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T CELL SENESCENCE

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1. ABSTRACT

The aging of the immune system, referred to as immunosenescence, is associated with a dramatic reduction in responsiveness as well as functional dysregulation. This deterioration of immune function with advancing age contributes to the increased incidence among the elderly of morbidity and mortality from infectious disease, and possibly autoimmunity and cancer. In mammals, the defense for fighting infectious agents is composed of the innate and adaptive immune systems. Macrophages, granulocytes, and natural killer cells are the major components of the innate system whereas T and B lymphocytes comprise the adaptive system. Although both compartments are affected, adaptive immunity is most susceptible to the deleterious effects of aging. Innate immunity functions immediately after birth and manifests little change throughout life. In contrast, adaptive immunity is immature at birth, peaks at puberty and progressively declines thereafter. Though marginal alterations in B lymphocytes are apparent, the dramatic decline in humoral and cell-mediated responses is predominantly the consequence of senescent T cells. The following review focuses on the aging effect on T cells as reflected in altered function, subset representation, development, lifespan and activation. Age-associated alterations in antigen presenting cells are also discussed since these cells are required for T cell activation and may impact T cell function.

2. INTRODUCTION

Age-related changes in T cell function is evident in proliferation to mitogenic and allogeneic stimulation, helper function in antibody responses (1,2), delayed type hypersensitivity responses (3), cytokine production (4), and cytotoxic function (3). Included among the more dramatic changes that contribute to diminished T cell function are a decline in the frequency of $CD4^+$ T cells producing IL-2 and a decreased expression of IL-2 receptors (5). This is coupled with a decrease in the early events of signal transduction (6-8) and an overall decrease in proliferation of $CD4^+$ T cells (9-11) in response to various kinds of T cell receptor (TCR) and costimulus mediated stimulation. Superimposed on these alterations is a shift toward an increased representation of $CD4^+$ T cells with a memory phenotype (10,12,13). Although some functional changes found in the aged may be attributed to the shift in the representation of T cell subsets (e.g., memory/naïve), other alterations are likely to be intrinsic to $CD4^+$ T cells from the aged. Even though the potential T cell repertoire remains relatively unchanged in the aged (14), due to the reduction in thymic output of mature, naïve T cells, the peripheral repertoire of the aged is constricted. Moreover, clonal outgrowths are common in both CD8 and CD4 T cells of the aged. The accumulation of these alterations is believed to lead to immune dysfunction such that individuals are prone to the consequences of infectious disease, autoimmunity and cancer.

3. AGE-ASSOCIATED ALTERATIONS IN T CELL FUNCTION

The activation of naïve T cells requires the engagement of TCR with peptide antigens presented by MHC class II on antigen-presenting cells (APC) and the ligation of T cell co-receptors with their costimulatory ligands found on APC. Upon stimulation a cascade of signaling events occur in the T cell that lead to IL-2 secretion and limited proliferation. Concomitantly, the expression of the IL-2 receptor alpha chain (CD25) is upregulated and IL-2 drives further expansion and differentiation into effector cells. The effector cells, defined as highly activated T cells that rapidly produce large amounts of cytokines (other than IL-2) upon restimulation, even in the absence of costimulation (15), are responsible for the ensuing T cell response.

In T cells of aged humans and rodents, there is a significant decrease in IL-2 production, lymphoproliferation, and upregulation of CD25 expression after TCR and costimulus mediated stimulation. Although the overall decline in responsiveness reflects a loss with age in the frequency of cells that can be induced to secrete IL-2 and express CD25 (5,16,17), analysis of the reduced number of immunocompetent cells suggest that these cells retain their function (16,18). However, examination of the antigen induced response by purified naïve CD4 cells in aged TCR transgenic mice revealed that the majority of effector cells generated were not fully differentiated, i.e., they expressed an intermediate phenotype and did not secrete significant levels of cytokines other than IL-2 (19,20). Even though some laboratories (4,20) have found that addition of exogenous IL-2 can sometimes overcome the age-related defect, most laboratories find IL-2 has only a limited ability to restore full reactivity (21-23).

Information about other lymphokines in the aged is not as well characterized and is less consistent. The inconsistencies observed between laboratories are likely due to variations in stimuli, culture conditions, and antigen-presenting cells. In general, several groups have found an age-related increase in IL-4 (12,24,25) and IL-5 production (25,26). Many studies of CD4 T cells in mice show an increase in IFN-gamma with age (9,25,26) while an almost equal number show a decrease (27,28). However, the production of IFN-gamma by CD8 T cells is increased with age (29). The production of IL-10 increases with age in mice (30) and several groups have reported an age-associated decline in IL-3 production (24,31,32).

The mechanism underlying the age-associated decline in CD8 T cell responsiveness remains less clear. Several studies of aged rodents and humans have shown that cytotoxic T cells exhibit decreased responses to mitogens and antigens, decreased cell division, and increased IFN-gamma production (10,11,17,29,33,34). These functional changes may be the consequence of alterations intrinsic to the T cells of the aged, the switch in T cell subset representation and/or a manifestation of extrinsic factors that are altered with aging and affect CD8 cell function. Although some or all of these factors may play some role in reduced CD8 responses, it is certain that the decreased frequency of relevant antigen specific naïve cells that emigrate from the thymus of the aged contributes to the overall decline in T cell responses (35,36).

In old mice and humans clonal T lymphocyte expansions become more frequent and lead to skewing of the T cell repertoire in TCR-V beta usage (37-39). Clonal outgrowths are observed first among the CD8 population and later in the CD4 population (40). Initially the expanded clones are among the memory phenotype cells; however, later clones expressing both memory and naïve phenotype can be detected (41). The findings of an increase in clonal T cell populations in conjunction with the decline in thymic export of naïve T cells predict a constriction in repertoire diversity in the periphery in the aged. However, the extent of repertoire diversity has yet to be measured in the elderly.

4. T CELL SUBSETS

In the periphery of the aged, the total number of T cells as well as the ratio of CD4 to CD8 T cells remain relatively unchanged (42). However, the representation of naïve vs. memory T cell subsets is altered with age such that aging in rodents and humans leads to increases in the proportion of T cells expressing markers typical of memory cells, and declines in the proportion of T cells expressing markers typical of naïve T cells (10,12,13,29,43). Naive and memory T cell subsets have been distinguished from one another based on differential expression of the cell surface glycoproteins, CD44, CD45RB, and CD62L (L-selectin), wherein memory cells (44,45) and T cells from aged mice express higher levels of CD44 and lower levels of CD45RB and CD62L. The age-related transition to higher proportions of cells with a memory phenotype affects both the CD4 and CD8 populations. The majority of these cells that accumulate in aged subjects seem to be resting lymphocytes based on their size, DNA profile, lack of activation markers, and requirement for further stimulation for cell cycle entry (43,46).

The switch to a greater representation of memory-phenotype cells is consistent with models of T cell ontogeny. As mentioned above, the thymus involutes with advancing age (47) and the rate of production of newly emerging naive cells from the thymus exported to the periphery decreases to very low levels by late life (48). Superimposed on the diminished supply of naive cells from the thymus is the continued antigen-driven conversion of naive to memory cells throughout life. Moreover, it has been reported that apoptosis among naïve T cells is increased and among memory T cells is decreased (49). Altogether, these could lead to a gradual transition from a predominantly naive cell to a predominantly memory cell population that is observed with aging. Indeed, within 13 weeks of thymectomy of adult mice, cells expressing a memory phenotype predominate in the periphery (45,50).

The extent to which functional changes with aging are the consequence of an accumulation of more differentiated *vs*. intrinsically defective T cells (or both) has been the focus of many studies over the past decade. A

large body of evidence suggests that the shift from naive to memory T cells contributes to the immunodeficiency observed in old age (reviewed in (42)). Aging leads to a decline in the proportion of T cells that respond to mitogen or superantigen by the production of IL-2 or IL-3, as well as a decline in the proportion of T cells that respond to mitogen plus IL-2 by proliferation or by generation of cytotoxic effectors (10,51). Since naive T cells respond more frequently than memory T cells to the stimuli used, it can be argued that the decline in responsiveness is attributable to the decrease in the representation of the responsive population with age.

It has been argued that the relative proportions of memory/naïve T cell subsets determine changes in cytokine secretion patterns. For example, the increase in IL-4 and IFN-gamma production with age is also coincident with the increase in memory cells with age, IL-4 and IFN-gamma being largely memory cell products (29,44,45,52-57). However, age-associated alterations in cytokine production are not determined solely by the subset changes, but also by alterations within each of those subsets. For example, IL-3 secretion is not necessarily prevalent in naïve or memory subsets, yet IL-3 secretion is decreased with aging (25,31,51).

Although much of the age-related alterations in T cell function may be attributed to changes in the representation of populations, in some studies decreases in immunoresponsiveness in the memory/naive cell populations have also been documented. Limiting dilution assays of IL-2 producing and IL-2 responding cells have revealed a loss in responder cell frequency within the memory cell subset (58). Analysis of subset-specific protein-kinase-dependent phosphorylation pathways also indicates age-dependent changes that cannot be explained by shifts in T cell composition (59). Li and Miller (60) have shown that although memory T cells from old or young mice produced far more IL-4 than naive T cells from mice of the same age, aging nonetheless led to a decline in IL-4 production by memory cells after stimulation by anti-CD3 together with IL-2. Similar to the findings of a functional decline in the memory phenotype cells from aged mice, are findings demonstrating that naïve CD4 cells are also hyporesponsive. Under conditions of optimal antigen presentation *in vitro*, naïve CD4 cells isolated from aged TCR transgenic mice produced significantly lower levels of IL-2 upon antigen stimulation resulting in decreased expression of CD25 and reduced proliferation (61,62). Thus, changes inherent to the cells from the aged result in diminished responses.

4.1. Other subset alterations: T_H1 *vs.* T_H2, Rhodamine 123^{bright} *vs.* $R123^{dim}$, CD8 $CD122^+$

Given the profile of cytokines produced in the aged, one may postulate that in $CD4^+$ T cells there may be a propensity toward T_H2-like cells with age. Long-term lines and clones have been divided into two subsets, T_H1 *vs*. T_H2, each secreting distinct patterns of cytokines which may dictate their functional ability (63). Cells of the T_H1 type produce IL-2, IFN-gamma, TNF-alpha, and lymphotoxin and predominantly mediate cell-mediated immune responses and inflammatory responses, whereas cells of the T_H2 type produce IL-4, IL-5, IL-6, and IL-10 and provide optimum help for humoral immune responses (45,64). However, the shift in cytokine patterns with age would only be partly explained by a predominance of T_H2-like cells in the aged since IL-3 is produced equally well by both T_H subsets (63) and IL-3 production decreases with age (25,31,51).

The naïve and memory subsets of the CD4 and CD8 populations can be further divided according to their ability to extrude the fluorochromes Rhodamine 123 (R123) and Rhodamine-6G (65,66). Both of these fluorochromes are known to be extruded by P-glycoprotein, the 170-kDa ATP-dependent plasma membrane pump encoded by the multiple drug resistance genes (67); thus, higher levels of P-glycoprotein expression correlates with $R123^{dim}$ staining cells. An increase in the proportion of $R123^{dim}$ T cells in the naïve and memory cell pools of CD4 and CD8 T cells in aged mice was observed (61,65,66). Further separation of memory cells based upon R123 staining revealed that, within the CD4 memory pool, only cells with low levels of P-glycoprotein (i.e., $R123^{bright}$ staining cells) are able to respond to stimulation with anti-CD3 plus IL-2 by producing IL-4 (68). Moreover, recent data suggests that the recruitment of signaling molecules to the immunological synapse, the site of APC:T cell interaction, is diminished in this subset of memory cells (69). Thus, the shift to increased representation of cells with higher levels of P-glycoprotein may account for some of the decline with age in IL-2 responsiveness. However, the correlation with R123 expression and naïve CD4 T cell function remains untested.

Some laboratories have reported an increase in extrathymic CD8 T cell development in the aged (70,71). These cells are $CD8^+$ IL-2R beta $(CD122)^+$ and increase in number in the liver, spleen and lymph nodes (71,72). Moreover, cells in this subset as opposed to the $CD8^+$ $CD122^-$ subset have been shown to produce large amounts of IFN-gamma after stimulation with anti-CD3 antibody (72), suggesting the increase in IFN-gamma by CD8 cells of the aged may be a consequence of an increase in the representation of this subset. However, previous studies have shown that the memory phenotype CD8 T cells ($CD44^{hi}$) secrete high levels of IFN-gamma and the increase in frequency of these cells accounts for the reported increase in IFN-gamma levels upon TCR stimulation in the aged (29). Whether the CD8 $CD122^+$ T cells and the memory phenotype CD8 cells represent the same or overlapping subsets is unresolved.

5. THYMUS

T lymphocytes differentiate and mature in the thymus, an epithelio-lymphoid organ that is comprised of a cortex and a medulla. The epithelial cells provide a microenvironment in which the bone marrow-derived thymocyte precursors develop. As the thymocytes mature, they migrate from the cortex to the medulla of the thymus and upon maturation, exit to secondary lymphoid organs as naïve T lymphocytes. The various stages of T cell

development in the thymus are defined by the surface molecules expressed on the thymocytes and the state of T cell antigen receptor (TCR) gene rearrangement.

5.1. Involution

Among the most striking changes that occur with age is thymic involution, the shrinkage of tissue mass. The age-associated involution of the thymus is directly correlated with the functional loss of T cell immunity; involution starts at puberty, preceding the onset of the decline in T cell immunity. The rate of atrophy is greatest just prior to mid-life and slowly but progressively continues with age. The decrease in tissue mass results primarily from the shrinkage of the thymic cortex (73). A decline in the number of all thymocytes after an early developmental stage, i.e., $CD3^-$, $CD4^-$, $CD8^-$, $CD25^+$, and $CD44^-$, was found upon examination of the thymus from aged mice (74). This stage of transition from $CD3^-$, $CD4^-$, $CD8^-$, $CD25^+$, and $CD44^-$ to $CD3^-$, $CD4^-$, $CD8^-$, $CD25^+$, and $CD44^+$ thymocytes is associated with the rearrangement and expression of the TCR beta chain genes.

Although many factors may influence the development of T cells in old mice, the precise mechanism underlying thymic involution has not been defined. It is known that genetic makeup influences thymic involution (75) as evidenced in particular rat strains that neither experience thymic involution or T cell senescence. Since thymocytes are derived from bone marrow-derived precursors, it is possible that at least part of the age-related alterations of the T cell compartment reflect qualitative and/or quantitative changes in bone marrow stem cells. However, no differences in the efficiency of bone marrow from young or old donors to repopulate the thymus have been noted (76,77). Several other factors, extrinsic to the thymus, may also influence thymic involution. These include various neuroendocrine hormones (78) and nutritional manipulations (79). Recent studies of alterations intrinsic to the aged thymus have focused on the microenvironment. Although the number of thymic epithelial cells may not change with age (80), the amount of IL-7 produced in the thymus declines with age (81). IL-7 is an essential cytokine for T cell development, supporting TCR beta chain gene rearrangement in T cell progenitors (82,83). Thus, age-associated involution may be a consequence of diminished IL-7 production.

5.2. Thymic productivity

The decline in thymus cellularity is accompanied by a decline in thymic output in both humans (84) and mice (48). Although the number of naïve T cells exported from the thymus to the periphery is markedly diminished, there is evidence to suggest that thymic function is still retained in the very old (85,86). Much of this data come from studies of peripheral T cell recovery after depletion. In general, the recovery of CD4 cells in humans and mice that have been treated to deplete peripheral T cells is inversely related to age. Thus reconstitution is delayed in older individuals after chemotherapy or CD4 depletion by monoclonal antibody treatment (87,88). These studies also revealed that independent of the influence of age, the rapid recovery of peripheral CD4 T cells in all patients is directly correlated with the appearance of naïve T cells and thymus tissue, providing strong evidence that thymic activity is essential for naïve CD4 T cell recovery (84). In contrast, CD8 cell recovery was more rapid and was not associated with age (87).

Thymic function may also be measured by the detection and quantification of recent thymic emigrants in the periphery. This has become feasible with the detection of TCR rearrangement excision circles (TRECs) which are episomal by-products of TCR V(D)J rearrangements that are produced during thymopoeisis (36,89,90). TRECs are stable and not duplicated during mitosis; therefore, their presence is diluted with each cell division. TRECs are present in the naïve T lymphocytes of the aged supporting the premise that the thymus, though less productive, is nonetheless functional. Moreover, the frequency of TRECs declines in the naïve CD4 population with age suggesting that this population becomes more heterogeneous in the elderly.

6. LIFESPAN AND HOMEOSTASIS

There are no overt changes with age in the total number or relative ratio of CD4 and CD8 cells in the peripheral lymphoid tissues, speaking to a coordinated level of homeostatic regulation within the immune system. While naive T cells are exported from the thymus throughout life (90), the rate of release of newly developed T cells is at its maximum early in life and drastically decreases post-puberty (48). Despite this decrease in naive cell development over time, such T cells are still present in the periphery during old age. These are most likely the long-lived progeny of T cells exported from the thymus earlier in life. Naive peripheral T cells do not appear to have a definitive life span (91) and may persist indefinitely in the absence of new thymic emigrants. The interphase lifespan of naive CD8 T cells has been measured in mice as at least 8 weeks (92,93). The majority of naive T cells are in the G0/G1 phase of the cell cycle. In contrast, a large percentage of memory-phenotype T cells are found to be undergoing DNA synthesis at any given time (93,94). Aging has little, if any impact on the fraction of cycling memory T cells (MLT, personal observation).

The pool sizes of memory and naive T cells are independently regulated (95-97), with the two subsets occupying distinct environmental "niches" and requiring unique conditions for survival. Although the precise composition of these "niches" has not been defined, it is clear that peripheral survival of T lymphocytes requires specific environmental elements. For example, naive cells require the continuous presence of the selecting MHC molecules for long-term survival (97-100). In an elegantly designed experiment, Tanchot and Rocha (97) demonstrated that the level of MHC Class I expression within the peripheral microenvironment controlled the number of naive CD8 T cells. In contrast, survival of memory cells requires MHC expression, but not the specific restricting element or peptide (97-100).

In addition to MHC molecule expression, various cytokines are known to impact T cell survival. The

peripheral survival of CD8 memory T cells is influenced by the relative abundance of IL-15 and IL-2, with IL-15 promoting memory expansion and IL-2 suppressing this process. This was demonstrated by transfer of memory T cells into host animals treated with anti-cytokine antibodies. In this system (101), CD8 T cells from aged mice appear to respond similarly to those of young mice. These conclusions are further supported by the findings utilizing mice with targeted disruption of either the IL-2 beta or IL-15 alpha receptor chain. Mice without effective high affinity IL-2 receptors have a peripheral T cell population that is hyperplastic and displays a "partially" activated cell surface phenotype (102). In contrast, mice without high affinity IL-15 receptors are lymphopenic and largely devoid of CD8 peripheral cells (103). IL-7 may also play a role in naive T cell homeostasis through its ability to induce naive cell proliferation *in vitro*, without causing a concomitant maturation shift to an activated state (104). However, it is not yet clear that this is the case *in vivo.*

In addition to the role of cytokines, the accessory molecules B7/CD28 has been implicated in homeostasis of T cell subsets. In mice displaying enhanced B7.2 expression, there is peripheral T cell hyperplasia and a shift in the CD4 to CD8 ratio, towards increased CD8 representation. Decreased B7 expression had the reciprocal effect on CD4/CD8 ratio (105).

Under certain conditions, T lymphocytes can undergo nonspecific expansion in the periphery. Memory cells have a well-documented capacity for expansion in conditions of low T cell numbers (58,106). Serial transfer experiments indicated that transferred cell populations may undergo a 10,000-fold increase (58). This expansion requires TCR signaling as clearly demonstrated using T cells derived from mice bearing transgenic TCR. When transferred into mice not expressing the appropriate stimulatory MHC molecules, no expansion of memory T cells occurs (107). Aging does not appear to alter this property of memory cells, as extensive peripheral memory T cell expansion has been identified in both aged humans and rodents made lymphopenic by irradiation, drug or antibody treatment (reviewed in (108,109)).

Naive T cells also can expand when presented with a lymphopenic environment. The signals necessary for triggering this expansion include appropriate MHC and peptide (110-113). This homeostatic expansion appears to result in an increased density of cell surface CD44, but not in CD25 or CD49d expression. Furthermore, these cells are not activated to express effector function (110-112).

Taken together, all these results indicate that the size of the peripheral T cell population and its composition are regulated by active processes requiring the interaction of the lymphocyte and the microenvironment, rather than to a fixed intrinsic property of the T cell. There have been few investigations which directly address the effect of age on T cell survival. However, age has a significant impact on the quantity and profile of cytokine production as well as more subtle effects on the expression of various "costimulatory" molecules. These changes may alter the type and number of "niches" in the aged and thus the survival of various T cell subsets.

6.1. Replicative Senescence and Sensitivity to Apoptosis

Replicative senescence, a phenomenon first described by L. Hayflick (114), is the loss of the ability to undergo cell division after a finite number of cell doublings. This process is now understood at the molecular level as being a reflection of telomere shortening with each division to the point that chromosomal replication cannot occur, halting cell division. Telomere shortening occurs at different rates in various cell lineages (115), and appears to occur at different rates over the lifespan of the organism (115,116). Immunosenescence has been suggested to result as lymphocytes reach critical telomere length and undergo replicative senescence (117,118). This hypothesis has considerable support in that memory cells have been found to have, in general, shorter telomeres than naive T cells (119), and older individuals yield lymphocytes with shorter telomeres than young individuals (120). Telomerase, the ribonucleoprotein enzyme which lengthens telomeres is induced in T lymphocyte populations following activation (121-123). The capacity for telomerase activation is not impaired by aging (115). However, repetitive stimulation *in vitro* results eventually in a state in which telomerase is not upregulated, and telomere shortening occurs. In cultures of human cells this is accompanied by a loss of CD28 expression (118). These data suggest a role for telomere shortening in T cell senescence.

Proliferative responsiveness, clonal expansion and survival may also be regulated by the sensitivity of lymphocytes to activation-induced cell death (AICD). Advancing age is accompanied by increased sensitivity to AICD. Both memory and naive-type CD4 and CD8 human lymphocytes grow more sensitive to TNF-mediated apoptosis with increasing age possibly due to increased constitutive levels of TNF receptors and the associated death domain protein (TRADD), as well as increased activation of caspases (124,125). Likewise, sensitivity to apopotosis induced through the Fas/FasL pathway is also heightened in aged human lymphocytes. Some investigators have reported higher FasL expression by aged CD4 and CD8 T cells than on their young counterparts, as well as other alterations in the Fas/FasL signaling pathways with age which would increase the sensitivity to apoptosis induction in these populations (126).

7. T CELL ACTIVATION

The dramatic decline in T cell responses of the aged has been attributed to the marked diminution in the production of IL-2 after stimulation with mitogens, antigen, or anti-CD3 antibody (42). These responses are generated upon signaling through the TCR or through the cytokine receptor. In addition to signals transmitted through either of these receptors, an additional second signal from APC costimulation via other surface molecules, such as CD28, is needed. Thus, aberrations in any of these molecules and/or their ligands would lead to compromised T cell responses.

Because the numbers of TCR, cytokine receptors and CD28 molecules do not decline in resting T cells with age (7,11), it has been postulated that changes in the signal transduction machinery with age might be responsible for the impairment of T cell function. Indeed, several studies have shown in T cells after stimulation significant changes in the activity or expression of many signal-related molecules (6-8) as well as defects in calcium signal generation (127,128).

7.1. TCR signaling

Upon contact with an activated APC, a quiescent T cell passes through several extracellular membrane events prior to reaching full activation and the induction of the TCR signaling machinery. The first stage is adhesion. Integrins and adhesion molecules are likely to provide the essential elements needed for the initial APC-T cell contact. The next stage is the aggregation of the signaling complex that allows the TCR to initiate signal transduction. This requires active cytoskeleton-driven clustering of accessory molecules and TCR as well as the exclusion of molecules that may inhibit peptide-MHC:TCR interaction (129-132). Glycolipid-enriched microdomains in the cell membrane (133,134) facilitate the organization of several interactive molecules that participate in the induction of T cell activation at the T cell: APC synapse. The final checkpoint to achieve the minimal threshold for activation is the maintenance of a stable contact cap, which is the fundamental signaling unit. With regard to the initial stage of T cell activation in the aged, Jackola *et al.* (135) have reported defects in cell-cell binding that is associated with the altered activation capacity of the integrin, LFA-1. In a recent study, Tamir *et al.* (8) have shown that the redistribution of kinase substrates and coupling factors to the microdomains in the contact cap is altered in T cells from aged donors.

TCR signaling events can be summarized as follows. Occupancy of the TCR causes the initial activation of Fyn and/or Lck. Considerable evidence suggests that Lck and Fyn mediate phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAM) on CD3-zeta (136-138). This leads to the recruitment and activation of ZAP-70 (139), which binds to the phosphorylated ITAMs (140,141). Lck or Fyn then phosphorylates the kinase domain of ZAP-70 and augments ZAP-70 activity (141,142). Subsequently, additional sites of tyrosine phosphorylation can be detected on ZAP-70. Accumulation of phosphotyrosine on proteins of the TCR complex sets the stage for all subsequent signaling interactions.

T cells from healthy old mice display multiple defects early in the signaling cascade initiated by TCR stimulation. Data on the induction of Lck and Fyn activity has been variable, with results suggesting either no change or a decline in activity with age (6,143,144). An age-related decrease in the phosphorylation of the TCR-associated CD3-zeta chain has been detected in resting T cells and after activation (145,146). The amount of ZAP-70 associated with CD3-zeta in resting T cells of the aged is increased, and upon activation, ZAP-70 phosphorylation increased only in the young (145,147) although no age-related changes were observed in ZAP-70 protein kinase activity (147). Many of these alterations in TCR signaling could be a consequence of structural changes in the signaling molecule itself, downregulation by other molecules, and/or dysfunction of other molecules upstream. Regardless, these findings point to an early defect in TCR signaling. Although the primary molecular cause(s) of age-related changes in T cell activation has yet to be identified, a picture of the overall scheme for altered signaling is emerging.

7.2. Costimulation

Two independent signals are required for T cell activation, the TCR provides the first signal and a costimulator molecule provides a requisite second signal. It is believed that costimulators function mainly to enhance or modify TCR signaling and do not signal independently by themselves. Costimulators may act by enhancing the strength or duration (or both) of signaling by the TCR by enhancing antigen presentation or the stability of the contact cap. This was suggested by the finding that CD28 was critical only for antigens that have a short half-life (148). CD28 may also function to potentiate T cell activation by helping to recruit signaling proteins or by enhancing the activation of tyrosine kinases. Moreover, cytoskeletal movement is regulated by CD28-B7 and LFA-1-ICAM-1 interactions (129). This active accumulation of receptor pairs and other cytoskeleton-linked molecules at the T cell-APC contact cap, and the signal amplification that would result from these increased receptor densities, further support the idea that costimulatory molecules function to increase the overall amplitude and duration of T cell signaling. Several alterations in costimulation molecule expression and/or function have been reported with aging but very little has been reported describing the consequence of these effects to the early TCR signaling mechanism (8,149). Proliferative responses through CD28 in naïve and memory CD4 T cells decrease with aging (11). Although CD28 expression is equivalent on cells from either age group (11), the mechanism by which CD28 responsiveness is diminished is unknown.

8. INFLUENCE OF THE AGED ENVIRONMENT

8.1. Cytokines

As alluded to above, there is much evidence that the cytokine network is altered in old age (150). Cytokines present during antigen stimulation are known to influence the type of effector cells generated. Consequently, the type 2 cytokine profiles that dominate in the elderly (151) may impact on the ensuing T cell responses. T cell differentiation in the presence of type 2 cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13) would inhibit the production of type 1 effectors while promoting the generation of type 2 effectors, which are considered anti-inflammatory and mediate humoral responses (45,63). Thus, the dominant type 2 cytokine profile found in the elderly might induce decreased production of type 1 cytokines, such as IFN-gamma, thereby resulting in the inhibition of cell mediated immune responses and CD8 responsiveness (27,152). Alternatively, or in conjunction with the switch in cytokine milieu with aging, is the possibility that the function of APCs (i.e., dendritic cells) is altered.

8.2. APC

There is some evidence for age-associated changes at the level of the accessory cell. In mice, the precursor frequency of memory cytotoxic T cells that respond to influenza is entirely dependent upon the age of the APC donor. These studies demonstrate that memory T cells from influenza-primed old mice showed a significantly higher response in limiting dilution cultures when stimulated with influenza-infected splenocytes from young as compared to old mice (153). Responses to trypanosome (154) and pneumococcal antigens (155) are also compromised in aged mice due to suboptimal accessory cell function.

Whereas a large number of studies have described the ability of several cell types to function as APC, e.g., monocytes and macrophages, B lymphocytes, Langerhans' and dendritic cells, very few studies have examined alterations in APC function with age. Of the aforementioned cell types, the accessory cell function of macrophages and monocytes has been studied most extensively in the aged and has been reviewed elsewhere (156,157). Although several studies of accessory cell function have found little evidence for an age-effect on the accessory cell's ability to support T cell activation (158-160), many studies have observed a decline in APC function (9,161). Studies of macrophage cytokine production have yielded inconsistent results (162). Very little data are available on the age-associated changes of costimulatory molecule expression on APCs. Although one study in humans failed to find any decreases in expression of CD86 on either resting or IFN-stimulated monocytes from the elderly (163), follicular dendritic cells in germinal centers of aged mice may lack expression of CD86 (164). The latter would encourage the induction of anergy or apoptosis in the antigen-specific cells with which they interact.

Information on the function of dendritic cells from the aged is very limited. Studies of aged mice have shown that the density of Langerhans cells in the epidermis declines (165-167). Detailed studies of age-related changes in the number or distribution of dendritic cells found in tissues other than skin have not been reported. No consensus has been reached concerning the function of dendritic cells in the aged. Whereas some studies report a decline in function (165,168,169), other studies report no change (167,170,171). Dendritic cells generated by culture of adherent peripheral blood mononuclear cells (PBMC) from young and old donors in GM-CSF and IL-4 exhibited no age differences in surface phenotype, morphology, IL-12 production, and tetanus toxoid antigen presenting function (170,172,173). Thus it appears that given optimal culture conditions, the generation of dendritic cells in the elderly may not be impaired. However, it is unclear if less physiological activation of dendritic cells in situ might take place in the elderly.

9. CONCLUDING REMARKS

It has long been appreciated that advancing age is accompanied by a host of changes in immune function, many of which have been attributed to altered T lymphocyte activity. Identifying and understanding the basis of these functional changes remains a work in progress. Although the most obvious aging effect on this population is the reduction in T cell output by the thymus, the decline of peripheral T cell function with age is not due to a decrease in number. Two hypotheses have been advanced to explain T cell immunosenescence. The first of these speculates that gradual changes in T cell biochemistry are evidenced by altered signal transduction and abberant responses in the individual aged T cell. The second hypothesis proposes that the age-associated changes in T cell activity are due to global changes in the frequency of specific T cell subsets, resulting in the alteration of the entire population, even though individual cells retain normal function. The most accurate description of the aging effect on T lymphocyte activity combines elements of both hypotheses. In addition it is increasingly clear that age-associated alterations in structural and functional characteristics of other cells in the immune microenvironment play a role in directing the activity of T cells. Therefore, any hypothesis must take into consideration not only alterations intrinsic to the T lymphocyte component but also those in accessory cells.

The ultimate goal of understanding the basis for age-related changes in immune reactivity is of course to intervene in such a way as to preserve vigorous immune reactivity into late life. Our increasingly detailed understanding of the mechanisms responsible for thymic involution, coupled with the knowledge that T cell lymphopoeisis occurs to a limited extent in the aged, suggests that one avenue for immunoreconstitution may be augmentation of T cell differentiation in the aged by means of hormonal and/or cytokine supplementation. Likewise, the understanding of how cytokine production profiles are altered with age leads to the possibility that reversal to "young-like" levels of cytokine synthesis might have a beneficial effect on immune function in the elderly. Enthusiasm for such approaches must be tempered by the knowledge that not only the T lymphocytes, but also other cells integral to the initiation and promotion of immune responses may express aging-related alterations affecting their function.

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