Nature Reviews Rheumatology **10**, 697 (2014); published online 4 November 2014; doi:10.1038/nrrheum.2014.190

AUTOIMMUNITY

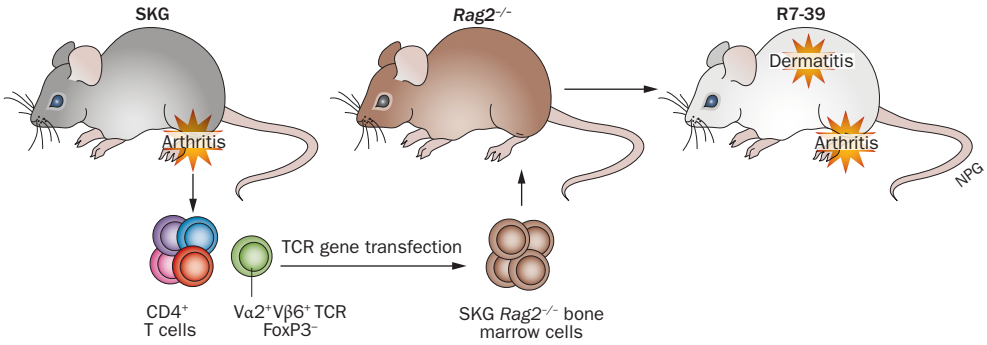
Engineering mice to identify autoreactive T-cell antigens

Now published in the journal *Science,* Professor Shimon Sakaguchi and colleagues present a novel method of autoantigen discovery and a proof-of-principle, showing that the 60S ribosomal protein L23a (RPL23A) is an arthritogenic T-cell autoantigen. "By attenuation of T-cell receptor (TCR) signal strength," says Sakaguchi, "T cells recognizing ubiquitous self-antigens, which are normally deleted in the thymus by negative selection, are selected as a dominant repertoire and are causative of systemic autoimmune diseases."

By engineering retrogenic mice to expand and isolate pathogenic TCR-expressing T cells, a method Sakaguchi says was pioneered by Dr Dario Vignali, the researchers were able to examine a number of individual TCRs for their pathogenicity. Adding to this technique, Sakaguchi says, "We then came up with the idea that by reconstituting arthritis-bearing retrogenic mice with B cells, autoantibodies thus produced by the 'help' of arthritogenic T cells would react with antigens recognized by the arthritogenic TCRs, circumventing other meticulous ways of directly identifying self-peptides."

Whereas autoantibody targets are relatively easy to identify in autoimmune diseases, the autoantigen targets of pathogenic T cells that might be central to rheumatoid arthritis (RA), for example, have been more elusive. Their scarcity in the periphery makes identification of autoreactive T-cell clones and their targets, in patients, a challenge, as $CD4^+$ T cells specific for ubiquitously expressed autoantigens, by definition, have high-affinity TCRs that tag them for thymic deletion.

To overcome these challenges, the researchers expanded pathogenic autoreactive T cells from SKG mice, a model of spontaneous autoimmune arthritis that is dependent on a mutation of a TCR signalling molecule, tyrosine-protein kinase ZAP-70.



Previously, Sakaguchi's group found that SKG mice have a missense *Zap70* mutation (encoding Trp163Cys) that, rather than causing T-cell deficiency, suppresses TCR signalling and thereby alters T-cell ontogeny. As a result, thymocytes with the strongest affinity for self-peptides are positively selected, instead of being deleted. Until now, however, the identity of these autoantigens was unknown.

To identify pathogenic T-cell clones, the researchers knocked-in (to the SKG mice) a fluorescent forkhead box protein P3 (FoxP3) fusion protein to ensure that protective regulatory T cells were not selected when isolating T cells from the arthritic joints of these mice.

Sakaguchi's team then cloned and transfected $Vα2^+Vβ6^+$ TCR genes from isolated $FoxP3^-CD4^+$ T cells into bone marrow cells from SKG *Rag2*$^{-/-}$ mice (which lack T and B cells). These cells were transferred back into *Rag2*$^{-/-}$ mice to generate retrogenic mice with Vα2Vβ6-restricted thymocytes.

Two arthritogenic TCRs (6–39 and 7–39) and one nonarthritogenic TCR were cloned in this way. 80.0% of retrogenic 7–39 TCR (R7–39) and 27.3% of 6–39 TCR (R6–39) mice developed arthritis with mononuclear cell infiltration, pannus formation and cartilage destruction. Furthermore, R7–39 mice developed psoriasis-like dermatitis.

To identify the target of the arthritogenic T cells, Dr Yoshinaga Ito, first author of the study, says, "We reconstituted the retrogenic mice with B cells (from TCRβ$^{-/-}$ bone marrow) to generate autoantibodies to the target antigen". Serum from these mice reacted with P3U1 cell extracts and mass spectrometry was used to identify RPL23A as the target, a finding functionally confirmed by stimulating arthritogenic TCR-bearing T-cell hybridomas with recombinant RPL23A.

RPL23A is 100% conserved in mice and humans, and the researchers showed that *RPL23A* mRNA is highly and ubiquitously expressed in healthy humans. Importantly, they also detected anti-RPL23A humoral and cellular immune responses in patients with RA, but not in patients with osteoarthritis ($n = 11$), systemic lupus erythematosus ($n = 30$), polymyositis or dermatomyositis ($n = 10$), although more patients might be required to see an effect.

Serum anti-RPL23 autoantibodies were detected in a substantially higher proportion of patients with RA (16.8%, $n = 374$, $P<0.001$), or with psoriasis (8.7%, not statistically significant, $n = 23$), than in healthy individuals (1.3%, $n = 74$), suggesting RPL23A autoreactivity is pathogenic, at least in a subset of patients.

Of ongoing work, Sakaguchi says, "We plan to further identify other self-antigens with the same method."

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Original article Ito, Y. *et al*. Detection of T cell responses to a ubiquitous cellular protein in autoimmune disease. *Science* **346**, 363–368 (2014)