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B cell repertoire and ageing

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A diverse B cell repertoire is essential for an effective immune response. Not only to provide a variety of antibodies to recognise the multiplicity of likely pathogen challenge, but also because B cells are important regulators of the immune response. In addition to their excellent capabilities as antigen presenting and activating cells, recent work shows that some subpopulations of B cells can have suppressive functions. The diversity of the B cell population as a whole decreases with age, and is associated with ill health. Whether decreased diversity is a feature of all B cells or a reflection of altered subpopulations is not clear, since different subsets of B cells have different functions and their repertoires are shaped by different selection pressures.

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Introduction

Older people are more susceptible to infectious disease, especially those where the route of pathogen entry is through the mucosa such as in influenza, pneumonia and urinary tract infections. The ability to respond to vaccines against these diseases decreases with age such that the percentage of older people able to produce an efficient antibody decreases [1,2], the pneumococcal vaccine can protect older people against bacteraemia but not against pneumonia [3] and there are similar age-related deficiencies in vaccines against Hepatitis A and B, Herpes Zoster, Tetanus, Tick Borne Encephalitis and diphtheria [4–7]. Serum antibodies are a well established, and easily measurable, endpoint of an antigen challenge-induced B cell maturation process that involves multiple cellular interactions [Figure 1]. A good humoral response, as measured by antigen-specific antibodies, is associated with recovery from pathogen challenge, and serum anti-

body titre is frequently used as a surrogate marker of protection in assessments of vaccine efficiency.

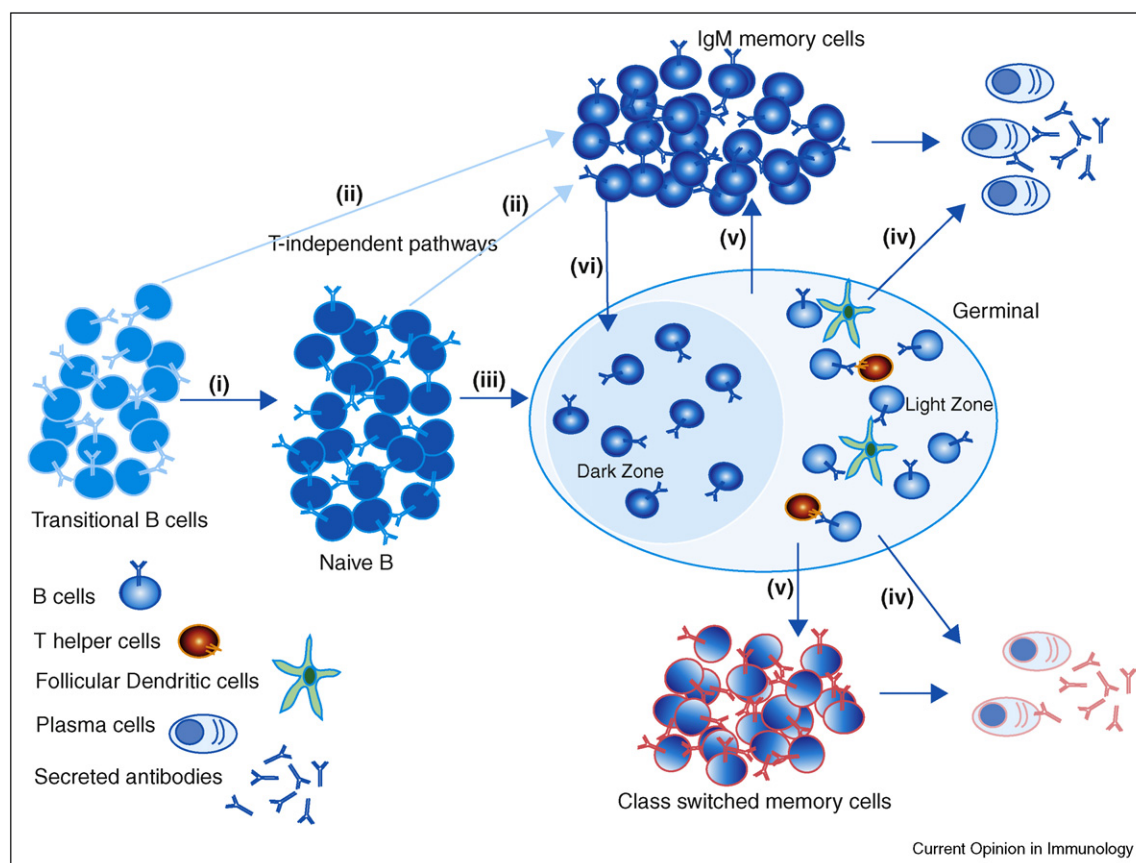
The B cell receptor (BCR) is encoded by the same rearranged gene as encodes the immunoglobulin (Ig) that its later developmental offspring will secrete. This gene rearrangement is crucial to the diversity of the B cell repertoire—enabling a large number of different specificities of Ig in order to improve chances of antigen recognition. It may also be the case that a more diverse response is more effective than a high affinity oligoclonal response, since it has been shown that a human response to even a relatively simple antigen, such as tetanus toxoid, can comprise up to 100 different clones [12]. In this review we briefly describe age-related changes in serum immunoglobulin, and additional roles of B cells in immunity, before reviewing the changes in B cell subpopulations and why these changes will affect the overall repertoire diversity.

Age-related changes in humoral immunity

A constant background of circulating antibodies with a broad range of specificity is an essential requirement as a first line of defence, their opsonising activity being a vital link between the innate and adaptive immune systems. It has been estimated that a healthy mouse has 16,000 different functional specificities [13]. Since antibodies have a limited life, a healthy population of plasma cells is required regardless of whether there are any specific pathogen challenges to generate new memory and plasma cells. That this is not always the case in older people is evidenced by the age-related increase in incidence of monoclonal gammopathies of undetermined significance (MGUS) where a single spike of immunoglobulin, representing the output of an expanded plasma cell clone, can be seen in 5.3% of the population over 70 years old [14]. Although MGUS is usually an early precursor of a haematological malignancy it is also associated with an increase in mortality from other causes such as bacterial infections, indicating a wider effect on the immune system [15,16].

A further consequence of the ageing humoral system is the change in background serum specificity to include a greater level of antibodies with autoreactivity. This is particularly true for antibodies against cardiolipin, DNA, antinuclear antibodies (ANA) and rheumatoid factor (RF). Although some autoimmune diseases, such as vasculitis, are age-related, more often than not the increase in autoreactive antibodies is not accompanied by autoimmune pathology [17]. It has been suggested that autoimmune antibodies are increased as a by-product of some

Figure 1



Different B cell populations in the periphery. Upon exit from the bone marrow, B cells pass through a transitional stage before becoming mature naive B cells. Some negative selection to remove autoreactive B cells occurs here [8] {1}. IgM memory cells may be generated from naive B cells after positive selection by T-independent antigen, and can be generated from transitional cells given the appropriate stimulation [9] {2}. In addition to positive stimulation from antigen and/or pathogen associated molecular patterns the generation of IgM memory cells also appears to involve negative selection to remove autoreactive cells [8]. T-dependent protein antigens positively select naive cells from the population and result in the generation of germinal centres {3}. In a germinal centre reaction the antigen-specific B cell loses Bcl2, becoming susceptible to apoptosis, and proliferates rapidly with the concomitant gain of mutations in its immunoglobulin (Ig) gene. Hypermutated Ig genes encode altered affinity for antigen, and the survival of the cells depends on their ability to compete for antigen binding and thereby receive help to survive from accessory cells. These cells are follicular dendritic cells, holding antigen on their surface in the form of immune complexes, and T cells, with the same specificity for antigen as the B cell. This limited cognate help provides survival and differentiation signals to the B cells with the best affinity so that they can differentiate into plasma cells secreting antibody {4} or into memory cells {5}. Some IgM memory cells are formed this way [10], and can re-enter the germinal centre in later antigen {6} responses [11]. Some cells receive T cell help and undergo class switching to become memory cells, usually of IgA or IgG isotype. IgG memory cells have been shown to encode autoantibody specificities acquired as a result of the germinal centre affinity maturation process [8].

other, specific, challenge, since an early study reported increases in anti-DNA antibodies in response to influenza vaccine in older people [18]. However, we do not see any challenge-related difference in the level of serum auto-antibodies after influenza and pneumococcal vaccines at any age (Ademokun *et al.*, unpublished).

B cell functions other than antibody production

Much work has been carried out to characterise the senescence of the T cell arm of the adaptive immune response, and many failings of the humoral immune system have been ascribed to the lack of T cell help for B cell function.

However, there are a number of B cell intrinsic changes in function with age that are not ascribable to a lack of T cell help [19]. It has also become increasingly clear that the contribution of B cells to the effective function of the immune system extends well beyond that of antibody production in response to cognate help from T cells. The B cell–T cell interaction is a two-way process, with B cell help playing an important role in T cell activation for both CD4⁺ and CD8⁺ T cells [20,21]. They can also modulate T cell function by inducing anergy, causing deletion or via cytokine-mediated suppression [22–24,25^{••}]. In addition, they secrete a variety of inflammatory cytokines, such as IL-6, TNF α , IFN γ and therefore are a

potential cause of the inflammatory milieu that occurs with age [26]. Importantly they can respond to T cell-independent antigens, such as pneumococcal polysaccharide—particularly important in an older population at high risk from pneumonia and other bacterial diseases. B cells also have Toll-like receptors (TLRs), that enable them to respond to pathogen associated molecular patterns such as LPS, and early indications are that the expression of these molecules might increase with age [27]. TLR's can be stimulatory in an immune response, sometimes inappropriately [28] but they can also activate immunosuppressive activity of B cells [29]. Many of the aforementioned properties of B cells have not been studied with respect to age and there is much scope for future investigation.

B cell repertoire—population influences

There are a number of peripheral development stages through which B cells can pass upon leaving the bone marrow. Each transition has selection processes such that the starting population is more diverse but further down the development pathway the population is selected and less diverse [Figure 1]. Thus any change in the proportions of different B cell populations will result in a change in overall repertoire. There are several age-related changes that occur in B cell population frequencies and numbers with age. In mice there is a decline in bone marrow output and impairments in haematopoietic stem cell (HSC) commitment to the B lineage [30,31[•]]. Parabolic experiments demonstrated that HSC commitment to the myeloid lineage at the expense of B lineage is not due to HSC intrinsic defects alone, defects occur in both blood-borne factors and bone marrow niche supporting cells [32^{••}]. This impairment to B cell commitment is manifested as a deficit in the numbers of early B cell populations such as the pro, pre, transitional and mature naive B cells. Others have suggested that it is not the BM output *per se* that is crucially impaired but rather the ability of naive B cells from older mice to colonise peripheral compartments that is reduced [33].

Despite the reduced influx of fresh naive cells into the periphery, the total numbers of peripheral B cells in mice remain the same. There are several mechanisms that can explain this anomaly. It has been demonstrated that those splenic B cells that do make it out of the BM in old mice also have a reduced turnover as compared to splenic B cells from young mice, and so live longer [34]. Another attractive idea is the homeostatic expansion of antigen-experienced cells, such as memory B cells, or antigen poised populations such as B1 cells [35] and marginal zone B cells [36]. In mice B1 B cells are identified as being CD5⁺ and there is evidence for a shift towards the B1 pathway in old mice, even from an early bone marrow stage [35,37]. B1 cells in mice produce antibody of low affinity and polyspecificity and have been associated with autoantibody production. A relative expansion of cells such as these would have the consequence of altering the

repertoire and is also consistent with the spontaneous elevation of autoantibodies in serum of older animals.

A rare population of CD5⁺ cells that are also CD1d⁺ have been described as potential regulatory B cells in mice owing to their production of IL-10 [38]. Downregulation of the immune response by anti-inflammatory cytokines such as IL10 is crucial for a balanced and effective immune system. The increase in CD5⁺ cells in old mice and the presence of IL-10 producing regulatory B cells in humans [25^{••}] makes the study of Bregs an interesting avenue for further research in immunosenescence.

In humans a decrease in total CD19 B cell numbers has been reported by several groups, although this is not always observed when defining B cells by CD20 expression [39[•]] and there may be some disconnect between CD19 and CD20 expression on B cells with age [40]. In addition to a reduction in total CD19⁺ B cell numbers, the proportion of different subsets is also altered. In general there is a skewing towards antigen-experienced B cells with a reduction in naive B cell numbers and frequency. Human B cell populations are routinely defined by CD27 and IgD expression and there is ambiguity about how the numbers and proportions of these populations change with age [39[•]]. Two interesting populations that can be differentiated by this analysis are the IgM⁺, IgD⁺, CD27⁺ (IgM memory cells) and the IgD[−]CD27[−] (double negative cells). Double negative cells are likely to be a heterogeneous population that contains at least some antigen-experienced cells as evidenced by rearranged IgG genes [41,42]. IgM memory cells are reduced in older individuals [40] whilst it is thought that double negative cells are increased with age [43]. It is interesting to speculate on the origins and specificity of these cells in this population and what the increase, in the case of double negative cells, might mean for the repertoire of the older individual. At the moment the expression of rearranged Ig genes in the absence of CD27 suggests they may be memory cells that have down-regulated CD27. Alternatively they might represent a different antigen-experienced pathway, independent of CD27 expression. There is circumstantial evidence that they may be involved in the serum autoreactivity seen in older people, since the population is increased in systemic lupus erythematosus (SLE) patients who have elevated levels of autoantibodies [44].

Since different subpopulations have different functions, and have been through different selection events in their development, it is clear that age-related alterations in the composition of the B cell population as a whole will impact on the overall diversity of the B cell repertoire.

B cell repertoire—BCR diversity

Each B cell, and any progeny arising from it, can be characterised by its unique Ig gene rearrangement. Thus the B cell repertoire can be measured by looking at Ig

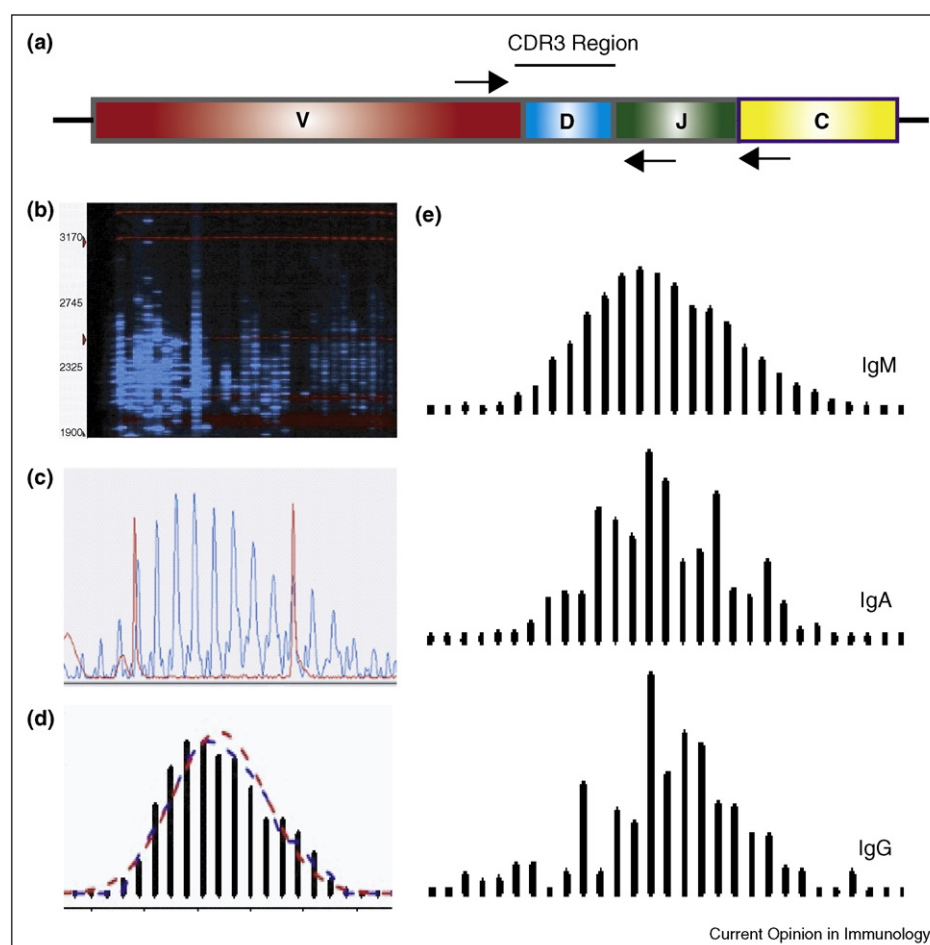
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gene diversity. Early reports of B cell repertoire indicated that older mice showed evidence of non-malignant clonal expansions [45], which in view of the homeostatic mechanisms that maintain the total numbers of B cells would lead to a decrease in diversity. Old mice have also been shown to have altered repertoires in response to challenge with the hapten phosphorylcholine [46–48]. The response in young mice was oligoclonal – dominated by use of one particular IGHV gene – but older mice had a more diverse response. A similar investigation in humans, looking at the response to pneumococcal polysaccharides 4 and 14 after vaccination also showed differences between their young and old volunteers, including a trend towards polyclonality with age [49], although this was not evident in their analysis of the light chains [50]. The presumption is that an age-related difference in the

basal repertoire causes the difference in response, although matching pre-challenge and post-challenge repertoires have not been investigated in reports so far. Previous studies on age variability in the basal human repertoire are limited, either by being restricted to particular families of genes [51–53] or by the numbers of sequences in the analysis [54]. Even the best analysis of the whole repertoire was limited to a total of 304 genes [55].

Methods other than sequencing can be used to determine B cell diversity. Age-related expansions of plasma cells resulting in MGUS can be easily detected by the presence of M protein in the serum. Expansions of B cells and plasma cells can also be detected by PCR amplification across the CDR3 region of the immunoglobulin

Figure 2



Spectratype analysis of B cell diversity. Immunoglobulin heavy chain genes comprise three different rearranged genes: IGHV, IGHD and IGHJ (A). The junction of the three genes is extremely diverse as a result of imprecise joining and by the addition of random nucleotides into the joins by terminal deoxynucleotidyl transferase. This region is known as the complementarity determining region 3 (CDR3). PCR amplification across this region results in a large number of different sized fragments, the distribution of which, in a healthy peripheral blood sample, is Gaussian. Separation of the fragments on sequencing grade gels (B) results in fragments spaced at 3 bp intervals (C). Measurement of peak heights enables proportional spectratypes of the distribution to be plotted and statistical metrics to be determined (D). If isotype specific primers in the C region are used instead of the 3' J region primers then spectratypes specific for IgM, IgA or IgG can be determined. The lack of diversity in class switched populations is evidenced by movement away from a Gaussian distribution (E).

heavy chain variable region and subsequent analysis using high resolution electrophoresis—known as spectratyping (Figure 2). In this way we have previously shown that the normal repertoire of B/plasma cells becomes less diverse with age, with clonal expansion of more than one cell occurring. This skewing of the B cell repertoire was associated with a general phenotype of frailty [56*].

It has been suggested that age-related differences in repertoire can occur at immature developmental stages in mice [57], although repertoire analysis of bone marrow samples has yet to be performed. In view of the normal clonal expansion that occurs in antigen-experienced cells and skews the repertoire (Figure 2) it is clear that a change in the proportion of naïve:memory B cell subsets, as discussed above, will result in a changed overall repertoire. In addition it has been shown that memory B cells in adults have been through more rounds of proliferation than in children, possibly as a result of repeated antigen exposure, and that naïve B cells themselves may undergo some homeostatic expansion [58]. These experiments have not yet been performed on elderly individuals, but it would be interesting to discover whether repeated proliferation within particular subsets of cells might be responsible for a decrease in diversity in the aged.

Future prospects

Repertoire analysis has previously been hampered by the lengthy process of sequence isolation and determination. However, as techniques of single cell isolation, cognate immunoglobulin gene pairing and deep sequencing become more widespread, more effective repertoire analysis is now possible. It is clear that there are changes in B cell repertoire with age in both mice and humans, and that these differences are related to poor health. However we also know that there are a number of different B cell populations that have a different repertoire of Ig genes [59] and so future studies will need to account for age-related subpopulation differences when determining age-related Ig gene repertoire differences. B cells in the ageing immune system have hitherto not been as well studied as other aspects of immunity. We predict this will change since there are some exciting developments in B cell biology that highlight multiple roles for the B cell and these present many areas for future investigation in immunosenescence.

Acknowledgements

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