

Loss of Vaccine Efficacy in Old Age

Vaccines are an extremely important tool in preventing deaths from infection and, since they are routinely administered as part of a normal health-care routine, are the main source of data on immune responses in man. It has been consistently shown that the effectiveness of vaccines is severely diminished in older people. Since specific antibody production is often used as a correlate of protection, vaccine studies are a useful way of observing the humoral immune response. An age-related reduction in specific antibody production occurs in response to many vaccines, including hepatitis B (Fourati et al. 2016), pneumonia (Simberkoff et al. 1986; Koivula et al. 1997; Örtqvist et al. 1998, 2007; Rubins and Janoff 2001), tetanus, diphtheria and tick-borne encephalitis (Hainz et al. 2005; Weinberger et al. 2013). The most commonly studied vaccine is that against influenza. The cellular response, i.e., T cells and release of cytokines, macrophages, and natural killer cells, is decreased with age, and the IgG antibody titer is significantly lower (Goronzy and Weyand 2013; McElhaney et al. 2016). While vaccination of older people against influenza is widely accepted as a valid health strategy to reduce disease incidence (Odelin et al. 2003; Keitel et al. 2006; Maciosek et al. 2006), disease mortality may not be affected (Simonsen et al. 2005; Rizzo et al. 2006). In agreement with previous studies, we have shown that older people fail to develop protective Haemagglutinin inhibition (HI) titers following vaccination (Ademokun et al. 2011; Reber et al. 2015). Recent studies have investigated different vaccination regimes in an effort to improve protection in the elderly. There is some evidence that boosting within 12 months of vaccination improves antibody responses and reduces innate responses (Kannan et al. 2015). Successive vaccination can improve the older immune response to levels shown by younger adults (Höpping et al. 2016). In the case of influenza, the response that is being studied is that of a recall response and may be complicated by the existence of slightly cross-reactive antibodies formed against previous strains of influenza. A study of vaccine responses against diseases that are not endemic in the country of origin can circumvent this. Some travel vaccines, such as hepatitis A, also show a reduced specific antibody response (Genton et al. 2006),

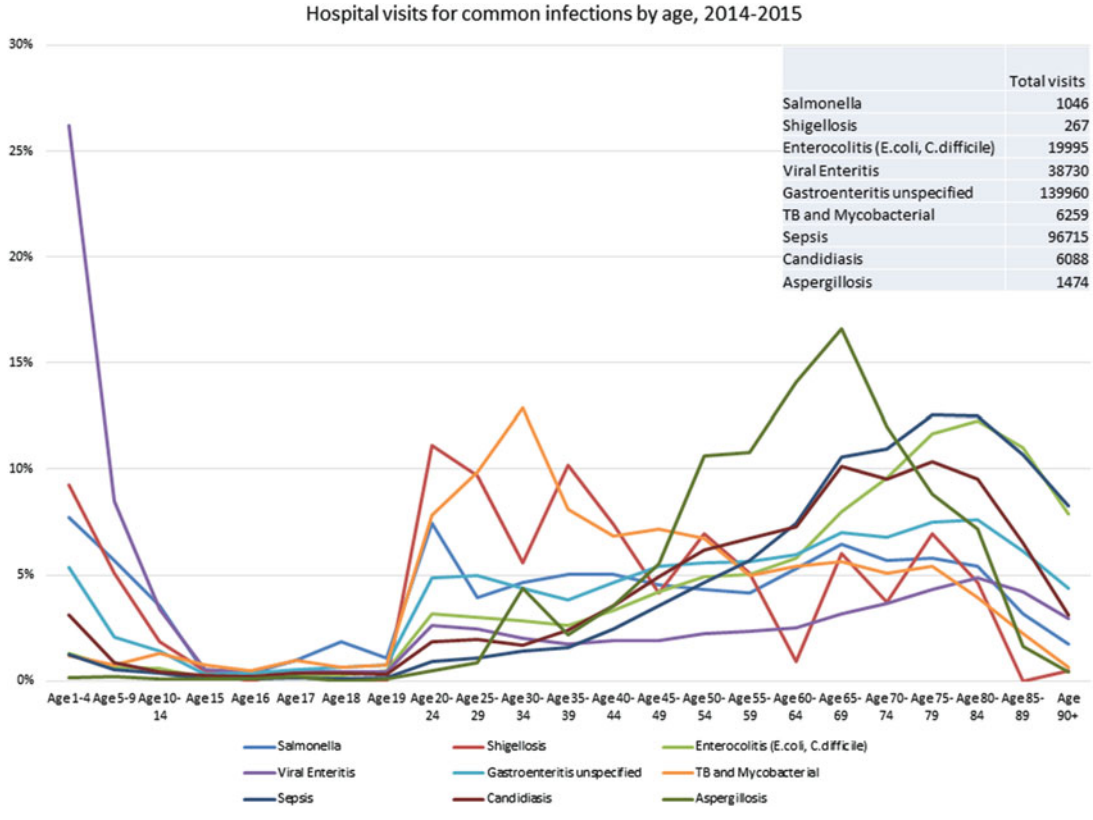

Fig. 2 Age-related incidence of common infections. The diagnosis at hospital visit of different types of common infections is shown according to age group (Data is taken from Hospital Episode Statistics, Admitted Patient Care – England, 2014–2015 (NHS Digital 2016))

while others such as yellow fever seem to show an undiminished antibody response but the response takes longer (Roukens et al. 2011), and there is an increased risk of adverse events in older people (Monath et al. 2005; Roukens et al. 2011; Thomas et al. 2012).

Age-Associated Inflammation and Loss of B Cell Tolerance

Aging is associated with an increased level of inflammatory markers such that this phenomenon which is sometimes referred to as "inflammaging." The most consistent data associates age and increased TNFα and IL6 cytokine levels in the serum; CRP is often also measured. IL-6 and CRP levels have been shown to predict physical and mental frailty and mortality risk (Puzianowska-Kuźnicka et al. 2016). Pre-existing inflammation can result in reduced responses to challenge; high levels of inflammatory response transcripts in human blood inversely correlate with responses to hepatitis B vaccine (Fourati et al. 2016). Evidence from mice suggests that the inflammatory environment causes a reduction in hematopoiesis in the bone marrow (Ratliff et al. 2013), which in itself is a major risk factor for frailty in the elderly. In addition, since inflammation is implicated in the pathogenesis in the majority of age-related diseases, and the immune system is the primary causative candidate, there is a great deal of interest in understanding the contribution of immunity to age-related inflammation. Similarly, aging is associated with an increase of autoreactive antibodies (Ademokun et al. 2011), and the significance of this is not completely understood.

B Cell Contribution to Inflammaging

Inflammatory cytokines can be secreted by many different cells, and it is worth noting that it is not only immune cells that can secrete inflammatory cytokines. Particularly with regard to age-related changes, the senescence-associated secretory phenotype of senescent cells must also be considered (Campisi et al. 2011). In the overall environment, with contributions from many other cells, it is not clear whether B cells make a significant contribution to the overall inflammatory milieu, although evidence is gradually emerging to show that their cytokine profile and responses are changed so they do contribute at some level. Older memory B cells have been shown to secrete higher levels of TNFα. TNFα in turn is negatively correlated with the overall immune response and, in particular, with a lower level of activity of the enzyme AID in B cells that is critical for the affinity maturation response (Frasca and Blomberg 2014). An increased production of IL6 has also been shown in older B cells (Bancos and Phipps 2010) and a reduction of IL6-expressing B cells in the clinic has been shown to ameliorate autoimmune disease (Barr et al. 2012) so the production of inflammatory cytokines from older B cells warrants further study.

Autoantibodies in Older People

There is a well-documented shift toward self-reactive antibody production with age, although generally not associated with autoimmune disease per se. Rheumatoid arthritis (RA) is an age-related inflammatory autoimmune disorder, and the increase in rheumatoid factor (RF) with age tempts one to assume a causal link. However, the increase in RF occurs regardless of whether the subject has RA or not (Andersen-Ranberg et al. 2004), and the presence of another autoantibody, anti-cyclic citrullinated peptide is associated with a higher risk of RA. This antibody is likely a result of environmental factors – specifically with smoking (Michou et al. 2008). The successful use of therapies such as rituximab, which utilize an anti-CD20 monoclonal antibody to ablate peripheral B cells, is ample evidence that B cells play an important part in the disease process of RA, but their role as antigen-presenting cells, activating T cells, and producers of cytokines may be more prominent than as autoantibody producers (Bugatti et al. 2014). A further complication in understanding the role of B cells in RA is their role as regulators, as outlined below. Another age-related autoimmune disease is ANCA-associated vasculitis, with a peak incidence at age 65–74 in the UK (Watts et al. 2000). The disease is characterized by antineutrophil cytoplasmic antibodies (ANCA), of which the two most common targets are myeloperoxidase (MPO-ANCA) and proteinase 3 (PR3-ANCA). In this instance, the role of the autoantibodies seems to be clear, in that they inappropriately activate neutrophils leading to systemic inflammation affecting joints, lungs, kidneys, and other tissues. The association with older age implies that B cell tolerance mechanisms with respect to ANCA are more likely to fail in the elderly.

However, as mentioned above, many autoreactive antibodies are increased in aging but are not pathogenic. Antinuclear antibodies (ANA) have consistently been found to be increased in the old (over 65) in the absence of disease; the Swedish longitudinal NONA immune study (Nilsson et al. 2006) showed significantly higher ANA levels in the oldest old (86–95 years) but found there to be no association nor any correlation to other immune risk factors (e.g., CD4/CD8 T cell ratio, CMV seropositivity). These findings are echoed by a Finnish study, where ANA positivity at the age of 90 did not show any correlation with survival or with the levels of serum markers of inflammation (Hurme et al. 2007). We saw a significant increase in IgG ANA autoantibodies with age which was unaffected by vaccination, so unlikely to be caused by bystander activation of polyspecific antibodies in a response (Ademokun et al. 2011). Other age-related increases occur in anti-ssDNA antibodies (Manoussakis et al. 1987; Xavier et al. 1995; Ioannidis et al. 2003), cardiolipin, and dsDNA.

Understanding the increase of autoantibodies without autoimmune disease becomes easier if we stop thinking about an antibody as being exclusively specific for one antigen – as in the lock and key hypothesis. Rather, we believe that all antibodies will have a certain amount of polyreactivity, perhaps particularly in the case of naïve B cells or T-independent responders such as IgG_2 B cells or IgM memory cells. The relative ability to bind self-antigen versus non self-antigen will

determine whether the antibody survives the tolerance mechanisms, and highly specific antibody can be generated later in a response – through affinity maturation in the germinal center T-dependent process. With this view in mind, then the increase in nonpathogenic autoantibodies in aging could be explained by a relaxation of the tolerance mechanisms, not causative of autoimmune disease in themselves, but perhaps a contributory factor toward increased incidence of disease such as RA or vasculitis. Data from our lab shows that there are immunoglobulin sequence characteristics that are removed from the repertoire in central tolerance, and the extent to which these are removed decreases with age (Martin et al. 2016).

Functional Subclasses of B Cells and Age-Related Changes

Most studies will agree that immune senescence does not involve a change in total B cell numbers, as assessed by the pan B cell marker CD19 (Ademokun et al. 2010; Melzer et al. 2015). We now know that there are many functions of B cells in addition to their role as antibody producers, and discovery of phenotypic markers to define functional subsets is an ongoing process. Most work in human is performed in blood, for obvious reasons, but we should not forget that the B cell microenvironment within tissues is extremely important and may result in a different cellular landscape than in PBMCs. Indeed, the fact that mouse studies are mostly from splenic B cells and humans are mostly PBMCs may confound attempts at extrapolation from mouse to human studies. In many instances, the starting point for distinction between human subsets is the expression of surface markers CD19 (pan B cell), IgD, and CD27. IgD is present on all mature B cells that have not been activated by exogenous antigen and also on a population of "IgM memory cells." CD27 is an activation marker on B cells and is present on most, but not all, memory cells. In this instance, B cell memory is generally confirmed by class switching and/or the presence of mutations in the Ig gene. Markers CD24 and CD38 are also often used to distinguish functional populations of cells. Comparing populations defined by IgD/CD27 with those defined by CD24/CD38 is broadly possible, although there are some cells showing exception which serves to illustrate the heterogeneity of many of these populations (Boyd et al. 2013).

CD27- Memory B Cells

There is a population of B cells that has lost IgD, has mutations in the Ig genes, and includes class-switched antibodies as well as a small population of IgM-only antibodies. All these are the hallmark signs of memory B cells, but the activation marker CD27 is not present on the surface (Wu et al. 2011). These cells are increased in aging (Bulati et al. 2011), as well as in autoimmune disease and chronic viral infections such as HIV (Wei et al. 2007; Moir et al. 2008), and have been associated

with frailty (Lu et al. 2016). It has been suggested that they are exhausted B cells, which have been a long time in activation and can no longer continue the response. However, the level of mutation in the Ig genes of these cells is generally lower than in the CD27+ cells (Wu et al. 2011), so it is difficult to argue that they have been activated for longer time. It has also been suggested that this population of cells contains tissue-like memory B cells that are activated by T-independent mechanisms as they are present in CD40-deficient individuals and therefore cannot receive T cell help to mature (Zelm et al. 2014). We can see evidence of related clones between the CD27- and CD27+ populations, but the lineage analysis does not show that one is consistently derived from the other (Wu et al. 2011). Coupled with our data that shows some CDR3 character differences between the populations (Wu et al. 2011), we are inclined to think of this population of cells as containing a "sin bin" for potentially autoreactive B cells that do not receive enough accessory cell help to allow them to continue the activation and maturation process. This does not exclude the possibility that T cell-independent responders, and potentially other types of cells, are included here. If this is the case, then an increase in this population with age implies a decrease in T cell help which allows potentially autoreactive antibodies to progress and/or loss of early stage tolerance mechanisms allowing more autoreactive B cells into the peripheral milieu. As with all these broadly gated populations of cells, it is likely that the population is heterogeneous, and so some detail of individual subset types may be lost in the averaging process when the population as a whole is measured.

Age-Related B Cells

There is a population of cells in mice identified as "age-related B cells" (ABCs) that accumulate with age and have very specific functional characteristics, including those predisposing toward autoimmune activation (Naradikian et al. 2016). ABCs are antigen presenters with high levels of CD86 and MHC II (Rubtsova et al. 2015). Work to place the human equivalent of these cells within the conventional human phenotyping of subsets is still ongoing. Since the CD27- memory subset increases with age and contains class-switched cells with hypermutation in their genes, then it is possible that that ABCs will fall in this category, particularly with the associations with infection and autoimmunity that have been made for both ABCs and human CD27- memory cells (Wei et al. 2007; Moir et al. 2008). The CD27- memory cells may be a tissue-related memory population with surface markers indicating trafficking to tissues including the mucosa (Bulati et al. 2014; Berkowska et al. 2015). The relationship of ABCs to the 4-1BBL+ B cells that have been identified by Lee-Chang et al. (2014) to be increased with age in human, macaque, and mice is not clear. 4-1BBL+cells also have increased CD86 and MHC, with high levels of CD40. The human 4-1BBL+ population appears to be divided into two groups with respect to CD27 positivity (Lee-Chang et al. 2014). More detailed phenotypical analysis of these populations is needed to reconcile the contradictions and place the ABCs in the context of other functional populations.

CD27+IgM+ B Cells

These cells are thought to be the recirculating equivalent of splenic marginal zone B cells, retaining IgM and IgD but also with mutations in their Ig genes and expressing CD27 on their surface (Dunn-Walters et al. 1995; Capolunghi et al. 2013; Bagnara et al. 2015). They are important in T-independent responses against antigens such as pneumococcal polysaccharides and therefore crucial in protection against bacteria (Shi et al. 2005). This population of cells decreases with age (Martin et al. 2015), which has serious implications for antibacterial immunity. The population itself is extremely heterogeneous, containing possible T-dependent relatives of switched memory cells, CD5+ B1 B cell equivalents (Rothstein and Quach 2015), and cells that respond to activation by neutrophils (Puga et al. 2011). We have shown that cells vary by the relative quantities of IgM and IgD on the surface within this population. The proportions of these different $IgM^{hi}IgD^{lo}$ versus $IgM^{lo}IgD^{hi}$ populations change with age and likely reflect a change in the proportions of different functional subsets (Martin et al. 2015).

Regulatory B Cells

B regs are potent suppressors of immune responses, both by secretion of IL10 and by other contact-dependent mechanisms that have yet to be fully elucidated. Although a phenotype for B regs has not yet been determined, and IL10 alone does not appear to be an identifier, it seems that B regs are mostly found in the transitional B cell population (Mauri and Menon 2015). This is the population identified by $CD24^{hi}CD38^{hi}CD27^{-}$ or by $IgD^{+}CD27^{-}CD10^{hi}$. This population of cells has been shown to decrease with age (Duggal et al. 2013; van der Geest et al. 2016), which will have deleterious consequences for control of inflammatory responses. Other B cell subsets can produce the suppressive cytokine IL10, in particular the IgM memory population defined by $IgD^{+}CD27^{+}$. If, it is the IL10 rather than the contact-dependent mechanism of suppression that is changed with age (Duggal et al. 2013), then the capacity of other B cells to produce IL10 is also important. A decrease in IgM memory cells with age, as mentioned above, will likely also result in reduced IL10 production capacity. Indeed the studies of van der (Geest et al. 2016) indicate that IL10 production from all B cells decreases with age, while the ability to produce the inflammatory cytokine TNFα is unaffected. Moreover there may be differential effects on different types of autoantibodies, since ANA correlated with IL10 production ability but RF did not (van der Geest et al. 2016).

Class Switching

A general increase in CD27+ memory cells has been reported with age (Macallan et al. 2005; Colonna-Romano et al. 2006). Memory cells are often described as "class switched" with no further distinction. IgG is by far the most common antibody

in circulation and therefore the most prevalent in the literature. However, in recent years, evidence is emerging to indicate that different classes and subclasses of antibody have critical roles in the immune response. IgA antibodies are thought of as mucosal antibodies, and the huge area of exposure to exogenous antigens at mucosal surfaces certainly requires specialized and efficient protection. The physiology of the gut undergoes significant changes with age, and much more work is required to understand the complex relationship between gut physiology, microbiota, and the mucosal immune system in aging (Mabbott et al. 2015). The character of the germinal center in Peyer's patches changes with age, with indications of less specific antibody selection (Banerjee et al. 2002). The serum IgA response to pneumococcal vaccine in older people is diminished with age, as is that of the serum IgM response (Ademokun et al. 2011). While these responses form a small part of the overall serum immunoglobulin response to the vaccine, it is thought that they are key to the antipneumococcal function of serum antibodies (Park and Nahm 2011). Furthermore, in high-throughput sequencing studies, some patients exhibited poor resolution of the immune response after vaccine challenge in terms of reduction of immunoglobulin clonality at day 28, and this failure was ascribed to IgA_1 only (Wu et al. 2012). In the same vaccine study, which compared adults aged 19–45 with those aged 70–89, there was a consistent increased level of IgG_2 relative to IgG_1 subclass in the older group. In human, IgG_2 is the class of antibody produced by T-independent antibodies such as pneumococcal polysaccharides and is limited in its effector abilities as a result of its poor affinity for Fcgamma receptors (Bruhns et al. 2009). Younger adults show a significant qualitative difference in the use of IGHV family genes in the IgG_1 and IgG_2 repertoire, possibly reflecting different activation and/or tolerance mechanisms in repertoire selection. In contrast, the repertoire of IgG_1 in older people is changed such that it more resembles that seen in IgG_2, indicating a possible change in activation/tolerance mechanisms with age (Martin et al. 2015).The textbook view of antibody function is that the specificity and affinity for antigen are dependent on Fab, and only the function of the molecule is changed by class switching to a different Fc. However, it has been shown that changing the class of an antibody can affect its fine specificity so class switch is important for more than just changing effector function (Torres et al. 2005). Together these results suggest that future studies on humoral responses should include measurement of all classes of antibody so as not to exclude investigation of some potentially significant functions of B cells.

Affinity Maturation of the B Cell Receptor

A possible explanation for a decrease in specific antibody is that the process of affinity maturation is defective. Affinity maturation is highly dependent on accessory cells and occurs in the specialized structure of the germinal center in secondary lymphoid tissues. The process involves the expansion of antigen-specific B cells and mutation of their Ig genes (resulting in altered antibody function), followed by selection of the B cells producing the best antibody (Gatto and Brink 2010; Zhang

et al. 2016a). Contained within the dynamic microenvironment of the GC are B cells, T cells, and follicular dendritic cells (FDCs) all in close proximity to allow the exchange of costimulatory molecules and cytokine signaling.

Germinal Center Environment

In GCs two compartments are established: the dark zone and light zone. Following antigenic stimulation, selected B cells migrate and converge on the GC FDCs, making contact with their long processes (Park and Choi 2005) and differentiating into centroblasts in the dark zone. At the light zone, there is a lower density of B cells (Park and Choi 2005; Gatto and Brink 2010; Bannard et al. 2013). The FDCs are the stromal cells of the GC and play a key role in regulating the humoral immune response (Tew et al. 1997; Gatto and Brink 2010). Unlike antigen-presenting cells (APCs), FDCs present intact antigen-antibody complexes on their cell surface (Zhang et al. 2016a), in the form of immune complexes which are highly immunogenic, and assist GC B cell proliferation (Qin et al. 2000; Li et al. 2000; Bannard et al. 2013). During centroblast proliferation, in the dark zone of the GC, hypermutation of the immunoglobulin (Ig) genes encoding antibody occurs. The B cells move into the light zone, as centrocytes, and will die by apoptosis unless they receive rescue signals conditional on efficient recognition of the antigen by the newly formed B cell receptor. Rescue signals are provided by FDCs and T follicular helper (T_{FH}) cells (Sage and Sharpe 2016). Since FDC and T cell help are limiting, there is competition between B cells, and therefore selection of those B cells with the highest affinity for antigen occurs. The resulting B cells can switch the class of their antibody, from IgM to IgG/IgA/IgE, which also requires T cell help. B cells with high-affinity antibody differentiate into either memory B cells, to provide for an efficient recall response, or plasma cells to secrete antibody. We have addressed the possible age-related changes in the GC reaction in three main areas: proliferation of B cells, hypermutation of the Ig genes, and selection of high-affinity antigen-specific antibodies.

A defect in B cell proliferation would have severe consequences for the GC reaction, since the loss of cells due to deleterious mutations acquired by hypermutation is extremely large and the pool of B cells required to counter this is therefore also large. For some cell types, proliferating cells can reach replicative senescence – where the telomeres at the ends of the chromosomes erode at each division and therefore there is a limit to the amount of proliferation one cell line can undergo set by the length of the telomere (Goronzy and Weyand 2012). Shorter telomeres in lymphocytes have been associated with higher risk for development of carcinomas (Wu et al. 2003) with higher chances of mortality infectious disease and heart disease in people over 60 years old (Cawthon et al. 2003; Halaschek-Wiener et al. 2008). It has been shown that telomere length decreases with age in T cells and to a lesser extent in B cells (Son et al. 2000; Martens et al. 2002; Weng 2008). However, we do not believe that the proliferative capacity of B cells in the GC is impaired in this way as a result of old age. Telomerase, the enzyme that elongates

telomeres, is upregulated in the GC, being high in centroblasts and higher still in centrocytes. This results in B cells leaving the GC for the periphery with substantially longer telomeres than when they first entered, up to 4 kb longer as determined by Southern blotting (Norrback et al. 2001). Further to this, memory B cells have telomeres on average 2 bp longer than naïve B cells (Martens et al. 2002).

There has been much debate as to whether the overall size and number of GCs change with age. Rodent models have shown a decrease in GC size and number (Gonzalez-Fernandez et al. 1994; Aydar et al. 2004; Kolar et al. 2006). Immunohistochemical studies measuring the size and overall number of B cell follicles in the human spleen, Peyer's patches (Banerjee et al. 2000), and lymph nodes (Lazuardi et al. 2005) have not shown any age-related difference. However, there have been two studies of the human tonsil, performed by flow cytometry rather than measuring individual GC sizes, which have both reported a decrease in GC B cells with age (Kolar et al. 2006; Mattila and Tarkkanen 1997). Tissue-specific differences may account for these discrepancies and further work would be needed to clarify the issue.

The mutations introduced during somatic hypermutation are generally point mutations, though some insertions and deletions may occur and tend to be in areas containing hotspot motifs (Rogozin and Kolchanov 1992; Spencer et al. 1999; Rogozin and Diaz 2004). There is conflicting opinion regarding whether there is a quantitative change in hypermutation in the aging individual. Reports have indicated no change, a decrease, or increase in mutation with increasing age. The fact that these studies do not agree is hardly surprising as they do not take into account patient health history, i.e. prior immune responses. The tissue origin of samples can also make a significant difference to the number of mutations observed, for example, we have shown consistently that B cells of mucosal origin have a higher level of mutations than those from, say, the spleen or blood (Banerjee et al. 2002).

We addressed these issues by assessing the frequency of SHM in individual B cell GC expansions. Histologically defined areas of GC from the spleen and Peyer's patch follicles of young and old humans were isolated so that only the mutations in lineages from that particular GC reaction were counted (Banerjee et al. 2002; Dunn-Walters et al. 2003). There was no difference in the frequency of mutation occurring in human GC reactions in the spleen and Peyer's patch with age. Lineage tree construction can furnish information on the affinity maturation dynamics by measurement of lineage tree shape parameters. Since a failure of adequate selection could result in the production of a population of cells with low affinity, such as is seen in the elderly, we investigated lineage trees from GC reactions in samples from patients of different ages for selection differences. We found a significant decrease in the degree of selection pressure acting on GC B cells in the Peyer's patch of the gut but not the spleen (Banerjee et al. 2002; Dunn-Walters et al. 2003).

An explanation for these apparent changes in selection is still elusive, but several factors could contribute. It may be solely a failure of the quality of B cells in terms of specificity or signaling function. However, since FDCs and CD4+ T cells are important in the selection process, they are also good candidates to investigate for

the failure of selection pressure. There is a well-documented age-related decline in thymus size and a reduced T cell output. Changes in the CD8+ T cell population are dramatic, more so in the face of chronic viral infection such as CMV (Wistuba-Hamprecht et al. 2015). The changes in CD4+ T cell numbers are much more modest, resulting in the CD4/CD8 ratio being a good marker of immune senescence. There is little information as to whether human T follicular helper cells (T_{FH}) of the GC are changed in aging. CD40 ligand on T_{FH} interacts with CD40 expressed on B cells, and this relationship is critical to T cell-dependent activation of B cell proliferation, memory formation, and class-switch recombination in the GC. Aged CD4 T cells in mice have shown reduced CD40L expression (Eaton et al. 2004) and higher production of IL-10 and IFN (Lefebvre et al. 2016), and these changes are correlated with a decrease in IgG levels reminiscent of the decreased IgG production in response to influenza vaccination in humans.

It has been suggested that the function of FDCs declines with increasing age (Aydar et al. 2004; Shikh et al. 2012). Defects may be intrinsic to the FDCs themselves or may be a failure of the FDC-B cell interactions. FDCs have Fc receptors and complement receptors 1 and 2 on their surface which retain antigen as immune complexes (Yoshida et al. 1993), and these interactions are crucial for the signaling and activation of antigen-specific B cells. Older FDCs fail to properly support the generation of memory B cells, and induce a reduced number of germinal centers (Aydar et al. 2004), likely as a result of a reduced capacity for holding immune complexes (Szakal et al. 1988). A decrease in immune complex retention and presentation to B cells would lead to lowered B cell activation in the GC.

Intrinsic B Cell Changes with Age

Although there is clearly a role for accessory cell failure in the age-related changes in germinal center responses, changes intrinsic to the B cell itself are also responsible. The key enzyme in affinity maturation of B cells is activation-induced cytidine deaminase (AID), which is directly responsible for both hypermutation of Ig genes and class switching. AID expression is regulated by the E2A-encoded transcription factor E47. E47 and AID expression are reduced in old B cells (Frasca et al. 2005), and this reduction is in turn due to the action of tristetraprolin, a controller of RNA stability (Frasca et al. 2008). The consequences of reduced AID function, given its importance in affinity maturation, would be predicted to be a reduction in high-affinity antibodies. Indeed the polyclonal antibody response produced in response to H1N1 vaccination is of lower affinity when levels of AID are lower, and the levels of AID were also seen to decrease with age (Khurana et al. 2012). Other intrinsic B cell changes occur. The decision of whether to differentiate into a memory cell or a plasma cell (PC) is dependent on the regulation of transcription factors such as Blimp-1 (Klein and Dalla-Favera 2008; Martins and Calame 2008). Blimp-1 has been shown to be significantly reduced in ex vivo B cells from older people (Frasca et al. 2016), and older people have less plasma cells in their bone marrow (Pritz et al. 2015).

The Older B Cell Receptor Repertoire

Evidence from our lineage tree studies on individual germinal centers (Banerjee et al. 2002) indicated that in some instances the founder B cells of a germinal center may have already been mutated. This occurred more often in the older samples and led us to postulate that B cells which have previously been through the affinity maturation process might be reused in subsequent immune responses. If the starting population of B cells has already been modified in response to a different antigen, then its ability to effectively change to accommodate a new antigen may be compromised. It is now well established, in mice, that naïve B cell output into the periphery decreases with age. It is known that B cell precursors in humans are also reduced with aging (Kuranda et al. 2011), which is likely to lead to reduced B cell output from the human bone marrow, and the fact that children reconstitute B cell function after bone marrow transplants more rapidly than adults do would support this (Savage et al. 2001).

Since B cell memory appears to be maintained by proliferation (Macallan et al. 2005), it is possible that proliferating memory B cell clones make up for any shortfall in immunological space caused by lower naïve B cell input. This leads us to postulate that GC reactions in older individuals may use "second hand" B cells more often, which would have consequences for B cell diversity. A diverse and functional repertoire of antibodies is essential to produce an effective humoral immune response. If the repertoire of B and plasma cells is reduced, then the ability to recognize foreign antigen is severely compromised. B cell diversity and antibody specificity are defined during the early stages of B lymphocyte differentiation, where the Ig genes are formed. The remarkable way in which gene segment rearrangement forms a complete Ig gene from different segments results in millions of different B cells, each with a unique Ig sequence capable of producing antibody with distinctive specificity (Fig. 3). Briefly, the Ig molecule consists of both heavy and light chains. There are three types of gene segments: variable (V), diversity (D, heavy chain only), and joining (J). The segments are randomly recombined to generate a VDJ for the heavy chain or VJ for the light chain. Thus a germline repertoire of 176 different V, D, or J genes can result in a possible 8,408 different gene rearrangements. Combination of the heavy and light chains results in over 3.7 million combinations. The region where the junctions join together is further diversified by an incomplete joining process. Addition and deletion of nucleotides by terminal deoxynucleotidyl transferase (TdT) activity at these joints lead to junctional diversity. The VDJ joining region of the heavy chain, the CDRH3 region, is so highly variable that it can be considered to be a fingerprint for that particular gene and the B cell (and its progeny) that carries it.

Dysregulation of the Repertoire in Older People

Prior to the advent of high-throughput sequencing methods, the best general assessment of repertoire diversity was by using spectratype analysis. This would give a quantitative estimation of repertoire disruption based on divergence away from a

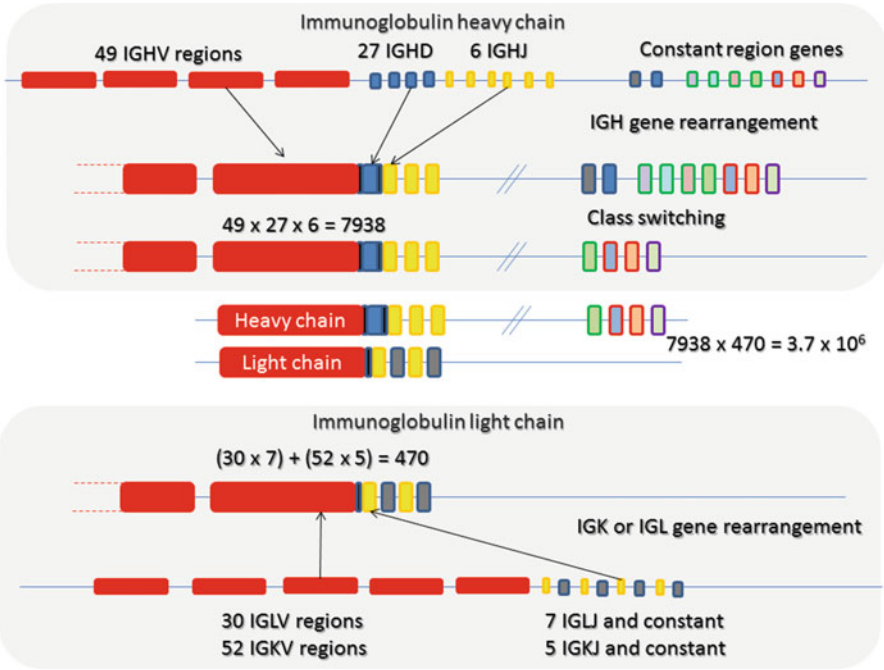

Fig. 3 Generation of immunoglobulin diversity. During germline Ig gene rearrangement, a variable (V) region is joined to a diversity (D) region and a joining (J) region for heavy chain. Light chain rearrangements can be either kappa or lambda and do not have D regions. During the rearrangement process, random N-nucleotides (N) are inserted into the junctions to form a unique CDR3 sequence. In this way, a huge number of different Ig genes are created, with correspondingly large antigen-binding possibilities. Selection processes will act on the repertoire to exclude potentially autoreactive sequences and favor sequences encoding useful antibodies. A study of the various characteristics within the repertoire can provide insight into age-related changes in B cell development (Adapted from Dunn-Walters (2016) with numbers of germline immunoglobulin genes taken from IMGT databases Lefranc (2016))

Gaussian distribution of CDRH3 lengths. In this way, we could show that dysregulated repertoires were more common in older people and were associated with frailty (Gibson et al. 2009). Spectratypes also indicated that a failure in the resolution of the immune response might occur in older people and that there were significant changes in CDRH3 length overall (Ademokun et al. 2011). However, although a spectratype is a relatively inexpensive way to investigate large cohorts of individuals, there is no qualitative information on the repertoire as a whole. Even high-throughput repertoire sequencing has its limitations when it comes to sampling the whole repertoire (Boyd et al. 2013), but a number of studies have confirmed the presence of age-related changes such as clonal expansions (Wu et al. 2012; Jiang et al. 2013), change in CDRH3 character (Wu et al. 2011; Wang et al. 2013; Martin et al. 2015), and change in class switching and subclass distribution (Rubelt et al. 2012; Galson et al. 2015; Martin et al. 2015).

Positive and Negative Selection

There is no evidence in humans that the mechanisms creating diversity in the antibody repertoire are changed with age. Therefore we assume that changes in repertoire that do occur with age will be as a result of subsequent environmental selection forces. Positive selection events can have a huge difference on the repertoire – where the antigen-specific cells are expanded and, in some instances, undergo affinity maturation in a germinal center. As outlined above, these events are all affected by age and will affect the memory B cell repertoire. One key difference in the repertoire after an immune response is in the size and physico-chemical character of the CDRH3 region, a region which is crucial for antigen binding (Wu et al. 2011). This occurs regardless of the activating antigen. At first sight, it might seem that a small CDRH3 size is beneficial to exogenous antigen binding. However there is also a decrease in CDRH3 size as B cells mature in the bone marrow so a more likely explanation is that larger CDRH3 sizes are deleterious in some way, presumably with autoreactive potential, and so are removed from the repertoire. Crucially, this decrease in CDRH3 size is not so obvious in samples from older people (Dunn-Walters 2016), indicating that the tolerogenic selection events are decreased in older people and perhaps providing an explanation for the observations of increased autoreactive antibodies with age discussed above.

Interaction of Humoral Immunity with Other Systems

There are a number of areas where other physiological systems interact with the immune system, and since aging is a whole body phenomenon, we need to take a more holistic view of the mechanisms of immune failure. Cross-disciplinary work that this requires is rare, but there are a few studies which indicate that there are important lessons to be learned and provide a tantalizing glimpse into the complexity of interrelationships.

Metabolic processes are important for all cells, and immune cells are no exception although data is limited. A transcript analysis has shown an association between memory B cell generation and cholesterol/lipid metabolic pathways in influenza vaccination (Haralambieva et al. 2016). Similarly, in mice, transcriptomic analysis identified metabolic pathways and the ER stress response pathways as being changed with age. Validation studies showed defects in glucose catabolism, oxidative phosphorylation, and increased reactive oxygen species in B cells with age (Kannan et al. 2016). Since obesity and metabolic syndrome are increased with age, the BMI of study subjects may be a confounding factor in age-related B cell studies. Obesity has been associated with attenuated influenza vaccine responses, decreased percentage of switched memory and transitional B cells, and an increased percentage of pro-inflammatory late/exhausted memory B cells (Frasca et al. 2016). Human B1 B cells have been identified in visceral

adipose tissue and may well have a protective effect (Harmon et al. 2016). However, it has also been suggested that B cells can be activated by products of lipolysis in visceral adipose tissue to release pro-inflammatory mediators, thus contributing to local and systemic inflammation (Nikolajczyk 2010). mTOR pathways, autophagy, and the unfolded protein response are all processes that have been associated with aging (Perl 2015; Taylor 2016; Zhang et al. 2016b) and have also been shown to be important in plasma cell function (Tellier et al. 2016; Arnold et al. 2016). Since plasma cell numbers in the bone marrow of older people have been reported to decrease with age (Pritz et al. 2015), then there may be common cellular changes with age that are particularly relevant to the humoral immune response.

Circadian rhythms have an effect on physiology and are known to change with age (Banks et al. 2016). Chronicity in blood lymphocyte composition, including of B cells, is observed in younger adults and decreases with age (Mazzoccoli et al. 2011). Chronicity is therefore likely to be important in determining responses to challenge; indeed a study of older adults (over age 65) found that the antibody response to vaccination against influenza was greater if the vaccine was administered in the morning than in the afternoon (Long et al. 2016).The exact mechanism by which chronicity affects the immune system is not known; many homeostatic physiological mediators also vary with time. One possibility is the effect of cortisol, and there are reports that stress can depress the efficacy of the immune system in old age (Duggal et al. 2015), a fact that is particularly relevant for the health and well-being of older people who are often subject to stresses of ill health and bereavement. Many different aspects of immunity are affected by an imbalance of DHEA/cortisol, including the lowering of antibody production from B cells (Buford and Willoughby 2008).

The exercise and nutritional habits of older people can also be affected by movement-restrictive comorbidity and issues of dentition. Humoral immunity is dramatically affected by lack of exercise (Hoff et al. 2015), and poor nutrition can affect a large number of different immune parameters (Lesourd 2004). Nutritional changes themselves will affect the microbiota. Experiments in mice clearly show changed B cell development in response to changes in tryptophan availability and microbial diversity (van Beek et al. 2016). It is clear that relatively simple changes in lifestyle factors can have substantial effects on immune function.

Cancer

B cells themselves are subject to malignant transformation, and many cancers such as leukemias/lymphomas can be age-related. In addition, tumor-infiltrating lymphocytes are critical in the control, or not, of tumor growth in malignancies of non-lymphocyte origin. The role of B cells is not yet fully understood, since they may contribute to the immune response against tumors in either a positive or a negative manner, depending on the type of B cell in the tissue. A systemic

antibody change might also affect tumorigenesis; it has been suggested that the increase in ANA antibodies seen with age may have beneficial effects by virtue of a possible antitumor activity (Torchilin et al. 2001). Whether there are tumor antigen-specific humoral effects is in question, but since most cancers increase with age, we should make efforts to understand the role of the aging B cell repertoire in cancer.

Monoclonal Expansions and Lymphomas

Expansions of B cells/plasma cells in the absence of any apparent exogenous challenge could be due to a number of factors. It may indeed be true that a decreasing naïve B cell output in the face of homeostatic mechanisms to keep the total number of B cells the same has resulted in the repertoire being increasingly made up of antigen-experienced expansions of cells. There may actually be an underlying infection, but the normal febrile symptoms by which we recognize as infection do not show due to the effects of immune senescence or a failure to resolve a response from a prior infection in a timely manner. A high-throughput repertoire study of individuals of known seropositivity status for the chronic viral infections CMV and EBV has shown that EBV, but not CMV, can cause an increase in clonal expansions in the repertoire (Wang et al. 2014). Alternatively there may be pathological monoclonal expansions of B cells, such as are seen in leukemia or lymphoma. Usually, these are diagnosed conditions, and individuals with this sort of medical history are excluded from studies on B cell diversity. However, it might be possible that a preclinical condition exists in some people. An increase in monoclonal expansions of B cells, both of CD5+ and CD5- phenotype, has previously been reported in older people (Ghia et al. 2004). Monoclonal gammopathy of undetermined significance (MGUS) is a predominant plasma cell disorder (Kyle et al. 2006) and has been shown to increase with age in both humans (Kyle et al. 2006) and mouse (Radl 1990). It is characterized by an increase in the presence of serum monoclonal Ig. MGUS is not found in young subjects, is prevalent in around 2% of over 50s, and has been reported to vary in the elderly from 11% to 38% (Kyle et al. 2002). There is an association between MGUS and onset of multiple myeloma or related malignant condition with average risk assessed at about 1% per year (Kyle and Vincent Rajkumar 2003). A vaccination study of MGUS patients showed poor vaccine responses and indicated that the recruitment of B cells to the immune response was compromised by the prior existence of expanded clones (Tete et al. 2015). Hence while one danger of MGUS is in potential transformation to malignancy later in life, another key risk is in susceptibility to infection. One might assume that in older people without MGUS, but with inappropriate expansions of B cells (as mentioned above), this would also be the case. Other malignancies of B cells other than myeloma are age-associated; a prime example is chronic lymphocytic leukemia (CLL), which is thought to arise from a premalignant monoclonal B cell lymphocytosis (MBL) that also carries a risk of susceptibility to infection (Whitaker et al. 2014; Scarfò and Ghia 2016). The B cell receptors encoded by CLL are autoreactive (Herve et al. 2005), and hence we think

that the expansion of potentially autoreactive B cells in older age is a likely contributor to malignant transformative events.

Role of B Cells in Antitumor Activity

The presence of B cells in several tumor types (colorectal, breast, lung, and gastric cancers) has been strongly associated with improved prognosis in a number of studies (Nelson 2010; Hennequin et al. 2015). This may be as a result of their role as antigen-presenting cells, thus aiding the tumor-infiltrating T cells, or may be a function dependent on their antibody (Nelson 2010; Lee-Chang et al. 2014). It is interesting to note that the age-associated 4-1BBL+ B cells make excellent antigen-presenting cells that can activate CD8+ T cells and therefore stimulate antitumor activity (Lee-Chang et al. 2016). B cell infiltrates may be diffused or aggregated into tertiary lymphoid structures, in some cases recapitulating germinal center morphology. Clonal expansions of switched B cells have been shown in melanoma, where the presence of B cells is also thought to be beneficial (Saul et al. 2016). However, experiments in mice have indicated that tumor-infiltrating B cells can be regulatory, causing downregulation of the antitumor response, and the presence of B regs in a number of solid tumors (e.g., pancreatic, ovarian, esophageal, hepatocellular carcinoma) has been predicted to aid tumor growth by inhibition of T cell activity and promotion of inflammatory growth effects (Schwartz et al. 2016). B regs in human PBMCs are of the IgM class, so perhaps distinguishing tumor B cells by class of antibody might aid in discriminating between the different functional effects. It has been suggested that aged human monocytes can convert B cells with regulatory capacity into antitumor 4-1BBL+ B cells (Lee-Chang et al. 2016). It seems that much more work is needed to elucidate the exact role of tumor-infiltrating B cells in different types of tumor.

Summary

In recent years, the role of the B cell in the immune response has been recognized as being much more than simply a factory to produce antibody. Thus the field of B cell immunology is in an exciting phase where there are many unanswered questions with respect to immune regulation and tumor immune responses; both these topics are of particular importance for older people. In addition, there is much scope for investigating B cell responses outside the textbook T-dependent IgG_1 antibody production. Many key age-related changes are in T-independent responses which would involve IgM, IgG_2, and IgA – particularly at mucosal surfaces mucosal surfaces which are often the first to encounter external pathogens and which and which may be involved in educating the host humoral immune system with respect to distinguishing inappropriate activation signals. The overall picture we currently have of B cell immunity in aging is that of a system sufficient in number and quality

at the very start of development, but with some breakdown in the selection controls balancing appropriate versus inappropriate activation signals.

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