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T CELLS AND AGING

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1. ABSTRACT

Deterioration of the immune system with aging ("immunosenescence") is believed to contribute to morbidity and mortality in man due to the greater incidence of infection, as well as possibly autoimmune phenomena and cancer in the aged. Dysregulation of T cell function is thought to play a critical part in these processes. Factors contributing to T cell immunosenescence may include a) stem cell defects, b) thymus involution, c) defects in antigen presenting cells (APC), d) aging of resting immune cells, e) disrupted activation pathways in immune cells, f) replicative senescence of clonally expanding cells. This review aims to consider the current state of knowledge on the scientific basis for and potential clinical relevance of those factors in immunosenescence.

2. INTRODUCTION

Scientific progress in immunosenescence research in general points more and more to a clinical relevance in man, although many areas remain controversial. The term immunosenescence designates that deterioration of immune function seen in the elderly which is believed to manifest in the increased susceptibility to cancer, autoimmune phenomena and infectious disease of the aged. There is increasingly good empirical evidence at least for the latter of these, showing that immunosenescence is clinically relevant for protection against infectious disease in the elderly. This suggests that failing immunity results in increased incidence of those diseases that the immune system evolved to protect against, ie. infectious diseases. Healthy life could be extended by preventing these

infections using interventions designed to prevent, ameliorate or reverse immunosenescence. Because the immune response is guided by T cells which must undergo extensive clonal proliferation on contact with antigen in primary responses and repeated clonal expansion for memory responses, replicative senescence may be particularly important for T cells. Memory responses and effective vaccination may be especially affected by T cell replicative senescence. Proliferative lifespans and replicative senescence have been extensively studied in certain tissues, especially fibroblasts, but relatively little is known about the behavior of T cells and the molecular biology of their growth control and senescence. The control of lymphocyte aging, proliferation, costimulation, apoptosis induction and memory cell maintenance at the cellular and molecular level *in vitro* can be studied using cultured human T cells. T cell clones *in vitro* could therefore provide a potential model to study "longitudinal" age-associated changes during clonal expansion and eventual replicative exhaustion. This review attempts to bring together recent publications in the area of T cell immunosenescence and to act as a resource for investigators from this and other fields.

3. WHAT IS IMMUNOSENESCENCE?

By most parameters measured either in the laboratory or *in vivo*, T cell function is decreased in elderly compared to young individuals. This perceived deterioration of immune responses is designated "immunosenescence" and is found in both long and short-lived species as a function of their age relative to life-expectancy rather than chronological time. Under certain special circumstances, immunosenescence may contribute to decreased pathology in elderly individuals, as in the lesser degree of acute rejection seen in clinical corneal and kidney transplantation [B. Bradley *et al* ., cited in (1)] and in murine models of systemic lupus erythematosis (2). However, under normal circumstances the effects of immunosenescence are likely to be primarily deleterious. There is increasingly good evidence that immunosenescence contributes to morbidity and mortality in man because of the greater incidence of infection, autoimmune phenomena and cancer in the aged. Most *in vitro* and *in vivo* tests of T cell function are depressed in elderly individuals (3), including even such very strong reactions as the rejection of allogeneic skin transplants (4). Moreover, prospective studies over the years have suggested a positive association between good T cell function *in vitro* and individual longevity (5-7). More recently, the first report of a Swedish longitudinal study of the very old has provided supportive data. Initially, data were collected on 102 donors aged 86 - 92. Two years later, 75 of these were still alive. A comparison of the two groups showed that non-survival was associated with the clustered parameters of poor T cell proliferative responses, high CD8 (cytotoxic/suppressor cell) cell fraction, and low CD4 (helper/delayed-type hypersensitivity [DTH] cell) and CD19 (B) cells. It was found that no single parameter was predictive for survival, but that a cluster of the above parameters was predictive (8). Other data illustrating the importance of the immune system in healthy aging come from studies on centenarians. By and large, unlike the "average" elderly, the healthy very elderly (centenarians) are found to have well-preserved immune functions, similar but not identical to, the "young" immune system (9).

Early observations on declining immune function with increasing age were made well over two decades ago, when it was reported that cytotoxic T cells were compromized in old mice (10). Since then many studies mostly performed in mice, rats and man but also including monkeys and dogs (11) have established that this immune decline is characterized in these diverse species by decreases in both humoral and cellular responses. The former may be a result of the latter, because observed changes both in the B cell germline encoded repertoire and the age-associated decrease in somatic hypermutation of the B cell antigen receptors (BCR) are now known to be critically affected by helper T cell aging (12). A basis for this may be the finding that a T cell product induces recombination-activating gene-1 (RAG-1) in athymic mice, which usually lack this in the bone marrow (BM) and therefore cannot rearrange BCR (13). Hence, the thymus is also necessary for B cell development, via its production of T cells and T cell-derived factors. *In vivo*, the T cell system of young and aged individuals is differently composed, particularly in terms of an increased proportion of memory cells in the aged. There is an overall decrease in mature $CD3^+$ T cells with age (14-16), although this may not be a continuous process, with T cells decreasing until the third decade, then staying constant until the 7th, after which decreases are again observed (17). Reciprocally, increased numbers of apparently activated T cells ($HLA\text{-}DR^+$, $CD25^+$), as well as increased numbers of natural killer (NK) cells (18) are seen. It must be borne in mind that all these data in human are relevant only to peripheral cells; the situation in the lymphoid organs is unexplored, although it is known that in rats the effects of aging on numbers and types of cells in spleen and periphery are different (19). In some more recent experiments in mice both secondary lymphoid organs and blood lymphocyte subsets have been studied. Thus, Poynter *et al* . reported that the proportion of T cells bearing the NK marker NK-1 increases with age in mice in blood and secondary lymphoid organs and that these cells rapidly produced large amounts of IL 4 on stimulation (20). There are strong arguments for the extrathymic nature of such NK-1+ T cells (21), which may therefore increase in compensation for decreased thymic output of conventional T cells.

Apparently paradoxically, despite declining immune function, aging is also associated with increased autoimmune phenomena. Because not only autoantibodies in general, but also clearly pathogenic autoantibodies, are routinely generated during normal immune responses to foreign antigen in the healthy young, the requirement for peripheral control of potentially damaging autoreactivity is paramount (22). This could be dysregulated in aging. Thus,

immunodeficiency on the one hand could be reconciled with increased autoimmunity on the other by postulating a compromized cellular regulatory activity with age. Data related to this point are controversial, but some are consistent with decreased cellular suppressive activity with age (23,24) or with increased resistance to suppressive influences in the aged (25). Decreased specific suppressor cell activity (26) in aged mice is associated with the appearance of MHC unrestricted T helper cells. The appearance of the same kind of MHC unrestricted helper activity has been observed in elderly humans (27), suggesting that altered suppression in aging may also occur in man. This has not been systematically investigated.

We believe that a better understanding of the causes of immunosenescence, particularly why a very small proportion of individuals successfully avoid it (currently about one person in 12,000 - 15,000 in developed countries has attained the age of 100), may offer the possibility of therapeutic intervention. Amelioration of the effects of dysregulated immune responses in the elderly may result in an enhancement of their quality of life, and significant reductions in the cost of medical care in old age. Whether an extension of healthy lifespan might theoretically follow is unknown and is not the main aim of this research, but is conceivable.

4. POSSIBLE CAUSES OF IMMUNOSENESCENCE

4.1 Hematopoiesis

Dysregulated hematopoiesis is seen in elderly individuals, raising the possibility that multiple lesions are responsible for altered immune function in the aged. Hematopoiesis may be compromized because of a severely reduced capacity to produce granulocyte/macrophage colony stimulating factor (GM-CSF) (28) and because lower numbers of progenitor cells are present in the BM (29). Normal cells with shorter telomeres possess less remaining replicative capacity than those with longer telomeres (see section 7). Accordingly, stem cells from adult BM have shorter telomeres than fetal liver-derived or umbilical cord-derived stem cells, and telomere lengths decrease on culture (30). This occurs despite low level expression of telomerase in these cells (31), although the rate of base-pair loss per population doubling of cells in culture is lower during the first two weeks, when telomerase is upregulated, than in the next two, when it is downregulated (32). The telomerase expressed is therefore functionally relevant. A survey of 500 autotransplant candidates (33) concluded that aging was associated with reduced numbers of committed hematopoietic progenitor cells, as measured by surface phenotype ($CD34^+$, $Thy1^+$, $CD38^{lo}$) and function (long-term culture initiation). In mice, the repopulating potential of murine fetal liver-derived stem cells is higher than that of adult BM-derived stem cells (34). Together, these sporadic results may suggest compromized ability to generate progenitor cells from BM in the elderly. Of direct clinical significance is the recent evidence that $CD34^+$ cells mobilize less effectively in cytokine-treated elderly compared to young donors (35). Moreover, the capacity of progenitor T cells from old BM to develop in the thymus may also be compromized (36). However, this has not been found in all models (37) where young and old BM was identical in reconstitution ability but age of the thymic stroma was found to be critical for the development of autoimmunity. The reasons for these differences are presently unclear. By studying thymocytes generated *in vitro* from young and old donor-derived progenitor cells, co-cultured in the presence of lymphoid-depleted fetal thymus, decreased generation of CD4/8-double negative thymocyte progenitors was demonstrated, along with a developmental arrest at the transition from CD44+ CD4/8-double negative to CD44-negative, CD4/8 double positive cells. This does suggest an intrinsic change in the stem cells with age (38).

Other studies in mice have found that hematopoietic stem cells are more frequent in old individuals and more likely to be in cycle, although they were less efficient at homing to and engrafting bone marrow of irradiated recipients (39). Some of the previously published inconsistencies in the data may be resolved by the study of de Haan *et al* . (40). These investigators showed that aging significantly alters the primitive hematopoietic compartments of mice in several ways: firstly, the proliferative activity of the primitive cells is greatly reduced over the first year of life; secondly, there is a (compensatory) increase in relative and absolute stem cell number with age; thirdly, the changes are strain-dependent and related both to the longevity of the strain as well as to the age of the individual mouse. A strong inverse correlation was observed between mouse lifespan and the number of autonomously cycling progenitors in 8 different strains of mice; a gene controlling this frequency was mapped to mouse chromosome number 18 (syntenic to human chr. no. 5, involved with various haematological malignancies, ref. 41). In outbred species such as humans, this type of variation would make analysis difficult. Therefore, more work needs to be done to definitively answer the question of whether any alterations in hematopoiesis in the elderly may contribute to immunosenescence.

4.2 Thymus

It is accepted that T cell differentiation is compromized with age because of thymic involution. However, even this generality may not be universally and incontrovertably true. Thus, a recent study of thymic samples from donors from one week to 50 years old showed an early decrease of cellularity but with two early peaks at 9 months and 10 years of age. Moreover, the adult thymus still contained thymocytes with similar surface phenotypes to those seen in young donors (42). This suggests that the thymus can remain active at leat up to middle age, but the functional activities of the thymus output could not be studied in this investigation. There may be a strong genetic contribution influencing thymic involution; for example, rats of the Buffalo strain do not experience thymic involution

and in parallel do not manifest decreased T cell function with age (43). Again, therefore, genetic heterogeneity in outbred populations might be expected to contribute to marked inter-individual differences. Thymic involution may itself be affected by the status of the T cells in the individual; for example, thymic involution is reported not to occur in T cell antigen receptor (TCR)-transgenic mice, leading to the conclusion that successfully matured T cells can maintain thymic integrity (44,45). In addition, reconstitution experiments indicate that the observed accelerated maturation of T cells to a memory phenotype in old mice is due to the aged environment and involves interactions via the TCR which are, however, not antigen-specific [(46) and M. Thoman, cited in ref. (1)]. T cells also affect the thymus itself via a feedback effect and provide survival signals for the medullary microenvironment. An important survival signal of this type may be IL 4 [M. Ritter, cited in (1)]. Thus, the aging of stem cells and/or T cell precursors may directly influence processes of thymic involution. CD4 T cells appear to be the most effective at maintaining thymic function and a decreased collaboration between thymocyte progenitors and mature CD4+ T cells from aged mice could also result in a defective feedback of aged CD4+ cells on thymocyte development and differentiation [(47) and A. Globerson, cited in (1)]. Signals controlling thymic status may also be derived from the nervous system, either directly from sympathetic innervation or indirectly via the hypothalamic-pituitary axis (48). There are increased numbers of noradrenergic sympathetic nerves and 15-fold increases in concentration of norepinefrine in the thymi of 24 month-old mice (49).

The phenomenon of thymic involution begins very early in life, even before puberty, and progressively continues. However, despite the assumption that thymic involution is essentially complete in adulthood, there are data to suggest that the replacement of thymic parenchyma with adipose tissue is a discontinuous process, reaching a maximum at around 50 years of age in humans and thereafter not progressing further (50). Moreover, the amount of non-fatty material in the thymus may not decrease further after the age of about 30 years (50). Secretion of the important immunoactive hormone, thymulin, continues throughout life, although blood thymulin levels do decrease with age (51). There is evidence here, however, that lower levels of thyroid hormones and insulin, rather than thymus dysfunction, are responsible for lower thymulin levels (51). These findings, together with the genetic heterogeneity of outbred populations probably influencing the occurrence and rate of thymic involution, make it difficult to assess the contribution of such involution to changes in T cell function in man. There is evidence to suggest that even in (some of) the very old, sufficient thymic function may be retained to allow for naive T cell differentiation (52). It has been estimated that complete thymic atrophy in humans would not occur until the age of about 120 years (53). The decrease in thymic size and alterations in thymic architecture and functionality for T cell differentiation which do occur up to middle age are the results of a controlled process independent of stress and lack of repair mechanisms. Thus, infection, pregnancy, stress, drug or hibernation-induced thymic involution are all reversible in younger individuals, leading to the suggestion that thymic atrophy is an energy-saving process according to the disposable soma theory of aging (54). According to this view, the evolutionary pressures on maintaining thymic function for constant full T cell repertoire generation were secondary to the generation early in life of a memory cell repertoire for a mostly tribally-limited pathogen presence. Thymic function did not need to be maintained beyond reproductive maturity because the number of new infections experienced by early humans in later life in the wild was too limited to make thymic maintenance worthwhile. This presupposes that early humans did not come into contact with very many new pathogens, suggesting a sedentary existence. However, early humans were nomadic, only recently becoming sedentary, so it is unclear whether this does apply. George & Ritter suggested (54) correlating thymic involution rates and function in animals and birds which migrate long distances, the hypothesis being that the more varied the environment, the more evolutionary pressure there would be to maintain the thymus. Another possibility to explain early thymic involution may relate to avoidance of undesired tolerization of newly generated T cells to pathogens which in later life have entered the thymus (55).

Whatever the reason, the effects of thymic changes, associated with increased age, on the immune system in mice are marked. As mentioned above, the situation in humans may not be so clear. Moreover, in mice, age-related changes in the thymus may influence other organ systems in some manner, as has been reported for effects on the liver (56). In mouse, the number of T cells exported from the thymus decreases with age, as does the ability of thymic epithelium from old animals to support the differentiation of T cells from young animals´ BM. The type of T cell produced is affected by aging. There may be a developmental block which results in an increase in the frequency of $CD3^+$ CD4/8-DN thymocytes (57). This may result in higher proportions of apparently immature T cells being present in old individuals (consistent with decreased thymic function; refs. 58-60). However, the markers used to discriminate immature T cells in these latter studies do not seem to have allowed for distinguishing between $CD2^+$ T cells and $CD2^+$ NK cells, so that the increase in immature T cells might actually represent an increase in NK cells. One group specifically tested this and concluded that such cells were indeed functionally active NK cells (61). Other important changes related to altered thymic function may include changed restriction repertoires of the T cells generated, such that even TCR2 (TCR-$\alpha\beta$) cells acquire responsiveness to antigen presented by non-self MHC in man (27) and mouse (26). Evidence has also been presented for increased levels of extra-thymically-differentiated T cells in elderly humans, as well as increased NK-phenotype (but not NK-functional) cells (62). The increase in extra-

thymically-differentiated T cells may also represent some sort of compensatory mechanism for decreased thymic integrity.

Studies on depletion of $CD4^+$ cells by CD4 mAb indicate that recovery of this population, which is dependent upon the presence of the thymus, is much slower in aged mice than young mice (63). In humans, CD4-depletion by mAb treatment in rheumatoid arthritis (RA) results in a very prolonged effect. T cell reconstitution is slow, there is a predominance of T cells with memory phenotypes, and there is limited TCR diversity (64). The ability to generate new T lymphocytes after chemotherapy is inversely related to the patients´ age, probably an indirect indication of thymic involution (65). During the first year of recovery after chemotherapy, the CD4 cells in adults also mostly carry memory markers, but in children they carry markers of naive T cell (66). Recovery of CD4 cells was inversely related to age of donor and was enhanced in patients with thymic enlargement after chemotherapy (67). However, CD8 cell recovery was much more rapid and was not associated with age or thymic enlargement. The CD8 cells were mostly CD57+ CD28-negative. All these data can be interpreted to imply that the prime source of reconstituting cells in adults is from peripheral expansion of pre-existing CD4 T cell subsets which survived conditioning, and not by thymus-dependent generation of new T cells. CD8 cell generation is thought to be extrathymic here (67). A similar phenomenon may be observed in HIV infection, where antiviral therapy results in an increase of naive CD4 cells only if some were still present before initiation of therapy (68). It is noteworthy that in bone marrow transplant (BMT) patients, even as long as 5 years after transplantation, CD4 cell counts are still depressed and cells with a naive phenotype are also rare. Cells with a memory phenotype ($CD45RA^{lo}$, $CD29^{hi}$, $CD11a^{hi}$) were abundant and many of these were CD28-negative. Moreover, there was a negative correlation between the ability to produce naive T cells after BMT and patient age (69), independently of the presence of graft-versus-host disease. These findings seem to apply only to naive T cells, as might be expected; thus, Koehne *et al* . reported that in human peripheral blood stem cell transplantation, only recovery of the $CD4^+CD45RA^+$ population, but not the $CD45RO^+$ population, was thymus-dependent (70). A bone marrow-transplanted young thymectomized patient mimicked this phentoype (71). The patient showed preferential recovery of CD45RO+ cells in the CD4 subset, although in CD8 cells, CD45RA+ cells were generated as well as in age-matchd euthymic patients. It was therefore concluded that a functional thymus was essential for the generation of naive CD4 cells, although extrathymic pathways for naive CD8 cell generation appeared functional (71). Following T cell-depleted BMT, loss of TCR diversity in slowly reconstituting cells is also seen, again consistent with peripheral expansion of a very limited number of T cells transferred with the graft (72).

4.3 Post-thymic aging

Finally, mature T cells are also subject to aging processes, either of the type affecting post-mitotic cells (when quiescent) or of the "replicative senescence" type (during clonal expansion for effective immune responses). This will be discussed at length in the following sections.

5. MECHANISMS CONTRIBUTING TO IMMUNOSENESCENCE

5.1 Accessory cells

Decreased T cell responses in the elderly may be due to decreased T cell function, decreased accessory cell function, or both. There is evidence for accessory cell changes (73) and monocytes are clearly compromized in their function in the elderly, eg. in that they secrete less IL 1 and have decreased cytotoxicity and protein kinase translocation (74). A more recent analysis suggested that lipopolysaccharide (LPS)-stimulated monocytes from the elderly produced less G-CSF, GM-CSF, IL 8, TNF-α, and MIP-1-α as well as less IL 1ß compared to those from young donors (75). In some clinically-relevant animal models, it is the accessory cells which seem to contribute critically to age-associated suboptimal responses, eg. in the response of mice to trypanosome antigens (76). Another example where T and B cell function appears to be normal, but accessory cell function is compromized in aged mice comes from a vaccination model using pneumococcal preparations (77). To distinguish between T cell and APC alterations may be difficult but can be approached in certain models by using purified T cells in the absence of accessory cells. For example, using mitogenic CD2 mAb and soluble costimulatory factors (cytokines, phorbol esthers, mAb), Beckman *et al* . (78) have shown that in $CD45RO^+$ CD4 cells, the only pathway not comparable between young and old donors was for stimulation by CD2 in combination with IL 7. Thus, signalling may be intact in old memory cells, except for IL 7-dependent pathways. In contrast, $CD45RA^+$ cells from old donors responded less well than young naive cells to CD2 + IL 2, IL 6, IL 7, IL 1 or phorbol esther, suggesting multiple deficiencies in the naive cells but not the memory cells of old donors.

On the other hand, dendritic cells (DC) obtained from elderly persons are reported to be able to present antigen at least as well, if not better, than DC from young donors (79). The same group also reported that DC from the elderly were able to inhibit apoptosis and stimulate proliferation in pre-senescent cultured T cells (80). These results suggest that at least a subset of APC in the elderly retain optimal function. On the other hand, it must be borne in mind that these results were obtained using DC generated *in vitro* using IL 4 and GM-CSF. Since the production of GM-CSF in the elderly is decreased (see section 4.1) there may not be so many functional DC available in old donors.

5.2 Alterations in signal transduction

That the earliest events in T cell activation are compromized in the elderly is reflected in findings that cell surface alterations associated with activation are affected, eg. CD69 and CD71 upregulation does not take place (81). Incomplete T cell activation may be caused in the first instance by disturbed signal transduction. Particularly in T cells there are multiple levels where lesions might be sought, since signal transduction through one, several or all of the TCR, costimulatory receptors, or growth factor receptors could be compromized with age. There is evidence for alterations in all three of these possible loci.

Damage to the cytoskeleton paralleling aging may have profound effects on cell function. In the case of T cells this may be relevant even to the earliest stages of T cell activation, because, for example, the signal-transducing TCR zeta chain component is associated at the cell surface with the cytoskeleton (82). If this has something to do with receptor recycling or maintaining correct three-dimensional structure of the receptor, cytoskeletal dysfunction could have profound effects on TCR signal transduction. Certainly, initial biochemical events following TCR triggering (formation of second messengers such as IP3 and DAG) are compromized, although the activity of PLC (which is responsible for IP3 and DAG generation) appears conserved in old T cells (83). However, the actual amount of PLC present in freshly isolated cells may be decreased with aging (84). Antibody against the signal-transducing CD3 zeta chain precipitates a series of tyrosine-phosphorylated proteins, the levels of which decline with age (85). Therefore, the level of expression of the TCR components and/or their ability to transduce signals may be compromized in old T cells.

Signalling pathways mediated by the family of mitogen-activated protein kinases (MAPK) are considered essential for normal cell growth and function; CD3 stimulated human T cells from 50% of old subjects were found to show reductions in MAPK activation. Stimulation with phorbol esther in combination with calcium ionophore resulted in greater MAPK activation in old cells, but still not to the same extent as young cells (86), suggesting signalling deficits between the TCR and the inducers of MAPK. MAPK are activated by another protein kinase, MEK; in mouse CD4 cells, there is an age-associated decrease in MEK (87). Moreover, kinases are commonly counter-regulated by phosphatases, and even if kinase decrease were not to occur, increase in phosphatase activity might have the same result. In the case of MAPK, it has been reported that expression of MAP phosphatase is indeed increased (in aged rat hepatocytes, at least) (88). In T cells, signalling through the TCR, CD4, CD8 or the IL 2R resulted in lowered protein tyrosine kinase activity in cells from old compared to young donors, although direct activation of protein tyrosine kinases (PTK) by pervanadate was normal in the old (89). It is therefore not yet clear whether the age-related decreased tyrosine phosphorlylation observed in CD3-stimulated human T cells is related to changes in PTKs or phosphatases (PTPases). Recent data from Whisler *et al* . indicate that CD45-PTPase activity in old cells after CD3-stimulation is not increased compared to young cells (90). They further found that fyn enzymatic activity but not lck activity was reduced in a high proportion of T cells from the elderly compared to the young, although protein levels were the same. They concluded that decreased fyn activation but not increased PTPase activity may contribute to lowered responses in the elderly (90).

Cell cycle analyses of PHA-stimulated cells from aged donors indicate a decreased frequency of cells entering S-phase with this age-related impairment of G1 progression correlating with decreased expression of c-jun, c-myc, c-myb, IL 2 and CD25 (91-93). The proportion of cells expressing c-myc (G0 to G1 marker) and c-myb (G1 to S marker) was decreased after PHA stimulation of old T cells, but the amount per cell seemed to remain the same as in young T cells (93). T cells retaining antigen recognition and effector function, yet apparently in a post-mitotic senescent or pre-senescent state have been described (94). These investigators also demonstrated that aged human T cells paralleled the senescent phenotype of fibroblasts in that on restimulation, fewer cells responded by entering the cell cycle, the remainder being arrested before S-phase. The cell cycle was also prolonged in those ca. 20% of senescent cells which could be restimulated (95). At least some of these results may reflect the situation *in vivo*, where PHA stimulation resulted in an earlier accumulation of cells in S phase in young donors´ T cells, and a significant delay, but eventually equivalent level, of S phase cells in the elderly (96).

In Fischer rats, the age-associated decrease in IL 2 mRNA and protein correlates with a decreasing ability of nuclear extracts of freshly isolated T cells to bind an oligonucleotide representing the transcription factor NF-AT (97), suggesting differences in transcriptional regulation in young and old cells. NF-AT forms an important family of at least four transcription factors; NF-AT DNA binding activity has been found in nuclear extracts of stimulated T cells (98) and is thought to be important for IL 2 gene transcription (99).

A set of transcription factors involving complexes of the various c-jun and c-fos proteins is involved in regulating transcription of many genes, including IL 2, and activation of AP-1 is detected a few hours after T cell stimulation (100,101). Specific defects in AP-1 activation have been reported in young T cell clones rendered anergic *in vitro* (102). The anergic phenotype is in some ways similar to the senescent phenotype (ie. cells can be stimulated via the TCR to secrete cytokines, be cytotoxic, but they cannot expand clonally via autocrine IL 2 production). AP-1 activation may be impaired in *in vivo*-aged human T cells as well (103). Using T cells from elderly donors rigorously selected for good health according

to the SENIEUR protocol, it was found that the PHA-stimulated activation of AP-1 was commonly impaired in the elderly. In many of these, addition of phorbol ester partially compensated for this defect, but a minority remained refractory. The defect appeared to be in the amount of AP-1 activity produced, since the AP-1 protein that was produced by cells from old donors behaved in the same way as that from young donors and also contained c-fos and c-jun (103). Thereafter, the same group reported that both AP-1 and NF-AT were reduced in elderly donors´ stimulated T cells (104). However, whether these changes were associated with alterations in T cell subset composition was not reported. These data are consistent with those of Song *et al* . (92) demonstrating decreased c-jun mRNA but normal c-fos mRNA responses to PHA in T cells from elderly donors. Moreover, fewer lymphocytes from elderly donors exposed to influenza virus *in vitro* expressed fos and jun compared to cells of younger donors, possibly as a reflection of compromized activation of anti-viral responses (105). Amongst the transcription factors of known importance for IL 2 production, CD3-stimulated induction of NF-kappa B was also found to be decreased in old mice (106) and humans (107). One reason for insufficient NF-kB activation may be that the natural inhibitor I-kB is not adequately degraded because of compromized proteosome function [Ponnappan, cited in ref. (1)]. Age-associated inactivation of proteosome function has been independently reported and attributed to the effects oxidative damage, which can be partly prevented by hsp90 (108). Hsp 90 levels are themselves decreased with age, in T cells (109). Whisler *et al* . (104) also found reduced NF-kappa B in some elderly human donors´ stimulated T cells, but they did not find a correlation with depressed IL 2 production (unlike their findings with NF-AT, see above). Interpretations may be complicated, however, by the unexpected finding that NF-AT may exert negative regulatory, not stimulatory, effects on the immune response (110). Finally, in rats, Pahlavani *et al* . reported that the induction of AP-1, NF-kappa B and Oct-1 DNA binding activity in nuclear extracts of spleen cells from old animals was significantly lower than that of young animals, and the decrease of AP-1was due to reduction of c-fos mRNA, whereas c-jun remained the same in young and old cells (111).

5.3 Defects in costimulatory pathways

Despite indications for the involvement of accessory cells in dysregulated immune function in the aged, most attention has been focussed on the T cell. T cells require stimulation via the antigen-specific TCR for activation. However, in addition they also require stimulation via non-polymorphic antigen-nonspecific costimulatory receptors. Abberations in these receptors would also lead to compromized T cell responses. Dobber *et al* . (112) reported that aged mouse $CD4^+$ cells stimulated with Con A or anti-CD3 + anti-CD28 mAb did show decreased IL 2 production compared with young cells, but when stimulated with immobilized CD3 mAb alone, they produced more IL 2 than young cells (but still very little compared to CD3 + CD28). This suggests that aged $CD4^+$ cells show diminished responsiveness to CD28 costimulation, whereas the limited response to stimulation via the TCR alone is retained in the old cells. Since memory cells in young (human) donors are easier to costimulate via CD28 than naive cells, whereas old donors´ cells are harder to stimulate with CD28 (113) this is consistent with alterations in aging not being solely explicable by accumulation of memory cells at the expense of naive cells. Moreover, in mice, the memory cells themselves function less well in old than in young donors (114-116). In this case, this may be related to their defective responses to signalling via the major costimulus receptor CD28, despite equivalent expression of CD28 on cells from young and old mice (117). Moreover, these cells, like certain other anergic (young) cells (118) may be able actively to suppress other cells in a mixed population, cells which otherwise would be capable of proliferation (119). Engwerda *et al* . have also more recently shown that activation-induced cell death (AICD) is increased in T cells from old mice, as a direct consequence of their decreased levels of CD28-mediated costimulation, which otherwise may protect stimulated cells from apoptosis (120) (although there are also other mechanisms of protection against apoptosis, some of which may not involve CD28 (121)) and conversely under certain circumstances CD28 costimulation may actually enhance apoptosis by upregulating apoptotic mediators such as bad (122). CD28 is a costimulator receptor twinned with a closely related second receptor CTLA-4 (CD152). This molecule delivers "off" signals to the T cell when ligated by the same structures as CD28 (CD80, CD86) (123). Old T cells may express increased amounts of CTLA-4, which may therefore make them harder to turn on even if CD28 functions normally (124). This is another property shared with young anergic T cells (A. Merl, unpublished results).

Not only CD28 costimulatory mechanisms but other important accessory/adhesion pathways may be compromized in aging. Thus, Jackola *et al* . (125) reported defects in cell-cell binding amongst healthy elderly donors, which was associated with altered activation capacity of the integrin LFA-1. Moreover, other surface receptors implicated in costimulation may be downregulated with aging. Preliminary evidence is beginning to show that the density of expression of the CD40 ligand CD154 is decreased on T cells aged *in vivo* (Lio *et al* . Mech. Aging Dev., in press) and *in vitro* (M. Adibzadeh, unpublished results).

5.4 Alterations in cytokine production and response

Once stimulated, T cells must transcribe T cell growth factor (TCGF) genes, secrete growth factors, upregulate TCGF receptors and respond to the cytokines. In this way, autocrine and/or paracrine clonal expansion, a prerequisite for successful immune responses, is effected. One result of poorer T cell function is decreased cytokine production. It has been long believed that a major

dysfunction in T cells from elderly donors is a selectively decreased ability to secrete T cell growth factors. Many studies have confirmed that T cells from aged humans can also show defects in IL 2R expression, IL 2 secretion and DNA synthesis after stimulation with mitogens like PHA. Data on cytokine secretion in the earliest studies were controversial, perhaps mostly due to employing apparently healthy elderly donors without rigorously excluding underlying illness, nutritional or psychological status. In addition, cytokine secretion may be affected by other (non-pathological) parameters such as exercise, as reported by Shinkai *et al* . (126). They found that although the numbers of circulating CD3, CD4 and CD8 cells were similar in sedentary and exercising elderly donors, and proliferative responses and cytokine secretion were reduced, these parameters were significantly more compromized in the sedentary group.

It was therefore anticipated that when data became available using donors selected according to standard criteria (namely, the SENIEUR protocol (127)), results on cytokine secretion and other immunogerontological parameters would become clearer. Such studies have confirmed dysregulation of cytokine production but not necessarily associated with lower IL 2 production. Thus, Sindermann *et al* . reported unchanged IL 2 and IFN-γ production in elderly German SENIEUR donors, but they did find significantly decreased IFN-α and soluble IL 2 receptor secretion (128). Others have found that IFN-γ production tended to be enhanced (18), and, as also shown in mouse, IL 10 production was enhanced as well (129,130). *In vivo* studies of plasma levels of factors such as IL 6 also reveal age-associated increases; in fact, it has been proposed that IL 6 levels may be a good overall biomarker of health in aging because plasma levels are correlated with functional status (131). Moreover, treating aged mice with IL 6- but not IL 1-neutralizing antibody resulted in a reversion of their cytokine production pattern to that characteristic of young animals (132). In American donors, Jackola *et al* . reported that the frequency of T cells responding to PHA by secreting IL 2 decreased with age, even in SENIEUR-selected donors (133). Clearly, despite the use of SENIEUR donors, discrepancies still arise. Unsuspected population genetic influences may be playing a role, since the distribution of MHC alleles differs even within different groups of the European population, and levels of immune responses and cytokine secretion as well as possibly longevity are known to be associated with MHC type (134). Nijhuis *et al* . agreed that IL 2 production in old Dutch SENIEUR donors (compared to young donors also selected with the SENIEUR protocol) was not decreased and presented evidence for increased IL 4 production. They also found that increased IL 4 production in elderly donors did not correlate simply with the measured increase in the fraction of memory (CD27-negative $CD45RO^+$) cells, although this was confirmed to be the case for young donors (135). This suggests that in young donors, different levels of IL 4 production are determined solely by antigen exposure and amount of memory cells, but that in aged donors, other regulatory mechanisms are operating. In contrast, Candore *et al* . reported decreased IL 2 and IFN-γ production but unaltered IL 4 and IL 6 secretion after PHA stimulation in Sicilian donors (136), and others have reported that both IL 2 and IL 4 secretion may be reduced even in (Italian) SENIEUR donors and have pointed out that decreased function may be linked to psychological factors (137), and suggested that these need to taken more into account in studies of immunosenescence (138). However, in this population, despite the relative decrease in cytokine secretion, T cells from these donors could proliferate well if supplied with exogenous growth factors (139). A more recent study with patients with Major Depression did not support a role for this condition in decreasing PHA-stimulated proliferative responses (140). Psychosocial factors may play a significant role in clinically-relevant situations also, eg. the humoral response to influenza vaccination. In one study, the effect of chronic stress (caring for a demented spouse) resulted in significantly lower antibody titers, as well as IL 1 and IL 2 production in the elderly caregivers (141). Some data are beginning to emerge on the regulatory effects of neuropeptides on cytokine secretion in young and elderly donors (142). Another study investigated the expression of dopamine receptors on human lymphocytes and revealed that precipitous loss of dopamine receptor D3 occurs between 40 - 50 years of age (143). Conversely, increasing levels of expression of cellular amyloid precursor protein (APP) by human lymphocytes are significantly positively associated with increasing age (144). Although the significance of these findings is unclear, studies of this type may begin to help shed some light on the mechanisms responsible for neuroimmunological communication and age-associated alterations. Other factors perhaps not sufficiently taken into account in previous studies may be not only differences between sexes but differences between females dependent on their reproductive history (145).

Few studies have compared the behavior of T cells from young and old donors at the clonal level. Paganelli *et al* . (146) reported on T cell clones (TCC) obtained from two centenarians (a highly selected population) compared to those obtained from three young donors. $CD4^+$ TCC made up 38% of TCC obtained from the young, but 53% of those from the old. Cytokine production from the CD8-TCC was the same in young and old-derived clones, but the CD4-TCC were different. Most TCC derived from young donors produced IFN-γ but not IL 4, whereas those from the centenarians produced both. This was interpreted to indicate a shift in the CD4 population from predominantly Th1 to Th0 phenotype in the centenarians. Similarly, Kurashima *et al* . (147) found that, as expected, naive cells produced mainly IL 2 and memory cells mainly IL 4, in young mice. However, the reciprocal was observed in old mice: naive cells produced more IL 4 than memory cells and memory cells produced more IL 2 than naive cells, although overall levels were reduced in old compared to

young mice. Thus, age-associated alerations in cytokine production are not determined solely by the subset changes, but by alterations within each of those subsets. On the other hand, although different investigators have confirmed the decrease in IL 2 production in old mice, they have concluded that this is solely a result of different subset composition. Thus, Engwerda *et al* . (148) reported that purified CD4 or CD8 cells from aged mice, stimulated with CD3-epsilon mAb and CD28 mAb, produced the same amounts of IL 2 and IL 4 as young cells of the same $CD44^{hi}$ or $CD44^{lo}$ phenotype (although they produced increased amounts of IFN-γ). Kirman *et al* . (149) reported that the age-associated increase in IL 4 secretion by mouse spleen cells was not caused by an increase in the numbers of IL 4-secreting cells, but the decrease of IL 2 secretion was associated with a decreased number of secretor cells. However, this could be prevented by exposing the mice constantly to high levels of IL 2 *in vivo*, which could therefore correct the age-associated cytokine imbalance in these mice.

Decreased soluble IL 2R secretion has been noted in elderly donors and may be of greater significance than possible IL 2 secretion defects (150). Other defects in IL 2R expression in aged T cells have also been reported: they are defective in their upregulation of the high affinity receptor for IL 2 (151), and even that lower proportion of cells expressing the receptor and retaining their ability to internalise the IL 2 still fail to respond properly (152). Thus, even under conditions where IL 2 secretion is apparently normal, and where IL 2R expression is also apparently normal in terms of receptor affinity and number (153), aged T cells may still proliferate less vigorously than young cells, even in the presence of exogenous IL 2 (153). The reasons for such suboptimal proliferation are still not clear, and signal transduction defects by the cytokine receptors seem not to have been investigated intensively yet. Such intrinsic decreased proliferative capacity is even seen in T cell clones established from aged SENIEUR donors´ cells, particularly for CD8+ cells (154).

On the other hand, the IL 2 secretion defect seen in vitro in many but not all studies may in fact be transient, with T cells from old donors re-acquiring this ability after a period in culture (155). Different donor states might then explain discrepancies found in cytokine secretion patterns even amongst SENIEUR donors, as noted above. Huang et al . found that old donors with apparent IL 2 secretion defects in vitro, in fact had relatively high serum IL 2 concentrations in vivo. Moreover, vaccination of young donors mimicked this effect and resulted in their T cells becoming refractory for IL 2 production shortly thereafter in vitro. These investigators therefore suggested that apparent defects in IL 2 secretion in elderly donors are a result of in vivo activation of their T cells by unknown mechanisms and reflect a normal event also seen in young donors after in vivo T cell activation by immunization (155). If these results are confirmed (and as far as the authors are aware, five years after publication this is not the case), a reassessment of the meaning of depressed IL 2 secretion by old T cells in vitro will be required. The immune "defect" observed here will then actually represent a normal consequence of activation, possibly a kind of refractory, temporary anergic state, or "exhaustion", which in animal models can even result in extra-thymic clonal deletion of the activated cells (156). On the other hand, "memory" cells accumulate in elderly donors, and may be in active division required for their maintenance of memory (157,158). Not only might these activated cells explain the data of Huang et al (155), but since they might eventually arrive at a post-mitotic state, this would also explain their eventual loss from the system altogether.

Direct measurements of IL 2 levels in the serum of SENIEUR donors have also failed to detect age-associated decreases in one study (159), although a second study suggested that serum IL 2 levels were reduced in the very old (160). However, both studies agreed that the level of soluble IL 2R in the blood was increased in the elderly, which could contribute to decrease of IL 2 function.

Other cytokines are also beginning to be examined, eg. spontaneous production of IL 8 by monocytes *in vitro* was reported to be lower in the elderly than in the young, but on stimulation with LPS more IL 8 was produced in elderly males than in young (161). Similarly, production of cytokines such as TNF-α may increase rather than decrease with age (162). Increases in TNF-α may be directly relevant for decreased T cell responses, because TNF-α can inhibit proliferation of some human TCC (163) and can attenuate TCR-signalling *in vivo* in mice (164).

As well as altered cytokine levels in aging, altered levels of cytokine antagonists might also influence cytokine networks. These possibilities are now beginning to be explored (for factors in addition to sIL 2R mentioned above). Thus, Catania *et al* . (165) reported a study of 122 healthy aged compared to 39 unhealthy (urinary tract infections) and 100 young controls regarding plasma levels of IL 1R-antagonist and sTNF-R. These were higher in the healthy than in young controls, and were even higher in the infected subjects.

6. CULTURE MODELS FOR IMMUNOSENESCENCE: THE HAYFLICK LIMIT APPLIES TO NORMAL T CELLS

Since the T cell response requires waves of clonal expansion followed by contraction and re-expansion on recontact with antigen, limits to the proliferative capacity of the T cell clones might impact deleteriously on the overall response. T cell clones *in vitro* may provide a good model for investigating age-associated changes in a longitudinal manner. They can be maintained for extended periods in tissue culture, but in most cases they have finite lifespans, eg. see refs. (94,154,166-172). In our comparison of the *in*

vitro longevity of T cell clones (TCC) derived from mature peripheral T cells and those derived from T cell progenitors of the same donor we found that both manifested limited lifespans. Progenitor-derived cells survived on average 20 populations doublings (PD) longer, but this corresponded to the length of time that they required to differentiate from progenitors into cells with a mature T cell phenotype (173).

Could there be exceptions to T cells with finite lifespans? Certain human T cell clones which have been cultured for many years must be presumed to have exceeded the Hayflick limit, although this has not been formally measured by any of the investigators working with such clones. How can these potential discrepancies be resolved? Human T cells infected with HTLV-1 or Herpes saimiri virus can become immortalized. In the latter case, the cells retain a normal functional phenotype, ie. they remain dependent upon exogenous growth factor for their continued proliferation, and they still respond specifically to stimulation via their antigen receptor and non-specifically via the alternative activation pathway (CD2/CD58-dependent). Therefore, inadvertant infection with H. saimiri would result in retention of apparently normal immunological attributes coupled with indefinite lifespan. However, the chances of inadvertant infection with this non-human virus are presumably very low, and can be excluded by screening for known viruses. Nonetheless, there always remains the possibility that rare events featuring transformation with unknown viruses might account for some examples of apparent immortality of TCC.

It may still be questioned whether suboptimal culture conditions for T cells are responsible for the short lifespans of the majority of TCC. Since the rare long-lived TCC are cultured under apparently very similar conditions to the normal, short-lived, ones, this may seem *a priori* unlikely. However, some simple manipulations of tissue culture conditions may be sufficient to affect longevity, and for some reason certain rare T cells might more successfully adapt than others. For example, it has become apparent that simply reducing the oxygen content of the culture environment from the supraphysiological tension commonly employed (air) to a more physiological level can result in considerable lifespan extension of fibroblasts (174,175). Whether this is the case for T cells and whether they are more sensitive to oxidative damage, has not yet been reported. Other possible manipulations which have been reported to extend the lifespan of fibroblasts, but which have not been tested on T cells, include using hydrocortisone, carnosine, anti-sense oligonucleotides for p53 and Rb, high albumin concentrations, additional growth factors and other hormones (reviewed in ref. (176)). Optimisation of culture conditions using capillary bed culture cartridges may also better mimic the *in vivo* environment and lead to extended lifespans, but this has also not yet been demonstrated (177).

Most human TCC are generated from cells obtained from peripheral blood, but such recirculating cells may not be truly representative of the T cell pool. The major lymphoid organs from which T cells can be obtained are skin and gut. T cells infiltrating the skin in various disease states can be cultured *in vitro* using the same techniques as employed for peripheral cells, and these have also been found to have limited lifespans (eg. ref. 178). However, skin-infiltrating cells cultured in the presence of IL 4 in addition to IL 2 but in the absence of antigen presenting cells have been reported to grow apparently indefinitely. These cells were found not to harbour HTLV-1 (although it cannot be formally excluded that some other, thus far unidentified, virus is involved). These long-lived T cells manifested various chromosomal abnormalities at different frequencies (179). Generation of these lines was reproducible in different donors with different diseases, suggesting that isolation of apparently immortal cells under these conditions was not a rare event. While some of the donors were cancer patients, perhaps displaying generalized genetic instability and chromosomal fragility, the majority were atopic dermatitis patients (not known to fall into this category). Moreover, one T cell line was established from a skin nickel patch test which retained a normal karyotype up to 300 PD (way beyond the Hayflick limit) and acquired an abnormal karyotype thereafter (K. Kaltoft, personal communication, May, 1997). Therefore, depending on the source of cells and the culture conditions, normal T cells may be able to proliferate indefinitely, but the majority of evidence accumulated thus far suggests that this is the exception rather than the rule.

7. DOES TELOMERIC END LOSS CONTRIBUTE TO THE REPLICATIVE SENESCENCE OF NORMAL T CELLS?

Loss of telomeric DNA, and gradual shortening of telomeres, has been proposed to result, after a certain number of cell divisions, in the inability of cells to divide again (180). In human monoclonal fibroblast cultures, telomere length was found to be reduced with culture age and was directly proportional to the remaining replicative capacity of the clone (181). Telomere shortening might therefore act as a mechanism counting the number of cell divisions that a cell population has experienced. Loss of telomeric DNA has also been demonstrated in blood cells *in vivo* and shown to be related to donor age (182). It occurs more rapidly in premature aging syndromes, eg. Hutchinson-Gilford progeria (183) or trisomy-21 (184). In culture, lymphocytes from normal donors allow an estimated telomere DNA loss rate of 120 bp/cell doubling, comparable to that seen in other somatic cells. More recently, Weng *et al* . (185) reported that $CD4^+$ memory phenotype cells showed consistently shorter telomeres than naive phenotype cells. Interestingly, this difference in telomere length between naive and memory cells was the same whether the cells were isolated from young or old donors. This may suggest that it is the T cell precursor rather than the mature T cell which has "aged", as defined by decrease of telomere length. Weng *et al* . also showed that telomere length

decreased during autocrine expansion of both naive and memory cells, and that the latter completed less PD than the former. The authors concluded from this that the replicative potential of memory cells was less than that of naive cells and that this might be related to telomere shortening. However, what they actually measured in their experiments was autocrine proliferative capacity, not replicative potential. Autocrine proliferative capacity relies upon the stimulation of growth factor secretion, upregulation of the growth factor receptor and correct signal transduction. As we have shown for culture aged T cell clones (173), exogenous factor-dependent growth of the cells (ie. replicative potential) is retained for a period far greater than the capacity to secrete interleukin 2 (autocrine proliferative potential). It is therefore unlikely that the cessation of growth noted by Weng *et al* ., which was only 10 PD for memory cells and 20 PD for naive cells, actually reflects shortened telomere-triggered blockade of autocrine proliferative capacity, not replicative potential.

Early data showed that telomere length in sperm DNA does not decrease with increasing age of the donor, suggesting that a mechanism for maintaining telomere length may be active in germ cells but not somatic cells (183,186). Such a factor, telomerase, an enzyme responsible for maintaining telomere length in unicellular eukaryotes, was previously found in immortalized human cell lines and tumor cells, but not in normal somatic cells. However, data obtained using more sensitive assays indicate that the presence of telomerase may be more widespread than previously thought (187). Moreover, Hiyama *et al* . (188) have shown that telomerase activity is detectable at very low levels in normal human T and B cells and that it increases greatly after mitogenic stimulation. Because the increase in telomerase activity was transient, it did not prevent the decrease in telomere length during long-term culture (189). Furthermore, the number of donors with telomerase activity detectable in their lymphocytes decreased with age. In the age range 0 - 19 years, all donors were positive, whereas this fraction decreased in the next group (20 - 39 years) before plateauing in the groups 40 - 59, 60 - 79 and 80 years. Therefore, lymphocytes may be unusual among somatic non-transformed cells in expressing telomerase, which is upregulated when they are stimulated to divide. This might represent one prerequisite for enabling T cells to avoid the Hayflick limit. It would therefore be worth testing this hypothesis by measuring telomerase activity in T cell cultures of different ages and different proliferative lifespans. In this respect, Effros´s group has presented preliminary data on the induction of telomerase in cultured T cells. They indeed found that the degree to which either CD4+ or CD8+ cells upregulated telomerase after PHA stimulation was inversely related to the length of time that they had been in culture (R. Effros, personal communication, Decemner 1997). Previous studies demonstrating decreased telomere length in *in vivo* or *in vitro* aged lymphocytes have examined uncloned (heterogeneous) populations. The results of these studies could be reconciled with a unique T cell longevity by hypothesizing 1) that only a small proportion of T cell clones manifest full telomerase function and are effectively immortal (consistent with most studies in the literature) and 2) that the reductions measured had not yet resulted in a critical low repeat number that would activate full telomerase function, as in the study by Weng *et al* . (185) discussed above, in which replicative potential was confused with autocrine proliferative capacity. Further studies will undoubtedly continue to contribute to this area: for example, Monteiro *et al* . (190) reported that telomere lengths in the CD28-negative CD8+ population were shorter than in the CD28+ CD8+ population, and that *in vitro* clonal expansion of CD8 cells is associated with TL shortening.

Telomerase may not be the only factor determining telomere length and cell survival. Strahl & Blackburn reported (191) that inhibitors of retroviral reverse transcriptase (telomerase itself is a specialized cellular reverse transcriptase) could cause progressive telomere shortening of immortalized human lymphoid cell lines *in vitro*. Telomerase activity was present in these lines and its activity was blocked by the agents tested. Telomeres in the blocked lines eventually stabilized and remained short. It was, however, suggested that telomere lengths in lymphoid cells lines (which were unstable even in the absence of inhibitors) are determined both by telomerase and telomerase-independent mechanisms.

8. ALTERATIONS IN T CELL SUBSETS AND MARKERS WITH AGING

Is the senescent phenotype due to increases in memory cells and to their "clonal exhaustion"?

Age-associated changes in T cell subsets occurring in rodents and humans have been repeatedly documented. Mice seem to show a relative loss of CD4 cells with age compared to CD8 cells. Whereas the TCR repertoire of the surviving CD4 cells is said to remain unchanged compared to young cells, the CD8 repertoire is markedly altered, suggesting expansion of a small number of CD8 (regulatory?) cells during aging (192). On the other hand, Rosenberg *et al* . failed to find quantitative differences in $CD4^+$ cells of old mice, but noted a striking decline in the ability of CD4+ cells to cause rejection of allogeneic skin grafts, whereas CD8+ cells retained fully their function as effectors (193). Moreover, the defect in CD4+ cells could not be overcome by treating the cells with soluble T cell helper factors (containing many kinds of cytokines as well as IL 2). The TCR1 repertoire may also change with age, although the meaning of this finding is unclear. Thus, Giachino *et al* . (194) demonstrated that the polyclonal Vδ1 and Vδ2 populations present in children changed to oligoclonal in the elderly (57 - 88 yr). This may reflect "memory" for common antigen. Although not observed by all investigators (195), age-associated changes in T cell

repertoire may apply to human as well, according to reports by Posnett *et al* . (196). Expansion of CD8 cells is common, but it may be the rarer CD4 expansions which are observed at increasing frequency in the aged (197). However, in some disease states, eg. RA, CD4 cells may show striking oligoclonal expansions (198). Whilst not seen in all normal donors, it is interesting to note that such CD4 expansions were also seen in unaffected siblings of RA patients, suggesting that they may be a genetic risk factor for rather than a consequence of RA (198). In $CD8^+$ but not $CD4^+$ T cells, up to >30% of the entire population may consist of oligo- or even monoclonal cells expressing the same TCR-Vß markers (196). Within the $CD8^+$ cells, these oligo- or monoclonal populations are prevalent within the CD28-negative subset (196) and the CD57-positive subset, which often overlaps with the CD28-negatives (199). It is interesting to note that it is this CD28-negative, CD8-positive subpopulation which was identified many years ago as containing suppressor cells (200). These data may explain the observation that alterations in proportions of different T cell subsets may also be more marked in $CD8^+$ than in $CD4^+$ cells of aged humans (201). However, this phenomenon may not be absolutely limited to CD8 cells. Recent data from Thorbeke´s group suggest that although "forbidden" CD4 clones are not present in 24 month-old mice (usually the uppermost limit in mouse aging studies), they do appear in those few mice reaching 30 months of age (202). It was suggested that these potentially self-reactive CD4 cells were derived extra-thymically because thymectomy increased rather than decreased their numbers. There is a considerable literature on extra-thymic T cell development in mice, and evidence for increased development of self-reactive extra-thymic, but not thymic-derived, T cells with age (203). The possibility of enhancing extra-thymic development with factors such as oncostatin-M may offer the opportunity for manipulation of this pathway (204). Moreover, the realization that mature thymus-derived T cells can re-acquire sensitivity to positive and negative selection outside the thymus, in germinal centers (205), indicates in theory the generation and selection of T cells may be effected even the absence of a functional thymus.

There are clearly more $CD4^+$ $CD45RO^+$ (and in mice, $CD44^{hi}$) "memory" cells and less $CD45RA^+$ (and, in mice, $CD44^{lo}$) "naive" cells in PBMC from elderly individuals. Whole blood analyses confirm the relative paucity of CD45RA+ cells in both the CD4 and CD8 populations of the elderly (129). If the $CD45RO^+$ cells represent memory cells, and if exportation of naive $CD45RA^+$ cells from the thymus decreases with age, then an accumulation of $CD45RO^+$ memory cells would be expected in elderly donors. This would be coupled with a predicted reduced ability to respond to new antigens, and a retained ability to respond to recall antigens, as long as the memory cells remained present. Certainly, the proportion of RA^+ cells decreases and RO^+ cells increases with increasing age (206). However, in the oldest old, decreases in memory cell phenotype RO^+ cells have also been recorded (160) and in whole blood analyses, a relative decrease of $CD45RO^+$ cells may also be seen in the CD8 but not in the CD4 population (129). However, in exceptional individuals (healthy centenarians) the decrease of RA^+ cells, especially in the CD8 subset, may be markedly less than in the ordinary old population (207). The meaning of this finding is unclear, because functional tests were not performed, and it is known from other studies that the $CD4^+$ cells responsible for the increase of RO^+ elements express lower levels of CD45RO than do young $CD45RO^+$ cells (208). Whether this is related to their impaired function (114,115) is not yet known (208). More subtle analyses may reveal further differences in surface phenotype and function, which remain to be collated and understood. R. Miller´s group has shown in mice, for example, that aging leads to an increase in the proportion of splenic cells expressing high activity P-glycoprotein (Pgp^{hi}) and therefore able to extrude rhodamine 123. Pgp is known also to participate in the transportation and secretion of cytokines including IL 2, IL 4 and IFN-γ (209). Moreover, mAb against Pgp block IL 2 release in PHA-activated T cells, demonstrating a critical role for this molecule in T cell function (210). Despite this, high Pgp expression in old T cells is linked to dysfunctional status. Possibly the Pgp itself is dysfunctional and over-expression represents an attempted compensation. There is an age-associated increase in the expression of MHC class I molecules on these high-Pgp CD4 memory cells. In spite of this, the levels of TAP1 decrease in old mice (TAP1 transporter is usually required for class I peptide loading). The Pgp and TAP1 molecules are related but whether Pgp is taking over the function of TAP1 in old cells and increasing class I remains an open question (211). The failure of Pgp^{hi} cells to respond to TCR-mediated stimulation cannot be overcome by CD28 signalling, PMA, IL 4 or IL 12 or combinations of these, and may therefore be considered "anergic". Not only do Pgp^{hi} cells fail to secrete IL 2 but also show impaired IL 5 and IL 10 production and proliferation. Oddly, however, their ability to secrete IFN-γ increases with age (212). However, all these data were gathered exclusively in murine systems and it is not known whether human cells behave similarly.

The question of whether antigen-independent functional changes in naive T cells can occur has also been addressed. As discussed above, differentiation of T cells to memory cells coupled with age-related changes in memory cell characteristics may be responsible for much of the altered functional phenotype of the aged individual. Linton *et al* . looked at TCR-transgenic mice with T cell specificity for pigeon cytochrome C antigen, for which they believed they had good evidence for lack of cross-reactivity with environmental antigens. They found that in aged animals, the TCR-transgenic CD4+ cells were decreased in number and in antigen responsiveness but that they maintained a naive cell phenotype. They concluded that the defects observed were therefore due to aging of the naive cells *per se* and not to environmental stimulatory influences (213). Such findings are clearly consistent with several studies

showing different patterns of cytokine production by young and old cells despite posession of the same "naive" phenotype (214).

In human, most T cells are CD7+, but the frequency of CD7-negative cells increases with age (215) and although isolated T cell clones retain stable expression of their CD7-positive or negative phenotype (216), repeated stimulation and propagation of uncloned lines results in accumulation of CD7-negative cells in the CD4 but not CD8 subset (217). Increased proportions of CD7-negative cells are found *in situ*ations of chronic antigenic stimulation *in vivo*, eg. in RA (218) and in kidney transplant recipients (219). Such CD7-negative cells show low proliferative responses to CD3-stimulation, low IL 2 secretion but high IL 4 and IL 10 secretion (220). These results suggest that loss of CD7 expression may be age-associated, but the fact that long-term cultured T cell clones retain high CD7 levels imply that factors other than merely the number of PD undergone are critical for CD7 expression.

Thus far, CD28 is perhaps the closest to a biomarker of aging found for human lymphocytes. Both *in vivo* and *in vitro*, the proportion of $CD28^+$ cells decreases with age. In monoclonal populations, the density of expression of CD28 decreases with age (221). Effros *et al* . were the first to observe a decreasing percentage of CD8 cells carrying CD28 in the elderly, paralleling their observations in T cell lines aging in culture (168). Others have confirmed that particularly the CD8 subset shows progressively decreasing CD28 expression with age (222). There is a correlation between shortening of telomeric repeats and age of the donor, which is not confounded by differences in white blood cell count (223). Moreover, telomere lengths (TL) in the CD28-negative cells were less than in the $CD28^+$ cells from the same donors, implying that the former had undergone more rounds of cell division than the latter (224) (see section 7). This type of proliferative senescence may therefore be responsible for the commonly observed accumulation of CD28-negative oligoclonal populations in elderly people (196). Although originally described only in CD8 cells, the number of individuals with such clonal expansions in both CD4 and CD8 cells was very similar (ca. 70% of individuals over 65); moreover, these expansions were stable over a two-year observation period (225).

In diseases with chronic antigenic stimulation, further circumstantial evidence in favour of the hypothesis of proliferative senescence indicated by downregulated CD28 expression can be garnered. To give some examples: the percentage of $CD28^+$ cells decreases during Chagasic progression (226); both CD4 and CD8 cells show decreased CD28 expression in chronic B lymphocytic leukemia (227); in rheumatoid arthritis, the percentage of CD4 cells carrying CD28 is reduced (228) and in both RA patients and normal controls the $CD4^+$ CD28-negative cells show TCRVB oligoclonality (229); in Crohn´s Disease, the ability of CD28 to mediate costimulation of CD4 cells is compromized (230). These examples suffice to illustrate the range of situations in which T cell proliferative senescence may play a role in modulating immune responses independently of the age of the host. The effects of this kind of "clonal exhaustion" in the elderly may simply be more noticeable than in the young because of thymic involution preventing effective generation of naive T cells and because T cells present in the old may already have undergone many rounds of division.

8.1 Longevity of naive and memory cells

An important question raised here concerns the longevity of naive and memory cells. There is some evidence that memory cells are not quiescent long-lived cells, but represent T cell clones in a constant dynamic state of activation. Beverly´s group used a method for measuring intermitotic time to investigate longevity of CD45RA and RO cells in cancer patients following radiotherapy. Irradiation induced dicentric chromosomal lesions which can be visualized cytogenetically. They found small numbers of $CD45RA^+$ cells with these lesions up to 10 years after irradiation, consistent with the belief that naive T cells can be very long lived. However, CD45RO+ cells with such lesions had all disappeared by one year, suggesting that they had all attempted unsuccessfully to divide, consistent with memory cells being in cycle (158). A later study extended these data to conclude that proliferation rates of naive cells were 8x lower than proliferation rates of memory cells. They estimated that, on average, naive cells divided once every 3.5 years, whereas memory cells divided every 22 weeks (231). However, as these authors themselves pointed out, there are some problems with these data, viz. they were dealing with cancer patients, whose T cells and immune status were not normal; irradiation can have indirect effects like induction of lymphopenia which further disrupts the system; possibly some cells with dicentric lesions can nonetheless divide; and the proportion of cells dying without attempting to divide is unknown. All these factors could lead to underestimates of memory cell longevity (232). A direct approach to whether memory cells continue to cycle *in vivo* has been taken using transgenic mice (233). Naive cells deliberately activated with specific antigen were transfered into athymic hosts in the absence of antigen and found to continue to cycle slowly for extended periods. Naive cells, on the other hand, did not begin to proliferate when transferred into the these recipients. The mechanism for the maintenance of slow cycling of the memory cells is unknown. It may be that unlike naive cells, memory cells do not require antigen to survive and proliferate, but only the self MHC molecule (ie. have a lower functional activation threshold, ref. 234). However, it is clear from these experiments that proliferative senescence could play a role in the eventual loss of the memory cells. The above data in man and mouse have to be reconciled with ealier results in the mouse showing that after thymectomy it is the naive population which disappears rapidly, whereas the memory cells are long-lived (235). The reason for this apparent discrepancy is unknown.

According to our *in vitro* data on T cell longevity, where TCC survive on average 35 PD and maximally 80 PD, this would put the life expectancy of a memory cell at 35 x 22 = 770 wk = 15 years average; 80 x 22 = 176 wk = 34 years maximum (without taking the primary immune response into account, for which the number of cell divisions required can only be guessed at (see ref. 236)). What might be the source of stimulation of the memory cells, which need to persist in the absence of antigen? Several groups have argued that the solution lies in the hypothesis that antigen does persist somehow. This is certainly true for many viruses, but it is unclear for which other antigens it may apply. For viral infection, there is clear evidence that memory T cells are indeed maintained in a constant activated state even for very long periods. Thus, Rehermann *et al* . studied patients up to 23 years after clinical and serologic resolution of HBV infection and still found evidence for recently activated HBV-specific CTL (237). This might well eventually allow enough time for "clonal exhaustion" of the originally responding T cell clones, in the absence of the generation of de novo responses (see calculation above). Data from other chronic infections such as HIV may be consistent with the scenario of clonal expansion leading to clonal exhaustion and lack of replacement by new thymic emigrants eventually resulting in diminution of the repertoire and loss of anti-HIV responses (238). CD8 cells of the same CD28-negative phenotype as seen in HIV-uninfected senescent cultures and possessing similarly short telomeres have also been described in young persons with AIDS, leading to the suggestion that replicative senescence of virus-specific T cell clones *in vivo* might contribute to disease progression (239). A marked decrease in telomere length has also been recorded in CD4, CD8 and B cells from HIV-infected patients with advanced immunodeficiency, supporting the notion of a high turnover of these cells and suggesting that replicative senescence may be involved in the final immunosuppression of these patients (240). There is also an increase in $CD4^+$ CD28-negative cells in HIV infection, albeit not so marked as in the CD8 subset (241). Indeed, disease progression in AIDS is reported to be marked by an accumulation of CD28-negative cells unable to secrete IL 2, whereas long-term non-progressor patients maintain CD28 levels and IL 2 secretion capacity (242). The rate of destruction of HIV-infected cells in young and old patients seems similar, leading to the suggestion that the elderly cannot replace CD4 cells as rapidly as the young (243). It has been found that T cell responses in HIV patients are characterized by severe TCRVB biases and clonal expansions in CD4 cells, and that such responses are exaggerated with disease progression (244). Despite this, others have found no evidence for increased CD4 turnover in HIV infection on the basis of lack of truncation of telomere length in CD4 cells during progressive disease, although this was clearly confirmed in CD8 cells (245). However, virally infected cells may conceivably express dysregulated telomerase. Independent evidence for enhanced cycling of CD4 cells in HIV derives from measurements of mutations in the hprt locus, which showed that mutation rates in the CD8 and CD4 cells were similarly high (246), consistent with an increased division rate in both subsets. The cytokine secretion profile of mutant CD4 clones (from healthy or HIV patients) was predominantly Th2-like, whereas the CD8 mutants had the same pattern as wild-type.

For non-viral antigens, and thymus-independent regeneration of T cells in general,it is probable that antigen-driven peripheral expansion commonly occurs (247), implying that a finite proliferative lifespan of the T cells would be of critical import for the functional intergrity of the immune system. Experimental data have implicated a sort of TCR cross-reactivity responsible for maintaining numbers of T cells, eg. in the case of CD4 cells via class II molecules regardless of their peptide loading (248).

8.2 Activation-induced cell death and aging

Further light has been cast on the aging of human $CD4^+$ T cells using the mAb PD7/26 specific for the CD45RB isoform. Salmon *et al* (249) reported that whereas CD45RA expression is lost rapidly after activation of naive cells *in vitro*, loss of RB expression is gradual, occurring over many cell cycles, and reciprocating the increase in RO expression. The progressive shift from RB^{hi+} to RB^{lo+} is paralleled by gradual loss of bcl-2 (which protects against apoptosis) and acquisition of fas (which can mediate apoptosis), as well as gradual loss of ability to secrete enough IL 2 to maintain autocrine proliferation (whereas IL 4 secretion remains intact). This eventually results in the cells becoming dependent on exogenous IL 2 not only for growth but for their survival, because without enough IL 2 they undergo apoptosis. Longevity extension for T cell clones might therefore be achievable by upregulation of bcl-2. However, suppression of apoptosis by bcl-2 or bcl-2 family-member bcl-x(L) results in enhanced radiation-induced mutagenesis, consistent with the original isolation of bcl-2 as an oncogene (250).

The first report describing the behavior of CD45RB *in vivo* in elderly humans confirmed that $CD4^+$ cells in old donors showed significantly decreased 45RB expression (251). In agreement with the *in vitro* data, the percentage of human CD4 and CD8 fas+ cells also increases with aging *in vivo* (252,253). The majority of these express CD45RO, and CD25 and CD69 activation markers (254). Parallel to this, the amount of soluble fas in the blood of elderly donors is significantly increased compared to young donors (255). Moreover, Phelouzat *et al* . (256) have shown increased CD3-mediated AICD and hence decreased proliferation in the elderly. This was found not to be due to IL 2 deprivation, nor was it associated with decreased bcl-2 expression (256). There is a possibility that increased AICD might be associated with decreased levels of IL 6 because IL 6 has been reported to protect neonatal T cells from AICD (257). However, IL 6 production seems not to be decreased in older individuals, as discussed above. Consistent with the

findings of increased susceptibility to AICD ex vivo, T cell clones aged in culture also become increasingly susceptible to AICD; moreover, T cell lines derived from old donors become more susceptible more rapidly than those derived from young donors (258). In mice *in vivo*, evidence for increased apoptotic death of superantigen-stimulated T cells has been forthcoming (259). In humans, similar phenomena may be reflected in pathological states such as chronic phase HIV infection, where the fraction of apoptotic cells is greatly increased, especially amongst those with an activated (CD45RO+, DR+, Fas+, CD38+) phenotype, suggesting that chronic stimulation leads to clonal exhaustion by increased susceptiblity to AICD (260). Consistent with this is the finding that *in vitro* anti-oxidant treatment, which can inhibit AICD, can to some extent restore the proliferative defect of HIV-infected CD4 cells (261). Also consistent with the idea of clonal exhaustion, monitoring HIV-infected individuals for strength and breadth of proliferative responses to HIV peptides revealed that patients with weaker responses progressed more slowly than those with higher responses (262). This could be interpreted to mean that stronger proliferative responses, while neuroprotective (262), result in more rapid clonal exhaustion and therefore disease progression.

However, the idea that increased levels of AICD are detrimental to functioning of the immune system must be reconciled with data from several sources suggesting an age-associated increase in resistance to apoptosis on the part of cells from various tissues including lymphocytes. Thus, Zhou *et al* . (263) generated fas transgenic mice and compared immunological status of young and old transgenics with wild-type littermates. They found that fas expression (like the expression of some other receptors in the same family, eg. TNF-R) and fas-induced apoptosis was decreased in old wt mice, but not old transgenics. In old wt mice, there was an increase in CD44+ fas-negative cells, decrease in autocrine proliferation, decreased IL 2 production and increased IL 4 and IL 10 production. In the transgenics these changes were not found. Even age-related thymic involution was prevented in the fas-transgenics. It was therefore suggested that some of the manifestations of aging on the immune system were related to downregulated apoptosis (263). However, the lifespans of the transgenics were not increased and this seemed to be associated with enhanced production of IL 6 and other factors in these mice (264). How might the transgenic fas expression exert these effects? Perhaps by removing defective cells by apoptosis and making room for fresh cells? More likely may be the alternative function of fas, ie. that of lymphocyte stimulation rather than killing. Thus, low to intermediate fas expression (and ligation) results in apoptosis, whereas high level expression can protect against cell death (265), and thereby result in enhanced responsiveness. Spaulding *et al* . (266) provided evidence partly consistent with that of Zhou *et al* . in normal mice, where they demonstrated that T cell apoptosis induced by irradiation, heat shock or CD3-stimulation was reduced in old compared to young mice, unless the former had been maintained on a calorically restricted diet. Polyak *et al*. 267) also reported higher levels of *in vivo* and *in vitro* lymphocyte apoptosis after irradiation in young compared to old mice. Some further supporting data for the concept of decreased apoptosis in aged cells may be found in the report of Lechner *et al* . (258) who found decreased inducibility of CD95 after CD3-stimulation of old persons´ T cells compared to young. However, as already discussed above, susceptibility to AICD of T cell lines established from old donors was greater than those from young donors (258). Other tissues may also show increasing susceptibility to apoptosis with age, as is the case with hepatocytes (268). Here, caloric restriction also reverses the age-associated effects and reduced apoptosis, the opposite of its effect on lymphocytes.

9. CLINICAL RELEVANCE OF IMMUNOSENESCENCE

Decreased T cell function in the elderly is shown most clearly *in vivo* by DTH tests to recall antigens (269) as well as to clinically relevant immunization procedures where T cell-dependent antibody production is depressed, eg. see refs. (270,271). Antibody responses following primary immunizations in the elderly are often reported to be decreased. However, immune responsiveness to the primary antigen Helix pomatia haemocyanin (HPH) was retained in healthy elderly SENIEURS (272). By contrast, elderly subjects not fulfilling the SENIEUR criteria had a decreased immune responsiveness to HPH.

Vaccination may also have a less long-lasting effect in older donors (273). Responses to secondary antigens may normalize after boosting in elderly donors, but the improved response is not sustained for the same duration as in the young (274). Consistent with this, the majority (62%) of elderly individuals vaccinated with tetanus had antibodies to tetanus up to 10 years after vaccination, but this halved (to 33%) for vaccination more than 10 years previously. In contrast, almost all young donors retained tetanus antibodies even > 10 years after vaccination (275).

There may be more subtle differences between the responses of young and old individuals, such as a selective impairment of particular classes of antibody production, for example, of IgG1 responses to inactivated influenza virus vaccine in the elderly (276,277). This possibly reflects less efficient or altered T cell help. This shift in immunoglobulin (sub)class distribution may be a reflection of altered cytokine patterns. The response to influenza vaccination may also be highly dependent on the ´flu strain involved, as several authors have shown (278-282). They found that while the majority of elderly people responded to strains of the influenza A H3N2 subtype, few responded to strains of the influenza A H1N1 subtype (278-282). These findings are probably due to pre-exposure of the elderly and young to different strains of ´flu viruses (283).

The elderly may have lower IL-2 production following *in vitro* stimulation with ´flu vaccine as was shown by McElhaney *et al* . (284). Subcutaneous low-dose IL 2 treatment prior to vaccination has been reported to enhance protection of the elderly to ´flu (285). Similar findings concerning antibody titer (but not lymphoproliferative responses) have been reported for tetanus toxoid vaccination (286). Another study has examined IgG responses of the elderly to pneumococcal vaccination and found no decrease in the old 4 - 6 weeks after vaccination with Streptococcus pneumoniae (287). Another recent study of the efficacy of ´flu vaccinations in the elderly found that annually repeated vaccination resulted in an improvement of humoral responses to several virus strains, rather than a decrease that might be predicted from clonal senescence (288). Thus, it may also be the case that in those studies where poorer responses of the elderly to vaccination were observed, this may reflect their state of health and consumption of medication more than anything else. This was illustrated in a recent study on ´flu vaccination, where response was correlated with the well-being of the vaccinees as assessed according to activities of daily living (ADL) scale (289) and in a study on responses to hepatitis B vaccination (290). However, it could also be that the 4 - 6 week period examined after immunization is not long enough to be informative. For example, old mice did not show a decline in antibody responses after an immunization with Streptococcus pneumoniae, but nonetheless, after a second immunization, the old mice showed a marked decrease in antibody production compared to the young. This was traced to a defective function of $CD4^+$ T cells (291).

However, despite these findings, it remains the case that the precise clinical relevance of T cell immunosenescence is hard to define. Indeed, there are studies suggesting that it is the NK status of subjects which is critical; Ogata *et al* . recently reported that not the numbers but the functional activity of NK cells was the only parameter correlating with death (due to infection) in the follow-up period (292). However, they did not test T cell function, only numbers. Inclusion of T cell functional parameters has been shown to predict mortality in a Swedish prospective study (8). In mice, there are several models where age-associated alterations in immune responsiveness correlate with a decreased ability to cope with infection, eg. by trypanosomes (293). In human, Varicella zoster reactivation is commonly quoted in support of the relevance of immunosenescence, and specific abnormalities in anti-viral immunity have been distinguished in the elderly in some studies (294). For example, the well-known age-associated increased incidence of shingles in the elderly is associated with a decrease in the frequency of VSV-specific T cells which produce IFN-γ and decreased amounts secreted compared to young immune donors. On the other hand, the production of IL 4 in the same donors was unchanged (295). Correspondingly, antibody levels to VSV are maintained in the elderly, but this is clearly not always enough to prevent reactivation of infection. These data suggest that the general state of health is not the only factor contributing to depressed immunity. On the other hand, the similarity of some infection- or cancer-induced and aging-induced changes can be striking. Moreover, these changes may be additive, resulting in severe clinical manifestations. Thus, Utsuyama *et al* . (293) reported that Trypanosoma musculi-infected mice exhibited a rapid change in the $CD4^+$ population in the direction of memory/activation with decreased cytokine production; this was associated with clearing of infection; however, old mice already having this phenotype were barely able or unable to clear the infection.

Autopsy data on the very old suggest that the accepted prime age-associated causes of death in the "younger old" (ie. cardiovascular disease, cancer) do not necessarily apply to the very old. Studies from Leiden, Geneva and Tokyo have found the prime cause of death to be infectious disease in the over 80´s (however, whether these were really opportunistic infections is still not clear). An extensive study on major causes of death in Japan between 1951 and 1990 suggests that unlike those causes showing deceleration or neutrality with advancing age, those showing acceleration in old age (ie. pneumonia, influenza, gastroenteritis, bronchitis) mostly involve infectious agents (296). According to some data, a major predictor of mortality in the elderly is lung function (297). Immunosenescence may also play an important role here, since the defense of this most common pathogen entry portal is critical. In support of this concept, Meyer *et al* . (298) have provided evidence for immune dysregulation in the aging human lung. On the other hand, allergic reactions may decrease with age for the same reason, as with the well-known "growing out" of asthma. In a rat model of asthma, it was found that the level of specific IgE antibody and eosinophils in bronchoalveolar lavage was markedly higher in young rats. This correlated with increased interferon-γ production and decreased IL 5 in old rats T cells (299).

10. PREDICTORS OF MORTALITY AND LONGEVITY

Lifespan is influenced by genetic makeup, most clearly demonstrated so far for the association between longevity and MHC type in mice (300) and possibly humans (301-303). One mechanism accounting for this association may be a relationship between MHC alleles and rate of thymic involution (304). The lifespan of allophenic mice produced from a long-lived and a short-lived strain (305) is positively correlated with the proportion of lymphocytes derived from the long-lived strain, showing the importance of the immune system to overall lifespan (306), perhaps via the effect of mature T cells on maintenance of the thymic environment alluded to above. The association may be by way of susceptibility to lymphomas: it has been shown that mice which have higher levels of memory cells, lower levels of naive cells and lower proliferative responses at 6 months of age retain these patterns in later life, and that in

genetically heterogeneous populations, mice with this phenotype have significantly shorter lifespans caused by an increased incidence of lymphomas (307). However, more recent data indicate that it may not be that death is caused exclusively by lymphomas in this system, because the CD4 memory correlation holds regardless of whether the mice died of lymphoma, fibrosarcoma, mammary carcinoma or other terminal disease (308). In inbred mouse strains, the vigor of T cell responses in old mice is influenced by MHC type, with those mice possessing "low responder" phenotypes succumbing at an earlier age than those with "high responder" phenotypes, mostly due to their increased susceptibility to lymphomas (309). Increased levels of IL 6 in old mice may play a role in the increased occurrence of lymphoma (310). Therefore, the mechanism of the genetic association of MHC with lifespan in mice may be a reflection of decreased immunosurveillance against lymphoma or other tumors, as a result of immunosenescence. Mice genetically selected for high antibody responses are found to have longer lifespans, and this is also associated with lower incidence of lymphoma (311). On the other hand, mice selected for high or low T cell responses to PHA also have lower and higher lymphoma incidences respectively, but do not differ in longevity; whereas mice selected for resistance to chemical carcinogenesis show altered tumor incidence and longevity without corresponding alterations in immunity (311). What these results may mean in longer-lived, less tumor-prone species like man is unclear.

There are certainly also other genetic influences on immune responsiveness, upon which the effects of aging may be superimposed, that must be taken into account. For example, the CD4:CD8 ratio, which is also affected by age, is different in males and females and in family segregation analysis has been shown to be under genetic control (312). The MHC type of the individual may also affect the absolute numbers of lymphocytes in the periphery; for example, it has been reported that HLA-B8, DR3-haplotype-bearing donors have lower levels of peripheral lymphocytes than other donors, possibly because of increased levels of spontaneous apoptosis (313). It may be interesting to note that this HLA-B8, DR3 haplotype was one of those found very rarely in a recent study of elderly Greeks (in 1.6% of SENIEUR elderly versus 10% of young controls, ref. 303). Several groups are searching for genetic markers of longevity, and some data are now beginning to appear, eg. the finding that a particular allele, C2, of the proto-oncogene transcription factor ETS-1 is represented at significantly higher levels in the elderly compared to teenagers (314). The relevance to mammalian aging of the recently defined mutations in "gerontogenes" in roundworms and other invertebrate species is unclear, but will be an exciting area of investigation (315).

In most aging studies in human, individuals > 60 years are commonly considered "old". Longitudinal studies are required to establish the critical changes within the immune system and what may be associated with "healthy aging". Nowhere is this clearer than in the study of centenarians, who may be considered to be that very small proportion of successfully aged individuals. An examination of healthy centenarians would show whether immunological aging is divorced from overall physiological aging. Were the immune system to be senescent despite health in these individuals, this would indicate the lack of importance of immunity for healthy aging. Not surprisingly, healthy centenarians are found to have well-preserved immune functions, much more similar to the "young" immune system than the average situation for less extremely old donors. Thus, as summarized by Franceschi (9), T cell proliferative responses are well-maintained (albeit taking place more slowly), the T cell repertoire still contains all Vß families, hematopoiesis is maintained, autoantibodies are absent, and interestingly, there is a high level of lymphocyte genomic stability (low spontaneous breaks etc., which otherwise are thought to increase with age in average, non-centenarian, donors (316). There were some data suggesting that healthy centenarians represented a group with the best retention of thymic structure and function and that these individuals were also characterized by lower DNA damage (52).

11. POSSIBLE APPROACHES TO INTERVENTIONIST MANIPULATIONS

11.1 Vitamins and minerals

Immunogerontological parameters may be affected by many outside influences rather than aging *per se*, particularly if donors are not selected for perfect health using strict clinical criteria. Some of these factors may be subject to manipulation. For example, much attention has been paid recently to the effects of exercise on immune function in the elderly, although details of the mechanisms involved in any observed improvement in immune function are completely unknown (317). It is difficult to dissect the effects of the many different interacting and confounding factors, including health status, nutritional status, psychological status etc., which overlay a background genetic influence. However, some factors are easily manipulated and have encouraged interventionist approaches simply because these are feasible. Even in carefully selected donors, for example, nutritional status may play a significant role in exacerbating immunological differences (318). Dietary supplementation studies are relatively easy to perform and several including immunological data have been reported. For example, vitamin C supplementation was reported to enhance the mitogenic responses of lymphocytes from elderly people (319) and Vitamin E supplementation has been known for some time to enhance lymphocyte proliferative responses and IL 2 production *in vitro* and DTH *in vivo* in elderly people (320). This correlated with a decline in prostaglandin E_2 (PGE_2) synthesis, which is known to increase with aging. The immunosuppressive effects of PGE_2 are predominantly mediated by increasing cAMP levels; therefore agents which decrease cAMP levels might also be expected to

enhance lymphocyte responses. These may include insulin and chromium (321). Unlike a number of other proposed factors, the benefits of vitamin E supplementation have been subjected to vigorous scientific testing. A recent substantial study concluded that vitamin E supplementation for 4 months improved a number of clinically-relevant indices of cell-mediated immunity in the healthy elderly, including DTH and antibody responses to hepatitis B and tetanus vaccines but without increasing autoantibody titers (322). In mice, some data indicate that certain senescence-associated alterations which can be measured on T cells are prevented by *in vivo* treatment of mice with the anti-oxidant vitamin E (323). Thus, vitamin E supplementation prevented the observed age-related decline of anion transport by lymphocytes in mice and inhibited the generation of the "senescent cell antigen" (SCA) from the anion transporter "band 3". Prevention or delaying of band 3 aging and subsequent generation of SCA has the consequence that the lymphocytes are not eliminated from the system via SCA-mediated interactions with the reticuloendothelial system. By analogy with the mouse, vitamin E supplementation might be expected to have a greater impact on the old than the young; thus, in a mouse influenza model, high-dose vitamin E significantly enhanced lung virus clearance in old mice, with little effect on young mice. However, it is not known whether these effects of vitamin E are attributable to its anti-oxidant or some other function.

Another supplement commonly believed to enhance immunity is often taken along with other factors, namely ß-carotene. However, results of two careful recent studies do not support immunoenhancing effects of either short (3 weeks) or long-term (up to 12 years) ß-carotene supplementation in randomized double-blind placebo-controlled longitudinal comparisons in human. There were no pre- to post-intervention changes measured in DTH, lymphocyte proliferation, IL 2 production, PGE2-production or lymphocyte subset composition (324).

Not only vitamin- but also trace element-deficiencies in the elderly may contribute to immunodeficiency. For example, levels of selenium decrease in rat lymphoid tissues with increasing age (325) and selenium supplementation has been reported to reverse low levels of proliferation and CTL generation in aged mice (326). In elderly people, selenium supplementation was reported to enhance lymphocyte proliferative responses to pokeweed mitogen (PWM) (327). Moreover, it is well established that the availability of certain essential micronutrients decreases with age; one very important such factor may be zinc (328,329). Zinc is necessary for the function of many hormones and enzymes, including those known to affect immune responses (eg. testosterone, ref. 330)). This has practical implications, because even if absorption is compromized in the elderly, sufficient supplementation might still overcome the problem (331). In rats, there is also a decrease in serum zinc, and the levels found in the thymus are lower compared to young animals as well (332). These observations on zinc levels may not be limited to rodents, because in humans, zinc supplementation studies have indeed suggested improvement of some parameters of immune function (333,334). One reason for this may be the zinc-enhancement of otherwise age-associated lowered levels of interferon-alpha production in the aged (335). Experimental zinc depletion and repletion of healthy humans revealed that secretion of the Th1-type cytokines IFN-γ and IL 2 was decreased during zinc deficiency, whereas Th2-type cytokines (IL 4, IL 6, IL 10) were not affected (336). In animals, Mocchegiani *et al* . (337) confirmed that oral zinc supplementation resulted in a recovery of thymic function (and also demonstrated its influence on extrathymic T cell differentiation pathways (338)) and showed that thymic regrowth was associated with a partial reconstitution of peripheral immune function (as measured by mitogen stimulated proliferation and NK activity). Moreover, low levels of activity of the zinc-dependent hormone thymulin were not dependent on the state of the thymus itself, but on decreased zinc saturation of the synthesized hormone. The authors concluded that age-dependent thymic involution and compromized thymic hormone function were not preprogrammed but were caused by the decreased availability of zinc. In this context it is interesting to note the claim that the beneficial effects of melatonin supplementation or pineal grafting are associated with increased plasma zinc levels in old mice in the absence of exogenous zinc supplementation (339) (although melatonin may have direct effects on lymphocytes, which express mRNA for melatonin receptors (340)). It is argued by Fabris *et al* . that the common pathway of several life-extending endocrinological manipulations is in fact via zinc bioavailability (341). Thus, even such a well-established concept as the inevitability of the age-associated process of thymic involution and the resulting perturbation of T cell generation may not be immutable. However, the beneficial effects of zinc supplementation are controversial and others have found no benefit of zinc replacement even in elderly populations confirmed to show serum zinc deficiency (129,342). In some studies, even inhibitory effects of zinc supplementation have been reported (343). It has also been found that the degree of decrease of lymphoproliferative responses observed in the elderly does not correlate with decreased levels of plasma zinc (or vitamin E, retinol or ß-carotene) (344). The situation is therefore not yet clarified.

11.2 Hormones

Aging of the immune system will most likely affect other organ systems and eventually impact upon the lifespan of the individual (345). Manipulations said to increase lifespan of mice by injecting melatonin or by transplanting pineal glands are accompanied by maintenance of T cell immune responsiveness (as measured by DTH) and prevention of thymic involution (346). Melatonin may have direct effects on CD4 but not CD8 cells because of a direct effect on gene regulation via binding the putative nuclear melatonin receptor (347). *In vivo* treatment with melatonin is reported not to reconstitute age-associated impairment of NK activity or lymphoproliferative responses in mice (348). Consistent with these results *in vitro* supplementation also failed to reconstitute proliferation or IL 2 production in old rat cells (349). It is therefore unclear whether

and how melatonin extends lifespan.

Age-associated changes in secretion of growth hormone (GH) and related hormones, releasing factors and binding factors may contribute to immunosenescence. Thus, GH substitution may reverse some immune defects in humans and primates, as reviewed in (350). Administration of low-dose GH to elderly adults for 6 months resulted in an increase in IGF-1 levels (which are reduced in aging) and an improvement in some physiological parameters, such as muscle strength. Immunological parameters were not reported. The same considerations may apply to other factors, eg. the native steroid dehydroepiandrosterone (DHEA), which, like most steroids, has immunomodulating activity. Whereas levels of cortisol increase with age in both men and women (351), in general the levels of DHEA decline with age (352). However, longitudinal studies suggest a great deal of inter-individual variation, and can even show age-associated increases in DHEA levels in a sizeable proportion of the population (353). It has been suggested that decreases of DHEA could be associated in some way with immunosenescence, because treatment of old mice with DHEA augments the otherwise decreased capacity of T cells to produce IL 2 and IFN-γ. It also decreases the spontaneous secretion of IL 6 (354) and IL 10 observed in old mice and reverses their hypersensitivity to endotoxin-stimulated release of both IL 6 and IL 10 (355). Analogously, it prevents the retrovirus-induced increased IL 6 and IL 10 secretion seen in old mice, prevents decreases of IL 2 and IFN-γ production and enhances their T and B cell proliferative responses (356). DHEA reverses the senescent phenotype (as defined by the pattern of cytokine secretion) in mice and enhances the effects of vaccination of old mice to hepatitis B (357). In man, DHEA also enhances IL 2 production (358) and supplementation trials have been carried out (eg. see ref. 359), but there are few immunological studies. Khorram *et al* . (360) found that DHEA administration resulted in a significant augmentation of serum IGF-1 and decreased IGFBP-1, which may contribute to immune enhancement. They also found an increase of monocytes during treatment, as well as increases in mitogenic responses of both T cells and B cells. The numbers and activity of NK cells were also enhanced.

In contrast to DHEA, dihydrotestosterone (DHT) downregulates IL 4, IL 5 and IFN-γ production but does not affect IL 2 (361). DHT levels also decrease with age, and a recent cross-sectional study found that bioavailable testesterone correlated best with significantly age-associated cognitive and physical parameters (362). However, a recent longitudinal study found no correlation between entry-point testosterone levels and death rates over the 15 year follow-up period in 77 men (363). Together, DHEA and DHT supplementation alter the cytokine profile of old mice such that it again resembles that of young mice; such an activity could be measured *in vivo* as well as *in vitro* (361). Exogenous hormone supplementation might correct age-associated defects insofar as these are dependent upon cytokine profiles. This has been tested in a mouse model of influenza virus vaccination. Danenberg *et al* . (364) reported that DHEA supplementation resulted in a reversal of the age-associated decline in immune responsiveness in mice, reflected by increased humoral responses in treated mice and increased resistance to challenge with live virus. In another study, Ravaglia *et al* . (365) reported on the relationship between DHEA levels and health in free-living people over the age of 90. They found five-fold lower levels of DHEA in both males and females aged 90-106, compared to young controls. Thus, even "successfully" aged persons had greatly reduced levels, leading to the question of whether this matters. Ravaglia *et al* . demonstrated that it can matter, because within the old male group, the level of DHEA correlated with their health, as measured on the ADL scale. On the other hand, DHEA levels are clearly reduced in the aged although the degree of reduction fails to correlate with health status as assessed by the strict SENIEUR protocol (366). A supplementation trial to assess the effects of DHEA on responses to tetanus and influenza vaccination in man did not yield as dramatic results as seen in mice (367): there was a trend toward increased antibody titers to influenza but not tetanus, and even this failed to reach significance (367). Danenberg *et al* . even reported a decrease in attainment of protective antibody titer in elderly volunteers given DHEA in a prospective randomized placebo-controlled double-blind study of the effects of DHEA on influenza vaccination (368). Thus, the decreased ´flu response in elderly humans, unlike that in mice, could not be reversed by DHEA, and a higher baseline level of DHEA was also not found to be predictive of better ´flu vaccination outcome (368).

Given the known or suspected interactions between the endocrinological and immunological systems and the well-established impact of sex and other hormones on immune responses, it is perhaps surprising that few studies have addressed the question not only of gender differences but also the effects of pregnancies on immunosenescence. Some investigators have begun to approach this by surveying leukocyte subsets in mice of varied gynecological histories. One such study concluded that both gender and pregnancies affect the age-related distribution of lymphoid and macrophage populations in the spleens of C57Bl/6 mice, for example (369).

11.3 Anti-oxidants

Thus far, there are few studies with SENIEUR aged human T cells aimed at clarifying the mechanisms of reduced function. It is important that donors selected according to standardized health criteria should be entered into immunogerontological studies, in an attempt to exclude alterations in immune parameters caused by underlying pathology rather than aging per se. The SENIEUR protocol is a strict donor selection procedure whereby only a small fraction of the elderly are classified as perfectly healthy. It has been established that the reduced proliferative responses stimulated by phytohaemagglutinin (PHA) are still observed even in perfectly healthy SENIEUR donors, although it has been reported that the response to immobilized CD3 mAb may not be reduced (370). The reason for this discrepancy is unclear. However, direct biochemical signalling with phorbol esther and ionomycin may also result in reduced responses in elderly donors, suggesting that even if signal transduction and second messenger production proceed normally in aged T cells, downstream events may still be dysregulated (371). In mice, T

cells from old animals stimulated by CD3 + CD4 ligation mobilize less calcium ions than T cells from young animals. They also perform less tyrosine phosphorylation of phospholipase C gamma 1 and other phosphoproteins. Moreover, these events appear to be sensitive to anti-oxidant levels, such that Grossmann *et al* . suggested that one reason for decreased PLC gamma-1-dependent signalling was the decrease in antioxidant levels in old cells in rats (372). The general importance of anti-oxidant systems is illustrated by the report that although resting young and old rat splenocytes did not differ in their content of the important anti-oxidant reduced glutathione, in proliferating cells from old animals, the expected increase in glutathione was delayed. This correlated with an increasing number of cells showing evidence of mitochondrial dysfunction in terms of depolarized membrane potential and decreased mitochondrial mass. Impairment was completely prevented by addition of extra glutathione to the medium (373). An *in vivo* relevance for these findings is suggested by the fact that reduced, but not oxidized, glutathione levels in the plasma are decreased in elderly compared to young donors (374). In mice, however, the age-associated reduction of GSH levels did not correlate with increased susceptibility of lymphocytes to oxidative damage. This was found to be due to a predominance of memory cells in the aged animals and the fact that memory cells, despite lower GSH, were more resistant (in young and old mice) to oxidative damage (375). Although it has proven difficult, as exemplifed above, to show age-related decreases in enzymatic anti-oxidant defences, most studies have concentrated on assaying levels of known anti-oxidants, rather than on measuring anti-oxidant function. A recent study investigating the latter in human plasma did provide evidence consistent with decrease with age, but only in males and then only over the age 74 (376). Another recent study confirmed decreased antioxidant activity in aged rat plasma but failed to show the same in man (377). Despite the above data, interventionist approaches with antioxidants remain attractive because of their cheapness and easiness.

11.4 Caloric restriction

Many studies have examined some aspects of the biochemical changes associated with T cell activation and their alteration with aging. In some animal models other than mouse, results comparable to those in humans have been obtained; thus, in rhesus monkeys, $CD4^+$ cells from old donors respond less well to CD3-stimulation, and this is partly associated with a decreased frequency of responding cells and is reflected in lower calcium-mobilization in old cells (378). The same investigators also reported that one of the major strategies to prolong rodent life, which is associated with improved immune function, namely caloric restriction (CR), did not alter the depressed calcium-mobilization rates in old monkeys. It did, however, retard the marked age-associated decline of DHEA levels in rhesus monkeys. It also ameliorated the levels of lipid peroxidation of lymphocytes, supporting the view that CR effects are at least partly mediated through reduced free-radical damage (379). Moreover, CR reduced levels of a marker of oxidative DNA damage in old rats (380). Limited biochemical studies employing SENIEUR donors have indicated that membrane lipid alterations in the elderly may be important for altered immunological function (381). Rather than the membrane lipid constitution per se that was different between young and old, it was the changes observed upon blastic transformation of stimulated lymphocytes which correlated with decreased proliferative function. CR has multiple effects which are only now being elucidated, eg. some data show that old CR mice retain better GH receptor function than old ad libitum-fed mice (382). A major mechanism may be via the lowering of nutritionally-driven insulin exposure which lowers overall growth factor exposure (383). One relatively clear finding in monkeys is that CR reduced body temperature, as a result of decreasing energy expenditure, consistent with the "rate of living" theory of aging (384). Further evidence that any longevity enhancement by CR in rhesus monkeys is not correlated with improvement of immune responses stems from the study of Roecker et al . (385). They showed that mitogen-stimulated proliferation, NK activity, and antibody production were all reduced in CR monkeys compared to controls, with no effects discernible on cell number or surface markers. On the other hand, in a long-lived rat strain, CR clearly resulted in improved T cell proliferation after mitogenic stimulation. This, and the cytokine (higher IL 2, lower IL 6 and TNF-α) and surface marker (higher OX-22) profiles of the T cells suggested that the CR animals had a higher fraction of "naive" cells compared to the controls with more "memory" cells (386). However, in two other rat strains, Konno (387) had shown accelerated thymic involution in CR animals, and either a slight decrease or no change in immune function. This suggests that the genetic background has a major impact on the effects of CR. In mice as well, CR results in decreases in the otherwise age-associated increased constitutive serum levels of IL 6 and TNF-α (388). Of course, CR may have many physiological effects which are nothing to do with immunity, for example, CR slows down the age-related increase in mutations (389), perhaps via effects on DNA polymerase-alpha (390).

11.5 Mutations and DNA repair

Mechanisms for recognition of DNA damage and its repair are important in maintaining cellular integrity. If these are reduced during aging, this would also contribute to failing function. Manipulations to enhance repair might therefore offer some benefits. Boerrigter et al . (391) found that the rate of disappearance of a particular kind of chemically-induced DNA damage was age-dependent in mice, and also varied between strains, with longer-lived strains having better damage repair capacity than shorter-lived strains of the same age. Cortopassi & Wang summarized various publications recently to survey agreement on rates of DNA repair in different species and the correlation between repair and maximal lifespan (392).

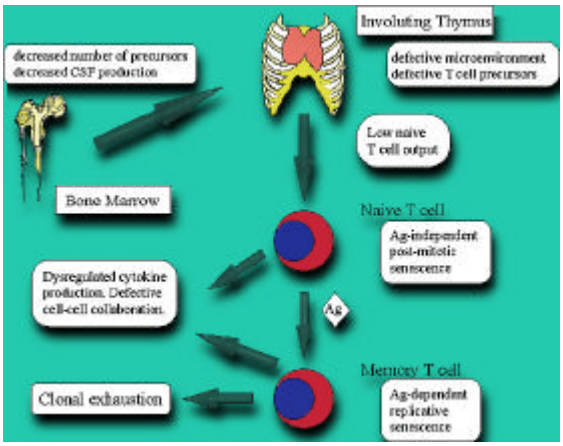

Figure 1. Senescence affects T cell differentiation and function at different levels. First, there is a decreased number of bone marrow precursors migrating to the thymus. In the involuting thymus the function of both the thymocytes and the cells from the microenvironment is compromized in the ability to support T cell differentiation, resulting in a decreased output of new naive T cells in the elderly. Long lived naive T cells suffer antigen-independent post-mitotic senescence even in their quiescent state. T cells which have been activated and become memory cells are maintained in a proliferative state and are, therefore, subjected to replicative senescence and clonal exhaustion. These changes result in a defective capacity of T cells to collaborate in other aspects of the immune response, hematopoiesis and lymphopoiesis.

They concluded that large differences in DNA repair capacity were found in different species and that the correlation between maximal lifespan and repair was indeed good, although not excellent. Moreover, DNA repair capacity within a particular species may correlate with age of the individual. Thus, there is an age-related decrease in post-UV-irradiation DNA repair capacity in cultured skin fibroblasts from normal human donors, estimated at -0.6% per year up to the age of > 90 years (393). Moreover, the same group estimated a corresponding increase in mutability of DNA in B cell lines from these donors of + 0.6% per year (393), suggesting that DNA repair decrease with age and this correlates with increased mutability. An underlying mechanism responsible for changes in DNA repair with aging may be decreased expression and function of DNA topoisomerase I, an enzyme that alters the superhelicity of DNA (394).

In T cells, studies of mutations revealed that background mutant frequency (MF) at the hprt locus increases with age up to advanced middle age (316). In parallel with the age-related increase in MF, there was an age-related decrease in DNA repair capacity against hydrogen peroxide-induced DNA damage (395). However, MF induced by X-rays was not increased with age, leading to the conclusion that in human T cells aging does not affect the cytotoxic or mutagenic effects of X-rays (396). If confirmed, this is significant, because it suggests that decreased effíciency of DNA repair may not be the sole reason for age-associated MF increases. However, when older aged individuals were examined, an increase in basal levels of DNA damage in lymphocytes from donors 75-80 years old compared to those 35-39 was no longer found. There was also no significant difference between frequency of mutation at the hprt locus in the young and more aged populations, nor was there any difference in DNA repair capacity after hydrogen peroxide-induced DNA damage (397). These findings, together with the increased levels of anti-oxidants glutathione peroxidase, catalase and ceruloplasmin in the elderly, suggest that those individuals with best retention of DNA repair mechanisms and anti-oxidant defences belong to the group with extended longevity.

It has been argued that one of the unifying factors shared by life-extension manipulations and mutations is the adjustment of the organism to low levels of stress. It is hypothesized that activation of stress-protective mechanisms early in life may result in their better function later in life and that stress-resistance is a determining factor of longevity (315). In man, senescent T cells do show a reduced stress response as reflected by decreased production of hsp70 after heat shock, associated with decrease in binding of nuclear extracts to the consensus heat shock element. The progressive decline in hsp70 response with increasing age of T cells in culture was found to correlate with the percent of proliferative lifespan already completed (398). An age-dependent decrease of heat shock factor-1 (HSF-1) binding in isolated human lymphocytes ex vivo, as well as gradual loss of heat-iducible HSF-1 in cultured T cells as they age has also been observed by Jurivich et al . (399). A member of the hsp70 family, mortalin, has been proposed as a marker for cells committed to apoptosis. Some recent data implicate hsp70 as a protector against apoptosis (400), others show that overexpression of transfected hsp70 enhances AICD in T cells (401). The expression not only of the hsp 70 family, but also hsp90 family stress proteins, has been reported to be reduced in aged T cells examined directly ex vivo, suggesting that results with cultured cells are relevant to the in vivo situation (109). Interventions which would enhance the stress response may therefore also be directly relevant to delaying immunosenescence (315). Even low-level irradiation may fall into this category. For example, Hyun et al . (402) found that low level irradiation, followed by a period of adaptation and then high level irradiation protected mouse spleen cells against the latter (as measured in mitogen-induced proliferation assays and in irradiation-induced apoptosis assays).

12. PERSPECTIVES

We suggest the following simple interpretation (figure 1):

As the organism ages, the output of T cell precursors from the BM decreases. Those precursors that enter the progressively involuting thymus are doubly compromized in their ability to generate new T cells: firstly because of their intrinsic deficiencies and secondly because of the reduced thymic function. There is therefore a quantitative and qualitative component to the dysregulated generation of naive T cells which becomes greater as the individual ages. In addition, naive cells produced by the thymus of the individual when young and surviving for extended periods in the periphery, themselves age even in the quiescent state. T cells which have been activated at some time during the life of the individual may remain present as memory cells and respond to rechallenge by antigen. However, because memory cells are maintained in a proliferative state even in the absence of specific antigen, they are subject to the aging limitations of proliferating cells and eventually undergo "replicative senescence". Even before they reach this terminal state, their function is altered and impaired compared to young cells, for example in terms of their increased susceptibility to activation-induced cell death. Since these cells cannot be so easily replaced by freshly activated naive cells as efficiently in old as in young individuals, the resulting immune response is reduced and generation of memory compromized in the elderly.

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