

Commentary

Immunosenescence: impact in the young as well as the old?

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1. Introduction

Immunosenescence is that state of dysregulated immune function contributing to the increased susceptibility of the elderly to infectious disease, and possibly autoimmunity and cancer. Because the adaptive immune system relies upon waves of clonal expansion and contraction of T lymphocytes, ageing processes may be doubly critical for T cells. That is, firstly, the phenomenon of replicative senescence may impact upon the ability of T cells to repeatedly undergo clonal expansion (this is independent of the age of the host); and, secondly, processes associated with the advancing age of the host (specific to the immune system, e.g. thymus involution, or generally associated with ageing, e.g. hormonal changes) may have deleterious effects. The present communication arose as a result of discussions and presentations at a recent meeting¹ which brought together many investigators from different disciplines to consider the impact of immunosenescence on disease in the elderly. In addition to deliberations on infectious diseases and vaccination in old age, autoimmunity and cancer, attention was also focussed on the impact of immune dysregulation on age-associated diseases such as Alzheimer's and atherosclerosis. This

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¹ Immunosenescence—a major cause of disease in the elderly: from molecules to patients, 3–5 December, Kühtai, Austria organized by Georg Wick and Beatrix Grubeck-Loebenstein.

commentary does not pretend to do more than briefly summarize these latter areas of discussion, nor the intensive discussions concerning the possibilities for collaborative funding in the context of the European Union Fifth Framework Program for Research and Development (1999–2002). An overall summary by the meeting organizers covering the scientific and financial aspects of the research discussed at the conference will appear in the journal *Experimental Gerontology*.

2. The innate immune system

The meeting addressed basic aspects of immunosenescence related to T cells, B cells and innate immunity. The adaptive immune system is dependent upon the functional integrity of the innate immune system, without which antigen presenting cells cannot be primed to present antigen in a stimulatory form to T cells. Humoural immunity is in turn dependent upon intact T cell responsiveness for B cell generation, differentiation and antibody production. In addition to its crucial role in adaptive immunity, the innate immune system is in its own right an effector system forming a first line of defense against pathogens prior to the development of the delayed adaptive response. Age-associated alterations in the human natural killer (NK) system were reviewed by R. Solana (Córdoba), who showed that although the numbers of NK cells increase in the elderly, their functional activity on a per cell basis decreases. These cells become less effective at lysing their target cells and have a decreased response to IL 2 stimulation. However, because of the increased numbers of NK cells, total lytic activity is probably not markedly changed in the elderly, perhaps emphasising the importance to survival of maintaining adequate NK activity. Indeed, one recent report has suggested that in a longitudinal study, the critical immune system factor associated with resistance to infectious disease and hence longevity in the elderly was NK function [Otaga et al., 1997. *Clin. Immunol. Immunopathol.* 84, 269]. Other speakers discussed mechanisms of inflammation in age-associated degenerative diseases, such as Alzheimer's (P. Eikelenboom, Amsterdam; P. McGeer, Vancouver) and atherosclerosis (P. Libby, Boston; G. Wick, Innsbruck), as well as rheumatoid arthritis (H. Moutsopoulos, Athens; R. Maini, London), and argued that, once triggered, the activity of the innate immune system may contribute to disease pathology so that these diseases might even be considered to a large extent immunopathologically determined. P. Morgan (Cardiff) pointed out that the innate immune system may also be of critical importance in cancer and cancer therapy. Tumours may resist complement attack by upregulating CD59, an inhibitor of the complement attack complex. Because the complement system is also dysregulated with age, this could contribute to increased cancer incidence in the elderly.

3. The interface between innate and adaptive immunity

In order to activate T cells, antigen presenting cells (APC) must be present and functional in the elderly. Dendritic cells (DC) are specialised in this function. Studies

of various aspects of DC function have suggested that they are very similar in the young and in the old (B. Grubeck-Loebenstein, Innsbruck). She showed that the kinetic of DC differentiation *in vitro* and the up and down regulation of DC surface markers was identical using monocytes from the blood of young or elderly donors. Long-term cultured DC (3 weeks) from the young or old were equally competent to stimulate T cells. Both young and old donors' DC responded better to exposure to whole influenza virus than to virus components. However, subtle differences were discernable in that DC from the elderly were slightly less able to stimulate T cell secretion of IFN- γ and very slightly less able to stimulate IL 2 secretion compared with DC derived from young donors. One possible alteration in DC with age might concern antigen processing mechanisms at the level of the proteasome, as discussed by H.-J. Schild (Tübingen) using yeast mutants to analyse proteasome subunit specificity in peptide generation. These basic concepts applied to studies of ageing may illustrate hitherto undetected differences at the molecular level.

4. The adaptive immune system

Given an essentially well-maintained ability to present antigen in the elderly, at least as far as most parameters of DC function are concerned, the next stage at which immune responses in the elderly might be compromised is at the level of the T cell. Each individual possesses a small number of T cells specific for a particular antigenic epitope, and these cells must undergo extensive clonal expansion in order to generate sufficient cells for a successful immune response. Using sensitive molecular methods to detect T cells with identical specificities (using TCR heteroduplex V-chain analysis with a sensitivity of detection of at least one cell in 10 000), P. Beverley (Compton) analysed T cell responses to EBV in acute infectious mononucleosis (AIM). It was found that a relatively small number of different clones, perhaps 50, was activated in acute phase disease, but these were present in very large numbers, estimated to be at least 10^8 , or more likely, 10^9 , cells per clone. This implies that at this time, each clone must have undergone something like 28–30 population doublings (PD). AIM patients at follow-up were found to retain some but not all of these clonal expansions; after one month, about half the clones had disappeared, but of those remaining, some could still be found up to one year later. The latter were shown to be antigen-specific by stimulation *in vitro* with autologous B-LCL. The CD8+ clones among these populations were found not only in the CD45RO (activated/memory cell population) but also in the CD45RA (resting/naive cell population) subsets; moreover, some RA and RO cells possessed the same TCR. However, the CD4 cell expansions were only found in the CD45RO+ populations. Intriguingly, telomere lengths in the clonally expanded CD8 cells were not reduced and the presence of telomerase was demonstrated in the cells at a high level (ca. 70% of that in the control 293 cells). In CD4+ clones, telomerase-positivity was also high, at around 50% of control level. These results suggest that long-lived clones *in vivo* are responsible for maintaining immunity to

specific antigen, and that activation of telomerase in these cells may enable them to maintain telomere lengths and avoid replicative senescence. Those cells not expressing telomerase may be the ones scheduled for replicative senescence and elimination by clonal deletion (accounting for the loss of half the clones during the first month of AIM, and the gradual loss of clones thereafter).

The question of the longevity of T cell clones (TCC) in culture, and whether TCC may constitute informative models for T cell ageing *in vivo*, was addressed by G. Pawelec (Tübingen). He showed that TCC derived from normal peripheral CD3 + T cells from young healthy donors had a wide range of life expectancies. Of those clones which could be established *in vitro* (requiring an estimated 10 PD, equivalent to ca. 10^3 cells), the average longevity in many different experiments with different donors was around 33 PD. However, of the total starting population, many of which could not be established as clones, the estimated mean longevity was only 17 PD. Nonetheless, the longest lived clones under these conditions achieved 170 PD. At the end of their very variable lifespans, TCC were deleted by apoptosis, but prior to their demise they had already altered their functional phenotype and surface markers. They progressively downregulated costimulatory receptors such as CD28, CD134 (OX-40) and CD154 (CD40-ligand), upregulated the negative costimulatory receptor CD152 (CTLA-4), greatly decreased IL 2 production, but increased IL 10 production whilst maintaining IFN-secretion at the same level. These findings *in vitro* parallel quite accurately what is known about alterations to T cell phenotype and function *in vivo*. Preliminary data from a European Union-supported collaboration, EUCAMBIS (see www.medizin.uni-tuebingen.de/eucambis/) suggest that one possible explanation for the variable lifespans of the TCC is related to levels of DNA damage and repair. In a small number of TCC, the accumulation of a critical level of DNA damage was associated with proliferative arrest, paralleled by upregulation of mRNA for the mitotic inhibitors p16-INK4a, p21-WAF1 and p27-kip2. In addition, it may be informative to assess the expression of the alternative p16 gene product, p14-ARF, because this also seems to play an important role in senescence, at least in keratinocytes and fibroblasts (P. Janssen-Dürr, Innsbruck). If the above findings can be confirmed on a larger series of clones, these results strongly support the damage accumulation/catastrophe hypothesis for replicative senescence of T cells. Moreover, cross-sectional *ex-vivo* studies performed in the context of the previous EU collaborative project EUCAMBIS, headed by G. Pawelec, showed that basal DNA damage and mutation frequency (of the *hprt* locus) in the young group and very old group were lower than in the middle-aged group. Reciprocally, DNA repair capacity was better in the young and very old than in the middle-aged (as shown by Y. Barnett (Coleraine), who was not present at the meeting). This suggests selective pressures eliminating those individuals with higher levels of DNA damage and mutations (unrepaired DNA damage). Intriguingly, in further studies together with M. Rea (Belfast), she found that in near-centenarians, the mutation frequency was again increased, suggesting that this population represents those whose DNA repair capacity first begins to decrease 30 or so years later than the average population. G. Pawelec argued that these *ex-vivo* results parallel very closely what happens to human TCC

in culture and that information concerning clonal heterogeneity and manipulation of functional clonal longevity *in vitro* may indeed be useful for the understanding and manipulation of the process *in vivo*.

A careful consideration of immunological parameters *ex vivo* in the elderly, particularly concentrating on centenarians as examples of successful ageing, was given by C. Franceschi (Bologna). Nonetheless, and possibly in parallel with the results mentioned above, it may be that centenarians have already progressed beyond the point at which they can resist selective pressures (which contributed to their extended survival), and by the time they are studied at 100 + years, their risk factors are already reverting again to those of a middle-aged population. A prediction from this would be that centenarians should again begin to appear like a middle-aged group, in this case, in terms of their immunological parameters, whereas the very old but not yet centenarian group should be different (still resistant). This has not yet been consistently tested, but it is known that many of the immunological parameters (biomarkers; risk factors) of centenarians are shared by the younger old, but not the young. Thus, Franceschi summarized that centenarians also possess increased numbers of CD28-negative T cells and of CD95 (fas) + T cells, telomere lengths in T cells are decreased, IL 3 and GM-CSF secretion is decreased, but IL 6 and SCF increased, and in general there are markers of a pro-inflammatory state. However, there are also still some notable differences, for example in the resistance of mitochondria to depolarization. Here, mitochondria from centenarians are more resistant than from the old, and even more resistant than those from the young, and the cells are more resistant to apoptosis.

How far these types of age-associated parameters impact on the humoral immune system was discussed by M. Weksler (New York). The age-associated decrease in antibody affinity and the increased production of autoantibody suggest dysregulation in the B cell compartment. The important finding that the pattern of autoantibody production is quantitatively but not qualitatively altered in aged individuals may suggest persistence of the B cell clones also present in the young, but their escape from regulation in the old. Moreover, generation of naive B cells in the old is decreased in parallel with decreased RAG enzyme expression, the induction of which is T cell-dependent. Thus, *in vitro* administration of a T cell factor secreted by CD8 but not CD4 cells induced RAG gene expression by bone marrow B cell precursors from nude or old mice. Incubation of the T cell factor with anti-IL 16 antibody markedly decreased its capacity to induce RAG gene expression in nude or old mice *in vivo*. Furthermore, the administration of rIL 16 to nude or old mice induced RAG gene expression in their bone marrow B cell precursors. Therefore, age-associated perturbations of CD8 cell function, which if anything are more extreme even than those of CD4 cells, would be expected to have an impact on the B cell system.

5. Clinical implications

What are the clinical consequences of immunosenescence? Much of the remainder of the meeting concentrated on problems of vaccination of the elderly, whether they

responded more poorly than the young, and what could be done to improve their responses. An overview of influenza vaccination in the elderly was provided by E. Remarque (Leiden), who showed that merely doubling the vaccine dose could already result in demonstrable improvement in antibody titers. T. Aguado (WHO, Geneva) outlined world-wide efforts to improve vaccination of the elderly, for example by using the newly approved adjuvant MF-59, which was reported in greater detail by R. Rappuoli (Sienna), describing improved flu vaccination results in the elderly at the first years vaccination as well as at the second and even third year. However, whether the increased titers observed will be markedly greater than those achieved by E. Remarque by doubling the vaccine dose is still unclear. Other novel approaches include the use of DNA itself as an adjuvant, as described in animal models by H. Wagner (Munich). He showed that much of the impact of BCG therapy for cancer could be attributed to sequence-dependent bacterial DNA products contained therein. This effect was so powerful that it could replace the requirement for CD80/86-costimulation in CD28-knockout mice, and was at least as effective as LPS in activating murine DC; however, the DNA sequence effective in mice does not work in humans, and the search is on for the appropriate human sequence. D. Lowrie (London) discussed DNA vaccination for tuberculosis using mycobacterial antigen-coding sequences, also in mice, in which the plasmid vector also acts as an adjuvant for the response to the antigen. This approach resulted in protection of the mice from challenge with virulent mycobacteria which was sustained for more than 12 months. The other main area of discussion of potential clinical applications of the knowledge gained in the studies reported at this meeting was in the treatment of Alzheimers and atherosclerosis with anti-inflammatories and rheumatoid arthritis with TNF-neutralising antibodies. These studies are summarised in detail elsewhere.

6. Conclusion

Several of the presentations at the meeting graphically illustrated the inter-disciplinarity of research on immunosenescence and the necessity for an integrative approach to age-associated clinical disease. Immunosenescence in the adaptive immune system, manifesting as a deficiency, is primarily a T cell problem because APC and B cells retain function in the aged very well; dysfunction of these components where it does occur may often be traced back to T cell-dependent events. Immunosenescence as a manifestation of clonal exhaustion may also occur in young individuals as a result of chronic antigenic stimulation and as such is not only a problem of the elderly. Therefore, research on immunosenescence, already important in the context of increasing numbers of the elderly in society, is also critical in the context of chronic infection, organ transplantation, and possibly cancer, in younger individuals as well, and not only in advanced age.

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