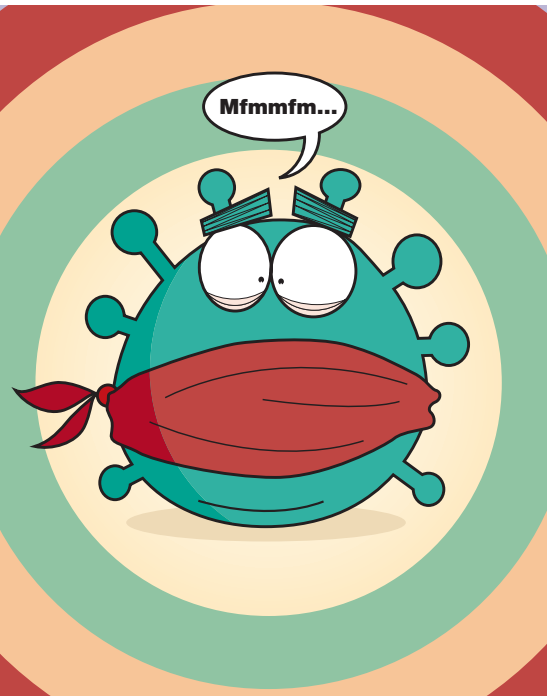
HIV

Silencing HIV the natural way



A recent paper in *Immunity* describes the first natural example of RNA silencing in mammalian host–virus interactions. This evolutionarily ancient form of nucleic-acid-based immunity has previously been found in plants and invertebrates. But the demonstration that human cells can physiologically silence HIV-1 RNA highlights the universal importance of this system.

RNA silencing (also known as RNA interference, RNAi) in invertebrates is based on the cleavage of double-stranded RNA precursors by the RNase Dicer to form short interfering RNAs (siRNAs) of ~21 nucleotides; siRNAs then induce the degradation of complementary target RNAs, leading to post-transcriptional gene silencing. Silencing of viral genes is a natural antiviral response in plants and insects, using siRNA derived from the viral genome by the host processing machinery.

To examine the relevance of this response in immunity to HIV-1, the authors looked for sequences in the viral genome that could form the RNA-duplex precursors necessary for cleavage by host Dicer. They found one sequence in HIV-1, in the *env* gene (which encodes the envelope surface glycoprotein), that formed a 19-base-pair duplex, and this sequence was functionally recognized by Dicer *in vitro*. Furthermore, the Dicer cleavage product — viral siRNA (vsiRNA) — was detected in HIV-infected T cells but not in mock-infected cells.

To test whether the vsiRNA could mediate RNAi, they used constructs of its target RNA sequence fused to enhanced green fluorescent protein (EGFP) such that cleavage of the target RNA would prevent transcription of the downstream EGFP-encoding RNA. The addition of vsiRNA precursors led to a dose-dependent silencing of EGFP expression, and these precursors could also silence expression of the cognate target HIV-1 *env* mRNA.

Because HIV-1 successfully replicates in human cells to high levels of cell-free RNA, it must be able to defend itself against this host-exploited RNAi. Synthetic siRNA precursors specific for the HIV-1 transactivation response element (TAR) were ineffective at suppressing EGFP expression from a TAR–EGFP transcript when the HIV-1 transcriptional transactivator (Tat) was included in the assay, and the suppressive function of Tat was also shown for other non-TAR sequences. This indicates that Tat is a general suppressor of RNA silencing (SRS). This

AUTOIMMUNITY

Homing in on the target

Although multiple pancreatic islet-cell molecules are targets of autoimmune responses in human type 1 diabetes and in mouse models, conclusive evidence of the autoantigens that are important for initiation of the disease has been difficult to obtain. Now, two articles in *Nature* show that insulin is likely to be a crucial autoantigen in the development of type 1 diabetes in both the mouse model and human patients.

Previously, it has been shown that most CD4$^+$ T cells infiltrating the pancreas in non-obese diabetic (NOD) mice recognize insulin, in particular peptide 9–23 of the insulin B chain. So, to test the role of this response in the development of disease, Nakayama *et al.* generated NOD mice in which both insulin genes (*Ins1* and *Ins2*) were deleted and replaced with a transgene encoding mutant pro-insulin. The mutant pro-insulin contained a single amino-acid change at position 16 of the insulin B chain, which preserves the metabolic activity of insulin but prevents recognition by the infiltrating T cells. None of the *Ins1*$^{-/-}$*Ins2*$^{-/-}$ NOD mice expressing the modified insulin showed signs of an immune response to the islet cells, and they did not develop diabetes (although autoimmune reactions were still evident in the salivary glands). By contrast, the presence of either insulin gene in these mice was sufficient to restore diabetes, confirming a crucial role for both insulin genes as targets of organ-specific autoimmunity.

Unlike mouse studies, the study of human autoimmune diseases is often hindered by the scarcity and lability of the relevant target tissues or draining lymph nodes. But in the second study, Kent *et al.* obtained viable pancreatic draining lymph nodes from three individuals with type 1 diabetes (two with long-term disease and one with recent-onset disease) and three control individuals. From these lymph-node samples, the authors generated single T-cell clones in a non-biased manner and analysed their T-cell receptor (TCR) repertoire and their antigen specificity. T-cell clones isolated from pancreatic lymph nodes of control individuals expressed heterogeneous TCR repertoires, indicative of polyclonal expansion. By contrast, more than half of the T-cell clones from the long-term diabetic individuals expressed identical Vβ chains, and of these clones, half had the same TCR α-chain, implying antigen-driven expansion of a common progenitor cell. The clones from the long-term diabetic individuals, but not those from control individuals, proliferated specifically in response to peptide 1–15 of insulin A in a dose-dependent manner. This response was restricted by the MHC class II allele HLA-DRB1*0401, which is known to confer genetic susceptibility to diabetes.

To rule out the possibility that the insulin-specific T-cell responses could have arisen from long-term use of daily insulin injections (to control blood glucose levels), Kent *et al.* showed that insulin-reactive T cells were not found in the spleen of one of the long-term diabetic individuals, and CD4$^+$ T-cell clones from pancreatic draining lymph nodes of

was not a consequence of the transcription of a downstream effector, as a point mutant of Tat without transcriptional activity could still function as an SRS. Instead, Tat was shown *in vitro* to target the host RNAi mechanism upstream of vsiRNA generation, at the level of Dicer.

Another Tat mutant was transcriptionally proficient but lacked SRS activity; recombinant HIV containing this mutated form of Tat replicated less efficiently than HIV containing wild-type Tat. However, the difference was only slight, indicating that HIV infection is controlled by a range of host factors, one of which is the balance between host-exploited RNAi and viral SRS. When this balance was disrupted by the addition of oligonucleotides that inhibit vsiRNA to HIV-1-transfected cells, there was a dose-dependent increase in viral replication.

The authors suggest that the finding of only one duplex sequence in the HIV-1 genome indicates that there is a strong selective pressure exerted by host RNAi against such sequences and that the remaining sequence must have a crucial non-mutatable function for HIV-1, requiring the maintenance of a viral SRS.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER Bennasser, Y., Le, S.-Y., Benkirane, M. & Jeang, K.-T. Evidence that HIV-1 encodes an siRNA and a suppressor of RNA silencing. *Immunity* **22**, 607–619 (2005)

an individual with type 2 diabetes did not recognize any pro-insulin peptide.

Knowing which autoantigens trigger autoimmunity is still out of reach for many researchers studying autoimmune disease, but the evidence from both of these papers that insulin has a crucial role will no doubt increase our chances of developing antigen-specific tolerization therapy for diabetic patients.

Lucy Bird

References and links

ORIGINAL RESEARCH PAPERS Nakayama, M. *et al*. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. *Nature* **435**, 220–223 (2005) | Kent, S. C. *et al*. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature* **435**, 224–228 (2005)

TUMOUR IMMUNOLOGY

B cells lead the way in tumour progression

Many studies have reported a link between chronic inflammatory diseases and cancer development. However, it has not been clear how the recruitment of inflammatory innate immune cells is initiated or sustained at sites of tumour development. Lisa Coussens and colleagues now report an important role for B cells in promoting innate-immune-cell inflammation in a mouse model of epithelial carcinogenesis, which is in contrast to the view that adaptive immune cells are involved in 'surveillance' against developing neoplasms.

K14-HPV16 **mice express oncogenes from human papillomavirus type 16 under the control of the human keratin 14 promoter/enhancer, leading to multi-stage epithelial carcinogenesis. The pre-malignant stage is characterized by infiltration of innate immune cells such as granulocytes and mast cells into the skin. However, when *K14-HPV16* mice were crossed with recombination-activating-gene-1-deficient mice (*K14-HPV16/Rag1*$^{-/-}$ mice), which lack T and B cells, this infiltration was significantly reduced. Decreased infiltration of the skin was associated with decreased activity of matrix metalloproteinase 9 (MMP9) compared with *K14-HPV16* mice. MMP9 is secreted by leukocytes and has a role in cancer development through its effects on tissue remodelling and release of the angiogenic growth factor vascular endothelial growth factor (VEGF) from the extracellular matrix. Correspondingly, *K14-HPV16/Rag1*$^{-/-}$ mice had reduced levels of VEGF in skin lysates compared with *K14-HPV16* mice, as well as decreased markers of angiogenesis.**

The lack of typical pre-malignant inflammatory characteristics in *K14-HPV16/Rag1*$^{-/-}$ mice was associated with decreased progression to epithelial carcinoma. Only 6.4% of *K14-HPV16/Rag1*$^{-/-}$ mice developed full-blown carcinomas compared with 47% of *K14-HPV16* mice. Therefore, the lack of T and B cells of the adaptive immune system inhibits pre-malignant inflammation and tumour progression in this model.

To examine the specific role of B cells in this process, the authors looked at antibody deposition. B cells do not infiltrate pre-malignant skin, but they might exert their effects through the systemic production of antibodies specific for antigens in the skin. Deposits of IgG and IgM could be detected in the skin of *K14-HPV16* mice by 1 month of age, and these increased up to 6 months of age in association with the development of chronic inflammation. The adoptive transfer of B cells from *K14-HPV16* mice, which would contain primed and/or memory B cells of the desired specificity, to *K14-HPV16/Rag1*$^{-/-}$ mice confirmed the important role of B cells by restoring leukocyte infiltration of the skin and other downstream characteristics such as angiogenesis. The transfer of serum from *K14-HPV16* mice had a similar effect, which is in line with the postulated systemic actions of B cells through the production of antibodies and/or soluble mediators such as cytokines.

The authors conclude that B cells are a crucial part of tumour progression in this model through the promotion of chronic inflammation in the pre-malignant state. Therefore, therapies that aim to stimulate B-cell responses, such as vaccination, should be used with caution in cancer-prone patients or patients with pre-malignant disease.

Kirsty Minton

References and links

ORIGINAL RESEARCH PAPER de Visser, K. E., Korets, L. V. & Coussens, L. M. *De novo* carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* **7**, 411–423 (2005)