IMMUNOGENETICS

Guilty by association

Graves' disease, autoimmune hypothyroidism (AIH) and type 1 diabetes (T1D) — what's the connection? These autoimmune diseases commonly occur in family clusters, and susceptibility to them is known to be associated with the same region on human chromosome 2q33. Similarly, the syntenic region on mouse chromosome 1 (the *Idd5.1* locus) is associated with susceptibility to T1D in nonobese diabetic (NOD) mice. Now, Ueda *et al.* have narrowed down the suspected region to a mutation of the gene encoding the inhibitory co-stimulatory molecule cytotoxic T-lymphocyte antigen 4 (CTLA4), and have described a possible mechanism for the effects of this mutation.

CD28, *CTLA4* and immune co-stimulator (*ICOS*) are the only three functional genes in this region of human chromosome 2q33, but until now, the association with autoimmune-disease susceptibility has not been refined any further. A 100-kb section of this region (comprising *CTLA4* and the 5′ region of *ICOS*) was found to contain a cluster of single nucleotide polymorphisms (SNPs) that are more common in patients with Graves' disease than in control individuals. This disease-associated cluster was then refined further to a non-coding region 3′ of the end of the *CTLA4* transcript. The SNP with the strongest association with Graves' disease in this region is known as CT60, with the A allele being protective and the G allele increasing susceptibility. CT60 was also shown to be associated with AIH to a similar extent, although it had a weaker effect in determining susceptibility to T1D.

As nucleotide variation at CT60, or its neighbouring SNPs, does not result in an amino-acid change in the CTLA4 protein, the authors looked for differences in the level of expression of *CTLA4*. There are two main isoforms of CTLA4 in humans — the full-length isoform (flCTLA4) and a soluble isoform (sCTLA4). The ratio of sCTLA4 to flCTLA4 messenger RNA was 50% lower in T cells from disease-susceptible CT60 G/G individuals than from disease-resistant CT60 A/A individuals.

In support of the results from humans, it was shown that there is also a difference in the level of expression of a *Ctla4* splice variant between congenic NOD mice having the

VACCINES

BCG — a work in progress

The *Mycobacterium bovis* bacille Calmette–Guérin (BCG) is one of the world's most widely used vaccines, which reduces the incidence of certain childhood forms of tuberculosis by approximately 70%. It has little or no effect, however, on the adult pulmonary disease, and with two million deaths from tuberculosis every year, there is an urgent need for an improved vaccine. One possible explanation for the modest efficacy of the BCG vaccine is a shortage of antigens that elicit a protective response against *Mycobacterium tuberculosis*. Indeed, the attenuated BCG vaccine was originally derived by serial passage of a virulent strain of *Mycobacterium bovis* and a crucial event in the attenuation process was the loss of many coding sequences, including a group of nine genes known as the region of deletion 1 (RD1). Reporting in *Nature Medicine*, Pym and colleagues now show that genes in the RD1 locus are required for the secretion of ESAT6 — a protein of unknown function that stimulates a strong T-cell response. Furthermore, they show that immunization with a recombinant BCG strain, with the RD1 replaced, improves protection against *M. tuberculosis* infection in animal models.

Working to the hypothesis that BCG efficacy could be improved by re-introducing lost *M. tuberculosis* antigens, the authors initially set out to unravel the genetic basis of secretion of the ESAT6 family of immunodominant T-cell antigens. The entire gene cluster of RD1 was shown to be essential for normal secretion of ESAT6 and its binding partner CFP10, indicating that the flanking genes encode a specialized secretion system. Secretion of ESAT6 (and probably CFP10) was essential for the induction of an optimal T-cell response, and a recombinant vaccine strain overexpressing the antigen intracellularly did not induce a marked specific response.

Immunization with a recombinant BCG strain that contains the RD1 elicited better protection against aerosol challenge with *M. tuberculosis* in animal models. Intriguingly, a markedly reduced bacterial load was observed in the spleens of vaccinated animals, indicating an improved ability to restrict spread of the pathogen from the initial site of infection. Primary tuberculosis occurs mainly in the central and lower

C57BL/10 *Idd5.1* locus and non-congenic NOD mice, which have haplotypes of the *Idd5.1* region that confer protection against or susceptibility to T1D, respectively. The newly identified ligand-independent isoform of *Ctla4* (*liCtla4*; a transmembrane isoform that lacks the CD80/CD86-binding domain) had a fourfold increase in expression by T cells from *Idd5.1*-congenic mice compared with NOD mice.

So, these results indicate that differential expression of alternatively spliced forms of *CTLA4* might have an important role in determining susceptibility to autoimmune disease. For example, the authors propose that reduced levels of sCTLA4 could increase T-cell activation by reducing binding of sCTLA4 to its ligands CD80/CD86 and allowing increased T-cell activation through CD28.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Ueda, H. *et al*. Association of the T-cell regulatory gene *CTLA4* with susceptibility to autoimmune disease. *Nature* 30 April 2003 (DOI: 10.1038/nature01621)

FURTHER READING Sharpe, A. H. & Freeman, G. J. The B7–CD28 superfamily. *Nature Rev. Immunol*. **2**, 116–126 (2002)

lobes of the lung and is often asymptomatic. To reach the upper lobes, the most common site of pathology, movement through the bloodstream is required. As such, a vaccine that inhibits the process of dissemination from the site of primary infection would probably influence the course of disease.

The precise mechanisms that underlie the ability of the RD1 locus to enhance protection against *M. tuberculosis* remain to be elucidated. The presence of two extra antigens (ESAT6 and CFP10) that induce a more robust $CD4^+$ T-cell response, coupled with a possible role for these molecules in allowing the vaccine strain to persist in the host, might be important in contributing to the improved efficacy. Ultimately, the marked protection that is observed after re-incorporation of the RD1 locus indicates a compelling reason to include this modification in any recombinant BCG vaccine, and work is now underway to characterize other missing immunodominant antigens of *M. tuberculosis* that might improve the BCG vaccine even further.

David O'Connell, Editor,
Nature Reviews Microbiology

References and links

ORIGINAL RESEARCH PAPER Pym, A. S. *et al.* Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. *Nature Med.* 14 April 2003 (DOI: 10.1038/nm859)

FURTHER READING Young, D. B. & Stewart, G. R. Tuberculosis vaccines. *Br. Med. Bull.* **62**, 73–86 (2002) | Kaufmann, S. H. How can immunology contribute to the control of tuberculosis? *Nature Rev. Immunol.* **1**, 20–30 (2001)

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AUTOIMMUNITY

Autoimmunity and tolerance — two sides of the same coin

Induction of disease-specific tolerance using autoantigens is the goal of many ongoing studies of autoimmune disease. The potential application of this approach has now been shown in two related studies from Len Harrison's laboratory, but the potential pitfalls of this approach are apparent in the second study.

Steptoe and colleagues used non-obese diabetic (NOD) mice to test whether the expression of autoantigen by syngeneic haematopoietic stem cells (HSCs) can prevent the spontaneous development of diabetes by the induction of tolerance. The autoantigen pro-insulin was targeted to resting antigen-presenting cells in NOD mice by expressing pro-insulin under the control of the MHC class II promoter (NOD–PI mice). When bone marrow from these mice was transferred to recipient wild-type NOD mice, the occurrence of insulitis was reduced, and the development of diabetes was prevented in most recipients. This also occurred in wild-type recipients of T-cell-depleted bone marrow, indicating that transferred T cells did not contribute to the effect. Moreover, transfer of purified NOD–PI progenitor cells reduced the incidence of diabetes, and purified NOD–PI HSCs prevented diabetes in recipients. This study establishes proof of principle that syngeneic HSCs expressing autoantigens might be a useful therapeutic tool, but the precise mechanisms of tolerance induction require further study. The approach would require some modification for use in pre-diabetic humans and would also require vectors that can effectively transduce HSCs for long-term gene expression after engraftment.

In the second study, Martinez and colleagues tested whether autoantigenic peptides that were administered mucosally could inhibit the development of autoimmunity. Intranasal treatment of young NOD mice with the pro-insulin peptide B24–C36 prevented them from developing diabetes, which is rapidly induced by injecting splenocytes from recently diabetic NOD mice, and this tolerogenic effect was due to the development of regulatory $CD4^+$ T cells. Spontaneous diabetes in male NOD mice was reduced, but in females, development of diabetes indicated the simultaneous induction of cytotoxicity by mucosal administration of B24–C36. The authors found that this peptide contained overlapping $CD4^+$ and $CD8^+$ T-cell epitopes. When the $CD8^+$ T-cell epitope was disabled by truncation of a binding anchor for $H{-}2K^d$, a single intranasal administration of the truncated peptide was sufficient to prevent the development of diabetes in this system.

Together, these two studies show both sides of the coin of antigen-specific immunotherapy: the potential and the pitfalls.

Elaine Bell

References and links

ORIGINAL RESEARCH PAPERS Steptoe, R. J. *et al.* Transfer of hematopoietic stem cells encoding autoantigen prevents autoimmune diabetes. *J. Clin. Invest.* **111**, 1357–1363 (2003) | Martinez, N. R. *et al.* Disabling an integral CTL epitope allows suppression of autoimmune diabetes by intranasal proinsulin peptide. *J. Clin. Invest.* **111**, 1365–1371 (2003)