Autoimmune diabetes

Retrovirus as trigger, precipitator or marker?

Christophe Benoist and Diane Mathis

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease that is characterized by the specific destruction of insulin-producing β -cells in the pancreatic islets of Langerhans^{1,2}. Despite decades of intensive investigation, the trigger for this self-attack has remained a mystery. But in the 25 July issue of *Cell*, Conrad *et al.*³ describe new findings which suggest that a virus may be responsible.

It has long been thought that human IDDM may be triggered by an infectious agent^{1,2}. The main evidence is that the concordance rate for monozygotic twins is only about 50 per cent; that incidence varies along a north-south gradient; and that the frequency of diabetes is increasing at a striking rate in some countries. But there are alternative explanations for these epidemiological phenomena — in fact, most of the variations within and between countries (for example, the higher incidence in more northern European countries) fit better with the idea that infections nonspecifically protect from,

rather than incite, disease. This idea would also be more consistent with observations on the non-obese diabetic mouse (the most popular animal model of autoimmune diabetes), which has the highest incidence of disease when it is housed under germ-free conditions. So, because there has never been direct evidence that an infectious agent triggers human IDDM, support for the hypothesis of an infectious trigger dwindled.

In 1994, Conrad *et al.*⁴ reported intriguing results that rekindled the debate on the involvement of a virus or bacterium in IDDM. They analysed the repertoire of T lymphocytes that had invaded the pancreatic islets of two recently deceased, acutely diabetic patients. According to information from animal models^{1,2}, T cells are the primary mediators of IDDM. The authors found that T cells whose antigen receptors (T-cell receptors; TCRs) carried the V β 7 variable region were strikingly enriched in these patients. This over-representation was peculiar to the infiltrating cells, because it

was not observed with T cells in the blood of the same patients.

Such an enrichment for a particular V β region evokes the behaviour of superantigens. These proteins, produced by certain viruses and bacteria, can stimulate an inordinately large number of T cells. They do this by binding directly to a subset of V β regions, rather than by interacting in the usual, much more restricted, fashion with a TCR α/β -chain combinatorial site. On the basis of these results, Conrad and colleagues proposed that viral or bacterial superantigens are the trigger for IDDM. But the significance of these findings was questioned on several counts, most notably because only two, rather atypical, patients were involved in the study. Moreover, the predicted superantigen — and whatever produced it — needed to be identified⁵.

In their new paper, Conrad *et al.*³ describe the isolation of a hitherto unknown endogenous retroviral genome from a patient with diabetes, and the identification of a fragment of one of its proteins as a superantigen. Given the lack of direct evidence for the action of an exogenous infectious agent as the trigger for human IDDM, the authors surmised that an endogenous retrovirus might be involved. Vertically transmitted retrovirus genomes (both complete and partial) are found in abundance in the genomes of vertebrates, and some of them encode potent superantigens. Moreover, the expression of particular endogenous retrovirus transcripts specifically in pancreatic-islet β -cells, has been correlated with disease in non-obese diabetic mice^{6,7}.

To assay for a culprit retrovirus, Conrad *et al.* measured virus-derived reverse-transcriptase activity in supernatants from cultures of freshly isolated pancreatic islets. They found activity in the samples from the two patients mentioned above, but not in those from non-diabetic people. The reverse transcriptase seemed to derive from leukocytes (such as B cells, T cells, macrophages and dendritic cells) rather than from β -cells, because supernatants from cultures of splenocytes had even more activity. The authors then used a sophisticated polymerase-chain-reaction strategy to isolate a complete retroviral genome from the islet supernatant of one of the diabetics. Transcripts derived from (or at least related to) this genome were found in blood from 10 diabetic patients, but not from non-diabetic controls. The retrovirus had never previously been identified, but it shared homology with mouse mammary tumour viruses, which produce superantigens. So they searched for — and found — a superantigen encoded by the newly identified viral genome, located within the gene for the envelope protein. Suggestively, this superantigen stimulated T cells that displayed TCRs with V β 7, but not those that presented other variable regions. ►

Cryobiology

Cold comfort for anglers and toxicologists

The ragworm *Nereis virens* is an object of some desire, for not only is it widely favoured as bait by anglers but it is also used in toxicity assays. Demand for *Nereis* outstrips supplies from natural resources, however, not least because breeding is highly seasonal.

Hence the interest in cryopreservation of ragworm larvae and in the specimen pictured here, which has been down to -196°C (the temperature of liquid nitrogen) and back again. The larva emerged from the process alive, well and ready for rearing to adulthood to do its commercial duty. The feat is reported by P. J. W. Olive and W. B. Wang of the University of Newcastle upon Tyne (*Cryobiology* 34, 284–294; 1997), who describe the painstaking permutation of experimental protocols and age of larvae necessary to achieve high rates of successful cold preservation and recovery of larvae. They are able, they say, to preserve over a million larvae from a single artificial fertilization of *Nereis*.

The technique is subject to a patent application by Seabait Ltd. It hinges on the rate of the initial cooling of the larvae to around -35°C before they are plunged into liquid nitrogen: too fast, and ice formation

will occur inside the larvae and kill them; too slow, and the cryoprotectant employed (in this case dimethyl sulphoxide) will poison them.

Cryopreservation methods are well established for gametes and tissues for transplantation, and for some years have been applied to the early embryonic stages of certain insects and mammals. But Olive and Wang believe that, apart from organisms that are naturally cold-resistant, the young *Nereis* they use are the most developmentally advanced multicellular creatures to have been successfully subjected to the process. The larvae have functional muscle, nervous and digestive systems, and eyes and other sense organs, and they can crawl or swim. **Tim Lincoln**

Conrad *et al.* propose a model to explain how this endogenous retrovirus provokes diabetes (Fig. 1a). For some reason, the retroviral genome starts to be transcribed in leukocytes, giving rise to superantigen. The synthesis of superantigen in 'professional' antigen-presenting cells that express major histocompatibility complex class II molecules provokes a broad, systemic response by most of the T lymphocytes that display particular V β s — especially V β 7. By chance, some of the activated T cells can also respond to antigens that are specifically found in pancreatic-islet β -cells. Before their activation by superantigen, these T cells do not attack the β -cells because, being naive, they cannot circulate through tissues.

Figure 1 Conrad *et al.*³ have isolated the complete genome of a hitherto unknown endogenous retrovirus in the supernatant of pancreatic-islet cultures from a diabetic patient. a, The authors believe that the retrovirus is the initial trigger for diabetes. An unknown event (stress, infectious agent, hormonal perturbations) induces activation of the endogenous retrovirus in the leukocytes found in lymphoid organs. The retrovirus-encoded superantigen (vsAg) activates V β 7-positive T cells (7), which can then travel to non-lymphoid organs. By mischance, some of the V β 7-positive T cells are reactive to pancreatic antigens, which home to the pancreas and destroy the islets. b, An alternative explanation is that the same unknown event induces expression of the superantigen (systemically or just locally) which then activates V β 7-positive T cells. The cytokines produced by these T cells directly destroy pancreatic β -cells and/or upset the immunoregulatory balance, starting a local chain-reaction. c, Another possibility is that glucose intake, together with defective pancreas function, provokes hyperglycaemia, which in turn induces production of endogenous retrovirus transcripts in haematopoietic cells.

But, on activation, they rapidly invade the islets and destroy the β -cells.

Although this is certainly an attractive hypothesis, some of the predictions that could have been made do not seem to hold. For example, one might have expected a systemic expansion of V β 7-positive T cells and, perhaps, some restriction in the repertoire of V β 7-positive cells in the islets of recently diagnosed diabetics. But neither was observed in the two patients studied⁴, and alternative models need to be considered.

First, perhaps the retrovirus-encoded superantigen is not the initial trigger but rather a downstream precipitator. A completely unrelated event could stimulate the T cells to invade the pancreatic islets (Fig. 1b). The resulting inflammation, known as insulinitis, would remain innocuous until provoked to destruction by any of several possible secondary events, including a systemic or local response to a superantigen. Studies of non-obese diabetic mice have already shown that the progression from insulinitis to diabetes is neither automatic nor immediate, but that it is highly regulated⁸. Second, expression of the retroviral genome and the superantigen that it encodes may be a marker of, rather than the trigger for, diabetes (Fig. 1c). In support of this theory, endogenous retrovirus transcripts can be induced by the activation of leukocyte subsets under diverse conditions⁹, and even by the elevated glucose

levels found in diabetic mice¹⁰.

By identifying an endogenous retrovirus-encoded superantigen in man, Conrad *et al.* have made an exciting advance. We now need to define its precise relationship to diabetes, and to know whether it is generally linked to this disorder or whether it is found only in particular variants, such as patients with the more acute forms. It will also be interesting to see whether endogenous retroviral superantigens operate similarly in other autoimmune diseases. There is already evidence that an endogenous retrovirus is found specifically in patients with multiple sclerosis¹¹ but, again, it is critical to establish whether the retrovirus is the initial trigger for, a downstream precipitator of, or just a marker for, this disease. □

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1. Bach, J. F. *Endocr. Revs* 15, 516–542 (1994).
2. Tisch, R. & McDevitt, H. *Cell* 85, 291–297 (1996).
3. Conrad, B. *et al. Cell* 90, 303–313 (1997).
4. Conrad, B. *et al. Nature* 371, 351–355 (1994).
5. MacDonald, H. R. & Acha-Orbea, H. *Nature* 371, 283–284 (1994).
6. Gaskins, H. R., Prochazka, M., Hamaguchi, K., Serreze, D. V. & Leiter, E. H. *J. Clin. Invest.* 90, 2220–2227 (1992).
7. Suenaga, K. & Yoon, J.-W. *Diabetes* 37, 1722–1726 (1988).
8. André, L. *et al. Proc. Natl Acad. Sci. USA* 93, 2260–2263 (1996).
9. Moroni, C. & Schumann, G. *Nature* 254, 60–61 (1997).
10. Leiter, E. H., Fewell, J. W. & Kuff, E. L. *J. Exp. Med.* 163, 87–100 (1986).
11. Perron, H. *et al. Proc. Natl Acad. Sci. USA* 94, 7583–7588 (1997).

Protein–protein interactions

Calcium turns turquoise into gold

Tullio Pozzan

Over the past few years, targeting of appropriately engineered and differently coloured green fluorescent protein (GFP) mutants has become popular for investigating many biological processes. These range from gene expression¹ to protein sorting², and from organelle shape and mobility^{3,4} to diffusion of proteins within membranes or the lumens of organelles^{4,5}. But it has been difficult to make GFP sensitive to its local chemical environment — for example, to fluctuating concentrations of important ions and messengers such as Ca²⁺, H⁺ and cyclic AMP.

Advances in the molecular engineering of a GFP-based Ca²⁺ indicator are now reported by Anthony Persechini's group in the *Journal of Biological Chemistry*⁶ and *Cell Calcium*⁷, and by Miyawaki *et al.* on page 882 of this issue⁸. Taking advantage of fluorescence resonance energy transfer (FRET) between differently coloured GFP mutants, new, molecularly engineered Ca²⁺ indicators have been generated. These proteins can not only be targeted to different cellular organelles —

and so solve classic problems in Ca²⁺ signalling — but they also represent prototypes of new probes to investigate protein–protein interactions in living cells.

The unique ability of GFP to illuminate the behaviour of proteins in living cells was first shown by Chalfie and colleagues¹. Using an approach pioneered by Heim and Tsien⁹, GFP fusion proteins — dubbed 'cameleons' by Miyawaki *et al.*⁸ — have now been generated (Fig. 1). The two groups^{6–8} have linked blue-shifted mutants of GFP (emitting blue or turquoise light) to longer-wavelength GFP mutants (emitting green or yellow light), through two types of connecting sequence. FRET takes place between the two GFP mutants if they are appropriately positioned. A non-destructive, spectroscopic phenomenon, FRET is highly sensitive to the distance and orientation of fluorophores. The excitation of the donor results in nonradiative excitation of the acceptor, which eventually emits its characteristic fluorescence. In the cameleons⁸, when FRET is maximal, yellow (gold) light is emitted.