The Ogryzlo Day Lecture

Premature Immunosenescence in Rheumatoid Arthritis

Presented at the University of Toronto Rheumatic Disease Unit Annual Ogryzlo Research Day, June 26, 2001

The common denominator of all therapeutic interventions that successfully treat rheumatoid arthritis (RA) is the ability to suppress the immune system. The more completely we suppress immunity, the faster and more efficiently we can reduce disease activity. This principle is exemplified by recent therapeutic modalities that have shown efficiency in treating RA, the anticytokine biologicals[1,2]. It may not be surprising that an autoimmune syndrome can be managed effectively by intensive immunosuppression. It also should be expected that profound immunosuppression comes with a price, the inability to properly combat infections once the minimally required immunocompetence to provide protection is compromised[3–5]. What remains an enigma is that, despite our ability to profoundly suppress immunity, we have not made progress in inducing lasting remissions of the disease. Continuous benefit from therapy is strictly dependent on the regular reinduction of iatrogenic immunodeficiency. If the available immunosuppressants were to affect disease-inducing pathways in the immune system, we should see longer-lasting therapeutic effects. Dismantling one or several components of the final pathway of chronic inflammation may provide symptomatic relief, yet this carries little chance of curative intervention. The ultimate goal of a rheumatologist lies in maintaining or reversing the abnormalities causing RA while balancing the associated risk of toxicity. As rheumatologists move toward this goal, we will find it indispensable to understand the pathogenic fundamentals of RA.

THE CURRENT PARADIGM — OVERREACTIVE IMMUNE RESPONSES CAUSE RA

Adaptive immunity is a highly complex process mediated by lymphocytes that recognize a specific antigen and initiate a response to eliminate the antigen. B cells produce specific antibodies and have the capability, if appropriately supported by T cells, to undergo affinity maturation, thereby optimizing the fit of the antibody to the antigen. Receptors on T cells are triggered when the receptors bind complexes of antigenic peptides embedded into self-MHC dimers on the surface of antigen-presenting cells. T cells respond to stimulation of their antigen receptors through several distinct mechanisms: they express regulatory molecules on their membranes; they release cytokines that regulate the function of immune effector cells; and they differentiate into effector cells that mediate cytotoxicity. The adaptive immune system has 4 cardinal features that facilitate the recognition and selective elimination of specific foreign molecules, microorganisms, and malignant cells. These are antigenic specificity, diversity, immunologic memory, and self/nonself-discrimination. A critical element in this system is the assembly of an enormous repertoire of $> 10^9$ different receptors/antibodies that can distinguish a seemingly unlimited number of nonself-antigens. Equally important are regulatory mechanisms that protect healthy host tissue from the powerful instruments of immunity.

There is experimental evidence that several of the cardinal principles of the adaptive immune system are abnormal in RA[6,7]. Antibodies reactive to self-antigens are assembled and undergo affinity maturation, making them potentially dangerous. T cells and B cells in the synovial lesion organize into sophisticated lymphoid microstructures, known to amplify antigen recognition[8-10]. T cells, by producing interferon-γ (IFN-γ), drive macrophages to secrete proinflammatory cytokines. T cells are also suspected to directly affect the process of bone erosion by activating bone-resorbing osteoclasts[11]. The dogma has been that the immune response driving RA is elicited by arthritogenic antigens, which induce immunologic memory and are the driving force of non-immune cells mediating tissue damage.

Considering the central position of CD4 T cells in adaptive immunity, they should be instrumental players in pathogenic immune responses and prove to be ideal therapeutic targets. Guided by this consideration, several experimental therapeutic approaches have been developed, including depletion of T cells and bone marrow ablative therapy with stem cell transplantation[12-14]. In essence, these experimental treatments did not lead to the abrogation of RA; rather, rheumatoid disease recurred or persisted in patients who were severely lymphopenic.

CHALLENGES TO THE CURRENT PARADIGM — DISEASE IN THE FACE OF SUPPRESSED ADAPTIVE IMMUNE RESPONSES

Patients experimentally treated with T cell-depleting antibodies, including CAMPATH-1H and anti-CD4, experienced transient improvement in inflammatory activity, only to have disease relapse while peripheral T cell numbers were

severely suppressed[15,16]. Patients with RA who have been treated with bone marrow ablative therapy and stem cell transplantation have had recurrent disease, despite the aggressive immunosuppressive intervention[13,14]. Patients treated with tumor necrosis factor-α (TNF-α) inhibitors experienced improvement, not impairment, in T cell reactivity as the burden of disease declined[17]. All these observations challenged the model of the antigen-specific T cell activated in the synovial lesion being the master player in RA. A possible explanation would be that the clinical manifestations of RA, including its destructive synovial lesions, are not related to T cell activity. This question has been studied by implanting rheumatoid synovium into severe combined immunodeficiency (SCID) mice. Depletion of human T cells in synovium-SCID mouse chimeras was used to test the requirement for T cells in maintaining synovitis. Antibody mediated depletion of tissue-infiltrating T cells eliminated the disease. Anti–T cell reagents reduced tissue IFN-γ production in a dose-dependent manner. In parallel, the proinflammatory cytokines interleukin 1ß (IL-1ß) and TNF-α declined. IL-15 production in the synovial infiltrates was also dependent on IFN-γ, and the matrix metalloproteinases (MMP)-1 and -3 were profoundly downregulated after T cell depletion. IFN-γ was identified as a critical survival factor for tissue-residing macrophages, the cellular source of the proinflammatory and tissue-destructive mediators. Vice versa, adoptively transferred T cells were able to amplify synovial inflammation multifold. From these studies, it can be concluded that T cells are obligatory in rheumatoid synovitis[18]. Why then can T cell depletion in patients with RA not remit the disease process? Depletion of mature T cells would fail to reset the immune system in RA if the newly formed T cells repeat the pathogenic immune responses, as would be the case if patients with RA had a generalized and persistent defect in the replenishment and the selection of the T cell pool[19].

T CELL HOMEOSTASIS IN RA — PREMATURE AGING OF THE T CELL POOL

RA preferentially affects individuals who are past the prime of their reproductive potential; the risk for disease increases with advancing age. In essence, RA manifests in hosts whose immune system has reached late adulthood. Lymphocytes, like all somatic cells, have a limited ability to divide. To maintain sufficient T cell numbers in the T cell pool, new T cells must feed into the compartment. The number of T cells needed for replenishment depends on the half-life of such cells. Recent technological advances have made it possible to measure the *in vivo* half-life of human T cells. Surprisingly, T cells were found to have half-lives of 77 to 87 days, implying that ~2×10^9 T cells must be added daily to maintain a T cell pool consisting of 2×10^{11} members[20]. The major, if not only, source of new T cells is the thymus. Thymic function is strictly age-dependent, with a decline of 90% to 95% between the ages of 20 and 60 in humans[21]. It has long been known that thymic size is maximal during puberty, to be followed by involution of the gland. Although recent studies in patients with HIV infection have suggested the possibility of persistent thymic production in adults, it is highly unlikely that thymic output contributes significantly to T cell homeostasis in individuals older than 40 years[22].

To examine how T cell regeneration is regulated in patients with RA, we measured the lengths of telomeric sequences in lymphocytes. Telomeres are positioned at the ends of chromosomes, and 50 to 100 base pairs (bp) of telomeric sequence are lost during each cell division. Telomere length can, therefore, help in determining the proliferative turnover of cells. In healthy individuals, telomeres in CD4 T cells undergo age-dependent erosion of ~1500 bp between the ages of 20 and 60[21]. In individuals with RA, telomeres are already severely shortened at age 20, with minimal reserve left for age-dependent shortening during the subsequent 4 decades. CD4 T cells in patients with RA must have cycled excessively to cause prematurely eroded telomeres. CD8 T cells are affected as well. More important, telomeric shortening is also evident in naive, antigen-inexperienced CD4 T cells[21].

One possible explanation for telomeric erosion in RA is that T cells, even naive T cells, are driven to proliferate due to excessive production of growth factors and cytokines. Alternatively, increased replication of T cells could be part of a feedback mechanism to ensure sufficient numbers of circulating T cells despite failing input of novel T cells. To address this issue, we determined the number of T cells bearing T cell receptor excision circles (TREC), episomal byproducts generated during T cell receptor (TCR) rearrangement. These TREC can be used to identify T cells that have recently emigrated from the thymus. In healthy individuals, TREC levels decline in relation to age, with a 90% to 95% reduction between the ages of 20 and 60[19,21]. In patients with RA, TREC levels are significantly below normal at age 20, only about one-third of those found in age matched controls. Low levels of TREC are maintained over the lifetimes of individuals with RA, most of them expressing < 10% of age appropriate concentrations. In essence, thymic production rates are insufficient in patients with RA, forcing peripheral T cells to hyperproliferate to fill the void (Figure 1). As a consequence of excessive self-replication, CD4 T cells become senescent. T cell senescence is associated with a series of functional deviations, one of which is autoreactivity of the "recycled" T cells[23].

FUNCTIONAL CONSEQUENCES OF PREMATURE AGING — THE LOSSES AND GAINS OF SENESCENT T CELLS

When T cells enter a state of senescence, they lose functions; more interesting, they also gain functions (Figure 2).

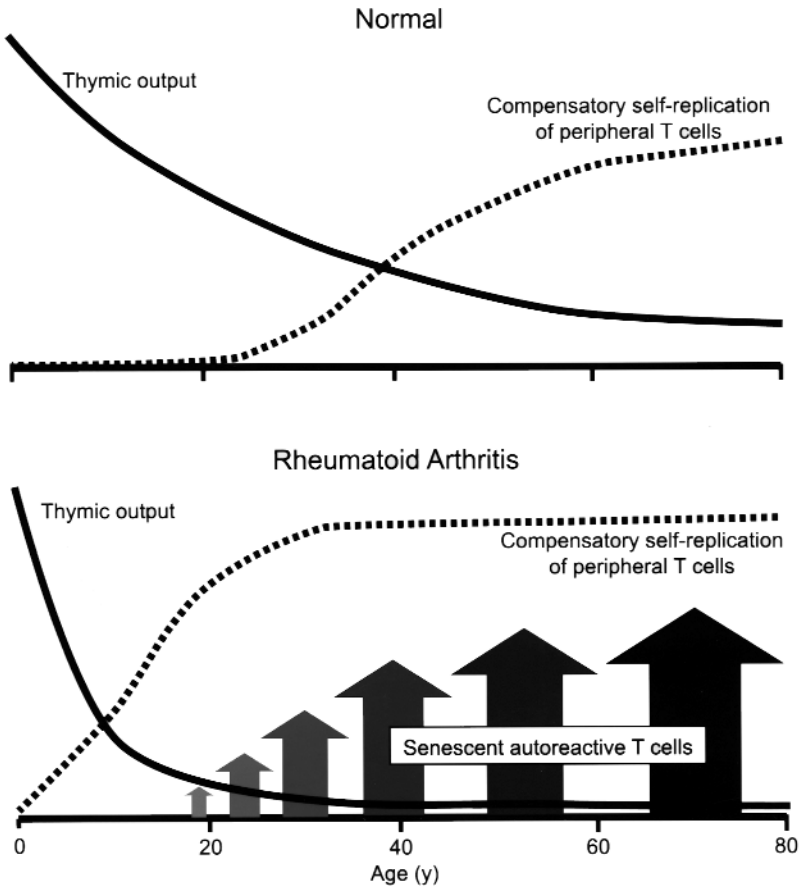

Figure 1. The emerging disease model for RA. In a healthy immune system, the age related decline in thymic T cell production is compensated by self-replication of mature T cells (upper panel). The resulting T cell pool is a mixture of "old" and "new" T cells. If thymic T cell generation ceases prematurely, the replicative pressure on peripheral T cells overwhelms the replicative potential of lymphocytes, leading to cellular senescence. As a consequence, senescent T cells, characterized by autoreactivity and proinflammatory capabilities, accumulate in the T cell compartment.

T cells "differentiating" in the periphery obviously are not exposed to the selection process imposed by the thymus. Thymic deficiency, in contrast, may also cause a lack of regulatory T cells that could prevent functional activity of anti-self responses. We have proposed that the dominance of senescent T cells leads to a fundamental shift in the functional profile of the T cell pool. Thus, premature aging in the immune system with RA could be considered a critical process in the loss of self-tolerance. Several consequences of premature senescence of T cells and their possible influence on the affected patient have been studied (Table 1).

One of the consequences of excessive compensatory self-replication of T cells is a contraction of the TCR repertoire. Instead of assembling an enormous array of different TCR, patients with RA generate a T cell pool with ~10% of the expected TCR specificities, expanding each of them to a degree that lymphopenia is prevented[24]. This contraction in diversity has obvious effect, because T cell responses to antigenic challenges may be compromised due to the lack of a perfectly-fitting antigen receptor.

Equally important are the restrictions imposed by the proliferative potential of a somatic cell. Lymphocytes must be able to expand promptly when triggered through their TCR. A single precursor gives rise to an expanding clone that can eliminate a dangerous antigenic stimulus. Naive T cells can proliferate to huge clonal populations. This clonal burst is restricted in naive T cells from patients with RA, impairing the ability of their immune systems to react to antigen with the appropriate expansion of antigen-specific effector cells[21].

Premature senescence of T cells in patients with RA, which essentially affects the entire T cell pool, is not only associated with functional loss. The senescence program in T cells is complex and includes a spectrum of genes that are downregulated or upregulated as the T cell progresses through its life cycle. The best studied genetic consequence of T cell senescence is the loss of CD28, a major costimulatory molecule[25] considered to be essential in complementing TCR signals. The underlying genetic mechanisms of CD28 loss have been explored[26]. CD28 deficiency occurs in T cells

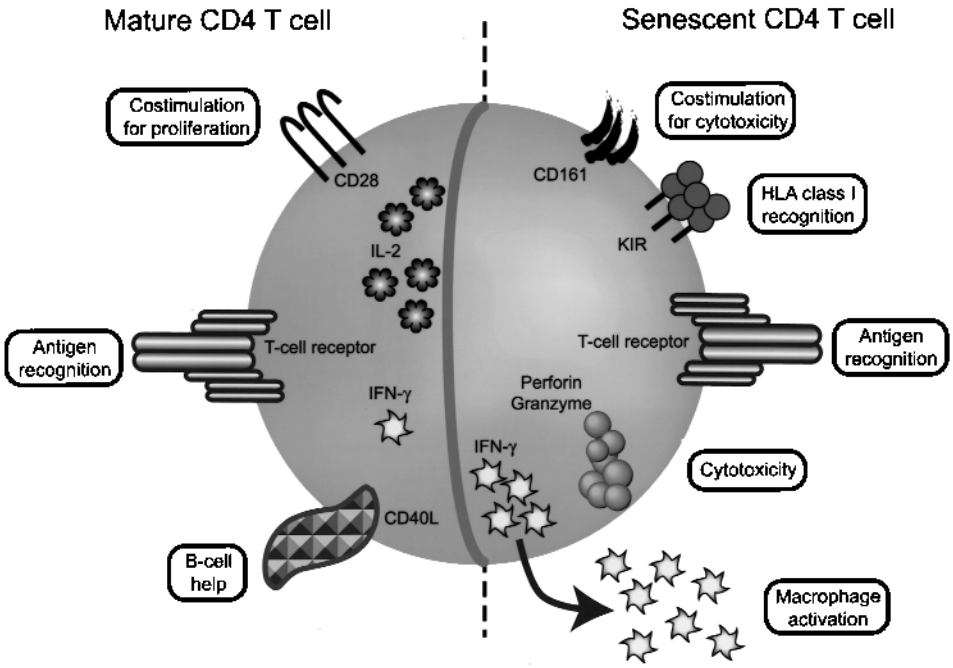

Figure 2. Phenotypic and functional profiles of "young" and "old" T cells. Classic helper T cells recognize antigens and provide help through communication with B cells and other effector cells. Activation of classic T cells can only be completed if the cell receives a costimulatory signal, usually through CD28 (left). In contrast, senescent CD4 T cells undergo a dramatic shift in expression of cell surface receptors and functions. They become independent from the CD28 mediated costimulatory signal and instead use several different receptors commonly expressed by NK cells (CD161, killer cell immunoglobulin-like receptors, KIR). KIR recognize HLA class I molecules, complementing antigen recognition by the T cell receptor with signals typically used by the innate immune system. Senescent T cells acquire cytotoxic granules and can kill target cells (right).

Table 1. Premature immunosenescence in rheumatoid arthritis.

Event	Consequence
Premature loss of thymic T cell production	Dominance of "recycled" T cells in the pool
Excessive self-proliferation of naive T cells	Restricted clonal burst (immunodeficiency?)
Compensatory proliferation of selected CD4 T cells	Monoclonality of T cells
Accelerated senescence of CD4 T cells	Autoreactivity
	Production of large amounts of IFN-γ
	T cell mediated cytotoxicity

that fail to assemble a protein complex that interacts with the CD28 transcription initiator sites. The precise nature of these regulator proteins has not been determined. Interestingly, TNF-α has been found to modulate these nuclear proteins, affecting the transcriptional control of CD28 and, through this mechanism, possibly inducing premature T cell senescence[27].

Several other events accompany the senescence program in T cells (Figure 2). CD4+, CD28^{null} T cells in patients with RA express molecules typically found on natural killer (NK) cells. The NK cell receptor, CD161, appears on CD4+ T cells that infiltrate into synovial lesions[28]. CD161 has been implicated in regulating NK cell cytotoxicity. Additional NK cell related receptors have been identified by comparing gene profiles of CD4+, CD28+ T cells and CD4+, CD28^{null} T cells. Most remarkably, CD28 deficient T cells from RA patients express killer cell immunoglobulin-like receptors (KIR)[29,30], which recognize HLA class I polymorphisms. Triggering of KIR on CD4+, CD28^{null} T cells amplifies the production of IFN-γ, assigning costimulatory potential to this receptor family. Evidence of an important contribution of KIR to the rheumatoid disease process has come from studies examining genetic polymorphisms of KIR genes. The KIR2DS2 gene is highly enriched among individuals with RA who have vascular complications, as in rheumatoid vasculitis. These patients are also likely to possess the complementary polymorphic HLA-C ligand[30].

THE FUTURE OF TREATING RA — IMMUNORECONSTITUTION INSTEAD OF IMMUNOSUPPRESSION

Evidence strongly supports an important role of senescent CD4 T cells in the rheumatoid disease process (Figure 2). These unconventional CD4 T cells, often equipped with NK cell related molecules, have proinflammatory capabilities, particularly so by virtue of IFN-γ production[31]. In addition, they express the cytotoxic proteins perforin and granzyme B and possess several receptor molecules capable of regulating the cytolytic machinery[32]. The accumulation of senescent CD4 T cells is a result of premature aging, most likely caused by insufficient production of novel T cells, inducing excessive compensatory replication of mature T cells in the peripheral T cell pool (Figure 1). The obvious questions concern how thymic function is altered in RA. It will be important to know when in a person's life T cell production is impaired. Reduction of TREC-bearing T cells is evident in patients with RA who are as young as 20 years, with no evidence of further acceleration in TREC loss with progressing disease. As well we need to determine whether defective rejuvenation of the T cell pool is reversible. This may lead to novel strategies of immunointervention. Specifically, immunoreconstitution instead of improvements in immunosuppressive therapy may advance our ability to treat autoimmunity. While counteracting the excessive proinflammatory capability of a prematurely aged immune system is symptomatically beneficial, fundamental improvement may only be achieved if senescent T cells are replaced by biologically young and functionally competent T lymphocytes.

CORNELIA M. WEYAND, MD;
JÖRG J. GORONZY, MD,
Departments of Medicine and Immunology,
Mayo Clinic,
Rochester, Minnesota, USA.

Address reprint requests to Dr. C.M. Weyand, Guggenheim 401, 200 First Street SW, Rochester, MN 55905. E-mail: weyand.cornelia@mayo.edu
This work was supported by grants from the National Institutes of Health (R01 AR42527, R01 AR41974, R01 AI44142, and R01 AG15043) and by the Mayo Foundation.

ACKNOWLEDGMENT

The authors thank James W. Fulbright for graphics preparation and editorial assistance and Linda H. Arneson for secretarial support. We are very grateful to our colleagues and the patients who provided samples for this study.

REFERENCES

1. Keystone EC. Tumor necrosis factor-alpha blockade in the treatment of rheumatoid arthritis. Rheum Dis Clin North Am 2001;27:427-43.
2. Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? Annu Rev Immunol 2001; 19:163-96.
3. Keane J, Gershon S, Wise RP, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001;345:1098-104.
4. Graninger WB, Smolen JS. One-year inhibition of tumor necrosis factor-alpha: a major success or a larger puzzle? Curr Opin Rheumatol 2001;13:209-13.
5. Baghai M, Osmon DR, Wolk DM, Wold LE, Haidukewych GJ, Matteson EL. Fatal sepsis in a patient with rheumatoid arthritis treated with etanercept. Mayo Clin Proc 2001;76:653-6.
6. Panayi GS, Corrigall VM, Pitzalis C. Pathogenesis of rheumatoid arthritis. The role of T cells and other beasts. Rheum Dis Clin North Am 2001;27:317-34.
7. Cope AP. Regulation of autoimmunity by proinflammatory cytokines. Curr Opin Immunol 1998;10:669-76.
8. Takemura S, Braun A, Crowson C, et al. Lymphoid neogenesis in rheumatoid synovitis. J Immunol 2001;167:1072-80.
9. Takemura S, Klimiuk PA, Braun A, Goronzy JJ, Weyand CM. T cell activation in rheumatoid synovium is B cell dependent. J Immunol 2001;167:4710-8.
10. Weyand CM, Kurtin PJ, Goronzy JJ. Ectopic lymphoid organogenesis: a fast track for autoimmunity. Am J Pathol 2001;159:787-93.
11. Gravallese EM, Galson DL, Goldring SR, Auron PE. The role of TNF-receptor family members and other TRAF-dependent receptors in bone resorption. Arthritis Res 2001;3:6-12.
12. Isaacs JD, Manna VK, Rapson N, et al. CAMPATH-1H in rheumatoid arthritis — an intravenous dose-ranging study. Br J Rheumatol 1996;35:231-40.
13. Tyndall A. Autologous hematopoietic stem cell transplantation for severe autoimmune disease with special reference to rheumatoid arthritis. J Rheumatol 2001;28 Suppl 64:5-7.
14. Bingham SJ, Snowden J, McGonagle D, et al. Autologous stem cell transplantation for rheumatoid arthritis — interim report of 6 patients. J Rheumatol 2001;28 Suppl 64:21-4.
15. Brett S, Baxter G, Cooper H, Johnston JM, Tite J, Rapson N. Repopulation of blood lymphocyte sub-populations in rheumatoid arthritis patients treated with the depleting humanized monoclonal antibody, CAMPATH-1H. Immunology 1996;88:13-9.
16. Jendro MC, Ganten T, Matteson EL, Weyand CM, Goronzy JJ. Emergence of oligoclonal T cell populations following therapeutic T cell depletion in rheumatoid arthritis. Arthritis Rheum 1995;38:1242-51.
17. Cope AP, Londei M, Chu NR, et al. Chronic exposure to tumor necrosis factor (TNF) in vitro impairs the activation of T cells through the T cell receptor/CD3 complex; reversal in vivo by anti-TNF antibodies in patients with rheumatoid arthritis. J Clin Invest 1994;94:749-60.
18. Klimiuk PA, Yang H, Goronzy JJ, Weyand CM. Production of cytokines and metalloproteinases in rheumatoid synovitis is T cell dependent. Clin Immunol 1999;90:65-78.
19. Goronzy JJ, Weyand CM. Thymic function and peripheral T-cell homeostasis in rheumatoid arthritis. Trends Immunol 2001; 22:251-5.
20. Hellerstein M, Hanley MB, Cesar D, et al. Directly measured kinetics of circulating T lymphocytes in normal and HIV-1-infected humans. Nat Med 1999;5:83-9.
21. Koetz K, Bryl E, Spickschen K, O'Fallon WM, Goronzy JJ, Weyand CM. T cell homeostasis in patients with rheumatoid arthritis. Proc Natl Acad Sci USA 2000;97:9203-8.
22. Douek DC, McFarland RD, Keiser PH, et al. Changes in thymic function with age and during the treatment of HIV infection. Nature 1998;396:690-5.
23. Schmidt D, Goronzy JJ, Weyand CM. CD4+ CD7- CD28- T cells are expanded in rheumatoid arthritis and are characterized by autoreactivity. J Clin Invest 1996;97:2027-37.

24. Wagner UG, Koetz K, Weyand CM, Goronzy JJ. Perturbation of the T cell repertoire in rheumatoid arthritis. Proc Natl Acad Sci USA 1998;95:14447-52.
25. Vallejo AN, Brandes JC, Weyand CM, Goronzy JJ. Modulation of CD28 expression: distinct regulatory pathways during activation and replicative senescence. J Immunol 1999;162:6572-9.
26. Vallejo AN, Weyand CM, Goronzy JJ. Functional disruption of the CD28 gene transcriptional initiator in senescent T cells. J Biol Chem 2001;276:2565-70.
27. Bryl E, Vallejo AN, Weyand CM, Goronzy JJ. Down-regulation of CD28 expression by TNF-alpha. J Immunol 2001;167:3231-8.
28. Warrington KJ, Takemura S, Goronzy JJ, Weyand CM. CD4+, CD28- T cells in rheumatoid arthritis patients combine features of the innate and adaptive immune systems. Arthritis Rheum 2001;44:13-20.
29. Namekawa T, Snyder MR, Yen JH, et al. Killer cell activating receptors function as costimulatory molecules on CD4+ CD28null T cells clonally expanded in rheumatoid arthritis. J Immunol 2000;165:1138-45.
30. Yen JH, Moore BE, Nakajima T, et al. Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis. J Exp Med 2001;193:1159-67.
31. Park W, Weyand CM, Schmidt D, Goronzy JJ. Co-stimulatory pathways controlling activation and peripheral tolerance of human CD4+ CD28- T cells. Eur J Immunol 1997;27:1082-90.
32. Namekawa T, Wagner UG, Goronzy JJ, Weyand CM. Functional subsets of CD4 T cells in rheumatoid synovitis. Arthritis Rheum 1998;41:2108-16.