Immunology and Cell Biology (2012) 90, 277–287

www.nature.com/icb

REVIEW

T-cell receptor signaling and the pathogenesis of autoimmune arthritis: insights from mouse and man

Shimon Sakaguchi[1,2], Helen Benham[3], Andrew P Cope[4] and Ranjeny Thomas[3]

The immune system has evolved to survey and respond appropriately to the universe of foreign pathogens, while at the same time deploying an intricate repertoire of mechanisms that keep responses to host tissues in check. For the adaptive immune system, specificity and sensitivity are provided by a large repertoire of antigen T-cell receptors (TCRs) constructed in their extracellular domain to recognize antigenic peptide fragments restricted and presented by histocompatibility complex molecules, and coupled through intracellular domains to signal transduction modules that serve to transmit environmental cues inside the cell. In this review we consider recent evidence that has provided insight into how altered TCR signaling thresholds could contribute to human autoimmune arthritis, including rheumatoid arthritis (RA), and the spondyloarthropathies (SpA). We also discuss mechanistic studies that demonstrate how perturbations of T-cell antigen receptor signaling in the SKG mouse model can promote systemic autoimmunity and the intersection with essential innate immune pathways that lead to the development of chronic inflammatory phenotypes.
Immunology and Cell Biology (2012) **90,** 277–287; doi:10.1038/icb.2012.4

Keywords: spondyloarthropathy; rheumatoid arthritis; T-cell receptor; innate immunity

The immune system has evolved to survey and respond appropriately to the universe of foreign pathogens, while at the same time deploying an intricate repertoire of mechanisms that keep responses to host tissues in check. Moreover, human populations have evolved with a genome shaped by interaction with infectious organisms. The need for protection against pathogens has driven evolution of the innate and adaptive immune system through selection pressure on a population basis, characterised by a highly polymorphic major histocompatability complex (MHC) combined with genetic variants that control the thresholds for, and the magnitude of immune responses.[1] For the adaptive immune system, specificity and sensitivity are provided by a large repertoire of antigen T-cell receptors (TCRs) constructed in their extracellular domain to recognize antigenic peptide fragments restricted and presented by MHC molecules, and coupled through intracellular domains to signal transduction modules that serve to transmit environmental cues inside the cell. The response to these environmental cues manifests in cellular activation, differentiation and lineage-specific effector or regulatory lymphocyte responses. As this pathway is one of the best defined in the immune system, and has been implicated in immunopathology for many decades, there has been great interest in defining to what extent inherited defects of TCR signaling might translate into immune pathology in general, and autoimmune diseases in particular. In this review we will consider recent evidence that has provided insight into how altered TCR signaling thresholds could contribute to human autoimmune arthritis, including rheumatoid arthritis (RA), and the spondyloarthropathies (SpA). We discuss mechanistic studies that demonstrate how perturbations of T-cell antigen receptor signaling can promote systemic autoimmunity and the pathways that lead to the development of distinct chronic inflammatory phenotypes in particular.

The components that make up the TCR signaling modules—protein tyrosine kinases, phosphatases and adaptor proteins—are essential for lymphocyte development and function.[2] For example, null mutations of the zeta-chain-associated protein kinase ZAP-70, arising either through inherited mutations or engineered by gene targeting, cripple antigen receptor signals and lead to a profound state of immune deficiency.[3–5] Early lymphocyte development in the thymus (for T cells) is blocked and, because positive selection is attenuated, a full complement of T lymphocyte specificities in the periphery is absent. Although null mutations of antigen receptor subunits, or their downstream signaling modules, is a rare event in man, it is becoming increasingly apparent that more subtle variants in genes encoding antigen receptor signal transduction pathways may contribute to immune pathology in ways that might not have been predicted.[6] Indeed, as we will discuss, data emerging from mouse models indicate that distinct thresholds of signaling can give rise to specific disease phenotypes, due in part to the fact that the TCR is

[1]Laboratory of Experimental Immunology, WPI Immunology Frontier Research Center, Osaka University, Suita, Japan; [2]Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan; [3]The University of Queensland, Diamantina Institute, Princess Alexandra Hospital, Brisbane, Queensland, Australia and [4]Academic Department of Rheumatology, Centre for Molecular and Cellular Biology of Inflammation, Division of Immunology, Infection and Inflammatory Diseases, School of Medicine, King's College London, London, UK
Correspondence: Professor R Thomas, The University of Queensland, Diamantina Institute, Princess Alexandra Hospital, Brisbane, Queensland 4102, Australia.
E-mail: ranjeny.thomas@uq.edu.au
Received 1 December 2011; revised and accepted 18 December 2011

pleiotropic, delivering quantitatively distinct signals in different cell lineages, including effector and regulatory cells.[7,8]

EVIDENCE FOR ABERRANT TCR SIGNALING THRESHOLDS IN RA

For decades it has been reported by many laboratories that T cells from patients with RA are anergic, or hyporesponsive to antigen receptor engagement (reviewed in Thomas *et al.*[9]). In the earliest studies, this conclusion was based on depressed DTH responses *in vivo* and impaired lymphocyte transformation *in vitro*.[10,11] This paradox has been difficult to reconcile with the observation of persistently activated T cells in peripheral blood and inflamed synovial joints, and enhanced autologous mixed lymphocyte responses when T cells are stimulated by RA synovial dendritic cells (DCs).[12] On the other hand, this state of impaired antigen responsiveness seems in line with the clinical state of immune suppression characteristic of RA patients with severe, active inflammatory disease. Although it was suggested that this immune state could be due to the effects of immunosuppressive therapy, this view cannot account for the observation that immune responses are often restored in patients following successful treatment, especially with biological therapy.[13–15] This implicates the inflammatory process in attenuating adaptive immunity. Indeed, suppressive influences on T-cell function may well derive from inflammation-driven upregulation of multiple regulatory pathways in antigen presenting cells, characterized by enhanced expression of interleukin (IL)-10, IL-1 receptor antagonist (IL-1Ra), transforming growth factor-β, indoleamine-2,3-dioxygenase and inducible nitric oxide synthase activity.[16–19]

ACQUIRED CHANGES IN TCR RESPONSIVENESS IN RA T CELLS

Insights into the molecular and cellular basis for altered TCR responsiveness have uncovered a panoply of mechanisms for the variable state of T-cell responses. Some studies have demonstrated clear hyporesponsiveness to TCR engagement, while others have demonstrated reduced thresholds of TCR signaling. Some of the more common aberrations of TCR signaling in RA T cells, derived from cellular or biochemical analyses *ex vivo* of isolated peripheral blood or synovial fluid lymphocyte samples, are summarized in Table 1.

The peak incidence of RA is in the fifth or sixth decade.[20] Accordingly, it has been proposed that distinct features of the ageing immune system, in the context of genetic variants that might serve to accelerate the ageing process, might contribute to RA pathogenesis. Evidence that the ageing immune system may perturb pathways of T-cell activation is suggested by the expression of terminally differentiated phenotypes,[21] shortened telomeres,[22] and, when comparing aged with young donor T cells, redistribution of phosphoproteins,[23] and impaired recruitment of signaling intermediates into lipid rafts, associated with rigidity of the plasma membrane due to increased cholesterol content.[24]

Although reduced TCR signaling should cause self-reactive thymocytes to be positively selected, such a decrease in TCR signaling for thymic deletion might be canceled out by a commensurable decrease in TCR signaling for the activation of T cells in the periphery, so that the whole system readapts to novel but fixed 'calibration set-points' (or 'tuning').[25] How could reduced TCR signals in peripheral T cells translate to immune pathology? There are several possibilities, none mutually exclusive. For example, reduced TCR signals in regulatory T-cell (Treg) subsets would permit unbridled reactivity of effector T-cell responses. This might become more likely because of enhanced selection in the thymus of Treg cells with intermediate affinity for self antigens in the repertoire. Such FoxP3+ cells have been shown to have high plasticity in the periphery, for example, as provoked by IL-1β for conversion to IL-17-expressing autoreactive effector T cells. Activation-induced cell death of effector T cells might also be perturbed. A third possibility is that impaired IL-2 production, as a consequence of reduced TCR signals in recently activated effector T-cell precursors, might lead to a failure of Treg fitness, activation-induced cell death, or both. Although studies of human T cells lag behind those in mice, one recent report has demonstrated an association between low TCR signal strength and increased production of IL-17.[26] Thus, although a wide variety of aberrations of T-cell responsiveness, defined at a cellular or biochemical level, exist in patients with RA, the extent to which these anomalies are the cause or the effect of the chronic inflammatory process is unclear.

DO HUMAN GENETIC STUDIES SUPPORT A FUNDAMENTAL ROLE FOR T-CELL ACTIVATION AND DIFFERENTIATION IN RA?

Analysis of large cohorts of RA patients has demonstrated that there are at least 30 gene loci, confirmed in validation cohorts or identified through meta-analyses, that contribute to disease susceptibility.[27] The MHC remains the locus most strongly associated with RA, especially *HLADRB1*. The major influence of this association appears to be in the presentation of disease-associated autoantigens. For example, disease-associated HLA-DR4 molecules (HLA-DRB1*0401) present distinct immunodominant epitopes when compared with the closely related disease non-associated HLA-DR4 counterpart (HLA-DRB1*0402).[28] Data from animal models also indicate that there may exist a preference for the binding and presentation of citrullinated peptides by HLA-DR4 and the activation of arthritogenic T cells,

Table 1 T-cell signaling abnormalities reported in RA

Mechanisms suggesting increased threshold of TCR signals (hyporesponsive)	*References*	*Mechanisms suggesting decreased threshold of TCR signals (hyper-responsive)*	*References*
Impaired IL-2 production	10	Increased CD70 in $CD4^+CD28^-$ T cells	108
Reduced TCRζ expression	109,110	Increased pErk	111
Reduced LAT expression	112	Lymphopenia and homeostatic proliferation	113
Increased levels of reactive oxygen intermediates	114		
Defective RAP1 activation	115		
Heightened predisposition to apoptosis *ex vivo*	116		
Immune senescence	Reviewed in Weyand *et al.*[117]		
Ageing	Reviewed in Fulop *et al.*[118]		

Abbreviations: IL-2, interleukin-2; RA, rheumatoid arthritis; TCR, T-cell receptor.

under circumstances where the presence of an Arg residue at P4 of the peptide would ordinarily preclude high-affinity binding to the disease-associated HLA-DR4.[29,30] It is of particular interest that in Asian populations, polymorphisms in the *PADI4* gene are associated with RA. This gene encodes one of the de-iminating enzymes responsible for post-translational modification of Arg to Cit.[31] Notwithstanding the evidence for presentation of disease-associated autoantigens by HLA-DR molecules bearing the shared epitope sequence, the shared epitope may also be having other roles in the predisposition to RA. First, there is evidence that calreticulin—a chaperone protein involved in apoptosis—is an alternate ligand for the shared epitope sequence, capable of stimulation of nitric oxide secretion, which in turn inhibits T-cell function.[32,33] Second, not only HLA-DR4+ RA patients but also HLA-DR4+ healthy individuals show evidence for increased cellular senescence and telomere shortening.[22] These varied roles of the HLA-DR genetic association in RA may not be mutually exclusive.

Besides the HLA association, candidate gene approaches and genome-wide association studies have identified an allelic variant in the protein tyrosine phosphatase Lyp, encoded by *PTPN22* to be one among those that are strongly associated with RA in patients of Caucasian ancestry.[34,35] The finding that *PTPN22* single-nucleotide polymorphisms are associated with not only RA, but also type 1 diabetes, systemic lupus erythematosus, Hashimoto's thyroiditis and Addision's disease, has generated great interest in the functional consequences of the disease-associated mutant, not least because of the data implicating a role for Lyp as a negative regulator of TCR signaling.[36] Genetic evidence points to the R620W mutant (C1858T) as being the causal variant in RA and type 1 diabetes. Interestingly, however, the association of *PTPN22* with Crohn's disease is in the opposite direction.[37] The R620W mutation reduces the interaction between the P1 domain of Lyp and the SH3 domain of an associated kinase Csk. Both proteins are recruited as a complex to the scaffold protein PAG, serving to dephosphorylate the Y394 within the activation loop of the kinase domain of Lck (through the function of the phosphatase Lyp/PEST-domain enriched tyrosine phosphatase (PEP)), while phosphorylating a negative regulatory Tyr (Y505) in the C-terminal tail of Lck (through the function of the kinase Csk; reviewed in Burn *et al.*[38]).

The notion that a causal variant in a phosphatase might predispose to autoimmunity through perturbations of TCR signaling is particularly attractive, and is supported by the phenotype of PEP-deficient T cells, which exhibit an activated phenotype.[39] However, the results of the first functional genomic studies in man indicated that R620W was in fact a gain-of-function variant associated with attenuation of TCR signaling, proliferation and TCR-induced IL-2 production.[40] Accordingly, it was proposed that the disease-associated variant might impair thymic-negative selection on the one hand, while perturbing Treg function on the other. Analysis of T cells from genotyped donors lends support to this view.[41,42] In contrast, co-transfection studies of Lyp together with Csk indicate that the R620W variant is a hypomorph.[43] More recent data indicate that the disease-associated Lyp, and its murine orthologue, may be an unstable loss-of-expression variant, due to increased susceptibility to calpain- and proteosome-mediated degradation.[41] Studies of R619W-expressing murine T cells demonstrated enhanced proliferative responses and TCR-induced phosphorylation of Lck and ZAP-70. Proliferation and TCR-induced pErk expression were also increased in T cells from R620W homozygous mutant human donors. According to these data, the variant is associated with reduced expression of Lyp, and presumably loss-of-function at a cellular level, and a state of T-cell hyperactivity or hyperresponsiveness. In view of these conflicting data, more work is needed to establish how autoimmune disease-associated variants of Lyp perturb TCR signaling thresholds. In addition, several studies in healthy individuals and those with autoimmune disease demonstrate dramatic effects of the R620W variant of *PTPN22* on B-cell memory and autoantibody production, which may be just as relevant as its effects on T-cell function.[42,44]

Allelic variants have been reported at the *CD28*, *CTLA4*, *PRKCQ* and *CD247* loci,[27,45] all of whose gene products regulate receptor proximal TCR signaling events, including very early events in the immune synapse.[46] The causal variants have yet to be defined and so until there are data describing the function of such variants, at least in RA, it is difficult to predict the impact of altered function in the context of TCR signaling thresholds. On the other hand, genetic polymorphism in *CD247*, which encodes the TCRζ chain invariant subunit (reported to be downregulated in autoimmune disease), has been studied in more depth in SLE, where disease-associated 3′UTR variants are thought to be loss-of-expression mutants, manifest in peripheral blood T cells as increased numbers of T cells expressing low levels of TCRζ protein.[47]

When taken together, these data support the view that allelic variants associated with RA induce rather subtle perturbations in pathways of T-cell activation to either alter signal input (regulated by MHC class II, the molecular modifications of antigenic peptide and expression of the TCR/CD3 complex), signal transmission (*PTPN22, CD28, CTLA4, PRKCQ*) or events downstream (signal output) arising through variation in the function or expression of STAT4, REL or IL-2/IL-2R (Figure 1).

WHAT HAS THE SKG MOUSE TAUGHT US ABOUT THE PATHOGENESIS OF AUTOIMMUNE ARTHRITIS?

A spontaneous mutation in a colony of BALB/c—the SKG mutant—was noted to be associated with the development of inflammatory autoimmune arthritis. The key features of this model are that (1) a genetic defect in ZAP-70, a protein tyrosine kinase expressed by T cells, is necessary and sufficient to cause T-cell-mediated autoimmune arthritis, and (2) these clinical manifestations require environmental

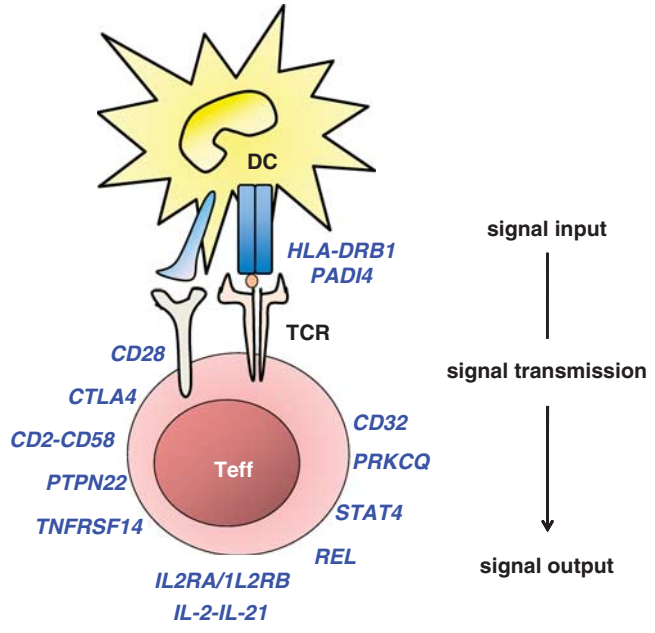

Figure 1 Genetic polymorphism points to perturbations of T-cell activation and alterations in thresholds of TCR signaling in RA.

stimuli. In the following sections, we described how a point mutation that perturbs TCR-proximal signaling leads to enhanced thymic production of pathogenic self-reactive (including arthritogenic) T cells, and of the Foxp3-expressing natural Treg cells that should normally keep them in check. We will then go on to describe peripheral activation, proliferation and effector T-cell differentiation leading to autoimmune arthritis, and the impact of environmental factors on these processes.

PATHWAYS TO THE GENERATION OF ARTHRITOGENIC T CELLS AND ALTERED NATURAL TREG CELL FUNCTION IN SKG MICE

In SKG mice, mutant ZAP-70, which attenuates TCR signaling, affects the TCR repertoire of developing T cells. Because of this signaling defect, only highly self-reactive T cells with strong TCR-proximal signaling that compensate for the mutation are positively selected. The same signaling defect attenuates strong self-peptide MHC ligand-induced TCR signals that would otherwise induce apoptosis, and so negative selection is also impaired.[7,48] These anomalies in the thymus skew the whole T-cell repertoire to become a highly self-reactive one, allowing arthritogenic clones to reach the periphery. The change in TCR repertoire depends on the degree to which the TCR signal is attenuated. Thus, *skg/null* mice, generated by mating homozygous SKG mutant mice with ZAP-70-deficient mice, develop more severe arthritis than SKG mice. In contrast, *skg*/+ mice (*skg* heterozygous) do not develop arthritis, even though their T cells are skewed to a self-reactive repertoire to an extent greater than wild-type mice. With their relative peripheral T-cell deficiency resulting from reduced positive selection, SKG T cells exhibit more robust proliferative responses than wild-type control T cells, through strong recognition of self-pMHCs expressed *in vivo*.[49] Consistent with this is the finding that *in vitro* autologous mixed lymphocyte reactions, in which $CD4^+$ T cells proliferate by recognizing self-pMHC presented by autologous DCs, $CD4^+$ T-cell proliferation was more vigorous if derived from SKG than from wild-type mice. In contrast, SKG T cells were much less responsive than wild type $CD4^+$ T cells in allogeneic MLR.[49] Thus, the SKG thymus is abnormal, as it generates T cells with a variety of anti-self specificities. This indicates that the signal intensity emanating from autoreactive TCRs is sufficient to promote proliferation in spite of the attenuated *skg* ZAP-70 TCR signal, which conveys hyporesponsiveness to non-self-antigens, such as alloantigen.

The skewing in the TCR repertoire in SKG mice promotes positive selection and attenuates negative selection of autoreactive arthritogenic T cells; other self-reactive T cells, which would be normally be positively selected in wild-type animals, fail to be selected.[48] This genetically alters the susceptibility of the background strain to particular autoimmune diseases, such that mice bearing the *skg* ZAP-70 mutation are more susceptible to some autoimmune diseases but more resistant to others. For example, depletion of Treg cells in BALB/c mice promotes autoimmune gastritis but very rarely autoimmune arthritis. Depletion of Treg cells in SKG mice, on the other hand, increases incidence of arthritis but reduces incidence of gastritis when compared with BALB/c controls. Similarly, diabetes prone non-obese diabetic mice are less susceptible to type 1 diabetes but more susceptible to arthritis by introducing the *skg* mutation. These changes to autoimmune disease susceptibility exhibit a gene dosage effect, such that *skg* heterozygous mice develop each autoimmune disease with intermediate incidence. Thus, the genetically determined SKG-induced TCR signaling impairment alters susceptibility to multiple autoimmune diseases by altering the self-reactive T-cell TCR repertoire. Furthermore, the more severe the TCR signaling defect and hence acquisition of a self-reactive TCR repertoire, the greater the likelihood of systemic autoimmune diseases such as arthritis, and the lower the likelihood of organ-specific diseases such as diabetes and autoimmune gastritis.

The *skg* ZAP-70 mutation influences the $Foxp3^+$ natural Treg cell TCR repertoire and function.[48] In wild-type animals, the TCRs of FoxP3+ Treg cells select thymic self-pMHC ligands with higher affinity than for conventional T-cell TCRs. The *skg* ZAP-70 mutation skews the Treg cell TCR repertoire towards even higher self-reactivity than the repertoire of conventional T cells, which are already more self-reactive than normal in SKG mice. As discussed above, $Foxp3^+$ Tregs require both TCR and IL-2 signals for suppressive functions, and thus impaired TCR signaling results in impaired suppressive function *in vivo* and *in vitro* in SKG ZAP-70 mutant mice.[50] For example, in cotransfer experiments, $Foxp3^+CD25^+CD4^+$ Treg cells from non-arthritic SKG mice were less capable of suppressing arthritis development than equal numbers of $Foxp3^+CD25^+CD4^+$ Treg cells from BALB/c mice.[48] The degree of impairment of suppressive function depends on the severity of signal reduction, such that $Foxp3^+$ Treg cells from *skg/*$^-$ mice are more self-skewed and much less suppressive *in vitro* than *skg/skg* Treg cells.[48]

Thus, given that both potentially self-reactive T cells and naturally occurring $Foxp3^+CD25^+CD4^+$ Treg cells undergo the same selection process in the thymus, the *skg* ZAP-70 anomaly alters the TCR repertoire of both the populations, leading to predominant development of arthritogenic self-reactive T cells and natural Treg cells with a similar self-reactivity, but impaired suppressive function. Put into context, the effects of altered TCR signaling thresholds in ZAP-70 mutant SKG mice on central and peripheral T-cell tolerance can be viewed as one point on a spectrum of TCR signal strengths.[48] It is difficult to determine exactly where the SKG mutant mice sit on this spectrum, but when the SKG mutation is compared with the effects of the *mrd* and *mrt* ZAP-70 kinase mutants, generated by mutagenesis in the Goodnow laboratory, one can begin to understand the complexity of TCR signaling and its impact on different cell lineages.[8] This is illustrated in Figure 2, where graded signaling defects generated by homozygous crosses (*mrd/mrd* and *mrt/mrt*) and compound homozygous mutations (*mrd/mrt*) are shown to induce quite distinct phenotypes, genetic background aside. Importantly, each genetic variant impacts on immune competence as well as promoting immune pathology. The subtleties of graded TCR signaling defects are further illustrated by the work of Weiss and colleagues who generated YYAA knock-in mice carrying engineered mutations of tyrosine residues Y315 and Y319 to alanine in the domain of ZAP-70 that serves to provide scaffolding function. Although these mice have anomalies of thymic selection and reduced numbers of thymic Treg similar to SKG mice and develop autoimmunity, as demonstrated by rheumatoid factor autoantibodies, they do not develop autoimmune arthritis.[51] It was suggested that differences in TCR repertoire could account for the distinct phenotypes observed.

SPONTANEOUS DIFFERENTIATION OF ARTHRITOGENIC EFFECTOR T CELLS IN SKG MICE

A repertoire of arthritogenic T cells in the periphery of SKG mice is not sufficient for the clinical expression of inflammatory arthritis,[52] because they need to be activated and differentiate into arthritogenic effector T cells. It turns out that IL-17-secreting Th17 cells are the major effector T cells in SKG arthritis.[49,53] Although both Th17 and Th1 cells are abundant in arthritic but not in unaffected joints in SKG mice, it is the IL-17-deficient SKG $CD4^+$ T cells that are unable to transfer disease to syngeneic T-cell-deficient recipients. IL-6, which

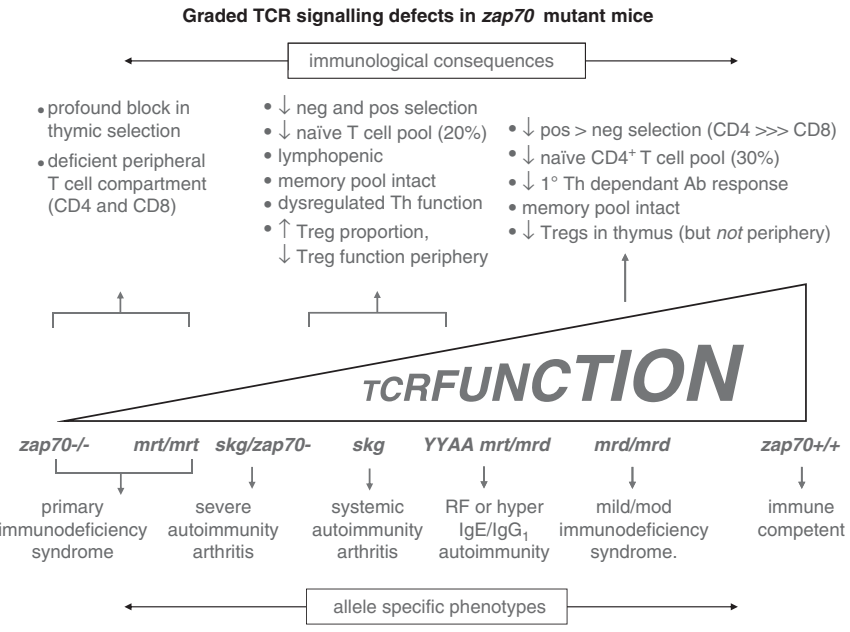

Figure 2 Graded TCR signaling defects in ZAP70 mutant mice.

promotes Th17 differentiation in mice, is required for disease development, as arthritis did not develop in IL-6-deficient mice,[54] and transfer of IL-$6^{-/-}$ SKG $CD4^+$ T cells to IL-$6^{-/-}$ immunodeficient $RAG^{-/-}$ mice failed to induce either Th17 cells or arthritis. In contrast, Th17 differentiation was intact in control IL-$6^{+/+}$ $RAG^{-/-}$-recipient mice to which IL-$6^{+/+}$ SKG $CD4^+$ T cells were transferred; arthritis occurred with all recipients. On the other hand, interferon (IFN)-γ deficiency enhanced arthritis—in part by facilitating Th17 differentiation. Arthritis developed spontaneously in IFN-γ-deficient SKG mice even in an SPF environment.[49] Thus, IL-6 facilitates whereas IFN-γ inhibits the differentiation of arthritogenic T cells to Th17.

In autologous mixed lymphocyte reactions with SKG T cells as responders, IL-6 was predominantly DC derived, while tumor necrosis factor (TNF) was produced by $CD4^+$ T cells/thymocytes and DCs, and IL-17 was produced by $CD4^+$ T cells but not by CD4SP thymocytes.[49] Cytokine production *in vitro* was blocked with anti-CD40L monoclonal antibody and to a lesser extent by anti-OX-40L. These *in vitro* data imply that, in SKG mice, thymic-derived self-reactive T cells are activated in the periphery in response to strong recognition of self-pMHC complexes, and thus stimulate upregulation of CD80/CD86 by APCs in a CD40/CD40L-dependent interaction, and that this feeds forward on T-cell proliferation. The pathway of Th17 differentiation is further enhanced, at least *in vitro*, by APC derived IL-6, TNF, IL-1β and IL-23, and tissue stroma-derived transforming growth factor-β.[49]

Th17 cells migrate to the joints in a chemokine-dependent manner, driven, at least in part through expression of the chemokine receptor CCR6.[49] Moreover, synovial fibroblasts from arthritic joints of both SKG mice and RA patients express the CCR6 ligand, CCL20, in abundance. CCL20 expression is increased *in vitro* by IL-1β, IL-17 or TNF, and suppressed by IFN-γ or IL-4.[49] Consistent with the dependence of SKG arthritis on Th17, anti-CCR6 blocking monoclonal antibody significantly inhibited arthritis development in SKG mice.

Owing to their high self-reactivity, SKG T cells differentiate into Th17 cells as a result of efficient cross-talk with APCs. This creates a positive-feedback loop: SKG T cells promote a cytokine milieu for their own differentiation to Th17. Furthermore, CCR6/CCL20-driven homing of Th17 cells to the joints is crucial for the development of Th17 cell-mediated autoimmune arthritis in SKG mice; it could be assumed that similar pathways may promote disease in patients with RA. It is still unclear why this chemokine/chemokine receptor profile is linked to specific synovial joint homing.

PROVOCATION OF CLINICALLY OVERT ARTHRITIS BY ENVIRONMENTAL AGENTS

Despite spontaneous differentiation of Th17 with arthritogenic antigen specificities, SKG mice houses under SPF conditions and do not suffer from arthritis, while severe arthritis occurs at high incidence under microbially conventional conditions.[53] Interestingly, when SKG mice born in an SPF environment are transferred to conventional conditions after weaning, their disease incidence is equal to that of mice born in conventional conditions, suggesting that environmental factors trigger disease. However, injection of several microbial-derived substances dose-dependently evoked arthritis in SKG mice under SPF conditions,[49,52,53] including zymosan (a crude yeast cell wall extract), β-glucans (glucose polymer β-1, 3-D-glucans) and mannan (poly-mannose). Polyinosinic-polycytidylic acid (Poly (I:C)), a double-stranded RNA construct with molecular features of some viruses, was mildly arthritogenic when injected every other day for 4 months.[52]

The most plausible mechanism by which these diverse microbial substances trigger autoimmune arthritis in SPF SKG mice is through activation of self-reactive T cells as a result of innate immune stimulation, rather than a direct arthritogenic effect. These direct effects are usually transient, mild, non-T-cell-mediated and absent after mannan injection.[49,52,53] Innate responses include DC maturation in response to zymosan through toll-like receptor 2 and 6. As a result, DCs upregulate expression of costimulatory molecules CD80/CD86, CD40, and class II MHC, and secrete a wide range of pro-inflammatory cytokines including IL-1, IL-6, IL-12, IL-23 and TNF.

Beta-glucans, the main constituent of zymosan, similarly stimulate DCs through the Dectin-1 receptor. Laminarin or curdlan, (linear or branching forms of β-glucan respectively) both induce SKG arthritis, and inhibition of Dectin-1 signaling prevents disease development. However, zymosan also activates the alternative pathway of complement (C). The β-glucan structure can also be recognized by ficolin-L, a key receptor of the lectin pathway of C-activation. Thus, substances containing β-glucan also contribute to triggering SKG autoimmune disease through complement activation. In this regard, injection of mannan, a potent activator of the lectin pathway of C activation, also induced arthritis in SKG mice when housed under SPF conditions.[53] Furthermore, C5a, a key anaphylotoxin of the complement pathway and a product of lectin, classical and alternative pathways, stimulated macrophages but not DCs, to produce inflammatory cytokines in synergy with toll-like receptor signaling, including IL-6. *In vivo*, C5a receptor (C5aR)-deficient SKG mice prevented differentiation and expansion of Th17 cells in spite of mannan or β-glucan stimulation, leading to attenuation of the development of arthritis.[53]

Collectively, these findings indicate that a broad spectrum of microbes, including fungi, bacteria and viruses, are involved in the initiation chronic autoimmune arthritis in SKG mice through their capacity to stimulate innate immunity in various ways, for example, stimulation of DCs and macrophages through toll-like receptor, Dectin-1 and C activation. Taken together, studies of autoimmune pathogenesis in SKG mice indicate that, in hosts bearing the specific SKG genetic alteration and autoimmune predisposition, a variety of environmental agents, rather than a specific etiological one, triggers a particular form of autoimmune inflammatory arthritis. Of importance, the degree to which such environmental factors are required to trigger disease depends on the magnitude of the genetic defect, as illustrated by the development of arthritis in *skg/−* mice in the absence of stimulation of innate immunity in SPF conditions. The model suggests the hypothesis that a one-time 'hit-and-run' exposure to environmental microbial agents may be sufficient to trigger chronic arthritis in individuals with an arthritogenic autoimmune T-cell repertoire as a consequence of genetic predisposition.

SPONDYLOARTHROPATHY: INFLAMMATORY ARTHRITIS DISTINCT FROM RA

SpA are common arthritic inflammatory diseases, affecting 1–3% of the population.[55] They consist of several rheumatic conditions including ankylosing spondylitis, reactive arthritis, psoriatic arthritis and arthropathy of inflammatory bowel disease (IBD). They have been previously grouped together on the basis of their shared distinctive clinical features and genetic susceptibility. Recent advances suggest that the IL-17 group of cytokines may be critical for the pathogenesis of SpA.

The distinctive clinical features of SpA are axial involvement, including inter-vertebral discs and sacro-iliac joints, and enthesitis (inflammation at sites of ligament and tendon insertions) together with certain extra-articular manifestations (Table 2). Although all the the entire SpA group shares common clinical features, each condition has a distinct set of symptoms. Notably ankylosing spondylitis presents with typical symptoms of inflammatory back pain in a young person, with males three times more common than females, which may be complicated by enthesitis (for example, plantar fasciitis or Achilles tendonitis), uveitis, IBD or psoriasis. On the other hand, reactive arthritis is characterized by inflammatory spinal pain and/or peripheral arthritis within 6 weeks of specific infections, including bacterial gastroenteritis and genitourinary infections: organisms include *Shigella*, *Yersinia* and *Chlamydia*. Enthesitis is common and dactylitis (diffuse swelling of a digit) may occur. Patients may also develop mucocutaneous ulcers, conjunctivitis and occasionally uveitis. Patients with psoriatic arthritis have a current, past or family history of psoriasis and peripheral inflammatory arthritis, with or without inflammatory back pain. Patients with arthropathy of IBD (enteropathic arthritis) have IBD and peripheral inflammatory arthritis, with or without inflammatory back pain.

Table 2 Clinical, etiological and pathologic manifestations of SpA and RA

Feature	*SpA*	*RA*
Axial skeleton disease	Inflammatory spinal pain, sacroiliitis	Cervical atlanto-axial inflammation
Peripheral Arthritis	Asymmetrical, predominantly lower limb, may be symmetrical and involve small joints of hands in PsA	Symmetrical, small and large joints
Entheseal involvement	Enthesitis, for example, achilles tendonitis	Tenosynovitis may accompany synovitis
Finger joints	Dactylitis: ligament, DIP, PIP involvement	PIP, MCP synovitis
Bone	New bone formation, ankylosis, osteoporosis	Bone erosion, osteoporosis
Skin	Psoriasis, balanitis, keratoderma	Subcutaneous nodules
Bowel	CD or ulcerative colitis	
Genitourinary involvement	Urethritis and/or cervicitis	
Eye	Uveitis or conjunctivitis	
Lung	Chest wall restriction, upper lobe fibrosis	Bronchiectasis, interstitial lung disease, emphysema (smoking related), pleurisy
Vessels	Vasculitis (ANCA+), carditis: aortic valve disease	Vasculitis (RF, immune complex-mediated), atherosclerosis, pericarditis
Autoantibodies	p or aANCA (UC), anti-fungal glycan (CD), RF (PsA), non defined in AS	RF, anti-CCP, 30% seronegative
Genetic association	HLA-B27 (AS), HLA-Cw*0602 (Ps), IL23R and other genes in IL-23/IL-17 pathway, NOD2 (CD), PGER (AS).	HLA-DR shared epitope, PTPN22, REL, CD40
Environmental triggers	GU or bowel (ReA), presumed micro-organism (PsA, AS, IBD)	Smoking, mineral oil, periodontal infection, EBV

Abbreviations: CD, Crohn's disease; CCP, cyclic citrullinated peptide antibodies; DIP, distal interphalangeal joint, EBV, Epstein-barr virus; GU, genitourinary; IBD, inflammatory bowel disease; MCP, metacarpophalangeal joint; NOD2, nucleotide-binding oligemerization domatin containing 2; NOD, non-obese diabetic; PIP, proximal interphalangeal joint; Ps, psoriasis, PsA, psoriatic arthritis; RA, rheumatoid arthritis; ReA, reactive arthritis; RF, rheumatoid factor; SpA, spondyloarthropathies; UC, ulcerative colitis.

CLUES TO SpA AND RA PATHOGENESIS FROM THEIR RESPONSE TO TREATMENT

Biologic agents that inhibit TNF, IL-6, B7-CD28 and B cells are all effective therapies for RA. Inhibitors of TNF have dramatically improved the quality of life of patients with SpA (ankylosing spondylitis, psoriatic arthritis and IBD). They are clinically effective for both peripheral and axial arthritis, and for extra-articular manifestations but do not affect the progression of ankylosis. In early clinical trials, inhibitors of B7-CD28 and IL-23 appear to improve psoriatic arthritis[56,57] and anti-IL-23 improved Crohn's disease.[58] Inhibition of IL-17 convincingly improved psoriasis and AS (although reported only in abstract form so far), but had modest effects in RA.[59,60] Anti-IL-23 has not been trialed in RA.

PATHOGENESIS OF SpA: HUMAN STUDIES

Early ideas about the pathogenesis of inflammatory arthritis, including RA and SpA, revolved around aberrant or dominant Th1 responses.[61] However, contradictory evidence emerged with several studies in animal models of autoimmune arthritis. A lack of protection or accelerated autoimmune arthritis in IFNγ-deficient mice, suggested a protective or beneficial role for IFNγ.[62,63] Both IL-4 and IFNγ-secreting T cells were demonstrated in human SpA lesions.[64] However, SpA patients were less able to generate IFNγ-producing T cells than controls.[65] The discovery of Th17 cells provided new insight into the contribution of T cells in SpA. A series of studies showed that experimental allergic encephalomyelitis or collagen-induced arthritis developed in mice deficient in IL-12p35 but not IL-23p19 or IL-12p40,[66,67] suggesting that IL-23 and not IL-12 was critical for the development of these autoimmune diseases. Complementing the relative lack of IFNγ-producing T cells in SpA, monocyte-derived macrophages generated from ankylosing spondylitis patients also demonstrated a defect in IFNγ production and signaling.[68] However, it is unclear whether this is cause or effect in disease pathogenesis.

Recent genetic studies demonstrated that genetic variants in *IL23R*, *IL12B*, *STAT3* and *Card9* are associated with ankylosing spondylitis, psoriasis and IBD (especially Crohn's disease), but not RA.[69,70] Of interest, not only is there considerable evidence of association of shared genetic polymorphism in multiple clinical forms of SpA, but experimental evidence from human diseased tissues and animal models is emerging that demonstrates common pathogenetic elements across SpA, including psoriasis and AS. One of the common denominators appears to be an IL-17 response following activation of the dectin-1-syk pathway.[71] IL-17 immunity towards micro-organisms, including commensal gut microflora, as well as pathogens such as *Chlamydia* species, is thought to be integral to the pathogenesis of the SpA diseases, which have long been proposed to be triggered by an abnormal immune response to infection. The heritability of ankylosing spondylitis in twins of >90% suggests that the disease trigger is ubiquitous—likely a micro-organism or set of organisms that triggers a common pathogenetic pathway, rendered more potent by associated polymorphic genes in distinct pathways.[72]

Dectin-1 is a receptor for fungal and bacterial β-glucan in innate immune cells and APCs, including neutrophils, monocytes, DCs and γδ T cells. Dectin-1 signaling promotes expression of IL-1β, IL-12p35, IL-12p40 and IL-23p19.[73,74] IL-23 is required for the expansion of IL-17$^+$ cells, and signaling through IL-23R activates Jak2 and STAT3 in T cells.[75,76] *Chlamydia*, a triggering genitourinary micro-organism associated with reactive arthritis, promotes expression of IL-23 by APCs through activation of the transcription factor C/EBP homologous protein.[77] Psoriasis is associated with staphylococcal colonization of the skin and streptococcal throat infections.[78] Staphylococcal and streptococcal superantigens have been implicated in T-cell activation and skin homing.[79] Recent studies demonstrate that innate immune cells have a critical role in the IL-23 and IL-17 pathway in SpA.

Neutrophils and mast cells were shown to be the predominant cell types expressing IL-17 in psoriatic skin, and to release IL-17 in the process of neutrophil DNA-anti-microbial extracellular trap formation, which was promoted by IL-23 and IL-1β.[80] Of interest, the frequency of IL-17-secreting cells in spinal facet joints was significantly higher in ankylosing spondylitis patients than in control osteoarthritis patients, and the majority of the IL-17+ cells were neutrophils, with a smaller proportion of mast cells, and very few T cells.[81] In synovial tissue of patients with undifferentiated SpA, the majority of the IL-17+ cells were mast cells and neutrophils.[82] Synovial neutrophil recruitment followed by deactivation has also been noted in the setting of reactive arthritis, following gastroenterological and genitourinary infections, suggesting an important local neutrophil effector role.[83,84] In ankylosing spondylitis, peripheral blood γδ T cells were the major IL-23R+ population, and relative to healthy controls, preferentially expressed IL-17 over IFN-γ upon mitogenic stimulation.[85]

In contrast, although innate immune cells expressing IL-17 are abundant at diseased tissue sites in SpA, CD4$^+$ Th17 cells have been more difficult to demonstrate. Although some papers found higher frequencies of Th17 cells in peripheral blood of ankylosing spondylitis patients relative to healthy controls,[86,87] Th17 cell frequencies were very low, and no differences were found in serum or intestinal IL-17 levels.[88,89] Taken together there is evidence for an IFN-γ defect in SpA and for enhanced signaling through dectin-1-associated Card-9 and then of IL-23 through IL-23R,[90] with overexpression of IL-17. However, the weight of evidence suggests that this IL-17 overexpression occurs in innate immune cells, which vary between disease subtypes within the SpA family. Given the critical role of Th17 in infection control, it remains unclear whether innate cells secreting IL-17 are similarly effective in the control of organisms such as fungi colonizing epithelial and mucosal surfaces.[91]

PATHOGENESIS OF SpA: MOUSE MODELS

There are few good animal models of SpA that reproduce human spondylitis, anterior uveitis, Crohn's disease and/or psoriasis. However, considerable progress has been made in psoriasis using human psoriatic skin explants grafted onto SCID mice.[92] HLA-B27/human β-microglobulin transgenic rats develop a CD4$^+$ T-cell-dependent multi-organ inflammatory disease with the clinical manifestations of peripheral arthritis, spondylitis, psoriasis-like skin lesions, epididymo-orchitis and colitis.[93] The IL-23/IL-17 axis has been shown to be strongly activated in the colon of B27 transgenic rats with SpA-like disease, and this is postulated to result from HLA-B27 misfolding and activation of the unfolded protein response, inducing IL-23.[94] The exact relationship of this finding to human HLA-B27-associated SpA is unclear, as the transgenic rats express a very high B27 copy number (disease severity is correlated with copy number), which might particularly favor the unfolded protein response and endoplasmic reticulum (ER) stress in these rats. In humans, HLA-B27 dimerizes in the ER that can provoke misfolding. However, *ERAP1* is genetically associated with both ankylosing spondylitis and psoriasis. These associations are significant only when co-expressed with HLA-B27 and the psoriatic HLA-C risk allele, respectively.[95,96] As the disease-protective *ERAP1* encodes a loss-of-function enzyme that trims peptides in the ER as they are loaded onto class I molecules, this implies that the functional HLA-B27 and HLA-C associations involve presentation of MHC class I-restricted peptides,[97] although it is possible that the unfolded protein response is involved.

Several interesting models highlight the critical role of innate immunity in the pathogenesis of SpA. Mice deficient in the DC-specific inhibitory c-type lectin receptor, DCIR, develop enthesitis, ankylosing ankle arthritis and sialadenitis, associated with DC expansion in lymphoid organs.[98] TNFΔARE mice overexpress TNF, and spontaneously develop SpA, including peripheral arthritis, spondylitis, enthesitis and Crohns-like ileitis.[99,100] In this model, arthritis is T- and B-cell independent, whereas IBD was abrogated on a RAG-deficient background, and non-hemopoietic cells expressing TNF-receptor I are sufficient for development of the pathology.[99,100] Recently, mice deficient in the ubiquitin-editing molecule A20 in DCs were shown to develop spontaneous $CD4^+$ T-cell-dependent colonic IBD and seronegative ankylosing arthritis and enthesitis associated with marked DC activation and myeloid hyperplasia.[101] The themes emerging from these models are the critical balance of innate immune activators and regulators, and the different pathogenetic mechanisms that contribute to clinical expression within the spectrum of human SpA.

INNATE IMMUNE ACTIVATION MEETS ABERRANT TCR SIGNALING IN SpA THROUGH THE SKG MODEL

As fungal β-glucan triggers dectin-1, and this receptor is upstream of the gene cascade associated with human SpA, the Thomas group investigated whether curdlan-treated SKG mice develop clinical evidence of SpA. One week after intraperitoneal curdlan injection, all SKG mice developed enthesitis, ankle and sacro-iliac arthritis, plantar fasciitis and spondylitis. Two months later, mice developed transmural ileitis resembling Crohn's disease and unilateral anterior uveitis.[102] Interestingly, $CD4^+$ T cells from SKG but not BALB/c mice transferred enthesitis/arthritis and spondylitis but not ileitis to SCID-recipient mice. Moreover, the nature of the trigger affected disease severity and clinical expression. Whereas curdlan triggered a rapid onset of arthritis, spondylitis and ileitis, which was more severe in females, mannan triggered mild arthritis and spondylitis, which was equivalent in males and females, but not ileitis. Thus, exposure of the innate immune system to systemic microbial components triggers inflammatory arthritis and spondylitis, when combined with the *skg* ZAP70 mutation reducing TCR signaling. The nature of the innate signals and microbial environment in which animals are housed, as well as the severity of the genetic lesion strongly affects disease severity, extra-articular organ involvement and gender bias.

Paradoxically, whereas RA is now known to be associated with genetic and functional alterations in TCR signaling, human SpA have not been associated with similar genes provoking deficient signaling of the TCR. On the other hand, the genetic associations with SpA strongly implicate signaling downstream of dectin-1 and IL-23R. However, in spite of a large increase in the number of genetic associations confirmed in ankylosing spondylitis over the past 5 years, only 25% of ankylosing spondylitis heritability has so far been explained. The situation is similar for other members of the SpA family, including psoriasis, psoriatic arthritis, Crohn's disease and ulcerative colitis, and indeed also for RA. As technology improves and decreases in cost, further genetic associations with SpA are emerging, including *RUNX3*, which may affect thymic selection and Treg function, and *LTBR-TNFRSF1A*, which may affect lymphoid organ development and innate immune responses.[103,104] What does emerge from studies in SKG mice is a reinforcement of current concepts of pathogenesis of human SpA, that is: innate triggers and the microbial microenvironment of the host (including gut, skin and genito-urinary tract) interact with multiple genetic lesions—some protective and

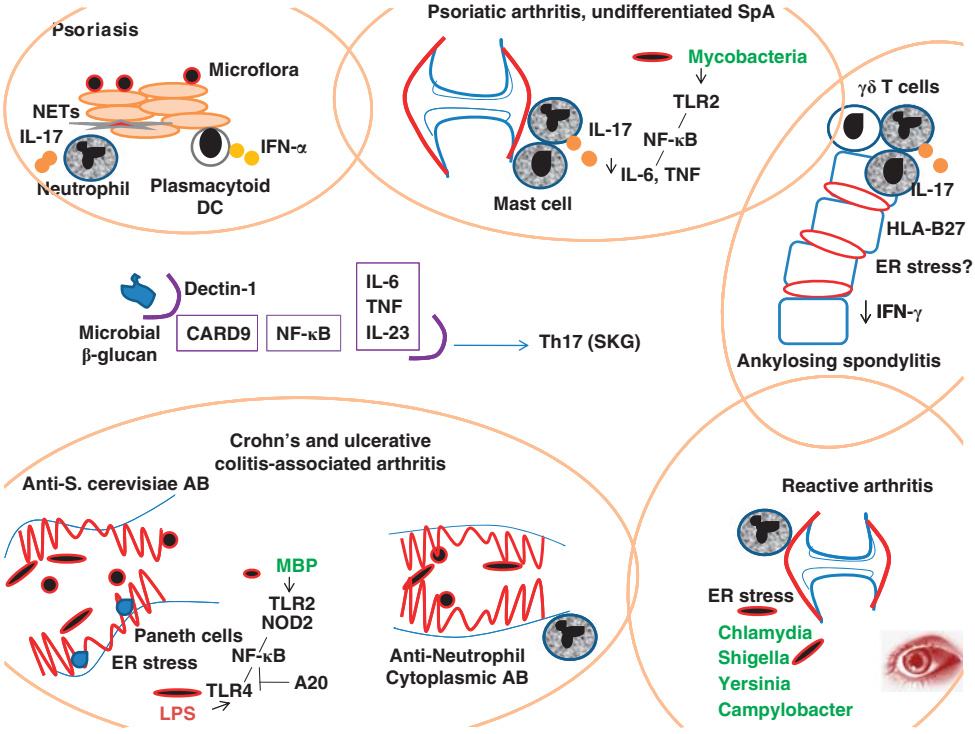

Figure 3 Genetic polymorphisms in SpA promote signaling downstream of dectin-1. The central signaling pathway exemplified in SKG mice and the polymorphisms determined in human SpA are depicted in the center of the picture. Innate immune pathogenetic mechanisms described for individual diseases, which make up the spectrum of SpA, are depicted within the overlapping ovals. Common mechanisms include stimulation by microbes, production of IL-17 by T cells or innate cells, involvement of neutrophils and ER stress. NETs, neutrophil extracellular traps; TLR, toll-like receptor.

others predisposing—to influence disease onset, severity, extra-articular organ involvement and gender bias.

Perhaps the most provocative aspect of the development of SpA-like disease in SKG mice is the development of multiple autoantibodies, including autoantibodies directed against cartilage, which are not a feature of human ankylosing spondylitis, and the dependence of arthritis and spondylitis on $CD4^+$ T cells. There is some scepticism as to the involvement of $CD4^+$ T-cell autoimmunity in human SpA, due to the paucity of joint-specific autoantibodies, the demonstration of strong innate responses, the lack of Th17 cells in blood or diseased tissues of SpA patients, and evidence from the TNFΔARE mouse model. Interestingly the arthritis in these transgenic mice remained clinically and histologically identical when crossed to a RAG-deficient background, but was more severe when NKT cells were deficient.[99,105] The observations emerging from several animal models, including SKG mice, demonstrate that multiple mechanisms can contribute to models with features of multisystem SpA, and that innate regulatory populations have a very important role in maintaining tolerance (Figure 3). Different mechanisms may underlie arthritis and IBD in SKG mice treated with curdlan. The genetics of human SpA strongly implicate multiple pathways in different patients depending on genetic background and environmental exposure. Furthermore, more than one abnormal signal is likely to be required to overcome normal innate and adaptive immune regulation.

CONCLUDING REMARKS

Despite some similarities in clinical features (peripheral inflammatory arthritis) and in treatment response, it is striking that almost no genetic associations are shared between RA and SpA, with the exception of a modest association of *IL23R* and RA. Indeed, in some cases where the same gene is associated, for example, *NFKB1* (encoding the p50 subunit of NF-κB) or *MHC* (RA and ankylosing spondylitis), *CD40* (RA and Crohn's) and *PTPN22* (RA and Crohn's), the associations have been found to have opposite effects.[106,107] This suggests that genetic associations with RA and SpA affect tuning around critical adaptive (for RA) and innate (for SpA) immune pathways, either leading to amplification or subtle modifications of the response to inflammatory or infectious triggers, such as smoking, resident oral, respiratory or gastrointestinal microflora and infection. Depending on the genetic variants and the environmental cues, these aberrations manifest as distinct clinical phenotypes associated with common end-organ tissue destruction in the musculoskeletal system. Given the striking propensity of the autoreactive SKG T cells for joints, there is a wealth of insight into RA and SpA pathogenesis yet to be obtained from further elucidation of the interaction of environmental and infectious influences in SKG mice.

1 Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007; **447**: 661–678.
2 Weiss A, Littman DR. Signal transduction by lymphocyte antigen receptors. *Cell* 1994; **76**: 263–274.
3 Gelfand EW, Weinberg K, Mazer BD, Kadlecek TA, Weiss A. Absence of ZAP-70 prevents signaling through the antigen receptor on peripheral blood T cells but not on thymocytes. *J Exp Med* 1995; **182**: 1057–1065.
4 Chan AC, Kadlecek TA, Elder ME, Filipovich AH, Kuo WL, Iwashima M *et al.* ZAP-70 deficiency in an autosomal recessive form of severe combined immunodeficiency. *Science* 1994; **264**: 1599–1601.
5 Rieux-Laucat F, Hivroz C, Lim A, Mateo V, Pellier I, Selz F *et al.* Inherited and somatic CD3zeta mutations in a patient with T-cell deficiency. *N Engl J Med* 2006; **354**: 1913–1921.
6 Goodnow CC. Multistep pathogenesis of autoimmune disease. *Cell* 2007; **130**: 25–35.
7 Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S *et al.* Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* 2003; **426**: 454–460.
8 Siggs OM, Miosge LA, Yates AL, Kucharska EM, Sheahan D, Brdicka T *et al.* Opposing functions of the T cell receptor kinase ZAP-70 in immunity and tolerance differentially titrate in response to nucleotide substitutions. *Immunity* 2007; **27**: 912–926.
9 Thomas R, Turner M, Cope AP. High avidity autoreactive T cells with a low signalling capacity through the T-cell receptor: central to rheumatoid arthritis pathogenesis? *Arthritis Res Ther* 2008; **10**: 210.
10 Emery P, Panayi G, Symmons D, Brown G. Mechanisms of depressed delayed-type hypersensitivity in rheumatoid arthritis: the role of protein energy malnutrition. *Ann Rheum Dis* 1984; **43**: 430–434.
11 Emery P, Panayi GS, Nouri AM. Interleukin-2 reverses deficient cell-mediated immune responses in rheumatoid arthritis. *Clin Exp Immunol* 1984; **57**: 123–129.
12 Thomas R, Davis LS, Lipsky PE. Rheumatoid synovium is enriched in mature antigen-presenting dendritic cells. *J Immunol* 1994; **152**: 2613–2623.
13 Cope AP, Londei M, Chu NR, Cohen SB, Elliott MJ, Brennan FM *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**: 749–760.
14 Maurice MM, van der Graaff WL, Leow A, Breedveld FC, van Lier RA, Verweij CL. Treatment with monoclonal anti-tumor necrosis factor alpha antibody results in an accumulation of Th1 CD4+ T cells in the peripheral blood of patients with rheumatoid arthritis. *Arthritis Rheum* 1999; **42**: 2166–2173.
15 Lorenz HM, Antoni C, Valerius T, Repp R, Grunke M, Schwerdtner N *et al. In vivo* blockade of TNF-alpha by intravenous infusion of a chimeric monoclonal TNF-alpha antibody in patients with rheumatoid arthritis. Short term cellular and molecular effects. *J Immunol* 1996; **156**: 1646–1653.
16 Cush JJ, Splawski JB, Thomas R, McFarlin JE, Schulze-Koops H, Davis LS *et al.* Elevated interleukin-10 levels in patients with rheumatoid arthritis. *Arthritis Rheum* 1995; **38**: 96–104.
17 Zhu L, Ji F, Wang Y, Zhang Y, Liu Q, Zhang JZ *et al.* Synovial autoreactive T cells in rheumatoid arthritis resist IDO-mediated inhibition. *J Immunol* 2006; **177**: 8226–8233.
18 Holoshitz J, Ling S. Nitric oxide signaling triggered by the rheumatoid arthritis shared epitope: a new paradigm for MHC disease association? *Ann NY Acad Sci* 2007; **1110**: 73–83.
19 Summers KL, O' Donnell JL, Heiser A, Highton J, Hart DN. Synovial fluid transforming growth factor beta inhibits dendritic cell- T lymphocyte interactions in patients with chronic arthritis. *Arthritis Rheum* 1999; **42**: 507–518.
20 Symmons D, Turner G, Webb R, Asten P, Barrett E, Lunt M *et al.* The prevalence of rheumatoid arthritis in the United Kingdom: new estimates for a new century. *Rheumatology* 2002; **41**: 793–800.
21 Vallejo AN, Bryl E, Klarskov K, Naylor S, Weyand CM, Goronzy JJ. Molecular basis for the loss of CD28 expression in senescent T cells. *J Biol Chem* 2002; **277**: 46940–46949.
22 Schonland SO, Lopez C, Widmann T, Zimmer J, Bryl E, Goronzy JJ *et al.* Premature telomeric loss in rheumatoid arthritis is genetically determined and involves both myeloid and lymphoid cell lineages. *Proc Natl Acad Sci USA* 2003; **100**: 13471–13476.
23 Eisenbraun MD, Tamir A, Miller RA. Altered composition of the immunological synapse in an anergic, age-dependent memory T cell subset. *J Immunol* 2000; **164**: 6105–6112.
24 Fulop Jr T, Gagne D, Goulet AC, Desgeorges S, Lacombe G, Arcand M *et al.* Age-related impairment of p56lck and ZAP-70 activities in human T lymphocytes activated through the TcR/CD3 complex. *Exp Gerontol* 1999; **34**: 197–216.
25 Anderton SM, Wraith DC. Selection and fine-tuning of the autoimmune T-cell repertoire. *Nat Rev Immunol* 2002; **2**: 487–498.
26 Purvis HA, Stoop JN, Mann J, Woods S, Kozijn AE, Hambleton S *et al.* Low strength T-cell activation promotes Th17 responses. *Blood* 2010; **116**: 4829–4837.
27 Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP *et al.* Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat Genet* 2010; **42**: 508–514.
28 Cope AP, Patel SD, Hall F, Congia M, Hubers HA, Verheijden GF *et al.* T cell responses to a human cartilage autoantigen in the context of rheumatoid arthritis-associated and nonassociated HLA-DR4 alleles. *Arthritis Rheum* 1999; **42**: 1497–1507.
29 Hill JA, Bell DA, Brintnell W, Yue D, Wehrli B, Jevnikar AM *et al.* Arthritis induced by posttranslationally modified (citrullinated) fibrinogen in DR4-IE transgenic mice. *J Exp Med* 2008; **205**: 967–979.
30 Hill JA, Southwood S, Sette A, Jevnikar AM, Bell DA, Cairns E. Cutting edge: the conversion of arginine to citrulline allows for a high-affinity peptide interaction with the rheumatoid arthritis-associated HLA-DRB1*0401 MHC class II molecule. *J Immunol* 2003; **171**: 538–541.
31 Barton A, Bowes J, Eyre S, Spreckley K, Hinks A, John S *et al.* A functional haplotype of the PADI4 gene associated with rheumatoid arthritis in a Japanese population is not associated in a United Kingdom population. *Arthritis Rheum* 2004; **50**: 1117–1121.
32 Ling S, Lai A, Borschukova O, Pumpens P, Holoshitz J. Activation of nitric oxide signaling by the rheumatoid arthritis shared epitope. *Arthritis Rheum* 2006; **54**: 3423–3432.
33 Ling S, Pi X, Holoshitz J. The rheumatoid arthritis shared epitope triggers innate immune signaling via cell surface calreticulin. *J Immunol* 2007; **179**: 6359–6367.
34 Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC *et al.* A missense single-nucleotide polymorphism in a gene encoding a protein

tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am J Hum Genet* 2004; **75**: 330–337.
35 Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW *et al.* Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet* 2005; **77**: 1044–1060.
36 Cloutier JF, Veillette A. Cooperative inhibition of T-cell antigen receptor signaling by a complex between a kinase and a phosphatase. *J Exp Med* 1999; **189**: 111–121.
37 Diaz-Gallo L-M, Espino-Paisán L, Fransen K, Gómez-García M, van Sommeren S, Cardeña C *et al.* Differential association of two PTPN22 coding variants with Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2011; **17**: 2287–2294.
38 Burn GL, Svensson L, Sanchez-Blanco C, Saini M, Cope AP. Why is PTPN22 a good candidate susceptibility gene for autoimmune disease? *FEBS Lett* 2011; **585**: 3689–3698.
39 Hasegawa K, Martin F, Huang G, Tumas D, Diehl L, Chan AC. PEST domain-enriched tyrosine phosphatase (PEP) regulation of effector/memory T cells. *Science* 2004; **303**: 685–689.
40 Vang T, Congia M, Macis MD, Musumeci L, Orru V, Zavattari P *et al.* Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. *Nat Genet* 2005; **37**: 1317–1319.
41 Zhang J, Zahir N, Jiang Q, Miliotis H, Heyraud S, Meng X *et al.* The autoimmune disease-associated PTPN22 variant promotes calpain-mediated Lyp/Pep degradation associated with lymphocyte and dendritic cell hyperresponsiveness. *Nat Genet* 2011; **43**: 902–907.
42 Rieck M, Arechiga A, Onengut-Gumuscu S, Greenbaum C, Concannon P, Buckner JH. Genetic variation in PTPN22 corresponds to altered function of T and B lymphocytes. *J Immunol* 2007; **179**: 4704–4710.
43 Zikherman J, Hermiston M, Steiner D, Hasegawa K, Chan A, Weiss A. PTPN22 deficiency cooperates with the CD45 E613R allele to break tolerance on a non-autoimmune background. *J Immunol* 2009; **182**: 4093–4106.
44 Menard L, Saadoun D, Isnardi I, Ng Y-S, Meyers G, Massad C *et al.* The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J Clin Invest* 2011; **121**: 3635–3644.
45 Zhernakova A, Stahl EA, Trynka G, Raychaudhuri S, Festen EA, Franke L *et al.* Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci. *PLoS genetics* 2011; **7**: e1002004.
46 Dustin ML, Depoil D. New insights into the T cell synapse from single molecule techniques. *Nat Rev Immunol* 2011; **11**: 672–684.
47 Gorman CL, Russell AI, Zhang Z, Cunninghame Graham D, Cope AP, Vyse TJ. Polymorphisms in the CD3Z gene influence TCRzeta expression in systemic lupus erythematosus patients and healthy controls. *J Immunol* 2008; **180**: 1060–1070.
48 Tanaka S, Maeda S, Hashimoto M, Fujimori C, Ito Y, Teradaira S *et al.* Graded attenuation of TCR signaling elicits distinct autoimmune diseases by altering thymic T cell selection and regulatory T cell function. *J Immunol* 2010; **185**: 2295–2305.
49 Hirota K, Hashimoto M, Yoshitomi H, Tanaka S, Nomura T, Yamaguchi T *et al.* T cell self-reactivity forms a cytokine milieu for spontaneous development of IL-17+ Th cells that cause autoimmune arthritis. *J Exp Med* 2007; **204**: 41–47.
50 Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M *et al.* Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998; **10**: 1969–1980.
51 Hsu L-Y, Tan YX, Xiao Z, Malissen M, Weiss A. A hypomorphic allele of ZAP-70 reveals a distinct thymic threshold for autoimmune disease versus autoimmune reactivity. *J Exp Med* 2009; **206**: 2527–2541.
52 Yoshitomi H, Sakaguchi N, Kobayashi K, Brown GD, Tagami T, Sakihama T *et al.* A role for fungal {beta}-glucans and their receptor Dectin-1 in the induction of autoimmune arthritis in genetically susceptible mice. *J Exp Med* 2005; **201**: 949–960.
53 Hashimoto M, Hirota K, Yoshitomi H, Maeda S, Teradaira S, Akizuki S *et al.* Complement drives Th17 cell differentiation and triggers autoimmune arthritis. *J Exp Med* 2010; **207**: 1135–1143.
54 Hata H, Sakaguchi N, Yoshitomi H, Iwakura Y, Sekikawa K, Azuma Y *et al.* Distinct contribution of IL-6, TNF-alpha, IL-1, and IL-10 to T cell-mediated spontaneous autoimmune arthritis in mice. *J Clin Invest* 2004; **114**: 582–588.
55 Braun J, Bollow M, Remlinger G, Eggens U, Rudwaleit M, Distler A *et al.* Prevalence of spondylarthropathies in HLA-B27 positive and negative blood donors. *Arthritis Rheum* 1998; **41**: 58–67.
56 Mease P, Genovese MC, Gladstein G, Kivitz AJ, Ritchlin C, Tak PP *et al.* Abatacept in the treatment of patients with psoriatic arthritis: results of a six-month, multicenter, randomized, double-blind, placebo-controlled, phase II trial. *Arthritis Rheum* 2011; **63**: 939–948.
57 Kavanaugh A, Menter A, Mendelsohn A, Shen Y-K, Lee S, Gottlieb AB. Effect of ustekinumab on physical function and health-related quality of life in patients with psoriatic arthritis: a randomized, placebo-controlled, phase II trial. *Curr Med Res Opin* 2010; **26**: 2385–2392.
58 Sandborn WJ, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, Katz S *et al.* A randomized trial of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. *Gastroenterology* 2008; **135**: 1130–1141.
59 Genovese MC, Van den Bosch F, Roberson SA, Bojin S, Biagini IM, Ryan P *et al.* LY2439821, a humanized anti–interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I randomized, double-blind, placebo-controlled, proof-of-concept study. *Arthritis Rheum* 2010; **62**: 929–939.
60 Hueber W, Patel DD, Dryja T, Wright AM, Koroleva I, Bruin G *et al.* Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci Transl Med* 2010; **2**: 52ra72.
61 Romagnani S. Lymphokine production by human T cells in disease states. *Annu Rev Immunol* 1994; **12**: 227–257.
62 Guedez YB, Whittington KB, Clayton JL, Joosten LA, van de Loo FA, van den Berg WB *et al.* Genetic ablation of interferon-gamma up-regulates interleukin-1beta expression and enables the elicitation of collagen-induced arthritis in a nonsusceptible mouse strain. *Arthritis Rheum* 2001; **44**: 2413–2424.
63 Chu CQ, Song Z, Mayton L, Wu B, Wooley PH. IFNgamma deficient C57BL/6 (H-2b) mice develop collagen induced arthritis with predominant usage of T cell receptor Vbeta6 and Vbeta8 in arthritic joints. *Ann Rheum Dis* 2003; **62**: 983–990.
64 Simon AK, Seipelt E, Sieper J. Divergent T-cell cytokine patterns in inflammatory arthritis. *Proc Natl Acad Sci USA* 1994; **91**: 8562–8566.
65 Rudwaleit M, Siegert S, Yin Z, Eick J, Thiel A, Radbruch A *et al.* Low T cell production of TNF alpha and IFN gamma in ankylosing spondylitis: its relation to HLA-B27 and influence of the TNF-308 gene polymorphism. *Ann Rheum Dis* 2001; **60**: 36–42.
66 Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA *et al.* Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med* 2003; **198**: 1951–1957.
67 Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B *et al.* Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 2003; **421**: 744–748.
68 Smith JA, Barnes MD, Hong D, DeLay ML, Inman RD, Colbert RA. Gene expression analysis of macrophages derived from ankylosing spondylitis patients reveals interferon-gamma dysregulation. *Arthritis Rheum* 2008; **58**: 1640–1649.
69 Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A *et al.* Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007; **39**: 1329–1337.
70 Reveille JD, Sims AM, Danoy P, Evans DM, Leo P, Pointon JJ *et al.* Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet* 2010; **42**: 123–127.
71 LeibundGut-Landmann S, Gross O, Robinson MJ, Osorio F, Slack EC, Tsoni SV *et al.* Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat Immunol* 2007; **8**: 630–638.
72 Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL *et al.* Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum* 1997; **40**: 1823–1828.
73 Gringhuis SI, den Dunnen J, Litjens M, van der Vlist M, Wevers B, Bruijns SC *et al.* Dectin-1 directs T helper cell differentiation by controlling noncanonical NF-kappaB activation through Raf-1 and Syk. *Nat Immunol* 2009; **10**: 203–213.
74 Bonifazi P, Zelante T, D'Angelo C, De Luca A, Moretti S, Bozza S *et al.* Balancing inflammation and tolerance *in vivo* through dendritic cells by the commensal Candida albicans. *Mucosal Immunol* 2009; **2**: 362–374.
75 Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J *et al.* A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. *J Immunol* 2002; **168**: 5699–5708.
76 Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; **201**: 233–240.
77 Goodall JC, Wu C, Zhang Y, McNeill L, Ellis L, Saudek V *et al.* Endoplasmic reticulum stress-induced transcription factor, CHOP, is crucial for dendritic cell IL-23 expression. *Proc Natl Acad Sci* 2010; **107**: 17698–17703.
78 Zhao G, Feng X, Na A, Yongqiang J, Cai Q, Kong J *et al.* Acute guttate psoriasis patients have positive streptococcus hemolyticus throat cultures and elevated anti-streptococcal M6 protein titers. *J Dermatol* 2005; **32**: 91–96.
79 Leung DY, Travers JB, Giorno R, Norris DA, Skinner R, Aelion J *et al.* Evidence for a streptococcal superantigen-driven process in acute guttate psoriasis. *J Clin Invest* 1995; **96**: 2106–2112.
80 Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S *et al.* Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. *J Immunol* 2011; **187**: 490–500.
81 Appel H, Maier R, Wu P, Scheer R, Hempfing A, Kayser R *et al.* Analysis of IL-17+ cells in facet joints of patients with spondyloarthritis suggests that the innate immune pathway might be of greater relevance than the Th17-mediated adaptive immune response. *Arthritis Res Ther* 2011; **13**: R95.
82 Noordenbos T, Yeremenko N, Gofita I, van de Sande M, Tak PP, Caòete JD *et al.* IL-17 positive mast cells contribute to synovial inflammation in spondyloarthritis. *Arthritis Rheum* 2011; **64**: 99–109.
83 Repo H, Koivuranta-Vaara P, Leirisalo-Repo M. Polymorphonuclear leucocyte function and previous yersinia arthritis: correlation of enhanced superoxide production with late manifestations. *Ann Rheum Dis* 1988; **47**: 452–457.
84 Repo H, Leirisalo M, Tiilikainen A, Laitinen O. Chemotaxis in yersinia arthritis. *In vitro* stimulation of neutrophil migration by HLA—B27 positive and negative sera. *Arthritis Rheum* 1982; **25**: 655–661.
85 Kenna TJ, Davidson SI, Duan R, Bradbury LA, McFarlane J, Smith M *et al.* Enrichment of circulating IL-17-secreting IL-23 receptor-positive γδ T cells in patients with active ankylosing spondylitis. *Arthritis Rheum* 2011 (e-pub ahead of print 5 December 2011; doi:10.1002/art.33507).
86 Shen H, Goodall JC, Hill Gaston JS. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis Rheum* 2009; **60**: 1647–1656.

87 Jandus C, Bioley G, Rivals JP, Dudler J, Speiser D, Romero P. Increased numbers of circulating polyfunctional Th17 memory cells in patients with seronegative spondylarthritides. *Arthritis Rheum* 2008; **58**: 2307–2317.
88 Romero-Sanchez C, Tsou HK, Jan MS, Wong RH, Chang IC, Londono J *et al*. Serum monocyte chemotactic protein-1 concentrations distinguish patients with ankylosing spondylitis from patients with mechanical low back pain. *J Spinal Disord Tech* 2010; **24**: 202–207.
89 Ciccia F, Bombardieri M, Principato A, Giardina A, Tripodo C, Porcasi R *et al*. Overexpression of interleukin-23, but not interleukin-17, as an immunologic signature of subclinical intestinal inflammation in ankylosing spondylitis. *Arthritis Rheum* 2009; **60**: 955–965.
90 Sarin R, Wu X, Abraham C. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. *Proc Natl Acad Sci USA* 2011; **108**: 9560–9565.
91 Thomas GP, Brown MA. Genetics and genomics of ankylosing spondylitis. *Immunol Rev* 2010; **233**: 162–180.
92 Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O *et al*. Plasmacytoid predendritic cells initiate psoriasis through interferon-α production. *J Exp Med* 2005; **202**: 135–143.
93 Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human beta 2m: an animal model of HLA-B27-associated human disorders. *Cell* 1990; **63**: 1099–1112.
94 DeLay ML, Turner MJ, Klenk EI, Smith JA, Sowders DP, Colbert RA. HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. *Arthritis Rheum* 2009; **60**: 2633–2643.
95 A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet* 2010; **42**: 985–990.
96 Evans DM, Spencer CCA, Pointon JJ, Su Z, Harvey D, Kochan G *et al*. Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat Genet* 2011; **43**: 761–767.
97 Kochan G, Krojer T, Harvey D, Fischer R, Chen L, Vollmar M *et al*. Crystal structures of the endoplasmic reticulum aminopeptidase-1 (ERAP1) reveal the molecular basis for N-terminal peptide trimming. *Proc Natl Acad Sci USA* 2011; **108**: 7745–7750.
98 Fujikado N, Saijo S, Yonezawa T, Shimamori K, Ishii A, Sugai S *et al*. Dcir deficiency causes development of autoimmune diseases in mice due to excess expansion of dendritic cells. *Nat Med* 2008; **14**: 176–180.
99 Kontoyiannis D, Pasparakis M, Pizarro TT, Cominelli F, Kollias G. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* 1999; **10**: 387–398.
100 Armaka M, Apostolaki M, Jacques P, Kontoyiannis DL, Elewaut D, Kollias G. Mesenchymal cell targeting by TNF as a common pathogenic principle in chronic inflammatory joint and intestinal diseases. *J Exp Med* 2008; **205**: 331–337.
101 Hammer GE, Turer EE, Taylor KE, Fang CJ, Advincula R, Oshima S *et al*. Expression of A20 by dendritic cells preserves immune homeostasis and prevents colitis and spondyloarthritis. *Nat Immunol* 2011; **12**: 1184–1193.
102 Ruutu M, Thomas G, Steck R, Degli-Esposti MA, Zinkernagel MS, Alexander K *et al*. Beta-glucan triggers spondyloarthropathy and Crohn's-like ileitis in SKG mice. *Arthritis Rheum* 2012 (e-pub ahead of print 10 February 2012; doi:10.1002/art.34423).
103 Klunker S, Chong MMW, Mantel P-Y, Palomares O, Bassin C, Ziegler M *et al*. Transcription factors RUNX1 and RUNX3 in the induction and suppressive function of Foxp3+ inducible regulatory T cells. *J Exp Med* 2009; **206**: 2701–2715.
104 Futterer A, Mink K, Luz A, Kosco-Vilbois MH, Pfeffer K. The lymphotoxin beta receptor controls organogenesis and affinity maturation in peripheral lymphoid tissues. *Immunity* 1998; **9**: 59–70.
105 Jacques P, Venken K, Van Beneden K, Hammad H, Seeuws S, Drennan MB *et al*. Invariant natural killer T cells are natural regulators of murine spondylarthritis. *Arthritis Rheum* 2010; **62**: 988–999.
106 Wang K, Baldassano R, Zhang H, Qu H-Q, Imielinski M, Kugathasan S *et al*. Comparative genetic analysis of inflammatory bowel disease and type 1 diabetes implicates multiple loci with opposite effects. *Hum Mol Genet* 2010; **19**: 2059–2067.
107 Blanco-Kelly F, Matesanz F, Alcina A, Teruel M, Díaz-Gallo LM, Gómez-García M *et al*. *CD40*: novel association with Crohn's disease and replication in multiple sclerosis susceptibility. *PLoS One* 2010; **5**: e11520.
108 Singh K, Deshpande P, Pryshchep S, Colmegna I, Liarski V, Weyand CM *et al*. ERK-dependent T cell receptor threshold calibration in rheumatoid arthritis. *J Immunol* 2009; **183**: 8258–8267.
109 Maurice MM, Lankester AC, Bezemer AC, Geertsma MF, Tak PP, Breedveld FC *et al*. Defective TCR-mediated signaling in synovial T cells in rheumatoid arthritis. *J Immunol* 1997; **159**: 2973–2978.
110 Zhang Z, Gorman CL, Vermi AC, Monaco C, Foey A, Owen S *et al*. TCRzetadim lymphocytes define populations of circulating effector cells that migrate to inflamed tissues. *Blood* 2007; **109**: 4328–4335.
111 de Launay D, van de Sande MG, de Hair MJ, Grabiec AM, van de Sande GP, Lehmann KA *et al*. Selective involvement of ERK and JNK mitogen-activated protein kinases in early rheumatoid arthritis (1987 ACR criteria compared to 2010 ACR/EULAR criteria): a prospective study aimed at identification of diagnostic and prognostic biomarkers as well as therapeutic targets. *Ann Rheum Dis* 2011; **71**: 415–423.
112 Gringhuis SI, Papendrecht-van der Voort EA, Leow A, Nivine Levarht EW, Breedveld FC, Verweij CL. Effect of redox balance alterations on cellular localization of LAT and downstream T-cell receptor signaling pathways. *Mol Cell Biol* 2002; **22**: 400–411.
113 Goronzy JJ, Weyand CM. Thymic function and peripheral T-cell homeostasis in rheumatoid arthritis. *Trends Immunol* 2001; **22**: 251–255.
114 Maurice MM, Nakamura H, van der Voort EA, van Vliet AI, Staal FJ, Tak PP *et al*. Evidence for the role of an altered redox state in hyporesponsiveness of synovial T cells in rheumatoid arthritis. *J Immunol* 1997; **158**: 1458–1465.
115 Remans PH, Gringhuis SI, van Laar JM, Sanders ME, Papendrecht-van der Voort EA, Zwartkruis FJ *et al*. Rap1 signaling is required for suppression of Ras-generated reactive oxygen species and protection against oxidative stress in T lymphocytes. *J Immunol* 2004; **173**: 920–931.
116 Abreu JR, Grabiec AM, Krausz S, Spijker R, Burakowski T, Maslinski W *et al*. The presumed hyporesponsive behavior of rheumatoid arthritis T lymphocytes can be attributed to spontaneous *ex vivo* apoptosis rather than defects in T cell receptor signaling. *J Immunol* 2009; **183**: 621–630.
117 Weyand CM, Fujii H, Shao L, Goronzy JJ. Rejuvenating the immune system in rheumatoid arthritis. *Nat rev Rheumatol* 2009; **5**: 583–588.
118 Fulop Jr T, Larbi A, Dupuis G, Pawelec G. Ageing, autoimmunity and arthritis: perturbations of TCR signal transduction pathways with ageing - a biochemical paradigm for the ageing immune system. *Arthritis Res Ther* 2003; **5**: 290–302.