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Quantitative and qualitative changes in the immune system during ageing

Introduction

It has been extensively reported that many immune functions decrease with advancing age after reaching a peak in young-adult life. This decline or even failure of the immune system has been associated with a number of diseases, which are known to have an increased incidence in old age. Infections, autoimmune diseases, immune complex diseases, amyloidosis and cancer have been mentioned in this respect. Elucidation of the mechanism(s) responsible for the declining immuno-competence may eventually lead to methods of delaying, lessening, or possibly preventing some diseases of the elderly.

An introduction to the normal immune system and a short review on the ageing immune system with emphasis on the humoral immune compartment will be presented in this paper. Age-related alterations in cell-mediated immune functions will be dealt with by the paper of Kruisbeek and Meihuizen (this volume, 1978).

The immune system and its functions

Two distinct types of specific immune reactions can be evoked when foreign material (antigen) enters the body and is recognized as such by the active cells of the immune system, the lymphocytes. The first type of reaction, the humoral immune response, is characterized by the synthesis and release of antibodies in the blood and other body fluids. These antibodies act by direct combination with the antigen. In the other type of reaction, the cell-mediated immune response, lymphocytes are the effector cells. This response is sometimes accompanied by the production of locally acting soluble mediators (lymphokines). Lymphocytes arise from the haemopoietic stem cells which in the adult reside mainly in the bone marrow. The stem cell is a pluripotent cell which gives rise to both new stem cells and differentiated cells of the erythrocytic, myelocytic, lymphocytic and megakaryocytic lines.

Antibody forming cells arise from the bone marrow-derived lymphocytes or B cells. In birds, B lymphocytes differentiate from primitive progenitors in the Bursa of Fabricius, which provides a unique inductive stimulus, just as the thymus in both birds and mammals provides a stimulus for the differentiation of another population of lymphocytes, the T cells. A bursa equivalent has not been identified in mammals. Two proposed sites for the origin of B cells are the bone marrow and the gut-associated lymphoid tissue. Extensive evidence has been obtained that, in most cases, T cells are required for B cells to produce an optimal antibody response. Both cell types recognize the antigen, but only the B cells give rise to antibody forming lymphocytes and plasma cells. T cells are considered to act as helper cells. Accessory cells, such as macrophages, dendritic cells and granulocytes are also involved in a humoral immune response. These latter cell types facilitate the response in a nonspecific manner.

Not all antigens require the participation of T cells in order to induce an antibody response. Antigens with a very large number of identical repeating antigenic determinants (such as polysaccharides) can by-pass the helper T cells and stimulate B cells directly. An important observation was that most of these thymus-independent antigens trigger antibody formation of a specific immunoglobulin class, namely IgM. From other studies, it was also concluded that B cells committed to IgM synthesis require a lesser degree of interaction with helper T cells than do B cells committed to IgG synthesis (Van Muiswinkel and Van Soest, 1975).

Over the past years, a vast body of evidence has accumulated which indicates that negative interactions exerted by the so-called suppressor T cells are of equal importance in immunoregulation as are the helper T cells (reviewed by Gershon, 1974).

A major characteristic of the immune system is its diversity. An extremely large repertoire of immunoglobulins exists in the individual as a result of all possible combinations among the constant and the variable parts of the immunoglobulin molecules. This number more or less reflects the number of B lymphocyte clones, since an antibody forming cell produces only antibodies with a single specificity. Immunoglobulins with this specificity are present on the cell surface of B cells and act as receptors for the antige-

nic determinants. T cells also display antigenic specificity, although the precise nature of the antigen receptor on T cells is still controversial.
In addition to their helper and suppressor functions in a humoral immune response, T cells are responsible for cell-mediated immunity. This type of response is important in host defense against many viruses, fungi and mycobacteria. Delayed-type hypersensitivity, graft rejection, graft-versus-host reaction and anti-tumour immunity are also examples of cell-mediated immunity.

Ageing and the immune system

The initial studies on the effect of ageing on immunocompetence concerned humoral immune functions. For instance, the level of natural serum antibodies in man of several age groups was determined by measuring antibody titres against blood group antigen B (Thomsen and Kettel, 1929) and against sheep red blood cells (SRBC) (Friedberger, Bock and Fürstenheim, 1929). A gradual decrease of the various titres with advancing age was found. Natural antibody is the term used for antibody detectable in serum without prior immunization with the corresponding antigen. Assuming that the stimulatory factors responsible for the occurence of natural antibodies are, in fact, antigens present in the environment, the decrease in natural antibody titres reflects a decrease in the activity of humoral immune system in man. The pattern of the age-related decrease in natural antibody titres in man resembles the pattern found when antibody titres to SRBC were measured in mice following specific immunization at various ages (Makinodan et al., 1971a). Whether the decrease in serum antobody levels could be attributed to a decrease in the number of cells producing antibodies has been investigated by Makinodan et al. (1971a), employing the plaque forming cell (PFC) technique (Jerne and Nordin, 1963). They reported that the number of PFC in the spleen of 2.5-year-old mice is about 10% of that in young adult mice. Subsequent studies indicated that 10% of the decline can be attributed to changes in the cellular milieu, while changes intrinsic in the old cells did account for about 90% of the decline (Price and Makinodan, 1972a and b).
Before presenting more recent data on changes in humoral immune responsiveness, firstly the effect of ageing on the stem cells, the thymus and their ultimate descendants, the B and T cells, will be discussed.

a. Stem cells during ageing

It has been investigated whether the impaired immunocompetence with advancing age can be attributed to changes in the stem cell population. These studies revealed that the total number of stem cells in murine bone marrow remains rather constant throughout the life-span (Harrison, 1975; Tyan, 1977). In addition, Toya and Davis (1973) have demonstrated that stem cells remain pluripotent during ageing, since no change in the differentiation capacity was found.

b. Thymus during ageing

The principal change seen in the thymus with advancing age is atrophy of the lymphoid tissue, chiefly in the cortex, and an increase in the number of macrophages and plasma cells (Hirokawa, 1977). It is accompanied by a decrease in volume and weight of the whole organ. Hirokawa and Makinodan (1975) reported that the ageing thymus loose the capacity to generate functional T cells when transplanted in young thymectomized mice. Although this loss is not reflected in changes in the overall number of T cells (see below), a clear decline in immune functions attributed to T cells strongly suggests that the involution of the thymus plays a central role in the phenomena related to ageing of the immune system. In accompanying papers in this volume (Kruisbeek and Meihuizen; Wijermans and Astaldi), the possible causes for the decline in thymus function and consequently in cell-mediated immune responses will be discussed.

c. T and B cells during ageing

A question to be resolved is whether the depressed humoral immune response during ageing can be

explained by a quantitative rather than a qualitative defect in immunocompetent cells. The effect of age on the number of T cells, as judged by the presence of the Thy 1 antigen on the cell membrane, in the spleen of mice has been studied by several investigators (see the review by Kay and Makinodan, 1976). In most long-lived, nonautoimmune mouse strains, the number of T cells remains constant with age. With regard to the B cells, either a constant number or a moderate increase have been reported for the various lymphoid organs of several strains of mice (see Benner and Haaijman, this volume). B cells are characterized by the presence of membrane immunoglobulins. It may be concluded from these data that no dramatic, quantitative changes occur in the size of the T end B cell compartments during ageing in long-lived mouse strains. The above results were obtained by using antisera against markers expressed on nearly all B and T cells. It cannot be excluded, however, that changes in the ratio of responsive and nonresponsive cells do occur.

d. Humoral immune response during ageing

In our studies regarding changes occuring wi ge in the capacity to mount a humoral immune response *in vivo*, the number of PFC (antibody forming cells) after immunization with antigens was determined.

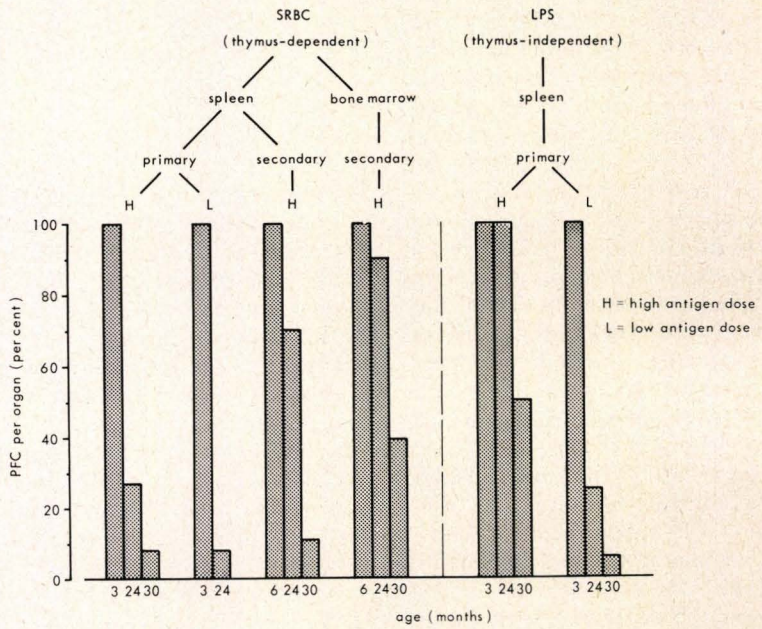

Fig. 1. Summary of the age-related changes in the humoral immune response of ageing CBA mice after immunization with the thymusdependent antigen SRBC (sheep red blood cells) and the thymusindependent antigen LPS (lipopolysaccharide of *E. coli*).
The number of IgM antibody forming cells (= plaque forming cells according to the method of Jerne and Nordin, 1963) is expressed in per cent of the response of 3- and 6-month-old mice respectively, which is taken as 100%. The primary anti-SRBC and anti-LPS response in the spleen was determined on the peak day (day 4) after immunization. Responses to both high (H) antigen doses (4×10^8 SRBC, 10 μg LPS) and low (L) doses (10^6 SRBC, 0,01 μg LPS) were compared. The secondary response to SRBC was determined on day 4 and day 6 after the booster injection in the spleen and bone marrow, respectivelely. For the secondary response, the mice received a booster injection of 4×10^8 SRBC 3 months after priming with 10^7 SRBC. The antigens were given intravenously. The figure is based on the average results obtained in 2-4 separate experiments. In each experiment, 5-10 mice were used per age-group.

Male CBA mice were used. The 90%, 50%, 10% and maximum survival ages of male CBA mice in our colony are 15, 29, 33 and 34 months, respectively. SRBC were employed as a thymus-dependent antigen. The use of lipopolysaccharide of E.coli (LPS) made it possible to study a humoral response exerted by B cells only (without the influence of helper or suppressor T cells). In addition to the primary immune response in the spleen to an optimal and low doses of both antigens, age-related alterations in a secondary response to SRBC in the spleen as well as in the bone marrow have been investigated. The results are summarized in Fig. 1 where the responsiveness of approximately 2- and 2.5-year-old CBA mice is expressed in per cent of the response of 3- and 6-month-old mice, respectively. The age-related changes observed in the above mentioned responses can be summarized as follows (see Fig. 1):

1. The primary response to an optimal dose of SRBC declines faster with age than the primary response to an optimal dose of LPS. Two-year-old mice still exhibit the same responsiveness to LPS as do young animals. A decrease of the anti-LPS response was observed only in 2.5-year-old mice.
2. The difference in responsiveness between young and aged mice becomes larger when a suboptimal dose of antigen is used. This applies for the anti-SRBC as well as for the anti-LPS response.
3. The primary response to SRBC in the spleen decreases earlier in life than does the secondary response to this antigen.
4. The bone marrow, which constitutes an important antibody forming organ during a secondary response to SRBC (Benner and Haaijman, this volume), shows a declining capacity to develop PFC in 2.5-year-old mice, whole the response in 2-year-old mice is still comparable to that of 6-month-old mice.

Fig. 1 only shows changes in the number of cells forming antibodies of the IgM class. In the same series of experiments, it was found (data not shown) that the generation of cells forming antibodies of the IgG class after a primary as well as a secondary stimulation with SRBC is more severely affected by age than is the generation of IgM-PFC. Some of the 2- and 2.5-year old mice failed to develop any IgG-PFC. It should be realized in this respect, that the IgG response requires more help from T cells than does the IgM response.

The afore mentioned results indicate that the capacity to mount an effective immune response towards a new antigenic challenge declines during ageing, especially when T cells are involved (e.g., anti-SRBC response and IgG antibody formation). The responsiveness to low doses of antigen, thymus-dependent as well as thymus-independent, decreases faster than that to optimal doses of antigen. A secondary response is less affected by age than is a primary response. The latter confirms the results reported by Makinodan et al. (1971b) and Haaijman and Hijmans (1978). Literature data indicate differences in the ageing behaviour of a thymus-independent humoral immune response in various strains of mice (see e.g. Blankwater, Levert and Hijmans, 1975; Mason Smith, 1976; Zharhary, Segev and Gershon, 1977; Friedman and Globerson, 1978). Nevertheless, in comparison with thymus-dependent humoral immune responses, the functional capacity of B cells seems to decline less rapidly with age in most strains studied (see also Segre and Segre, 1977).

Since macrophages, the third cell type involved in humoral immune reactions, show only minimal changes in mice with age (Kay and Makinodan, 1976), functional changes in the T cell population must play an important role in the impaired humoral immune responsiveness before changes in the B cell compartment occur. As discussed above, T cells, if involved, exert their influence on humoral immune reactions in two ways: as helper or as suppressor cells. Therefore, the decline can be interpreted as less helper activity and/or increased suppressor activity. Evidence for both possibilities has been obtained. Using a hapten-carrier system, Krogsrud and Perkins (1977) demonstrated that carrier-primed helper T cells show an earlier decline during ageing in their ability to initiate a response than do hapten-primed B cells. Studies on suppressor cell activity in mice suggested an increase during ageing (see e.g. Segre and Segre, 1977). To what extend both processes contribute to the decline remains to be investigated.

So far, changes in the humoral immune system as determined after a specified antigenic challenge have been discussed. Another approach can, however, be envisaged for a study of the humoral immune system. This approach consists of the determination of the levels of immunoglobulins (Ig) in the serum and/or the number of Ig-containing cells (C-Ig cells) in the various lymphoid organs, irrespective of the antigens to which these Ig's are directed. These parameters can be considered to be a reflection of all ongoing humoral immune responses. The results of such a study revealed that the total numbers of C-Ig cells and the overall Ig levels do not decrease with age (Benner and Haaijman, this volume). The discrepancy with the observation of an age-related decline in immune responsiveness after antigenic stimulation may be explained by the fact that the Ig levels and number of C-Ig cells result mainly from secondary and later immune reactions, which decline less rapidly with age. It can be assumed that primary responses, the most affected type of response, will represent only a small part of all ongoing responses, since fewer new environmental antigens are encountered at old age.

Although the overall Ig levels in the serum was not altered during ageing, some findings point to changes in the quality of the Ig's, i.e. in the extent of the antibody repertoire. This conclusion follows from the observations of an increasing variability in IgM and in the subclasses of IgG, an imbalance in the / ratio and of a restricted heterogeneity of the Ig's expressed ad maximum in the appearance of idiopathic paraproteins in the serum of old humans (Radl et al., 1975). Likewise, all mouse strains investigated so far, most notably C57BL/Ka show an increased tendency to produce homogeneous Ig with advancing age (Radl and Hollander, 1974). Preliminary results (Van Camp and Radl, this volume) indicate that the heterogeneity of the Ig is regulated by the thymus, since thymectomized mice display an earlier loss of this heterogeneity than do control mice.

The data presented so far lead to the conclusion that qualitative rather than quantitative changes occur in the humoral immune system with advancing age. Changes in the T cell compartment are most prominent, although a decrease in B cell activity certainly plays an additional role later in life.

The involvement of T cells as helper, suppressor and effector cells in many immune reactions makes it extremely important to study in detail how ageing affects the maturation of stem cells into these various types of T cells. Elucidation of this process must await more basic immunological studies on the various steps leadings to T cell maturation.

Summary

A review on the present knowledge of the effect of age on the humoral immune response of mice is presented. It is shown that the capacity to mount an antibody response decreases with age, especially when the immune system is challenged for the first time by low doses of antigen, and when T cells are required for the response. Literature data are presented indicating that the decline is not accompanied by quantitative changes in the number of stem cells or the overall number of B and T cells. The impaired immunocompetence may rather be attributed to alterations in the helper and suppressor T cell functions with age in addition to changes in B cell functions later in life.

Samenvatting

Een overzicht wordt gegeven van het effect van veroudering op de humorale immuunrespons van muizen. De daling in humorale immuunfuncties is het grootst wanneer het immuunsysteem voor de eerste maal moet reageren op suboptimale hoeveelheden antigeen en wanneer T cellen betrokken zijn bij de respons. De achteruitgang kan niet worden toegeschreven aan kwantitatieve veranderingen in de stamcellen en in het aantal B en T lymfocyten. Vermoedelijn functionele veranderingen in de helper- en suppressor T cel populaties en later in de B cel populatie verantwoordelijk voor het verlies van immunologische capaciteit.

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