*For debate***What future for therapeutic prevention of Type 1 (insulin-dependent) diabetes mellitus?****P. Pozzilli, A. Signore and D. Andreani**

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A number of promising therapeutic approaches aimed at the prevention of Type 1 (insulin-dependent) diabetes mellitus are briefly discussed here with particular reference to the various phases of Beta cell destruction in the natural history of the disease. These preventive approaches cover a wide range of strategies, including the maintenance of immunological anergy, peptide competition for Beta-cell antigen presentation, vaccination using specific antigens or T-cell clones, the use of immunotoxins to T-cell subsets and the possibility of inducing Beta-cell regeneration.

The debate concerning the relative merits of therapeutic prevention of Type 1 diabetes is now timely given the recent identification of new markers which could provide extensive and accurate information for prediction [1–3]. Progress in delineating the natural history of the disease during its pre-diabetic phase now permits the development of what could be defined as a stage-related immunotherapeutic approach [4] based on peripheral and local changes occurring during the pre-diabetic period (Fig. 1). Clinical trials in pre-diabetic subjects have already commenced and the International Diabetes Immunotherapy Group (IDIG) has recently published a list of ongoing studies [5].

What type of intervention?

The choice of strategy for intervention is very much dependent on the corresponding phase of the disease process before clinical onset. For instance, the maintenance of an anergy state to specific Beta-cell antigens in those genetically susceptible subjects in whom the absence of aspartic acid in position 57 of the DQ beta gene and presence of arginine in position 52 of the DQ alpha gene can be identified [1], before the autoimmune process has taken place, is a very interesting development (Stage 1) (Fig. 1). During a later phase, when clonal activation and expansion of specific T-cell clones have occurred (Stage 2) (Fig. 1), therapy with a synthetically made peptide competing for the Beta-cell antigen may be more appropriate. During a subsequent stage corresponding to Beta-cell destruction by autoreactive T-lymphocyte clones

which recognise the specific antigen (Stage 3) (Fig. 1), injection of autologous T cells capable of suppressing the autoreactive T-lymphocyte clones can be envisaged. Administration of an immunotoxin or an immunosuppressive agent to T-lymphocyte subsets involved in Beta-cell killing may be considered during the final phase of the pre-diabetic process (Stage 4) (Fig. 1). Finally, a combination of immunosuppressive therapy and agents capable of inducing Beta-cell regeneration may be adopted at the time of clinical diagnosis (Stage 5) (Fig. 1). The principal features of each of these therapeutic approaches are briefly discussed below, and readers are encouraged to consider the research findings and their implications for clinical practice.

Is the maintenance of immunological anergy applicable in Type 1 diabetes?

This is one of the most fascinating new areas in the therapy of autoimmune diseases in general, with possibly a specific application to Type 1 diabetes. Maintenance of a state of immunological anergy to Beta-cell antigen(s) may be possible only at a very early stage (i.e., at birth or within the first 2 years of life). Subjects with a genetic predisposition to Type 1 diabetes who possess the non-asp/non-asp haplotype at the DQ beta gene and arginine 52 at the DQ alpha gene [1] are considered at risk of developing the disease, and they are consequently ideal candidates for the condition of "non aggression". It could be argued that tolerance to specific antigen(s) may be induced by using a Beta-cell extract or even intact Beta cells. The specific Beta-cell antigen, once ultimately identified (glutamic acid decarboxylase GAD?) [2], could be injected into the thymus. Animal models indicate that it is possible to prevent rejection of transplanted islet cells in diabetic rats, suggesting that generation of tolerance to specific Beta-cell antigens can be achieved [6]. Furthermore, animal experiments using monoclonal antibody (MoAb) therapy suggest that tolerance may be induced by injecting anti-CD4 MoAb; their administration is followed by a state of immune unresponsiveness to autoantigens and also to heterologous proteins including the CD4 MoAb itself.

Fig. 1. The natural history of "pre-diabetes". Stages 1 to 5 indicate different phases of the natural history of the disease which can be identified on the basis of genetic, histological, immunological and metabolic parameters. Therapeutic intervention varies according to the phase of the disease. The shaded area in the upper part of the figure highlights the time when immunotherapy trials have been performed

Is there a role for peptide competition for antigen presentation?

The use of an artificially constructed peptide nearly equal to the putative antigen has been used in the preventive therapy of autoimmunity [7]. The capacity for blocking the autoimmune process in vivo by injecting a competitor peptide has been shown to be effective in allergic encephalomyelitis [8, 9].

The principal feature of peptide competition is the use of a peptide which shares similarities with the antigen, or is a part of it; usually 8 to 10 amino acid peptides are most effective as this number shows the highest affinity for MHC binding. When administered in vivo, the peptide is able to displace the binding of the native antigen. However, it should also be considered whether this effect would disappear if an immunotolerance towards the peptide developed and if, consequently, a non-specific immune response to the peptide was generated. The in vivo blockade of the MHC class II binding sites is a promising approach not only in the prevention but also in the treatment of some autoimmune diseases with a chronic pre-clinical phase, as may be the case for Type 1 diabetes. The rationale behind this approach is that most peptides bind to MHC molecules of the shared structural motives present at the level of the primary amino acid sequence. The characterization of these motives is quite wide-ranging as a large number of amino acids at any given position in the peptide are normally compatible with binding to MHC molecules. One

consequence of this is that peptides with different sequences may compete for presentation by the same MHC molecule to T lymphocytes and this phenomenon has been demonstrated in vitro and in vivo [10]. The high efficiency of peptide competitors in inhibiting antigen presentation in vivo raises the question of how all MHC class II binding sites might be blocked using a low concentration of competitor peptide. In animal models, in vivo competition for presentation by MHC class II molecules does not require a massive amount of competitor when the mouse lysozyme is employed [11]. Attempts of this kind in experimental Type 1 diabetes have not been reported so far, but the rationale is sound and a peptide competing for GAD antigen might be, for instance, synthesised to block antigen presentation and the autoimmune response to Beta cells.

T-cell vaccination, a promising approach?

Recent data in autoimmunity have indicated that sub-pathogenic doses of T cells specific for the autoantigen may improve the clinical state, possibly through the formation of anti-clonal typical T cells which have the capacity to inhibit the patient's autoreactive T cells [12]. The most significant problem related to the use of T cell vaccination is the need for HLA compatible antigen-specific T cells to prepare vaccines. In Type 1 diabetes it is practically impossible to obtain specific T cells from the pancreas due to the fact that only lymphocytes which infiltrate the islets of Langerhans should be collected because of the very low frequency of Beta-cell antigen-specific T cells in circulating blood. However, it has been shown that non-specific stimulation of peripheral lymphocytes in vitro with interleukin-2 (IL-2) and Concanavalin-A for 48 h may induce the growth and expansion of antigen-specific T cells [13]. It should also be noted that autoimmune CD4+ cells generated in vitro will not vaccinate when injected in the donor unless they are activated in the presence of the immunogenic cell antigen; therefore, the putative antigen must be incubated with expanded clones of peripheral lymphocytes before introduction in the donor. Resistance to the disease after vaccination with CD4+ is accompanied by the appearance of clone-specific anti-idiotypic T cells of CD4+ and CD8+ phenotype. By this process, the CD8+ anticlonal typical T cells may block the specific autoimmune effector T cells, and this may well be one of the mechanisms involved in inducing resistance. Activated T cells (antigen specific) may indeed migrate from peripheral blood to the thymus, thus carrying information for directing positive and negative selection of CD4+ and CD8+ clones [14]. Results from autologous lymphocytes obtained from the spinal fluid of patients with multiple sclerosis are encouraging [15], and the application of this technique is now envisaged in animal models of Type 1 diabetes using lymphocytes obtained from both the pancreas and the periphery.

Immunotoxin to T cells, a possibility worth trying at diagnosis?

Immunotoxins, known as conjugates of a toxin by an enzymatic method to MoAbs, are directed towards antigen expressed on the cell surface and are at present used

mainly in the therapy of tumours. When injected, the immunotoxin reaches its target and the toxin is internalised in order to facilitate its toxic effect [16]. The translocation inside the target cell is triggered by the acidification of the endosomes due to the action of ATP-driven protein pumps in the vesical membrane [17].

The use of MoAb anti-T lymphocytes (CD5) conjugated with ricin in early phases of Type 1 diabetes has been proposed by Skyler et al. [18] in a pilot trial. A serious limitation to the use of immunotoxins is that foreign proteins induce the formation of neutralizing antibodies, thus precluding prolonged and repeated treatment. This may significantly limit the duration of therapy, but the use of chimeric CD4 MoAbs and a panel of immunologically irrelevant toxins may resolve some of the inherent difficulties. Some IL-2 conjugated toxins have been also developed (such as diphtheria toxin conjugated IL-2, DAB 486 IL-2) which kill only activated lymphocytes, thereby avoiding the use of an MoAb as a carrier [19]. Anti T-cell immunotoxin has already been introduced successfully in the treatment of another autoimmune disease (myasthenia gravis) with an anti-idiotypic antibody to the acetylcholin receptor to eradicate B lymphocytes [20]. It is most likely that in the future drug targeting will play an increasingly important role in the study of Type 1 diabetes, at least in the final phase of Beta-cell destruction.

Is Beta-cell regeneration still possible even when destruction is nearly complete?

Recent data have shown the presence of a novel gene (reg gene) in Beta-cell regeneration in vitro [21]. The reg gene codifies for a 165 amino-acid protein which requires 15 to 30 days for replication when cultured under optimal conditions. Once isolated, islets were exposed to nutrient and non-nutrient growth factors and the reg gene expression directly correlated with an increased rate of islet-cell replication. This was parallel to a rise in specific messenger RNA expression compared with islets not incubated with stimuli. Although the product of this gene has not yet been studied, data from research of this kind may have important implications for intervention in the final process of Beta-cell destruction. During the cytotoxic process, the capacity of Beta-cells to regenerate may be limited by the persistence of the autoimmune process. Therefore, therapy in the early stages of overt Type 1 diabetes, when some Beta-cell mass is still present, could involve suppressing the immune response by immunosuppressive drugs, while at the same time facilitating Beta-cell replication (Stage 5) (Fig 1).

Conclusion

Stage-related immunotherapy may well represent the most promising development in the prevention of Type 1 diabetes. The vast contribution to the body of knowledge in this field in the last few years has introduced a wide range of therapeutic possibilities, and the outcome of this research has now begun to transcend speculation and offer increasingly clinically valuable strategies aimed at intervention in each phase of the pathogenic cascade of the disease.

References

1. Khalil I, D'Auriol L, Gobet M et al. (1990) A combination of HLA-DQ beta Asp 57 – negative and HLA-DQ alpha Arg 52 confers susceptibility to insulin dependent diabetes mellitus. *J Clin Invest* 85: 1315–1319
2. Baekkeskov S, Aanstoot HJ, Christgan S et al. (1990) Identification of the 64K antigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 347: 151–156
3. Signore A, Parman A, Pozzilli P, Andreani D, Beverley PCL (1987) Detection of activated lymphocytes in the endocrine pancreas of BB/W rats by injection of ¹²⁵I-interleukin-2: an early sign of type 1 diabetes. *Lancet* II: 537–541
4. Andreani D, Di Mario U, Pozzilli P (1991) Prediction, prevention and early intervention in insulin dependent diabetes. *Diab Metab Rev* 7: 61–77
5. International Diabetes Immunotherapy Group (IDIG) (1990) Newsletter, "Diabetes prevention and therapy", Vol 4. John Wiley Chichester
6. Poselt AM, Barker CF, Tomaszewski JE, Markmann JF, Choti MA, Naji A (1990) Induction of donor-specific unresponsiveness by extrathymic islet transplantation. *Science* 249: 1293–1295
7. Adorini L, Nagy ZA (1990) Peptide competition for antigen presentation. *Immunology Today* 11: 21–24
8. Adorini L, Muller S, Cardinaux F, Lehmann PV, Falcioni F, Nagy ZA (1988) In vivo competition between self peptides and foreign antigens in T-cell activation. *Nature* 334: 623–625
9. Adorini L, Appella E, Nagy ZA (1989) The immune response to structurally defined proteins. In: Smith-Gill S, Sercarz (eds) *The lysozyme model*. Adenine Press pp 257–266
10. Adorini L, Appella E, Doria G, Nagy ZA (1988) Mechanisms influencing the immunodominance of T cell determinants. *J Exp Med* 168: 2091–2104
11. Wraith DC, McDevitt HO, Steinman L, AchaOrbea H (1989) T cell recognition as the target for immune intervention in autoimmune disease. *Cell* 57: 709–715
12. Cohen IR, Weiner HL (1988) T cell vaccination. *Immunology Today* 9: 332–335
13. De Berardinis P, Londei M, James RF, Lake SP, Wise PH, Feldman M (1988) Do CD4-positive cytotoxic T cells damage islet beta cells in type 1 diabetes? *Lancet* II: 823–824
14. Angus DB, Surt CD, Sprent J (1991) Reentry of T cells to the adult thymus is restricted to activated T cells. *J Exp Med* 173: 1038–1046
15. Sun D, Quin Y, Chluba J, Epplen JT, Wekerle H (1988) Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-cell interaction. *Nature* 332: 843–845
16. Olsnes S, Sandvig K, Peterson OW, Deurs B (1989) Immunotoxins-entry into cells and mechanisms of action. *Immunology Today* 10: 291–295
17. Olsnes S, Moskaug JO, Stenmark H, Sandvig K (1988) Diphtheria toxin entry: protein translocation in the reverse direction. *Trends Biochem. Sci* 13: 348–351
18. International Diabetes Immunotherapy Group (IDIG) Newsletter (1991) Diabetes prevention and therapy. Vol 5. John Wiley, Chichester, p 8
19. Finberg RW, Wahl SM, Allen JB et al. (1991) Selective elimination of HIV-1 infected cells with an anti interleukin-2 receptor-specific cytotoxin. *Science* 252: 1703–1705
20. Brown RM, Krolick KA (1988) Selective idiotype suppression of an adoptive secondary antiacetylcholine receptor antibody response by immunotoxin treatment before transfer. *J Immunol* 140: 893–898
21. Okamoto H (1991) Islet B-cell regeneration and reg genes. In: HRifkin, JA Colwell, SITaylor (eds) *Diabetes*. Elsevier Science, Amsterdam, pp 268–270

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