Review
Ageing, autoimmunity and arthritis
T-cell senescence and contraction of T-cell repertoire diversity – catalysts of autoimmunity and chronic inflammation
Jörg J Goronzy\textsuperscript{1,2} and Cornelia M Weyand\textsuperscript{1,2}

\textsuperscript{1}Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota, USA
\textsuperscript{2}Department of Immunology, Mayo Clinic, Rochester, Minnesota, USA

Correspondence: Jörg J Goronzy (e-mail: goronzy.jorg@mayo.edu)

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Abstract

Rheumatoid arthritis (RA), like many other autoimmune syndromes, is a disease of adults, with the highest incidence rates reported in the elderly. The immune system undergoes profound changes with advancing age that are beginning to be understood and that need to be incorporated into the pathogenetic models of RA. The age-related decline in thymic function causes extensive remodeling of the T-cell system. Age-dependent changes in T-cell homeostasis are accelerated in patients with RA. The repertoire of naive and memory T cells is less diverse, possibly as a result of thymic insufficiency, and it is biased towards autoreactive cells. Presenescent T cells emerge that are resistant to apoptosis and that often expand to large clonal populations. These cells are under the regulatory control of nonconventional costimulatory molecules, display potent effector functions, and appear to be critical in the synovial and extra-articular manifestations of RA.

Keywords: costimulation, immunosenescence, pathogenesis, rheumatoid arthritis, T-cell homeostasis

Introduction

During thymic development, large arrays of clonally distributed $\alpha$–$\beta$ TCRs are generated that mediate the recognition of foreign peptides in the context of the appropriate MHC molecule. The theoretical diversity of the TCR repertoire is between $10^{15}$ and $10^{18}$ TCRs \cite{1}. Thymic selection mechanisms impose significant restrictions on this diversity \cite{2}; however, the resulting functional TCR repertoire is still extensive. Arstila and colleagues \cite{3} have estimated that the functional T-cell repertoire in the human adult is composed of $>2 \times 10^6$ different TCR $\beta$-chains, each of which may combine with $>100$ TCR $\alpha$-chains. Wagner and colleagues \cite{4} established even higher estimates of $2 \times 10^7$ different TCR $\beta$-chains in the naive T-cell compartment of young human adults. Given that the human body harbors $\sim10^{11}$ T cells, these estimates imply that each naive T cell has a clonal size of 100–1000 cells (Table 1).

Studies using the frequency of TCR excision circle (TREC)-positive T cells as an indirect measure of diversity are consistent with the higher estimates of diversity \cite{5–7}. TRECs are generated during TCR rearrangement, are not replicated, and are diluted during subsequent cell divisions \cite{8,9}. The frequency of TREC\textsuperscript{+} cells within the naive T-cell compartment can, therefore, be taken as an indirect measure of clonal size. Studies have suggested that this clonal size is strictly regulated at 10–20 cells per clonotype in the newborn and that it then slowly but steadily increases with age \cite{7}. Compared with the naive population of T cells, the memory compartment is clearly contracted in diversity. However, even memory T cells are very diverse. Estimates of diversity within the memory compartment range from $1 \times 10^5$ to $1 \times 10^6$ different TCR $\beta$-chains, each combined with one or very few different TCR $\alpha$-chains \cite{3,4}.
It is generally assumed that this high degree of TCR diversity is necessary to guarantee recognition of the universe of antigenic peptides. In fact, the T-cell repertoire is capable of responding to virtually any foreign organism. In spite of its structural diversity, however, the repertoire of functional TCR is still greatly outnumbered by potential antigenic peptides, particularly in small mammals such as the mouse. Plasticity in the TCR–peptide–MHC complex may account for the recognition of multiple antigenic peptides by the same TCR [10,11].

### T-cell diversity, tolerance, and autoimmunity

Recent studies have interpreted the need for repertoire diversity within the T-cell compartment from a totally different perspective, namely, one of regulation of immune responsiveness [12,13]. The immune system is under strict homeostatic control [14,15]. T-cell responses to self-antigens are prevented in the majority of individuals. Also, the magnitude of T-cell responses to foreign antigens is regulated. Generally accepted control mechanisms include the induction of apoptosis in the responding T-cell population, and feedback control by inhibitory receptors and regulatory T cells. Remarkably, diversity of the repertoire of naive and memory T cells has now been established as a major additional way to control unwanted clonal expansions, presumably functioning by means of clonal competition for space and resources.

A characteristic example of a breakdown in this control mechanism is the lymphopenic mouse [16–20]. Thymectomy shortly after birth is generally sufficient to induce an autoimmune syndrome. Similarly, adoptive transfer of small numbers of naive T cells into a T-cell-deficient host induces a wasting disease that has many features of the autoimmune disease, inflammatory bowel disorder. These autoimmune phenomena have been initially attributed to the absence of regulatory cells in the thymectomized mouse or in the lymphopenic host [16]. Experiments by Barthlott and colleagues [12], however, have shown that these autoimmune manifestations can be prevented by naive T cells that lack any features of regulatory cells but that have the potential of homeostatic expansion. Clonal competition is in part antigen specific, and clonal T-cell populations can selectively inhibit the division of T cells of their own specificity [21]. Equally important, regulatory control can also be exerted by T-cell populations of completely unrelated specificities, so long as these populations have the propensity for homeostatic proliferation [12]. These studies emphasize the intrinsic regulatory mechanism that is inherent in a diverse population of T cells and that keeps autoreactive T-cell responses in check while not curtailing immune responses to exogenous antigens.

### Threats to T-cell diversity

T-cell diversity is continuously challenged [2]. Antigenic stimulation induces rapid expansion of antigen-specific T cells that expand to large clonal sizes. This expansion is counterbalanced by subsequent clonal contraction, which appears to be preprogrammed. Clonal contraction is robust and is usually sufficient to maintain a diverse memory T-cell compartment. However, clonal T-cell populations can emerge, and they have been associated with chronic infection such as cytomegalovirus or HIV [22]. These clonal expansions are usually limited to the memory T-cell compartment and do not affect the diversity of naive T cells because naive and memory T cells underlie different homeostatic control mechanisms and compete for different resources [14].

One additional biological variable that has a profound impact on T-cell homeostasis is age. The generation of...
new T cells in the thymus is highest in the newborn and then progressively declines [23]. Thymic involution progresses at the rate of ~3% per year, and individuals older than 50 years have <1.5% of their thymic tissue remaining [24]. However, the demand for production of new T cells remains high in the adult. In studies using endogenous labeling of DNA, the daily fractional replacement rate is 0.1–0.6% for naive T cells, and memory T cells turn over at a daily rate of 0.9–3.1% [25]. In essence, adults need to produce 1.5 x 10^8 naive T cells and 1.5 x 10^9 memory T cells every day (Table 1). New naive T cells are only produced in the thymus. Therefore, the formation of new T cells declines sharply with age. The frequency of TREC+ cells, which gives an upper estimate of all (intrathymic and extrathymic) newly generated T cells, declines by >95% between the ages of 20 and 60 years. This decline demonstrates that thymic production in a 60-year old is, at most, 5% of the capacity that existed at the age of 20 years [5,26]. Consequently, the need for the replenishment of naive T cells must come from the autoproliferation of existing T cells [27]. Homeostatic proliferation of naive T cells is dependent on the recognition of self-antigen [28–30]. As a result, the generation of ‘new’ naive T cells by autoproliferation is under selective pressure and ultimately leads to TCR diversity contraction.

Studies on the impact of age on the repertoire diversity of naive T cells are not available; however, the continuous decline in the frequency of TREC+ cells indicates a steady increase in the average clonal size. Preliminary evidence suggests that the contraction accelerates markedly at approximately age 65 years, after which 95% of the CD4+ T-cell diversity is lost (unpublished observations). Data for CD8+ naive and memory T cells are not available because of the lack of a reliable phenotypic marker to distinguish these subsets.

The mechanisms underlying this accelerated contraction are unknown. Uneven homeostatic proliferation, which favors CD4+ T cells with higher avidity for self-antigens, may be one factor. An additional factor may be increasing competitive pressure from memory cells and a breakdown of distinct naive and memory cell compartments. Also, the phenotypic distinction of naive and memory cells based on CD45 isoforms, which is relatively reliable for CD4+ T cells, may be less distinct with age. The observed repertoire contraction may, in part, represent a shrinkage in size of the naive compartment.

Contraction in diversity and dominance of clonal T-cell populations is a relatively common finding in the memory compartment of elderly healthy individuals [31–33]. These clonal expansions predominantly involve CD8+ T cells, but they can also be found in CD4+ T cells [33,34]. These clonal expansions appear to resemble T-cell oligoclonality that is associated with chronic infections. Indeed, clonally expanded CD8+ T cells in otherwise healthy individuals may be specific for cytomegalovirus [22].

**T-cell diversity in rheumatoid arthritis**

Early evidence that T-cell homeostasis is not intact in patients with rheumatoid arthritis (RA) came from the observation that these patients carried large clonally expanded populations of CD4+ and CD8+ T cells [35–37]. TCR studies demonstrated some degree of preference for certain TCR variable region β-chains [38,39]. However, sharing of the third complementary determining region of the TCRs among different patients was not found, suggesting that these T cells were not specific for a common antigen. Also, the expanded T-cell clones were present in the circulation as well as in inflamed tissues. Frequencies of expanded clonotypes were independent of disease activity and were stable over time, again suggesting that these clonal expansions were not simply a consequence of an antigen-driven activation event in the synovial tissue [40].

Studies by Wagner and colleagues [4] and by Koetz and colleagues [26] examined whether the clonal expansions were indicators of a more profound defect in T-cell homeostasis (Fig.1). Specifically, these authors examined whether repertoire contraction also involved the naive T-cell compartment. Koetz and colleagues [26] stated that the frequency of TREC+ T cells was significantly lower in patients with RA compared with age-matched controls. One possible interpretation of these data is that patients with RA have a premature diminution of thymic production. In this model, the immune system in patients with RA would be prematurely aged by 20–30 years and would increasingly rely on autoproliferation to fill the void.

de Boer and colleagues [9] proposed an alternative model; namely, these findings may be the consequence of a primary increase in the turnover of naive T cells that would result in dilution of TREC+ T cells. The time of increased turnover must have preceded the onset of RA. By the time the patients have developed RA, they have reached a steady state as indicated by two observations. First, the frequency of cycling Ki-67+ T cells in the peripheral blood of patients with RA is not increased, but is even slightly decreased, indicating a reduced peripheral turnover. The second observation is that the concentrations of TREC+ cells are already reduced in 20-year old patients with RA, and the subsequent age-dependent annual loss is not different from age-matched healthy controls. This again suggests that the turnover at the time of disease is not increased [26]. Ponchel and colleagues [41] have confirmed the reduction in TREC+ T cells in patients with RA, and have correlated this with phenotypic changes of naive T cells that may be the consequences of increased homeostatic proliferation.
Irrespective of the primary defect, these data suggest that patients with RA have a history of increased homeostatic proliferation of naive T cells that predated their disease, that may have occurred to compensate for a lymphopenic state, and that has imposed major phenotypic changes. Increased homeostatic proliferation should lead to repertoire contraction and to signs of replicative stress; indeed, this is the case.

The history of replicative stress can be assessed by measuring the telomere length. Telomeres in CD4+ T cells in healthy individuals are relatively intact until the age of 40 years, when they begin to progressively erode until they plateau at a rather short length at the age of 65 years [26,42]. In contrast, patients with RA have nearly complete erosion of their telomeric ends in their early twenties. Most notably, the telomeric erosion in patients with RA affects naive T cells as well as memory T cells. Memory T cells in healthy individuals have lost ~1000 base pairs in telomeric length compared with naive T cells, which is consistent with an increased replicative history of more than 20 generations. In contrast, the telomeric lengths of naive T cells from patients with RA are only slightly longer than those of their own memory cells, and these telomeres are as short as those in memory cells of healthy age-matched individuals.

This increased replicative history is associated with a significant contraction in TCR diversity [4]. A contraction in diversity is to be expected if T-cell loss from the naive compartment is compensated by homeostatic proliferation, and this is further accelerated if homeostatic proliferation is not random. Diversity of the TCR was estimated by determining the frequency of arbitrarily selected TCR \( \beta \)-chain sequences derived from either CD45RO– (naive) or CD45RO+ (memory) CD4+ T cells. Compared with age-matched controls, the diversity of TCR \( \beta \)-chains was contracted approximately 10-fold (median frequency of a TCR \( \beta \)-chain of 2 \( \times \) 10–6 compared with 2 \( \times \) 10–7 in controls). The naive T-cell compartment, which is the primary contributor to TCR diversity, was affected in addition to the memory T cells. Contraction of diversity in the naive T-cell compartment could not be attributed to contamination of memory cells that reverted to the CD45RA phenotype. Based on sequence analysis, the distinction between naive CD4+ T cells and memory CD4+ T cells was maintained. The impact of a relative lymphopenia with subsequent increased homeostatic proliferation and repertoire contraction in RA is unclear but, in light of the experiments in the lymphopenic mouse, it is tempting to speculate that this scenario represents a major risk factor for breaking tolerance and developing autoimmune diseases such as RA.

**Cellular T-cell senescence: a gain and loss in function**

The immune system is a highly proliferative system because of homeostatic proliferation as well as antigen-specific responses. It is not surprising that, with advancing
age, the immune system has evidence of high replicative stress. Multicellular organisms have evolved a mechanism to prevent the dysregulated growth and transformation of proliferating cells. One such mechanism, cellular senescence, was first described as a process that limits the proliferation of senescent fibroblasts.

Based on these studies, three cardinal features of cellular senescence have been defined [43]. The first is that, after repeated divisions, the proliferative capacity of a cell starts to dwindle and eventually ceases. One reason for this proliferative arrest is the shortening of telomeres. T cells have the ability to upregulate telomerase and they are able to prolong their lifespan; however, they are not resistant to telomere erosion. The second cardinal feature is that senescent cells develop resistance to apoptotic cell death. Finally, senescent cells undergo multiple phenotypic and functional changes. Notably, these changes are not necessarily a consequence of loss of gene expression, but they are frequently associated with a gain in function, such as the production of inflammatory cytokines in senescent fibroblasts. This latter finding has led to a model of senescence, the evolutionary theory of antagonistic pleiotropy [44]. This model implies that genes selected to enhance the fitness of young organisms have unselected deleterious effects in the aged organism if aberrantly expressed.

Consistent with this model, replicatively stressed CD4+ and CD8+ T cells undergo multiple phenotypic and functional changes (Fig.2) [45]. The most widely acknowledged phenotypic change is the loss of CD28, which increases in frequency in the CD8+ T-cell population with age but which also occurs in CD4+ T cells to a lesser degree [46–48]. CD28 expression is regulated at the level of a CD28-specific initiator complex that includes the nuclear proteins nucleolin and hnRPNP [49,50]. Replicative senescence and chronic exposure to tumor necrosis factor alpha induce a loss of this initiator complex, particularly in CD8+ T cells [51]. This loss is partially reversible by IL-12 [52]. However, CD28 loss is not the only, and possibly not the most prominent, change in gene expression in senescent T cells. Senescent CD4+ and CD8+ T cells acquire the expression of many genes that are generally expressed on natural killer (NK) cells and that are associated with effector functions [53]. Even CD4+ T cells can acquire cytotoxic activity through the expression of perforin and granzymes [54,55]. Also, senescent CD4+ T cells express a number of new regulatory molecules instead of the traditional ones, such as CD28 and CTLA-4, that control their activation or inhibition.

In particular, CD4+CD28null T cells express immunoreceptors of the killer immunoglobulin-like receptor (KIR) family [53,56–58]. This receptor family is usually expressed on NK cells and often displays specificity for MHC class I molecules. The family is highly polymorphic, and individuals differ in the number of genes as well as allelic polymorphisms. The KIR family includes stimulatory and inhibitory members. The stimulatory receptors require an adapter molecule (DAP12) to be functional, but they then constitute an independent recognition unit. T cells lack this adapter molecule, and KIRs expressed on T cells are not stimulatory on their own. However, the KIRs are able to provide a costimulatory signal for T-cell effector functions in the absence of DAP12 [59]. This costimulatory signal functions through the activation of the c-Jun N-terminal kinase pathway, and it is important in lowering the threshold in response to TCR stimulation.

In essence, the aging T-cell compartment is characterized by the increased frequency of highly competent effector T cells that are under the control of regulatory molecules found on NK cells. It can be envisioned, based on their unique properties, that these T-cell populations play an important role in tissue injury and in loss of self-tolerance as the biological system ages.

**Senescent T cells: facilitators of inflammation**

Expansion of CD4+ and CD8+ T cells that have lost the expression of CD28, and are presumably senescent, has been observed in several autoimmune diseases including diabetes mellitus, RA, Wegener’s granulomatosis, multiple sclerosis, and ankylosing spondylitis [60–64]. In general, these cells were clonally expanded and included autoreactive T cells, implicating them directly in the pathogenesis of these diseases. In RA, specifically, increased frequencies of CD4+CD28null T cells are associated with more severe disease, again providing evidence for a direct role of these cells in the disease manifestations. In early RA, the frequency of CD4+CD28null T cells is a predictor for erosive progression [65]. In the established disease, the frequency correlates with extra-articular manifestations [66]. Increased frequencies are seen in nodular disease, and the highest frequencies are found in patients with rheumatoid vasculitis. Also, the T-cell type of large granular lymphocytes seen in Felty-like conditions appears to be directly related to the senescent CD28null T cells [67].

At first sight, the loss of CD28 would suggest that these cells are functionally anergic and prone to apoptosis; however, the opposite is the case. These cells are very potent effector cells, and at least CD4+CD28null T cells are resistant to apoptosis (the data on CD8+ T cells are contradictory) [68–70]. Resistance to apoptosis-inducing signals cannot be attributed to a single mechanism but is acquired and multifactorial, consistent with the senescent phenotype of these cells. CD4+CD28null T cells express more bcl-2, which renders them less sensitive to growth-factor withdrawal [68]. CD4+CD28null T cells are also resistant to Fas-mediated apoptosis. These cells fail to degrade FLIP following T-cell activation and/or IL-2 stimulation. They, therefore, do not activate the death pathway...
upon Fas-ligand engagement [69]. The resistance to growth-factor withdrawal and Fas signaling may prevent the usual clonal downsizing *in vivo* after antigen-specific stimulation.

The accumulation of oligoclonal T-cell populations appears to be more the consequence of a prolonged survival than increased proliferation, again consistent with the concept of cellular senescence. Given the central role of T-cell apoptosis in T-cell homeostasis and peripheral tolerance, the prolonged survival of these cells may contribute to their role in inflammatory diseases. Specifically, overexpression of c-FLIP has been shown to induce autoimmunity [71].

In addition to resistance to apoptosis, other functional and phenotypic changes in senescent T cells in RA are of importance for their role in perpetuating chronic tissue inflammation. First, the shift in regulatory molecules, from the classic CD28–CD80/CD86 pathway to alternate immunoreceptors, changes the cellular context in which T-cell stimulation is facilitated. There is no longer a unique role for professional antigen-presenting cells that express CD80/CD86, but other cell types can be T-cell stimulatory. More importantly, CD4+CD28null T cells are very potent effector T cells and can cause tissue injury by virtue of their high cytotoxic activity and their excessive production of proinflammatory cytokines, including tumor necrosis factor alpha and IFN-γ. There is evidence that both dimensions are of functional importance in RA. Weissman and colleagues [72] were the first to postulate a role for perforin/granzyme-positive CD4+ T cells in the synovial inflammation of patients with RA, and also in one patient...
with ankylosing spondylitis. Namekawa and colleagues [54] demonstrated the presence of these cells in the synovial tissue of patients with RA, again postulating that the gain in cytotoxic function is of functional importance in maintaining chronic synovitis.

Regulatory genes of the KIR family have been identified as disease risk genes in RA and in psoriatic arthritis [73,74]. In patients with RA, in particular those who have extra-articular manifestations, oligoclonal T-cell populations were found to preferentially express the stimulatory KIR2DS2 gene, often in the absence of inhibitory KIRs or inhibitory receptors of the c-type lectin family, CD94/NKG2A [75]. Indeed, expression of KIR2DS2 had functional implications in that it sensitized the T cells to respond to subthreshold TCR stimulation. The KIR2DS2 gene, present in only 40% of a healthy Caucasian population, was found in association studies to be a risk factor for rheumatoid vasculitis [73]. Association studies also suggested a role for the stimulatory immune receptors, KIR2DS1 and KIR2DS2, in the risk of developing psoriatic arthritis [74].

Senescent T cells: shifting the balance from tissue homeostasis to tissue inflammation in coronary artery disease

Acquisition of new functions by senescent T cells appears not only to be important in autoimmune disease manifestations but also in more subtle inflammatory reactions that are associated with tissue homeostasis and repair. One characteristic example is coronary artery disease (CAD).

It is well established that activation of systemic inflammatory responses, as exemplified by elevated C-reactive protein levels, is a risk factor for adverse outcome in patients with CAD [76]. The atherosclerotic plaque is now understood to be an inflammatory lesion. Inflammation may lead to plaque rupture and subsequent thrombosis, and it may cause the clinical manifestations of acute coronary syndromes (ACS) such as myocardial infarction and unstable angina [77–79]. Patients with ACS have highly elevated frequencies of CD4+CD28null T cells, consistent with the notion that they have a pre-aged immune system [80]. CD4+CD28null T cells have been isolated from ruptured coronary plaques that have caused fatal myocardial infarction or have been isolated from plaque material that was harvested during angioplasty of unstable plaques [81]. CD4+CD28null T cells from patients with ACS produce large amounts of IFN-γ in vitro [82], and increased IFN-γ activity in vivo can be demonstrated. IFN-γ-inducible genes are upregulated in the peripheral blood of patients with ACS, and circulating monocytes show evidence of nuclear translocation of STAT-1 homodimers, indicative of IFN-γ receptor triggering. CD4+CD28null T cells are also cytotoxic towards endothelial cells, and this activity can be significantly enhanced by C-reactive protein [83].

Taking the data together, CD4+CD28null T cells appear to be instrumental in plaque rupture, either indirectly via IFN-γ-mediated activation of macrophages or directly via their cytotoxic activity. Again, as seen in patients with RA, the activity of CD4+CD28null T cells can be modulated by regulatory receptors of the KIR family [84]. CD4+ T cells frequently express KIRs, specifically stimulatory isoforms, in patients with ACS. Most interestingly, T cells in patients with ACS can also express the adaptor molecule, DAP12. The coexpression of DAP12 and the stimulatory receptor encoded by the KIR2DS2 gene is sufficient to form an independent antigen recognition unit that confers the ability to fully activate a T cell, even in the absence of TCR triggering. Such activation potential in T cells should have detrimental consequences for maintaining tolerance and tissue integrity, a characteristic example being the plaque rupture in a coronary artery lesion.

Sharing of immunosenescent mechanisms between ACS and RA provides a pathogenic framework for the recent clinical observations that the increased mortality of patients with RA can be attributed to coronary atherosclerosis and its complications [85]. In a case–control study, patients with RA were more likely to have multivessel coronary involvement at the first coronary angiogram compared with the general population (KJ Warrington, PD Kent, RL Frye, JF Lymp, SL Kopecky, JJ Goronzy, CM Weyand, manuscript submitted). The risk for accelerated CAD conferred by RA remained significant after adjustment for traditional risk factors. This example also illustrates how the distinction between the autoreactive response leading to autoimmune disease and the local inflammatory response of tissue repair can be blurred. The same mechanism, in this case immunosenescence, is responsible for the chronic destructive inflammatory disease itself as well as for its seemingly unrelated comorbidities.

Conclusion

RA is a disease that predominantly occurs in adults and has its highest incidence rates in the elderly [86]. This coincides with a period when the generation of new T cells is minimal and the ability to mount a naïve T-cell response to new exogenous antigens starts to decline or is already severely compromised. Studies in patients with RA have shown that immune aging is accelerated, raising the question of whether the breakdown in tolerance can be truly explained within the classic models of an autoreactive T-cell response to a disease-inducing antigen or whether age-dependent changes of the immune system represent a critical factor.

The repertoire of naïve T cells in RA is contracted and shows evidence of senescence, which may predispose the system to autoimmune responses that mirror the mechanisms in the lymphopenic mouse. In RA, presenescent memory T cells emerge that have acquired many
functions of NK cells and are proinflammatory cells. We propose that the distinction between self and nonself requires a functional and competent immune system. Age-related degeneration of immunocompetence imposes an immediate risk on the complex processes of self-tolerance (Fig. 3). With premature immune aging in RA, failure of self-tolerance may occur more easily and earlier in life. Effector functions of presenescent T cells are critical for the autoimmune manifestations of RA, including some of the comorbidities of RA, such as CAD.

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Pathomechanisms in rheumatoid arthritis. The diagram illustrates how aging, altered T-cell homeostasis, and cellular senescence may be involved in the pathogenic events leading to rheumatoid arthritis.
Comparing Interests
None declared.

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