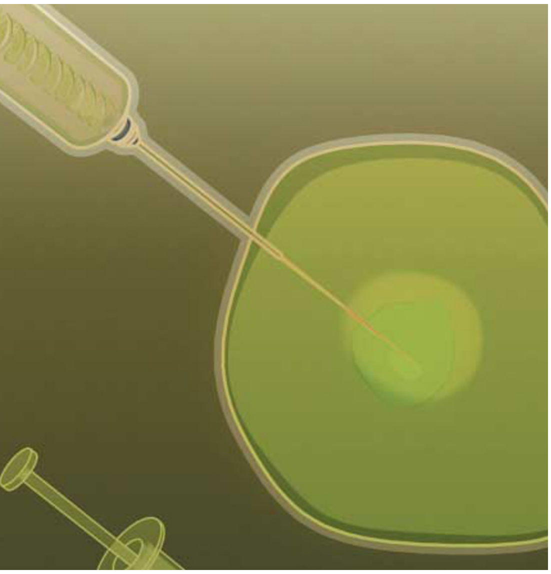
AUTOIMMUNITY

Gene therapy for diabetes



Type 1 diabetes mellitus (T1DM) occurs as a result of T-cell-mediated destruction of the insulin-producing β-cells in the pancreas. Non-obese diabetic (NOD) mice, which spontaneously develop diabetes, are a useful model system of this disease. In this study, the authors show that expression of diabetes-resistant MHC class II alleles by NOD mice, using retroviral transduction of autologous bone-marrow cells, is sufficient to prevent the development of diabetes.

In humans, the development of T1DM is associated with the inheritance of particular MHC class II alleles that lack a charged amino acid at position 57 of the β-chain. In NOD mice, the single MHC class II allele present is I–A^{g7}, which also lacks a charged residue at position 57. It is thought that lack of a charged residue at this position prevents the formation of a salt bridge between the α- and β-chains of the MHC class II molecule, which could affect the ability of these molecules to mediate negative selection of autoreactive T cells.

Previous studies using transgenic mice and allogeneic bone-marrow chimeras have shown that it is possible to prevent diabetes in NOD mice, but it has not been possible to determine the preventative mechanism.

Retroviral constructs with green fluorescent protein fused to the cytoplasmic tails of diabetes-resistant I–A β-chains (which have a charged residue at position 57) were shown to be expressed at the cell surface of MHC-class-II-positive cells and could pair with endogenous α-chains. Young NOD mice were lethally irradiated and transplanted with bone marrow retrovirally transduced with the I–Aβ constructs, and their blood-glucose levels were monitored each week. All of the mice were protected from diabetes, compared with four of six mice that received bone marrow transfected with a control construct. Even when diabetes was aggressively induced using cyclophosphamide, the mice that received I–Aβ-transduced bone marrow were resistant to the development of diabetes for up to 46 weeks after transfer, and the level of insulitis was reduced.

To address the mechanism of resistance, the authors used enzyme-linked immunosorbent spot (ELISPOT) assays to look at the frequency of T cells that produced cytokines in response to peptide 206–220 from glutamic-acid decarboxylase, an immunodominant self-antigen peptide in NOD mice. No cytokine production was detectable in NOD mice that received

T-CELL MEMORY

Memories are made of this…

Infection with the parasite *Leishmania* causes considerable morbidity and mortality, against which there is no effective human vaccine. Both humans and mice can resolve primary infections and become resistant to further infection, but some parasites persist and might contribute to long-term protection by maintaining the presence of effector T cells. If persistent antigen is required for long-term protection, then developing a non-live vaccine against leishmaniasis will be difficult. In this study, Phillip Scott and colleagues characterized the CD4$^+$ T-cell response during infection and showed that protective central memory T (T_{CM}) cells, which are not dependent on the presence of parasites, can develop in infected mice.

Memory T cells are a heterogeneous population thought to contain two distinct subsets — effector memory T (T_{EFF}) cells, which migrate to tissues and produce cytokines, and T_{CM} cells, which circulate through the lymph nodes. Scott and colleagues investigated the development of CD4$^+$ memory T cells during infection of mice with *Leishmania major*. CD4$^+$ T cells were labelled with CFSE (5,6-carboxyfluorescein diacetate succinimidyl ester) — which allows proliferative responses to be analysed by flow cytometry — and then transferred into naive recipients. After parasitic challenge infection of the recipient mice, some of these donor T cells migrated to the draining lymph nodes (dLNs) and proliferated, indicating that immune mice contain a T_{CM}-cell population. During proliferation, most cells downregulated their expression of the lymph-node homing molecule CD62L, indicating that these cells had differentiated into T_{EFF} cells. To confirm that T_{CM} cells present in the donor T-cell population from immune mice could differentiate into T_{EFF} cells and mediate protection, CD4$^+$CD62L^{hi} T cells — which do not produce interferon-γ (IFN-γ) when stimulated with leishmanial antigens — were purified and used in transfer experiments. Again, the donor T cells proliferated in the dLN; they also developed the capacity to produce IFN-γ and could be detected at the site of infection within two weeks. Importantly, the mice that received these T_{CM} cells were protected from challenge infection.

To address the role of persistent antigen in the maintenance of memory T cells, a mutant *L. major* strain that is unable to persist in mice was used. Mice infected with this mutant *L. major* had no detectable parasites by 15 weeks after infection. At 25 weeks after infection, no T_{EFF} cells were detectable, as shown by the lack of both an IFN-γ response to leishmanial antigens *in vitro* and a delayed-type hypersensitivity response *in vivo*. However, T_{CM} cells were detectable at 25 weeks; CFSE-labelled CD4$^+$ T cells from these mice, when transferred to naive recipients that were subsequently challenged with *L. major*, could migrate to and proliferate in the dLN. Furthermore, 25 weeks after the initial infection with mutant *L. major*, mice were protected against infection with virulent *L. major*, showing that the T_{CM} cells conferred protection.

This study shows that both T_{EFF} and T_{CM} cells contribute to immunity to *L. major* infection, but T_{CM} cells can be maintained in the absence of parasites and confer protection. Targeting T_{CM} cells could therefore be the basis for the development of a successful non-live vaccine against leishmaniasis.

Elaine Bell

References and links

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I-Aβ-transduced bone marrow, indicating that self-reactive T cells had been functionally inactivated or eliminated. To determine whether this was owing to deletion of self-reactive T cells in the thymus or to peripheral tolerance, the authors used I–A^{g7} tetramers loaded with a peptide known to stimulate pancreatic-islet-reactive T-cell clones. The frequency of CD4^{+} T cells labelled with the tetramer was significantly reduced among thymocytes from NOD mice reconstituted with I–Aβ-transduced cells compared with NOD mice that received cells transduced with the control construct, supporting the idea that I–Aβ mediated the removal of self-reactive T cells by negative selection.

This study offers the prospect that T1DM could be prevented by providing susceptible individuals with protective MHC class II alleles, using autologous bone marrow. This approach would be preferable to the use of allogeneic cells, because graft-versus-host disease would be avoided and it might be possible to use milder conditioning regimens before transplantation.

Elaine Bell

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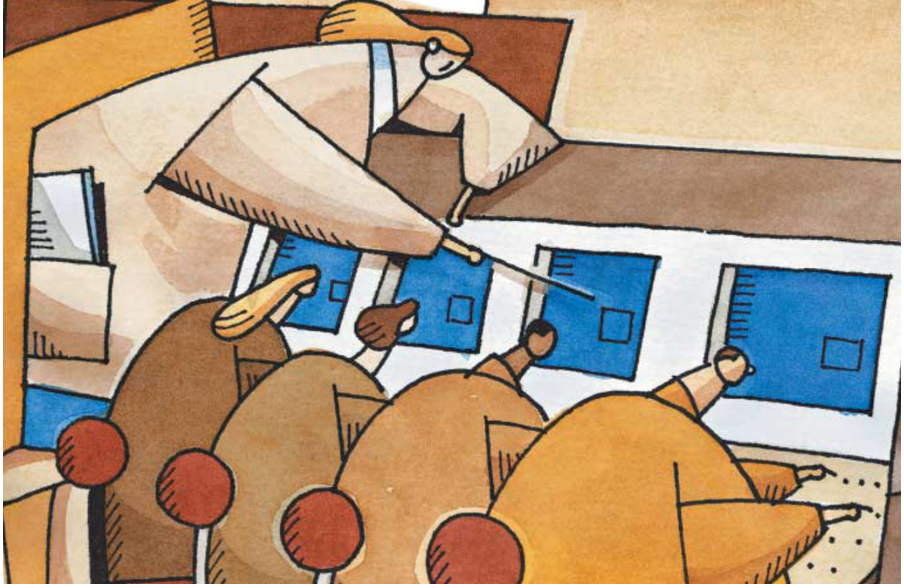

B-CELL DEVELOPMENT

BCL-6 recruits new team member

Delegation is the key to success, and just as every boss needs a team to ensure that the work gets done, this paper in *Cell* describes an important member of the team of cofactors that control B-cell differentiation in association with the master regulator BCL-6 (B-cell lymphoma 6). The transcriptional repressor BCL-6 is expressed by germinal-centre B cells and functions to antagonize plasma-cell differentiation controlled by BLIMP1 (B-lymphocyte-induced maturation protein 1). Now, this study shows that MTA3, a cell-type-specific subunit of the Mi-2/NuRD co-repressor complex, associates with BCL-6 to inhibit the terminal differentiation of B cells to plasma cells.

Immunohistochemical analysis of human lymph-node and tonsil sections showed that MTA3 is highly expressed by a population of germinal-centre B cells that also express BCL-6, and the co-expression of MTA3 and BCL-6 was observed in B-cell lines but not in plasma-cell lines. Furthermore, MTA3 and BCL-6 could be co-precipitated from a B-cell line together with other subunits of Mi-2/NuRD, indicating that BCL-6 stably interacts with an MTA3-containing Mi-2/NuRD co-repressor complex. Protein–protein interaction assays showed that the central region of BCL-6 interacts directly with the carboxyl terminus of MTA3.

A GAL4 tethering assay was then used to test the functional effects of the BCL-6–MTA3 interaction. BCL-6 fused to the DNA-binding domain of GAL4 represses transcription of a luciferase reporter construct containing GAL4-binding sites in HeLa cells, which constitutively express high levels of MTA3. But when RNA interference was used to block the expression of MTA3, transcriptional repression of the luciferase reporter mediated by BCL-6 was reduced. MTA3 was also shown to be required for BCL-6-dependent transcriptional repression in a more physiological setting; the depletion of MTA3 protein from B-cell lines, using RNA interference, resulted in the expression of plasma-cell-specific proteins, such as BLIMP1, that are known to be repressed by BCL-6. Using adenoviral vectors expressing BCL-6 and/or MTA3 to infect plasma-cell lines, the authors showed that marked repression of plasma-cell-specific transcripts and upregulation of B-cell-specific transcripts only occurred when both BCL-6 and MTA3 were co-expressed. This reprogramming of the plasma-cell transcriptional pattern to a B-cell pattern was accompanied by cell-surface expression of B-cell markers, such as CD19, CD20 and HLA-DR, and decreased cytoplasmic staining for the κ-light chain of immunoglobulins.

The evidence therefore points to a model in which BCL-6 recruits MTA3 to form a complex that prevents the differentiation of germinal-centre B cells to plasma cells until appropriate signals are received. Additional experiments showed that acetylation of the central domain of BCL-6 prevents interaction with MTA3 and abolishes repressive function, which indicates that such post-translational modification of BCL-6 might be one way in which signals for plasma-cell differentiation are transduced.

Kirsty Minton

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