Immunosenescence: potential causes and strategies for reversal
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Abstract
Age-related deterioration in immune function has been recognized in many species. In humans the clinical manifestation of such immune dysfunction is age-related increases in the susceptibility to certain infections and in the incidence of some autoimmune disease and certain cancers. Laboratory investigations reveal age-related changes in the peripheral T cell pool, in the predominant phenotype, cytokine production profiles, signalling function and in replicative ability following stimulus with antigen, mitogens or anti-CD3 antibody. These changes in the properties of peripheral T cells are thought to be causally linked to an age-associated involution in the thymus. Our analysis reveals that thymic involution is due to a change in the thymic microenvironment linked to a reduction in the level of available interleukin 7. Treatment with interleukin 7 leads to a reversal of thymic atrophy with increased thymopoiesis. This provides the potential to reverse the immune

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Abbreviations used: IL-7, interleukin 7; TCR, T cell receptor; TN, triple-negative.

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dysfunction seen in the peripheral T cell pool by replacing old cells with new output generated in the thymus. Problems to overcome in order for such an experimental therapy to be successful require careful analysis in order to provide an optimal strategy to ensure that new T cell emigrants from the thymus have a broad range of specificities and are able to enter the peripheral T cell pool.

**Background**

Surveys correlating the onset of specific diseases with specific age profiles reveal that in the older population (over 60 years) there is an increased incidence of some cancers, an increased likelihood of developing specific autoimmune disease and an increased risk of dying from certain bacterial and viral illnesses. Such age-related susceptibilities would appear to indicate deficiencies in the immune system.

Early thoughts on age-associated immune dysfunction centred on the number of T lymphocytes. These cells are central to the correct functioning of the immune system, as diseases in which they are compromised have revealed [1–3]. T cells are produced by the thymus, an organ known to atrophy with age in many species; as a consequence, the output of new T cells to the peripheral T cell pool declines with age. Although from this decreased output one would expect a reduced number of T cells in the peripheral T cell pool, comparison of the total numbers of T cells in old and young individuals failed to show any significant difference [4,5]. Maintenance of population numbers independent of the declining output of the thymus could be achieved by a combination of induced proliferation of memory T cells and a reduction in the losses from the peripheral T cell pool. Since T cells have a finite replicative lifespan [6,7], their continued induced proliferation with age may lead to an accumulation of T cells at or close to their replicative limit [7,8]. Since the immune response is based around clonal expansion to antigen, the consequence of reaching a replicative limit would be immune dysfunction.

Analysis of populations of the T lymphocytes from older individuals reveals that, as a whole, they are unable to proliferate to stimuli capable of inducing normal proliferative responses in similar cells from younger individuals [9–11]. This may be associated with an inability to undergo the activation process because of a decline in function of crucial cell surface molecules [12–16], defects in the signalling pathway [17,18], low cyclin-depen-

dent kinase (cdk)-1 activity [19], or changes in the cytokines produced [10,20,21]. All of these may be features of cells at or close to their replicative limit.

These multiple age-associated deficiencies within the T lymphocyte pool makes intervention to reverse aging effects directed towards peripheral T cells a difficult prospect. Since the initial step in the progression to immune dysfunction seems to be involution of the thymus, it may be more profitable to consider a therapy directed at reversing thymic atrophy and renewing thymic output. This could provide the peripheral T cell pool with functionally competent cells which, in addition, would extend the peripheral T cell repertoire.

**Thymic atrophy**

Analysis of thymic atrophy in the mouse and in humans reveals that the rate of loss of active thymic tissue is greatest towards the mid-life period (by 12 months of age in C57BL/10 mice and 30–40 years of age in humans), after which the rate of decline is less apparent [22,23]. The major subpopulations within the thymus are defined by their expression of CD4 and CD8; the early stages of the developmental pathway do not express the CD3 molecule and are located within the CD4+CD8− population. This led to their identification as triple negative (TN) cells [24,25], although the earliest progenitor was found to express very low levels of CD4 [26]. This was not thought to be functional, since CD4 knockout mice show normal early T cell development [27].

The earliest progenitors identified in the adult mouse thymus are within the population defined as CD4+CD25− TN cells, an oligopotential population with the capacity to produce T cells, dendritic cells or natural killer cells. In the next stages of differentiation the CD44 molecule is lost, and there is transient expression of the CD25 molecule (see Scheme 1). During the stage of CD25 expression there is rearrangement of the T cell receptor (TCR)β chain, and subsequent expression of this chain at the cell surface with a TCRα chain equivalent (the pre-TCR chain) [28, 29]. Progression past this stage involves selection then rearrangement and expression of the TCRαβ chain. Thymocytes which express a competent TCRαβ chain pair are chosen, and further maturation and selection occurs before the cell can leave the thymus to enter the periphery [30].

Recent results suggest that age-associated thymic atrophy found in mice is linked to tran-
sition from the CD44+CD25− TN to the CD44+CD25+ TN population (see Scheme 1), since the absolute number of CD44+CD25− TN cells does not change with age, but the number of cells at phenotypically identifiable stages after this initial stage declines. It is easy to envisage how a ‘bottleneck’ at this early stage in the T cell developmental pathway could lead to reduced numbers of cells at downstream stages. For example, in male mice the end result is a decrease of more than 10^6 cells in the overall number of thymocytes between 3 and 20 months of age.

Several hypotheses have been proposed to account for this thymic involution. An early suggestion was that the atrophy is due in part to an age-related decline in the number of thymocyte progenitors in the bone marrow that are able to migrate to the thymus [31]. Two later studies, one showing no difference in the efficiency of bone marrow from young and old donors to repopulate the thymus of animals of similar ages [32] and one showing no change with age in the absolute number of early T cell progenitors in the mouse thymus [22], both argued against this hypothesis. A second hypothesis suggesting an intrinsic defect in the thymic progenitor cells in the bone marrow of old animals which prevented them from differentiating as efficiently as those from young bone marrow [33–35] was not supported by a recent study showing that populations of CD44+CD25− TN cells from thymuses of young and old animals have the same capacity to differentiate in vitro in foetal thymic organ cultures (R. Aspinall and D. Andrew, unpublished work). A more recent hypothesis suggests that age-related thymic atrophy is due to alterations in the thymic microenvironment, leading to its decline in competency in supporting thymopoiesis.

One of the major elements contributing to the environment are the thymic epithelial cells, and reports that their numbers appear not to alter significantly with age (R. Aspinall and D. Andrew, unpublished work; [37]) argue against any hypothesis suggesting that atrophy may be related to their loss [38]. Characterization of these epithelial cells has been undertaken [39] and the analysis of changes within this population with age is currently at an early stage, with descriptions of histological changes and a decrease in expression of some cell surface molecules [40]. Recent work in the mouse suggests that, whereas the number of epithelial cells may not change significantly with age, there may be a reduction in the amount of interleukin 7 (IL-7) produced by these cells (R. Aspinall and D. Andrew, unpublished work). The cytokine IL-7 has an essential role in the T cell developmental pathway [41–43] and appears to be linked with the stages of T cell development associated with rearrangement and expression of the TCRβ chain. An initial report suggested that IL-7 was a cofactor for the V(D)J rearrangement of the TCR chain gene [44], but a later study suggested that IL-7 was important in D–J rearrangement for the TCR chain [45]. A more recent report showed that IL-7 was needed to induce TCRβ gene rearrangement in T cell progenitors, and suggested that this was presumably by supporting the survival of these cells [46].

A reduction in the intrathymic level of IL-7 with age would therefore fit well with the observed age-related changes in the intrathymic T cell developmental pathway, which appear to be associated with problems with the production of a TCRβ chain [22]. Supporting evidence comes from a study with F5 transgenic mice [22]. F5 transgenic mice carry a transgene which is a complete TCR specific for an influenza nucleoprotein presented in the context of H-2D^β, derived from a CD8^+ T cell clone inserted into a CD2
minigene cassette. This ensures directed expression of the transgene in thymocytes and T cells, since the transgene expression is induced after the multipotent stem cell enters the thymus. The expression of the transgene therefore does not require productive rearrangement of gene components contributing towards the TCR because of the expression cassette system. T cell development in F5 animals is apparently normal, albeit skewed towards the production of CD8 thymocytes, with the demonstration of positive selection in C57Bl/10 mice and negative selection in animals expressing Class II MHC IE molecules and the endogenous Mtv ligand. The expression of the TCR transgene follows the normal rules of expression, being present at lower levels on the CD4+CD8– subset and at higher levels on the single-positive cells [47].

F5 transgenic mice show none of the age-associated thymic atrophy seen in the normal non-transgenic mice. Analysis of their peripheral blood reveals that throughout the life (at least between 3 and 20 months of age) of the animal the majority of CD3+ cells are CD3+CD4+, a phenotype which is associated with naive T cells [48]. In normal animals, as the thymus atrophies with age and its output drops, the number of cells with a naive T cell phenotype declines. Thus with age the T cells in the peripheral pool change from being predominantly naive to being predominantly memory T cells [49]. The presence of these naive T cells in the transgenic animals may be due to the absence of the specific antigen for the transgenic TCR, but also provides some indication that prevention of thymic atrophy may prevent a wholesale shift in the peripheral T cell pool to containing mainly memory T cells.

Reversal of thymic atrophy
The association of thymic atrophy with puberty led to a series of experiments to determine whether alterations in hormone levels would affect thymic involution. Implantation of an epithelial cell line derived from the pituitary was shown to reverse thymic involution in aged rats [50,51], an effect which may be due to the growth hormone produced by these cells. Analysis of the effect of testosterone also revealed an intricate involvement with thymic cellularity. Surgical or chemical castration of old rats was shown to lead to the reappearance of a histologically normal thymus, in contrast with sham-operated animals [52,53], and the regeneration of the thymus in castrated animals was inhibited by testosterone implants [54]. Such treatments may be of value in reversing thymic atrophy, renewing thymopoiesis and providing a means of ensuring that higher levels of naive T cells emerge from the thymus of old individuals. However, their effect on the thymus may not be direct but rather indirect, producing an effect by altering the expression of certain genes within the epithelial cells, which would then change the thymic microenvironment, permitting renewed thymopoiesis. In seeking to rescue the thymus from involution a more direct approach, to replace any factors lacking, may be more preferable. A recent study suggests that treatment with IL-7 may be of benefit in reversing age-related thymic atrophy and inducing new thymopoiesis. Old animals treated with IL-7 showed increased thymic weight and cellularity compared with their saline-treated controls [36]. Preliminary studies revealed that IL-7-treated animals show a higher percentage of naive T cells in their blood compared with saline-treated controls, when analysed approx. 6 weeks after treatment. However, the difference was slight, albeit significant, and limited to the CD4+ T cell pool. No significant difference was found within the CD8 T cell pool, and this may be due in part to the presence within the naive CD8+ pool of the murine equivalent of the CD8+CD45RA–CD28– found in humans [16]. Any strategy directed towards increasing the output of the aging thymus may have to consider ways of ensuring that the population of new T cells can displace immuno-incompetent cells in the peripheral T cell pool.

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References


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