Commentary

Altered signalling thresholds in T lymphocytes cause autoimmune arthritis

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Abstract

The development of spontaneous autoimmunity in inbred strains of rodents has allowed us to investigate the molecular basis of chronic inflammatory disease in ways that would not be possible in humans. Recently, two new mouse models of autoimmune inflammatory polyarthritis have been reported that demonstrate how alterations in signalling thresholds sufficient to perturb central T-cell tolerance lead to inflammatory arthritis. These mice provide new insights into the complexities of what may turn out to be a heterogeneous group of diseases that we call rheumatoid arthritis. They will also provide unique tools for dissecting precisely how chronically activated T cells contribute to the effector phase of arthritis through mechanisms that may be less dependent on antigen receptor signalling.

Keywords: autoimmune arthritis, signalling, T cells, thymic selection

In the 1980s it was proposed that clonal expansion of T cells reactive to specific tissue antigens was a key event in the initiation and perpetuation of autoimmune disease [1]. This model gained much support from investigators in arthritis research, and so for more than a decade much effort focused on investigating the molecular basis of inflammatory arthritis induced in rodents following immunization with antigens [2]. The lack of robust spontaneous arthritis models, however, hindered progress toward unravelling the relationship between the adaptive immune response and the terminal effector phase of joint inflammation, and cartilage and bone destruction in a more physiological setting. Understanding this relationship has important implications for defining the contribution of lymphocytes to the pathogenesis of rheumatoid arthritis (RA) at different stages of the disease, as well as for defining therapeutic targets.

Two new mouse models have provided insight into how aberrations in T-cell development can generate a repertoire of autoreactive effector T cells that are necessary and sufficient to promote spontaneous inflammatory arthritis. In 2002, investigators from the laboratory of Hirano reported that a genetically engineered point mutation in the IL-6 family receptor signalling subunit gp130 was sufficient to induce an autoimmune arthritis [3]. The gp130 subunit is shared by receptors not only for IL-6 itself, but also for leukaemia inhibitory factor, ciliary neurotropic factor, oncostatin M, IL-11 and cardiotropin-1, and facilitates the recruitment of Janus kinase (JAK)-1, JAK-2 and tyrosine kinase-2 to the receptor complex (for review [4,5]). Receptor engagement leads to phosphorylation of tyrosine residues in the cytoplasmic domain of gp130. Phosphotyrosine within the YxxQ motif serves as a site for recruitment and activation of STAT3 [6]. STAT3 activation and dimerization is required for nuclear translocation and regulation of gene expression. This pathway is regulated by SHP-2, which associates with gp130 through Y759, and through recruitment of SOCS-3 it attenuates gp130 mediated signals [7,8]. Hirano and colleagues disrupted this negative regulatory loop by generating knock-in mouse lines expressing the 759Y to F mutation.

IL = interleukin; JAK = Janus kinase; RA = rheumatoid arthritis; SHP = src homology 2 domain-bearing protein tyrosine phosphatase; SOCS = suppressor of cytokine signalling; STAT = signal transducer and activator of transcription; TCR = T-cell receptor.

Although gp130$^{F759/F759}$ mice are normal at birth, they develop splenomegaly and lymphadenopathy associated with increased generation of T-helper-1 cells and hypergammaglobulinaemia. By 8 months of age gp130$^{F759/F759}$ mice develop a symmetrical and peripheral inflammatory arthropathy, progressing from toes and wrists to larger joints by 16 months. Disease occurred earlier and with greater severity in females. Histological analysis revealed a proliferative synovitis comprised predominantly of neutrophils, with few lymphocytes or plasma cells, associated with severe bony destruction, ankylosis in many joints, and reactive sclerotic changes. IgG rheumatoid factor, anti-single and anti-double stranded DNA antibodies, and antibodies to ribonucleoproteins were detected in serum. More detailed investigation of lymphocyte development uncovered a reduced frequency of CD4$^+$CD8$^+$ double positive thymocytes in the mutant mice, associated with increased CD4$^+$ or CD8$^+$ single positive thymocytes as compared with wild-type littermates. In peripheral lymph nodes CD62L$^-$CD44$^+$ activated memory T cells predominated, with increased expression of activation surface antigens CD69 and CD25, and hyperresponsiveness to T cell receptor (TCR) ligation with anti-CD3 antibodies.

The finding of increased numbers of activated T cells in otherwise naïve gp130$^{F759/F759}$ mice suggested that mechanisms of tolerance might be perturbed. Central tolerance was assessed by examining thymic selection of gp130$^{F759/F759}$ mutant male antigen-specific T cells using the H-Y TCR transgenic mouse model [9]. The frequency of CD8$^+$ H-Y specific thymocytes in female mutant mice was comparable to that observed in wild-type mice, indicating that positive selection was not affected by the gp130 mutation. In contrast, impaired negative selection of CD8$^+$ T cells was suggested by increased numbers of CD8^{lo} thymocytes, indicative of mutant H-Y specific cells that have escaped negative selection, as well as by increased double positive thymocytes. Defects in clonal deletion were also suggested by the observation of impaired reduction in Vβ8 T cell numbers after injection of superantigen. Biochemical analysis of IL-6 signals in gp130$^{F759/F759}$ mutant T cells confirmed sustained and prolonged tyrosine phosphorylation of JAK-1 and STAT-3 and the absence of IL-6 induced SOCS-3 induction. Prolonged STAT3 activation was associated with IL-6 induced inhibition of activation induced cell death and failure to upregulate Fas ligand upon anti-CD3 stimulation. The fact that arthritis did not develop in RAG-2 deficient gp130$^{F759/F759}$ mice suggested that aberrations in lymphocyte gp130 signalling in this model were particularly important for the development of arthritis. We learn from these mice that a single amino acid substitution, capable of promoting gp130 signalling, is sufficient to perturb central tolerance, leading to increased numbers of self-reactive T cells in the periphery.

In the 27 November 2003 issue of the journal *Nature*, Sakaguchi and colleagues [10] reported spontaneous arthritis developing in their inbred Balb/c colony (named SKG mice hereafter). The disease had many features of human RA, including a peripheral symmetrical arthritis leading to pannus eroding cartilage and subchondral bone, and joint destruction that was more severe in females. Although the synovitis was typical of RA with a predominant lymphocytic infiltrate, a systemic inflammatory disease was also evident in older mice characterized by pneumonitis, dermal infiltration, subcutaneous nodules and vasculitis – features not dissimilar to recognized extra-articular manifestations of RA. In keeping with this phenotype was a serotype characterized by high titres of IgG rheumatoid factor, autoantibodies to collagen II and hsp70, hypergammaglobulinaemia and increased circulating immune complexes, but no anti-DNA antibodies. The chronic inflammatory arthritis was T-cell dependent, as demonstrated in adoptive transfer experiments of splenic, lymph node or thymic CD4$^+$ T cells into Balb/c nude or severe combined immunodeficiency mice. By contrast, transfer of sera from arthritic mice was not arthritogenic.

Analysis of offspring from a series of SKG crosses implicated a genetic anomaly of a single gene locus inherited in an autosomal recessive fashion, with near 100% penetrance of homozygotes. Linkage analysis using microsatellite markers mapped the *skg* locus to the centromeric portion of chromosome 1. A single G/T transposition within the ZAP-70 coding sequence was subsequently found to segregate with *skg* disease. The mutation encodes a switch at codon 163 from tryptophan to cystein (W163C), corresponding to the initial amino acid residue of the carboxyl-terminal SH2 domain of ZAP-70. Transgenic expression of the ZAP-70^{skg} allele on a ZAP-70 deficient background confirmed that the ZAP-70 mutation was necessary and sufficient for the development of arthritis.

Biochemical analysis demonstrated that the ZAP-70^{W163C} mutation attenuated membrane proximal TCR signalling, leading to profound proliferative hyporesponsiveness of T cells to TCR engagement in spite of expressing an activated CD44^{hi}, CD45RB^{lo}, LFA-1^{hi}, ICAM-1^{hi} phenotype. This biochemical TCR signalling deficit translated to functional aberrations of thymic development manifest by reduced thymocyte apoptosis *in vivo* following anti-CD3 injection, increased numbers of TCR^{lo} thymocytes, and reduced numbers of peripheral CD4$^+$ or CD8$^+$ T cells. Impaired positive selection was observed in *skg*/*skg* DO11.10 TCR transgenic mice, as well as in *skg*/*skg* female H-Y TCR transgenic mice, whereas impaired negative selection of H-Y specific CD8$^+$ T cells was demonstrated in male H-Y TCR transgenic *skg*/*skg* mice, when compared with H-Y TCR transgenic littermates. Arthritis developed in the majority of either H-Y or

DO11.10 TCR transgenic mice that were also homozygous for the ZAP-70^{W163C} mutation, implying that this mutation leads to the selection of arthritogenic T cells expressing transgenic TCRβ chains pairing with endogenous TCRα chains. These data pointed to a shift in thymic selection of the peripheral T-cell repertoire as the primary cause of autoimmune arthritis as a direct consequence of a point mutation in a tyrosine kinase pivotal for transmitting signals from the TCR to downstream pathways.

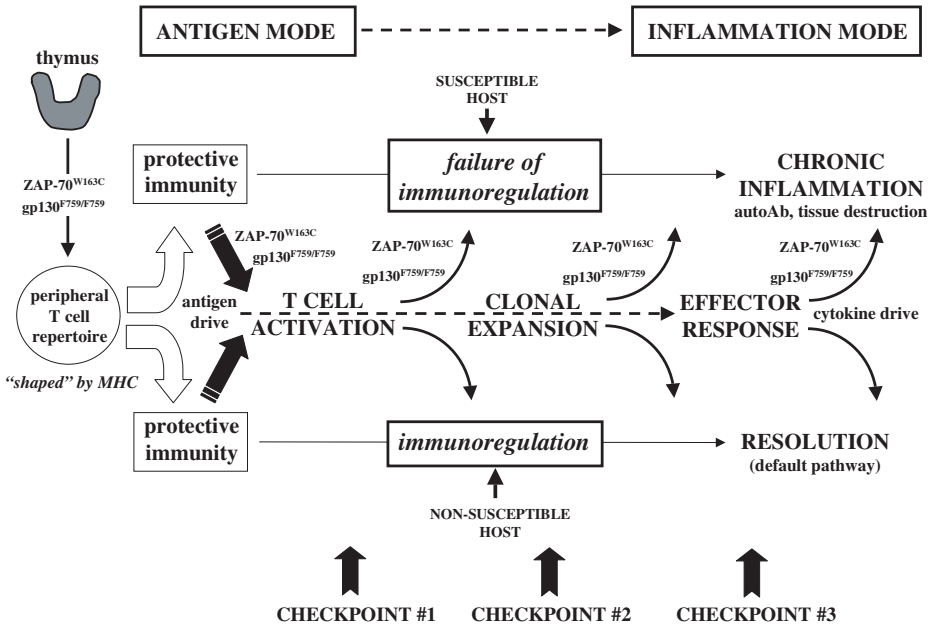
Both the SKG and gp130$^{F759/F759}$ mice develop spontaneous autoimmune arthritis because the signals that normally promote deletion of autoreactive T cells in the thymus are defective, but there the similarity ends. One striking immunological phenotype that distinguishes these models is the responsiveness to TCR stimulation; T cells from gp130$^{F759/F759}$ mice are hyperresponsive, whereas SKG T cells are profoundly hyporesponsive. So how can extremes of T-cell reactivity both promote inflammatory arthritis? The development of arthritis in gp130$^{F759/F759}$ mice is perhaps easiest to digest. First, there is unbridled gp130-mediated signalling and chronic STAT3 activation in all cells because SHP-2 mediated regulation is impaired [8,11]. Peripheral T cells express a memory/activated phenotype, differentiate toward T-helper-1 cells, and are refractory to peripheral clonal deletion. Persistence of activated clones *in vivo* would certainly account for hypergammaglobulinaemia and the extensive panel of autoantibodies detected in the sera of gp130$^{F759/F759}$ mice. On the other hand, Hirano and colleagues [11] previously showed that stimulation of gp130$^{F759/F759}$ mutant B cells with anti-CD40 alone enhances immunoglobulin production, which is further augmented with IL-6, whereas B-cell proliferative responses are no different from those of B cells from wild-type mice. This suggests that other cell types must be contributing to the pathological process. For example, it is equally plausible that aberrant gp130 signalling in endothelial cells, macrophages, or fibroblast-like synoviocytes contribute to the inflammatory synovitis through mechanisms independent of T cells. Generation of tissue specific gp130$^{F759/F759}$ mutant mouse lines would address this directly.

The SKG mouse is also intriguing but for different reasons. Here the mutation is highly specific not only for CD4$^+$ and CD8$^+$ T cells but also for targeting the TCR signalling pathway. ZAP-70 is the most membrane proximal protein tyrosine kinase. It is recruited to the TCR–CD3 complex upon ligation-induced phosphorylation of YxxL immunoreceptor tyrosine activation motifs in the cytoplasmic tails of TCRζ and CD3ε, γ and δ chains [12]. By binding to tandemly arranged immunoreceptor tyrosine activation motifs, ZAP-70 SH2 domains couple the receptor complex to downstream Ras/ERK and calcium signalling pathways [13]. Thus, the effects on negative thymic selection and the development of a repertoire of highly self-reactive T cells could be predicted. Indeed, Sakaguchi and colleagues [10] documented that physical association between mutant ZAP-70 and TCRζ is reduced. What is harder to reconcile is how impaired TCR signalling and proliferative responses can translate into an arthritogenic T-cell effector response that induces chronic inflammation in a disease model of RA in which MHC associations and lymphocytic synovial infiltrates support the concept of chronic antigen stimulation. On the other hand, more recent data demonstrate that T cells in rheumatoid synovium are paradoxically hyporesponsive to TCR ligation, leading to attenuation of proliferative and cytokine responses (for review [14]).

A number of possible explanations spring to mind. Perhaps the simplest is that TCR signals in peripheral *skg* T cells are sufficient for activation and terminal differentiation of effector T cells. Indeed, T-helper-1 and T-helper-2 cells are known to have distinct signalling thresholds for activation, at least in mouse models of T-cell differentiation [15,16]. Sakaguchi and coworkers [10] speculate that resident synovial cells, as distinct from stromal cells derived from other tissues, are especially sensitive to signals generated by chronically activated T cells, including cytokines. Interestingly, in tumour necrosis factor or IL-1 gene deficient SKG mice the incidence of arthritis is reduced to 20%, whereas arthritis is not observed at all on an IL-6 deficient background (Sakaguchi S, personal communication). Although ZAP-70 is not expressed in fibroblast-like synoviocytes, it is conceivable that the effector function of mutant T cells promotes fibroblast proliferation, as well as the secretion of cytokines and other effector molecules such as matrix metalloproteinases, through cell contact dependent mechanisms *in situ*, which are less dependent on T-cell antigen receptor signals.

More controversial would be the idea that effector responses of terminally differentiated ZAP-70 mutant T cells are independent of antigen signals. According to this model, *skg* T cells would stimulate synoviocytes to proliferate and activate B cells to produce antibody, perhaps through cell contact dependent mechanisms or by secretion of T-cell products on engagement of receptors other than the TCR [17,18]. Data suggest that subsets of RA synovial T cells, as well as having proximal TCR signalling defects [14], may well behave in this way, being sustained *in vivo* by chronic cytokine rather than antigen stimulation [19]. Another possibility, and one that must appeal to Sakaguchi, would be that ZAP-70^{W163C} impairs the generation and/or function of CD4$^+$CD25$^+$ regulatory T cells. A progressive imbalance of effector and regulatory cell numbers could account for the systemic

Figure 1



Checkpoints in T-cell development, differentiation and effector function in the pathogenesis of chronic inflammatory arthritis. A repertoire of arthritogenic T cells is shaped during thymic selection on MHC molecules complexed to self-antigenic peptides. Signalling thresholds will dictate which cells undergo positive and negative selection, as well as those cells that become activated and undergo clonal expansion in the periphery ('antigen mode'). Persistence of chronically activated T cells *in vivo*, augmented by failure to undergo activation-induced cell death (or propriocidal regulation), will promote effector function through cytokine overexpression and cell contact dependent mechanisms, leading to activation of monocytes, fibroblasts and B cells *in situ* ('inflammation mode'). This terminal phase is manifested by chronic cytokine expression, invasion of cartilage and subchondral bone by pannus, and autoantibody production. Proposed pathways of activation and differentiation perturbed by ZAP-70^{W163C} or $gp130^{F759/F759}$ mutations are indicated.

inflammation and autoimmunity in SKG mice, not dissimilar from that in mice following depletion of $CD5^{high}$ or $CD25^{high}$ $CD4^+$ T cell subsets *in vivo*, which was reported by Sakaguchi and colleagues some years ago [20,21]. The finding that depletion of $CD4^+CD25^+$ T cells exacerbates arthritis is consistent (Sakaguchi S, personal communication). Finally, the possibility that the ZAP-70^{W163C} mutation alters the repertoire of peripheral $CD4^+CD25^+$ T regulatory cells needs exploring.

Whatever the mechanism, this mouse will provide an invaluable tool with which to dissect the progression through key checkpoints during the evolution of arthritis. These checkpoints of T-cell activation, clonal expansion and differentiation are illustrated in Fig. 1. In particular, the model should be applied to address the critical question of why alterations in T-cell repertoire lead specifically to arthritis but not to other autoimmune diseases. Thus far the autoantibody profile seems remarkably similar to that seen in human RA, and so it will be of considerable interest to know whether reactivity to specific antigens and clonal expansion of *skg* T cells is of importance in this model.

In their report, Hirano and colleagues [3] reflect upon the diverse array of engineered mutant strains that induce spontaneous inflammatory arthritis and consider whether this might reflect either the heterogeneity of the human disease we recognize as RA, or the great complexity that underlies the disease process. On the face of it, both ZAP-70^{W163C} and $gp130^{F759/F759}$ mutations induce autoimmune arthritis because of a breakdown in central and peripheral T-cell tolerance. Similar defects in tolerance have been described in the K/B × N TCR transgenic arthritis model of Benoist and Mathis [22], even though passive transfer of *skg* serum does not elicit arthritis. However, the biochemistry, the cellular phenotypes, the serology and the histopathology are all quite distinct in these models; indeed, the clinical features are also different. Notwithstanding the strain differences, analysis of these mouse models suggests that there exists both great heterogeneity of disease and complexity at the molecular level. We now face the daunting challenge of reclassifying, at a molecular level, the inflammatory arthritis of our patients in the clinic. This is important not just to satisfy our intellectual curiosity. Indeed, if the molecular basis of inflammatory arthritis differs for subsets of RA patients, then it follows that therapy should be selected to match the disease. The observed responder rates of RA patients to different biological therapies seem consistent with this notion.

Competing interests

None declared.

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References

1. Janossy G, Panayi G, Duke O, Bofill M, Poulter LW, Goldstein G: **Rheumatoid arthritis: a disease of T-lymphocyte/macrophage immunoregulation.** *Lancet* 1981, **2:**839-842.
2. Holmdahl R, Andersson M, Goldschmidt TJ, Gustafsson K, Jansson L, Mo JA: **Type II collagen autoimmunity in animals and provocations leading to arthritis.** *Immunol Rev* 1990, **118:**193-232.
3. Atsumi T, Ishihara K, Kamimura D, Ikushima H, Ohtani T, Hirota S, Kobayashi H, Park SJ, Saeki Y, Kitamura Y, Hirano T: **A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis.** *J Exp Med* 2002, **196:**979-990.
4. Hirano T, Nakajima K, Hibi M: **Signaling mechanisms through gp130: a model of the cytokine system.** *Cytokine Growth Factor Rev* 1997, **8:**241-252.
5. Taga T, Kishimoto T: **Gp130 and the interleukin-6 family of cytokines.** *Annu Rev Immunol* 1997, **15:**797-819.
6. Hirano T, Ishihara K, Hibi M: **Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors.** *Oncogene* 2000, **19:**2548-2556.
7. Nicholson SE, De Souza D, Fabri LJ, Corbin J, Willson TA, Zhang JG, Silva A, Asimakis M, Farley A, Nash AD, Metcalf D, Hilton DJ, Nicola NA, Baca M: **Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130.** *Proc Natl Acad Sci USA* 2000, **97:**6493-6498.
8. Lehmann U, Schmitz J, Weissenbach M, Sobota RM, Hortner M, Friederichs K, Behrmann I, Tsiaris W, Sasaki A, Schneider-Mergener J, Yoshimura A, Neel BG, Heinrich PC, Schaper F: **SHP2 and SOCS3 contribute to Tyr-759-dependent attenuation of interleukin-6 signaling through gp130.** *J Biol Chem* 2003, **278:**661-671.
9. Kisielow P, Teh HS, Bluthmann H, von Boehmer H: **Positive selection of antigen-specific T cells in thymus by restricting MHC molecules.** *Nature* 1988, **335:**730-733.
10. Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S, Sakihama T, Matsutani T, Negishi I, Nakatsuru S, Sakaguchi S: **Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice.** *Nature* 2003, **426:**454-460.
11. Ohtani T, Ishihara K, Atsumi T, Nishida K, Kaneko Y, Miyata T, Itoh S, Narimatsu M, Maeda H, Fukada T, Itoh M, Okano H, Hibi M, Hirano T: **Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses.** *Immunity* 2000, **12:**95-105.
12. Chan AC, Iwashima M, Turck CW, Weiss A: **ZAP-70: a 70 kd protein-tyrosine kinase that associates with the TCR zeta chain.** *Cell* 1992, **71:**649-662.
13. Weiss A, Littman DR: **Signal transduction by lymphocyte antigen receptors.** *Cell* 1994, **76:**263-274.
14. Cope AP: **Studies of T-cell activation in chronic inflammation.** *Arthritis Res* 2002, **Suppl 3:**S197-S211.
15. Hosken NA, Shibuya K, Heath AW, Murphy KM, O'Garra A: **The effect of antigen dose on CD4$^+$ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model.** *J Exp Med* 1995, **182:**1579-1584.
16. Tao X, Constant S, Jorritsma P, Bottomly K: **Strength of TCR signal determines the costimulatory requirements for Th1 and Th2 CD4$^+$ T cell differentiation.** *J Immunol* 1997, **159:**5956-5963.
17. Yamamura Y, Gupta R, Morita Y, He X, Pai R, Endres J, Freiberg A, Chung K, Fox DA: **Effector function of resting T cells: activation of synovial fibroblasts.** *J Immunol* 2001, **166:**2270-2275.
18. Burger D, Dayer JM: **The role of human T-lymphocyte-monocyte contact in inflammation and tissue destruction.** *Arthritis Res* 2002, **Suppl 3:**S169-S176.
19. Brennan FM, Hayes AL, Ciesielski CJ, Green P, Foxwell BM, Feldmann M: **Evidence that rheumatoid arthritis synovial T cells are similar to cytokine-activated T cells: involvement of phosphatidylinositol 3-kinase and nuclear factor kappaB pathways in tumor necrosis factor alpha production in rheumatoid arthritis.** *Arthritis Rheum* 2002, **46:**31-41.
20. Sakaguchi S, Fukuma K, Kuribayashi K, Masuda T: **Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease.** *J Exp Med* 1985, **161:**72-87.
21. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M: **Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases.** *J Immunol* 1995, **155:**1151-1164.
22. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D: **Organ-specific disease provoked by systemic autoimmunity.** *Cell* 1996, **87:**811-822.