MINIREVIEW

Age-Associated Changes in Antibody-Forming Cells (B Cells) (43186)

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Classic studies by Walford (1) and Makinodan *et al.* (2) have demonstrated the progressive decline in immune responsiveness associated with aging. For example, there is a consistent decrease in the amount and in the average affinity of antibody produced by aged animals (1–6). Aside from these alterations at the antibody level, there are several changes in effector functions of the T cell compartment (7–10). Age-associated increases in autoantibody production have also been demonstrated (11). This review will focus specifically on the humoral arm of the immune system and describe changes that can be linked to senescence. Because of the demonstrated interaction between B and T cell populations, it is important to separate the effects of T cells (help or suppression) from age-associated characteristics that are intrinsic to B cells. Other reviews have concentrated on these age-related changes in the T cell compartment (11–13). Another difficulty associated with the study of aging is the large amount of individual variability that can be detected. This variability can actually be caused by the ever-present problem of tumors, which of course increases with age, affecting individuals' immune systems in a nonuniform manner. An additional characteristic usually associated with a discussion of aging is the loss of proper regulatory mechanisms. Such a loss of regulatory potential could cause the greater variability in measurements observed.

The cells central to the discussion of antibody production are the B cells. These cells have been intensely studied because they can be isolated from spontaneously occurring plasmacytomas (14) and also since B cell lines can be easily immortalized by transformation with Abelson virus (15) and simple fusion techniques (16). When B cell lines were initially analyzed, it was demonstrated that these cells produce enormous amounts of immunoglobulin mRNA and protein. The immunoglobulin proteins are the most extensively studied molecules in the field of immunology (17). Additionally, immunoglobulin genes have been altered using *in vitro* techniques and have been introduced into cells using numerous transfection or transgenic systems (18, 19) in order to delineate their function and regulation.

A conservative estimate is that B cells have the ability to produce an estimated 10^8 different antibodies (20), however, each B cell can produce only a single heavy and a single light chain, which interact to make an antibody with a unique specificity. The antibody combining site of the immunoglobulin molecule is constructed by rearrangement of germline-encoded DNA sequences (21). Using such a rearrangement process, a small number of germline elements can be rearranged in many different combinations to produce the vast B cell repertoire.

Another important characteristic is that the B cell population has a remarkable capacity to turn over quite rapidly, in that the half-life of many B cells is short. Newly arising cells are continually being produced from a self-renewing stem cell population present in the bone marrow. This property permits the continuing regeneration of the pool of peripheral cells that produce antibody. On the other hand, there is a subpopulation of B cells that can be quite long-lived, and these memory B cells give the individual an increased ability to respond more rapidly when an antigen is reintroduced.

The decline in antigen responsiveness associated with aging has been characterized in qualitative and quantitative terms (1–6, 22, 23). Although the kinetics of the immune response are similar, the magnitude of the response to thymic-independent and thymic-de-

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pendent (TD) antigens are both generally depressed (24–27). The increased affinity of antibody seen in sera after antigenic stimulation (known as the maturation of the immune response) with a thymic-dependent antigen in the young adult is profoundly altered in the immune response of the aged. In the aged, the expressed antibody repertoire is principally composed of low-affinity antibody with an apparent absence of medium- and high-affinity antibody (22, 25, 28, 29).

Along with this apparent lack of maturation of affinity in the aged, there occurs a change in the expressed repertoire of antibodies to a given antigen (30, 31). That is to say that the predominant response to a defined antigen in animals of different ages produces antibodies that utilize different genes. Concomitant with these changes in the expressed antibody repertoire in aged animals, regulatory mechanisms involving the idiotypic network are markedly increased in the immune response of the aged (26, 27, 32). Simply stated, it has been suggested that antibodies that recognize the variable portion of another antibody can affect the immunoglobulin level present in the serum. This auto-anti-idiotypic antibody produced during the normal immune response of the aged animal is markedly enhanced when compared with that seen in the immune response of the young adult. Autoimmunity is also an earmark of the aged immune response as witnessed by a rise in the titers of autoantibodies (reviewed in Refs. 33 and 34), but this age-related increase in autoantibodies is generally not associated with clinically relevant symptoms (35). The etiology of the increase in antibodies that recognize self-components could be explained either by inappropriate cross-reactive low-affinity antibodies such as displayed by aged individuals or to an alteration in the regulation of the T cell compartment. Further discussion of aging and autoimmunity is beyond the scope of this review.

Aside from the alterations at the antibody level, there are several changes in effector functions of the T cell compartment (36). These changes have proven easier to document than changes in the B cell compartment. Following the progressive involution of the thymus, there is a continuous decline in the number of T cells leaving the thymus, and aging is characterized by a decline in T helper cell function (7–12). In other aspects of the T cell compartment, such clinical tests as delayed type hypersensitivity reactions, which are used to evaluate normal immune status in the young, now prove unreliable in assessing the immune potential of the aged (37).

In this review, we shall consider the changes seen in the B cell compartment of the immune system of the aged. These changes include data that have come to light in the last 2 or 3 years and that we believe bring us closer to an understanding of the molecular events leading to the declining immune system of the aged.

Nonspecific Changes in the B Cell Compartment in Aging

In this section, different nonspecific immunologic parameters will be described that differentiate young from aged animals. As it occurs in most emerging disciplines, it is at the present time difficult to determine, in view of our incomplete knowledge, which of these changes will ultimately prove to be of biologic importance to the process of aging.

Several studies observed a decline in B cell proliferation in response to stimulation by lipopolysacchride or by an antibody to the constant region (38–40). In general terms, proliferation measurements correlate with measurements of B cell antibody responses. In recent studies, serum concentrations of several immunoglobulin isotypes and subclasses were determined in mice of different ages (41). Numerous inbred and hybrid strains of mice were studied. There is a gradual increase in serum immunoglobulin concentration associated with increasing age. This is generally true, although each strain presents a distinctive idiosyncratic pattern. For example, for the 2-month-old BALB/c mouse, serum levels for IgG_1, IgG_{2a}, and IgG_{2b} are 459, 814, and 202 μg/ml. respectively; by 19 months of age, these values have risen to 2390, 2341, and 1125. This increase in serum concentration was even seen for IgD, an isotype that is usually found at a very low concentration (200–500 ng/ml) in the serum of the young adult mouse (42). Fewer than 10% of young animals express concentrations of IgD greater than 500 ng/ml. In contrast, at 20–25 months of age, between 40 and 75% of mice in most strains studied had more than 500 ng of IgD/ml. It is not clear whether this increase in serum immunoglobulins represents an actual change in the production of antibodies by B cells or an alteration in the catabolism of these proteins in the aged animal. However, one should point out that data have also been obtained demonstrating a similar increase in serum IgD levels in the athymic BALB/c *nu/nu* mouse (42). This would indicate that the age-associated increase in serum immunoglobulin levels may represent an intrinsic change in B cells and that this change may be a thymic-independent event. On a more general level, these increases in serum immunoglobulin levels may be related to the antigenic experiences of the individual. Skewing to certain isotypes and to certain subclasses of antibodies has been reported in responses to carbohydrate, proteins, and viral antigens and parasitic infections (43, 44).

Immune Responses to Thymic-Independent and Thymic-Dependent Antigens

Antigens can routinely be segregated into those that need T cell help to induce significant antibody levels and those antigens that require minimal help. T-independent antigens are usually polysaccharides with

multimeric structures. It is thought that their potential for cross-linking B cell surface receptors leads to activation and immunoglobulin production, which requires minimal T cell help. T cell-dependent antigens do not lead to B-cell activation and significant antibody production unless T cell help and the cytokines they secrete are available.

Thymic-Independent Antigens. Although not generally recognized, the magnitude of the immune response to thymic-independent antigens is considerably diminished in the aged (26). In the murine model, the response to the haptenated thymic-independent antigen, 2,4,6-trinitrophenyl-lysyl-Ficoll (TNP-F), shows a progressive decline throughout the life of the animal. For example, the BALB/c mouse at 6–8 weeks of age responds with 70,000 plaque-forming cells (PFC)/spleen, whereas at 96–100 weeks of age the response decreases to about 10,000 PFC/spleen. Similarly, in the C57BL/6 strain there is a 4-fold decrease in anti-TNP-F responses between the ages of 8 and 78 weeks of age. Both C57BL/6 and BALB/c mice are standard, long-lived strains with life-spans ranging from 2.5 to 3 years (45). It is not clear whether this decline in immune response to thymic-independent antigens is caused by an intrinsic defect in B cells, a change in regulatory mechanisms, or both. Elucidating the mechanisms leading to the decline in the immunity of the aged is obviously important when one would like to impart to the aged the capacity to ward off microbial infections.

Thymic-Dependent Antigens. The magnitude of the immune response to TD antigens, which routinely comprise proteins and cell surface determinants, is also markedly diminished in the immune response of the aged and is qualitatively altered as well (1–4). For example, for the response to 2,4-dinitrophenylated (DNP) bovine γ-globulin young (2 months of age) BALB/c mice have been found to produce some 11,000 anti-DNP PFC/spleen, whereas 24-month-old mice gave responses of only 2000 PFC/spleen (22). A similar decrease was seen for C57BL/6 mice. The anti-TD antigen response of the aged is characterized by low-affinity antibody with a peculiar lack of medium to high-affinity antibody (22, 25, 28, 46). Therefore, antibody affinity maturation is apparently impaired in the aged. Since it is generally believed that high-affinity antibody is more efficient in helping fight off infectious microorganisms, this qualitative change toward low-affinity contributes further to the diminished capacity of the immune response in the aged.

Changes in the Regulatory Activities of the Immune Network in Aging

Jerne (47) proposed a network of interactive elements that might contribute to the regulation of the immune response. This network would be established following antigen stimulation by the anti-antigen antibody response (the idiotype or Ab1) leading to the production of a second antibody made against the combining site of the first antibody. This anti-idiotypic antibody (Ab2) could down-regulate the production of idiotypic antibody. We and others have shown that with aging there occurs a marked increase in the production of auto-anti-idiotypic antibody (Ab2) in mice (26, 27, 32) and in adult humans (48). At the serologic level, we were able to demonstrate that, following immunization with the same antigen, animals of different ages show a change in the expression of the antibody repertoire. To complicate this change, B cell recruitment following antigen administration is probably as efficient in the aged as it is in the young. This is supported by the finding that considerably greater heterogeneity of antibody affinity of the immune response of the aged is observed once the auto-anti-idiotypic antibody is removed from immune cell populations obtained from aged mice (49). In practical terms, this means that most medium- to high-affinity antibodies produced in the immune response of the aged are not secreted because they are presumably down-regulated by high production of anti-idiotypic antibody.

One hypothesis is that the increased production of Ab2 down-regulates Ab1 that may cross-react with self-antigens. This is supported by several lines of evidence: (i) there is an increase in circulating autoantibodies with aging; (ii) monoclonal antibodies obtained from aged immune mice tend to display broad reactivities, which include self-antigens; and (iii) the kinetics of production and the isotype of auto-anti-idiotypic antibody in the aged probably identify this response as an immunologic recall (50). It has been observed that similar properties of the idiotypic regulatory cascade described above have been demonstrated to be present for the fetal and neonatal idiotypic repertoire (51).

We have described two roles of the auto-anti-idiotypic antibody in the immune response of the aged, a beneficial one in the down-regulation of anti-self cross-reactivity and a potentially negative role in contributing to the decrease in the magnitude of the response. It is not clear at this time how the balance between the two roles is regulated.

Bone Marrow and Aging

As described in previous sections, with age there appears to be a general decline in the effectiveness of the immune system. Does a natural degenerative process occurring within the bone marrow contribute to the decrease of immunity seen in senescence?

The bone marrow is the source of newly arising cells of the erythroid, myeloid, and lymphoid lineages for the adult, whereas in fetal life the source of these cells is the liver. These various cell lineages are generated from pluripotent stem cells (PSC) in the bone

marrow (52, 53). The capacity of these PSC to repopulate has been demonstrated in experiments in which mice are reconstituted following lethal irradiation (54). More recently, the PSC have been identified and enriched from the bone marrow by unique cell surface characteristics, and the frequency of these stem cells that can regenerate the total peripheral cell population has been estimated to be about 0.1–0.3% of the bone marrow cells (55). The frequency of PSC for aged mice has yet to be determined. Obviously a change in the number of progenitor cells could well be the cause of the impaired or less effective performance of the lymphoid system, in particular the B cell compartment (56).

In the process of studying bone marrow cells, the observation was made that there is a distinct decrease in lymphoid cellularity with aging (57, 58). As this cellularity decreases, there is a concomitant increase in adipocyte-like cells (59). These adipocyte-like cells have most of the biochemical and histologic characteristics of classic adipocytes. The various cell lineages that are derived from the PSC can be expressed in different ratios when aged and young mice are compared. For example, in humans there is a marked increase in granulopoiesis as the cellularity of the bone marrow decreases (57).

Early experiments used reconstitution with bone marrow cells as a method to study stem cells. The bone marrow cells from either young or aged mice can be used to reconstitute lethally irradiated syngeneic recipients (54). Interestingly, when a mixture of differentially allotype-marked young and aged bone marrow cells compete in the reconstitution of a lethally irradiated young mouse, the bone marrow shows no predominance of either marker, but the peripheral lymphoid system displays a predominance of the cells characteristic of the young donor (60). These results suggest that homing and early proliferation in the bone marrow for young and aged cells are similar, but cells derived from the young animals are more successful in repopulating the periphery of the young recipient. In another autoreconstitution study of aged mice, the long bones (which contain most of the marrow) were shielded from the lethal irradiation, and bone marrow reconstituted the periphery together with T cells from young or aged donors. It was found that mice that received young T cells displayed auto-anti-idiotypic antibody profiles and a response to TNP-F like that of the young donor (61, 62), whereas those that received aged T cells responded like the aged donors (61, 62). These results strongly implicate the T cells in the quantity and quality of the immune response.

When the precursor frequency for aged versus young animals is determined for B cells that recognize particular antigens, a wide range of results is obtained. In aged individuals, the precursor frequency for phosphorylcholine (PC) is increased (63), whereas that for DNP and another hapten, 4-hydroxy-3-nitrophenyl acetyl (64), is decreased and that for influenza antigen PR8 is unchanged in aged mice (65). In a study comparing the bone marrow of young and aged mice, an age-related decrease in the number of cells in the pre-B cell compartment as described by cell surface phenotype was noted (61). In studying the function of bone marrow derived cells from aged donors, there was a decreased capacity to generate surface Ig^+ B cells both in numbers and in proliferative capacity as measured by tritiated thymidine incorporation following mitogenic stimulation. In addition, *in vitro* culture systems using bone marrow-derived cells demonstrated lower amounts of surface immunoglobulin and a decreased mitogenic response in the aged animals when compared with young animals (66). In mixing experiments, it was concluded that suppressor T cells were not the cause of this effect. It was also determined that a supportive cell might be missing or deficient in the bone marrow environment of the aged (66).

Antibody Repertoire and Aging

As described earlier, expression of antibody molecules of seemingly limitless variety is one of the most important functions of B cells. An individual mouse contains an estimated 10^8 B cells. The antibody repertoire, the large number of different specificities, is generated by mechanisms that involve rearrangement of sets of sequence elements in different combinations in each B cell. Rearrangement at the DNA level between three distinct sets of elements occurs on the heavy chain locus and between two sets of elements in the light chain loci. In this process, the elements from different sets of sequences rearrange because of the highly conserved sequences that flank each set of elements (67). Recently the enzymatic activity that can ligate these elements has been identified (68). In this process, the intervening DNA between these elements is usually excised to produce a combination of sequences that will characterize that B cell and its progeny. During this seemingly efficient process that shuffles DNA sequences, there is also an increase in the diversity through the joining process of the elements when random sequences can be added. The rearrangement process occurs in a progressive fashion until functional heavy- and light-chain proteins are produced. Each B cell can only produce a single heavy and a single light chain, and they associate on the cell surface to form the antigen receptor.

Aged animals may not display large differences in the magnitude of the antibody response when compared with that of young animals (i.e., the anti-PC antibody response) at the total antibody level, but the affinity of these antibodies is usually lower than that of the young (22, 23, 28, 29). When the antibody genes are studied in specific immune responses generated in young and

aged animals, different sets of genes appear to dominate in the response. For example, a gene of the *S107* gene family for the heavy chain and V_κ 22 for the light chain predominates in the anti-PC response in young mice (69). When the genes are studied in the anti-PC response in aged mice, other V_H genes are also utilized (30). In a second study of the anti-TNP response, a similar difference in the V_H genes used was seen when the responses of young and aged mice were compared (31). Is this change in predominant antibody response due to an intrinsic difference in the rearrangement of the genes in the aged animals? There is a precedence for such a skewing of variable region gene rearrangement frequency in early development. When V_H genes are studied in B cells obtained from fetal sources, there is a preferential rearrangement of those segments closest to those with which they can rearrange. This preference in V_H rearrangement frequency has been demonstrated in Abelson virus transformed cell lines (70), hybridomas, and in total RNA prepared from fetal B cells (70–72). However, in the young adult, when the rearrangement frequency is measured in splenocytes using several different approaches, V_H gene rearrangement frequency is essentially random (73–75). The only study of splenic B cells in aged animals analyzed the total antibody repertoire available. This polyclonal activation was accomplished by mitogenic stimulation of B cells and resulted in a nearly random rearrangement of V_H gene segments (76). This study does not support an intrinsic change in B cell rearrangement frequency for aged mice, but rather implicates a change in regulatory mechanisms.

The antibody repertoire in the adult is continually being affected by newly arising B cells that emigrate from the self-renewing stem cell population in the bone marrow. When V_H gene analysis is performed on bone marrow-derived cells, two different sets of results have been obtained. When total RNA was analyzed from newly arising B cells in the bone marrow, one group demonstrated a skewing in V_H gene expression similar to that described in fetal RNA (72). A second study, which analyzed gene expression at the individual B cell level, described random V_H gene expression of the bone marrow-derived cells (75). Our work, which utilizes a B cell colony assay to determine V_H expression levels and hybridomas from aged mice, supports random V_H gene rearrangement in bone marrow B cells in both young and aged mice (76). The problem with all of these studies is that each determines the frequency of expression for individual V_H gene families, and subtle changes could easily be overlooked due to the variability within the methods of analysis.

Conclusion

Despite considerable experimental effort, it is not possible to state unequivocally that with aging there occur some intrinsic changes in B cells. We have some indication that the number of pre-B cells may be affected in the bone marrow of the aged. If these changes extend to a decrease of the actual number of pluripotent stem cells, then this would indeed contribute to a lack of self-renewing capacity and affect the total peripheral B cell population. In those instances when actual precursor cell frequencies have been enumerated, even though for the majority of antigen systems studied B cell precursor frequency decreases in the aged, the responding B cells showed no individual functional decline (i.e., amount of antibody synthesized). Evidence has been obtained that changes in regulatory mechanisms are responsible, in part, for the decline in the immune response of the aged. This is particularly obvious when V_H gene usage in antibody is compared between the total available repertoire (i.e., following B cell polyclonal activation) and that seen after antigen stimulation. The T cell compartment plays an important role in the expression of the antibody repertoire in both the young and the aged (77). Thymic involution, decrease in T cell function, and changes in T cell receptor repertoire may lead to altered T cell regulation as the animal senesces.

We have presented evidence that in aging there is a decrease in the magnitude of antibody responses to both thymic-independent and TD antigens. There is also a decline in the affinity of antibody produced to TD antigen. An age-related change in antibody repertoire has been documented at both the serologic and molecular levels. Changes in regulatory mechanisms involving T cells and the immune network have also been described. Along with all of these changes, an intrinsic alteration in B cells may also occur in senescence. Both intrinsic and regulatory changes may contribute to the immune response as seen in the aged.

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