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Ageing, defence mechanisms and the immune system

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Introduction

Of all the systems in the body, the immune system is probably the best understood, both in mechanistic terms and in the ways in which it changes during ageing. Studies of the ageing immune system (immunogerontology) can be traced back to the early part of this century (and even earlier), and the literature has burgeoned in the last three decades. Perhaps the first publication was of the investigation undertaken by Peter Ludwig Panum (a Danish physician and discoverer of endotoxin) on the outbreak of measles in the Faroes in 1846. The Faroes had been free of measles since the previous epidemic of 1781. The 1846 outbreak affected 75–95% of the population although "of the many aged people still living on the Faroes who had had measles in 1781, not one was attacked a second time" [1]. This experiment of nature seems out of step with traditional thought about ageing and immunity, which views ageing as an immunodeficiency state that predisposes the host to infectious diseases (and possibly neoplasms).

The nature of immunity

Two interactive immune systems are recognized: innate (natural) immunity and acquired (adoptive or specific) immunity. Innate immunity comprises polymorphonuclear leukocytes, natural killer cells and mononuclear phagocytes and uses the complement cascade as its main soluble protein effector mechanism. It also utilizes numerous recognition molecules, including C-reactive protein, serum amyloid protein and mannose-binding protein. These and other molecules have been selected during evolution to bind carbohydrate structures that do not occur on eukaryotic cells and thus differentiate potentially harmful invaders from innocuous self.

Acquired immunity employs several sub-types of lymphocytes and utilizes antibody as its effector protein. Antibody and the T cell receptor are the recognition molecules that are able to recognize up to 1011 distinct antigenic structures. B lymphocytes can recognize protein, carbohydrate and simple chemical structures, whereas T lymphocytes appear to recognize only peptides. Clones of lymphocytes with receptors of sufficient affinity are triggered by antigen to proliferate and differentiate into various effector cells: T helper cells, cytotoxic T cells, suppressor T cells and plasma cells.

After a systemic immune response subsides, specific antibody often persists in the blood for many years and even decades. This implies that humoral effector cells (plasma cells) continue to secrete antibody. In contrast, the antibody response at mucosal surfaces is short-lived (a few months to a year). Effector T cells do not persist long, but antigen-specific clones remain expanded as memory lymphocytes which can differentiate into effector cells whenever the antigen is encountered. In general, memory responses are most effective in protecting against systemic infections (like the measles described above). Localized infections at mucosal surfaces can recur before memory lymphocytes can differentiate into effector cells, although subsequent episodes of disease are usually less severe.

The flexible nature of specific immunity poses the problem of differentiating innocuous antigens from harmful ones, a problem that innate immunity does not have. One way to overcome this problem is to delete potentially self-reactive clones during maturation of the cells (in the thymus for T cells and at an as yet unknown site for B cells). Further specificity is ensured by interactions with innate immunity. For T cells, antigen presentation is the critical step. Uptake of antigen into antigen-presenting cells (e.g. mononuclear phagocytes, dendritic cells) is determined by the presence of the carbohydrate moieties that are recognized by innate immunity. For B cells, the interaction with innate immunity seems to be their membrane receptors for the C3d component of complement which is found in association with CD19. CD19 is needed for antibody production with T cell-dependent antigens and amplifies signalling after the antigen binds membrane immunoglobulin. This process is facilitated by covalent binding of C3d to microbial carbohydrate structures.

The aged immune response

The bone marrow

All haemopoietic cells derive from a common pluripotent stem cell in the bone marrow which continues to supply precursor cells for the immune systems throughout life. Bone marrow stem cells seem to be little affected during ageing [2]. Although there is normal responsiveness to early multilineage regulators such as interleukin (IL)-3 and GM-CSF and normal numbers of myeloid progenitor cells, there is evidence of decreased responsiveness to the late-acting unilineage stimulator for the differentiation of neutrophils, G-CSF [3]. This implies that granulocyte production in an unstressed state may be unaffected by ageing but that the aged bone marrow may fail to meet demand when sufficiently stressed.

After precursor cells migrate from the bone marrow, altered immune/inflammatory responses might reflect intrinsic changes in the cells or altered regulatory influences in peripheral microenvironments. At present, it is not possible to generalize about the relative contributions from these two possibilities. However, it is clear that bone marrow from aged mice, while not identical to young bone marrow [4, 5], retains the ability to respond almost normally when transplanted into a young bone marrow microenvironment [6, 7]. Similarly, when young bone marrow repopulates an aged recipient, responses resemble those of old animals.

Acquired immunity

The dominant change in acquired immunity is involution of the thymus gland. The impaired T cell functions, so characteristic of old age in several mammalian species, are often attributed to thymic involution. There is certainly reduced proliferation of T cells in response to various stimuli, as well as reduced T helper cell activity with consequent impairment of both cellular and humoral responses. These changes are generally attributed to impaired production of the cytokine IL-2 by T helper cells as well as reduced responsiveness to this cytokine. The reduced production of IL-2 seems to result from a large number of cells failing to increase the intracellular calcium concentration in response to appropriate stimuli (also observed in neutrophils and parotid acinar cells), but with a substantial population which respond normally [8]. The altered responsiveness to IL-2 is associated with both reduced receptor number and affinity [9–12].

Age-related changes in B lymphocyte responses are altogether more subtle, in that responses to foreign antigens decline but responses to self antigens increase. Thus, antibody responses to pathogens and vaccines tend to be attenuated in the old, although influenza vaccine still confers considerable protection. Circulating autoantibodies and benign monoclonal gammopathies are common in most aged mammals, although the monoclonal gammopathies are rare in old people in Japan [13]. Like T cells, B cells appear to exist as discrete sub-populations. B1 lymphocytes seem to be important in the formation of autoantibodies and increase in number in old mice. Interestingly, chronic lymphocytic leukaemia, a neoplasm of old age, is a B1 neoplasm.

Innate immunity

The natural killer cell compartment enlarges in old age and these cells are functionally active [14]. Interestingly, both old and young people have greater numbers of natural killer cells than middle-aged people [15]. There have been few studies of complement activity in old age but they have shown no significant decline; occasional studies have even shown an increase. Circulating granulocyte numbers are maintained but there is controversy about whether ageing affects their functional activities. Malnutrition [16], co-morbid conditions and drugs [17] probably explain the most significant impairments described.

Ageing as a pro-inflammatory state

Old rats are exquisitely sensitive to the toxicity of bacterial endotoxins [18] and die after the systemic administration of doses that do not kill young control animals. Detailed light- and electronmicroscopic studies have shown that these old animals develop lung [19] and liver [20] injury very soon after endotoxin administration and that the tissue damage is associated with a very marked inflammatory infiltrate. Others have made similar observations in aged mice [21]. Similar data would be very difficult to obtain in humans, and much of our evidence is indirect. Perhaps the most convincing of this evidence is the clear age-related vulnerability of the severely ill and injured elderly to develop the systemic inflammatory response syndrome and multi-organ failure [22, 23]. The only direct evidence of which we are aware comes from our own studies on experimental wound healing in man.

Wound healing: an *in vivo* model of inflammation

The effects of human ageing on cutaneous wound healing are poorly understood and many studies must be criticized because of a lack of subject (and wound) characterization. Most studies describe cutaneous wound healing, which comprises a number of overlapping phases. The first of these is inflammation. The inflammatory response is an obligatory sequel to cutaneous injury in order to re-establish cutaneous homeostasis. When endothelial integrity is disrupted, platelets aggregate and induce haemostasis. Platelets

also release a number of cytokines which facilitate inflammatory cell migration and proliferation [24]. Neutrophils and macrophages remove cellular debris by phagocytosis and macrophages and lymphocytes also produce a further cascade of cytokines which stimulate cell migration, proliferation and extracellular matrix production [24].

There are specific *in vitro* age-related changes in the coagulation and immune systems which may influence wound repair. The adherence of human granulocytes to nylon increases with age, as does the ability of aged murine peritoneal macrophages to adhere to the extracellular matrix components fibronectin and collagen I [25, 26]. In contrast to these potentially pro-inflammatory changes, other reports suggest that neutrophil chemotaxis and respiratory burst declines with age [27]. Moreover, a functional decline of macrophages in mice may contribute to impaired wound repair with age [28].

Despite these observations, the belief that ageing impairs the inflammatory response has not been tested *in vivo*. In particular, reports of delayed and less intense acute cutaneous inflammatory reactions in aged humans contradict other reports of similar or even increased numbers of inflammatory cells [29]. A further compounding factor is that, in response to cytokines released at sites of inflammation, a number of cell adhesion molecules (CAMs) are expressed on the surface of endothelial cells. The cytokine cascade may differentially regulate these endothelial CAMs and thus may selectively recruit cell types which express specific ligands for these adhesion molecules. There are at least five families of adhesion molecules including the selectin family, of which E-selectin is a member and the immunoglobulin gene superfamily, of which ICAM-1 and VCAM-1 are members [30]. The expression of specific endothelial adhesion molecules can therefore modulate the inflammatory profile early in the wound healing process. The potential for age-related differential regulation of adhesion molecules *in vivo* is unknown.

Our own studies have tested the hypothesis that an age-related delay in wound healing is associated with an altered inflammatory response and endothelial CAM profile, as CAMs influence the temporal and lineage profiles of extravasated leukocytes within a wound. Cutaneous punch biopsies were taken from 132 health-status-defined subjects, aged 19–96 years (equal numbers of men and women) and the wounds re-biopsied at fixed time-points from day 1–6 months post-wounding. Wound healing was delayed in elderly subjects (especially in aged men) in terms of re-epithelialization, basement membrane and extracellular matrix deposition. However the visual quality of scarring was superior both macroscopically and microscopically in the aged group. Quantitative image analysis showed that there was a marked early increase in the neutrophil response in older subjects and a less pronounced peak in the wounds of younger subjects. Monocyte/macrophage and lymphocyte appearances were delayed in the older group, with a peak in cell numbers at day 84, compared with day 7 for monocytes and day 21 for lymphocytes in the young group, but with increased numbers of mature macrophages in the aged group. E-selectin was strongly expressed in a perivascular distribution in the early wounds of the old group, however only faint staining was seen from day 3 to 7 in the wounds of the young group. The expression of the adhesion molecules ICAM-1 and VCAM-1 was delayed and the staining less intense.

A number of factors could account for this early age-related increase in granulocyte numbers, including an altered cell phenotype, increased circulating numbers and changes in the wound microenvironment such as the cytokine or vascular adhesion molecule profile. Neutrophils are a major source of serine proteinases and metalloproteinase-9 which degrade a variety of extracellular matrix and basement membrane molecules [31]. Of significance is our recent report of an increase in these proteases in the wounds of aged humans, associated with a delayed rate of healing [32, 33]. The increased neutrophil response in old women compared with old men has, to the best of our knowledge, never previously been reported.

The marked delay in the appearance of monocytes/macrophages in the aged may again reflect changes in the circulating cell numbers, cell phenotype or wound milieu. In addition to their role in phagocytosis, macrophages also produce a variety of cytokines important in cell migration, proliferation and extracellular matrix production. Thus the age-related delay in macrophage infiltration may well be related to the delay in cytokine appearance and extracellular matrix deposition observed, findings paralleled by those observed in an aged mice [34–36]. An early increase of 25F9 cells (a sub-population of mature macrophages which normally appear in the late stages of inflammation) in the older group indicates that, despite a reduction in overall macrophage numbers at day 7, the majority are mature cells. This difference between old and young subjects is potentially important since the state of macrophage differentiation determines the expression of certain cytokines [37] and specific monocyte sub-populations express increased protease levels [38].

The delay in lymphocyte appearance with increasing age may also contribute to an age-related delay in wound repair. A physiological role for T lymphocytes was inferred from studies showing that lympholytic agents impaired wound fibroplasia, whilst lymphotrophic agents such as IL-2 increased wound collagen and breaking strength.

The early up-regulation of E-selectin with a delay in ICAM-1 and a delay and marked decrease in VCAM-1 in older subjects is compatible with the early neutrophil and delayed monocyte/macrophage and lymphocyte infiltration. Moreover, the early infiltration of

inflammatory cells in the young could be explained by the increased expression of ICAM-1 and VCAM-1. In age-associated pathological chronic wound healing states, such as venous ulcers, adhesion markers have been reported to be differentially regulated. VCAM-1 was reportedly absent, with the appearance of a strong staining intensity for E-selectin on capillaries below the ulcer [39]. These changes are consistent with our findings in the early acute wounds of healthy aged subjects, suggesting that intrinsic ageing *per se* predisposes to a cell profile associated with chronic wound healing states and subsequent changes in the proteolytic profile which increase tissue degradation [32, 33]. The age-related changes we report may suggest means of therapeutic modulation of the inflammatory response by manipulating the adhesion marker profile and thereby modifying the process of wound repair in aged humans.

Conclusion

The cells and tissues of the immune system can be affected in many ways during ageing. There are undoubtedly changes in the cells themselves, but all cells in a population are not necessarily affected. Studies on an unfractionated population of cells might well reveal an age effect, although the correct nature of the effect could be overlooked. For example, ageing is associated with reduced production of IL-2, but a substantial population of cells persists with normal responsiveness.

Most age effects are generally rather modest and do not compromise the organism, at least in a basal state. However, the capacity to withstand stressors is reduced, particularly when complicated by malnutrition and co-morbidity. Moreover, the way in which cells behave is highly dependent on the microenvironment in which they find themselves. Thus, cells such as neutrophils, even if functionally compromised, could well induce serious tissue damage if attracted there in large enough numbers. Sacher could well have been right when he speculated on the systemic aspects of ageing being more fundamental than the molecular.

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