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Inhibition of Diabetes in NOD Mice by Idiotypic Induction of SLE

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The present study was undertaken to investigate whether active induction of systemic lupus erythematosus (SLE) in non-obese diabetic (NOD) mice could affect their development of insulin-dependent diabetes mellitus (IDDM). NOD mice were immunized with a human IgM mAb carrying the 16/6 idiotype (MIV-7) or with control human IgM. The mice were bled monthly and tested for SLE-associated autoantibodies in the serum and for the presence of leukopenia, thrombocytopenia, proteinuria and immunoglobulin deposits in the kidneys. The development of diabetes was determined by a blood glucose level exceeding 15 mM on two consecutive weekly determinations and by the presence of insulitis in the pancreas. The NOD mice immunized with MIV-7 developed high and persistent levels of autoantibodies, including anti-DNA, anti-histones and anti-cardiolipin, untreated mice and those immunized with normal human IgM did not produce these autoantibodies. The MIV-7-immunized mice also manifested an elevated erythrocyte sedimentation rate, leukopenia, thrombocytopenia and significant proteinuria, as well as deposits of Ig in their kidney glomeruli. Thus, NOD mice immunized with MIV-7 developed both autoantibodies and clinical features of SLE. The MIV-7-treated mice, however, showed a significantly lower incidence of IDDM (25% *vs.* 90%, $P<0.003$), accompanied by amelioration of the insulitis. The present study indicates that the induction of SLE by idiotypic immunization can protect NOD mice from developing IDDM, pointing to the importance of immune dysregulation in shift from one autoimmune disease to another.

Key words: SLE, NOD, diabetes, idiotypes, autoantibodies

Introduction

Systemic lupus erythematosus (SLE) and insulin-dependent diabetes mellitus (IDDM) are distinct autoimmune disease that manifest very different immunological pathologies. Nevertheless, it has been reported that administration to non-obese diabetic (NOD) mice of a BCG vaccine can arrest the spontaneous development of IDDM, but that the treated mice then may go on to develop an SLE-like disease [1, 2]. It was reported that the SLE-like disease could be prevented by carefully adjusting the dose of BCG [3]. Although the development of SLE in NOD mice seemed to be triggered specifically by BCG, the observation of a possible 'trade-off' between two different autoimmune diseases could be meaningful. Indeed, a shift from one autoimmune disease to another is a phenomenon termed 'kaleidoscope of autoimmunity' [4–6]. Examples include the development of SLE [7], or anti-phospholipid syndrome [6], following thymectomy to treat myasthenia gravis, transition between SLE and Hashimoto's thyroiditis [8] and the development of chronic active hepatitis following splenectomy to treat thrombocytopeic purpura [9]. The present study was undertaken to investigate whether the active induction of SLE by idiotypic immunization of NOD mice could affect their development of IDDM.

We have developed a method to induce SLE in mice of various strains by immunizing them with a human anti-DNA mAb (MIV-7) that bears a pathogenic idiotype called 16/6 [10–14]. According to the theory of Jerne [15, 16], the idiotypic determinant of an autoantibody may induce an anti-idiotypic antibody, leading to an idiotypic network through which immunoglobulin expression might be controlled. We, and others, have shown that upon stimulation with an autoantibody carrying the 16/6 idiotype (Ab1), naïve mice can develop an anti-autoantibody (anti-Id; Ab2), and after several months the immunized mice may also produce an anti-anti-autoantibody (anti-anti-Id; Ab3), that may have similar binding characteristics to Ab1 [13]. Such immunized mice can develop a clinical

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SLE-like disease manifested by the appearance of additional autoantibodies, leukopenia, thrombocytopenia, proteinuria and immune-complex glomerular kidney disease [10–14].

In this paper, we report that 16/6-immunized NOD mice developed SLE, and were protected from developing IDDM.

Materials and Methods

NOD mice

Female mice of the NOD/Lt strain were raised and maintained under veterinary supervision in the Animal Breeding Center of the Weizmann Institute of Science (Rehovot, Israel) from breeders kindly supplied by Dr E. Leiter of Jackson Laboratories. These mice manifest insulitis beginning at about 1 month of age which progresses to overt hyperglycemia beginning at about 3.5 months of age. The cumulative incidence of IDDM rises to 80–95% by 6–7 months of age.

Immunizations

The mice were immunized at 8 weeks of age with 10 μg of human IgM mAb (MIV-7) [10] or control human IgM in MPL +TDM adjuvant (RIBI; Immunochem Research, Hamilton, MT, USA) administered subcutaneously into the hind foot pads. The RIBI adjuvant was used in these studies in place of the complete Freund's adjuvant (CFA) used in other SLE studies, because preliminary investigation showed that RIBI, unlike CFA, does not by itself inhibit the development of IDDM in NOD mice [17]. Three weeks after the primary immunization, the mice were boosted in the foot pads with 10 μg of MIV-7 or control IgM in PBS. A control group of female NOD mice was not immunized. Each experimental group contained 12 mice.

Antibody assays

Mice were bled monthly and tested for the presence of serum antibodies. Antibodies to calf thymus ssDNA and dsDNA (Sigma, St Louis, MO, USA) and antibodies to total histones were determined by an enzyme linked immunosorbent assay (ELISA) as previously described [10]. Anti-cardiolipin and anti-phosphatidylserine antibodies were detected by ELISA using 96-well ELISA plates (Nunc, Kamstrup, Roskilde, Denmark), coated with either cardiolipin or phosphatidylserine (Sigma) 50 μg/ml in ethanol. Following blocking with 5% bovine serum (BS), mouse sera (1:200 in TBS +2%BS) were added and incubated for 2 h at room temperature. Bound mice antibodies were detected using goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma) and the addition of its substrate P-nitro-phenylphosphate (Sigma). The colour reaction was read using a Titertrek ELISA reader (SLT; Labistruments, Austria) at an OD of 405 nm. Each step was followed by extensive washing with TBS. Antibodies to hsp60 or to peptide p277 were measured using an ELISA assay as described [18].

Blood cell counts

White blood cell and platelet counts from individual blood samples were quantified in diluted blood using a single optical cytometer (Coulter Counter HC Plus Cell Control Counter Electronics Ltd., UK).

Immunoglobulin deposits in kidneys

The mice were killed 6 months after receiving the booster injection of MIV-7 or control IgM. The kidneys were removed and frozen immediately in liquid nitrogen. Frozen cryostat sections (5 μm thick) were dried and fixed in acetone for 5 min and then washed with PBS–Tween 0.05% in BSA 0.5%. For the detection of immunoglobulin deposits, fluorescein isothiocyanate (FITS) conjugated goat anti-mouse immunoglobulin antibodies (Sigma) were applied for 60 min. After extensive washing in PBS–Tween 0.05% in BSA 0.5% the sections were analysed using a fluorescence microscope by an observer blinded from the source of the kidney sections.

The development of diabetes

Diabetes was determined by a blood glucose level exceeding 15 mM on two consecutive weekly determinations, tested using a Beckman II glucose analyser, and by insulitis.

Pancreas histology

Mice were killed at monthly intervals, three mice each time from each treatment group. The pancreases were fixed in Bouin, cut and stained by standard hematoxylin and eosine (H&E). At least 10 islets of each pancreas were scored, as described previously [18].

Results

Induction of experimental SLE

Figure 1 shows the kinetics of anti-dsDNA autoantibody levels in the sera of NOD mice in the months following immunization with MIV-7. It can be seen that NOD mice immunized with MIV-7 developed high and persistent levels of anti-dsDNA following immunization. Figure 2 displays the titers of SLE-associated antibodies 3 months after completion of immunization. It can be seen that the mice immunized with MIV-7 developed high titers of autoantibodies

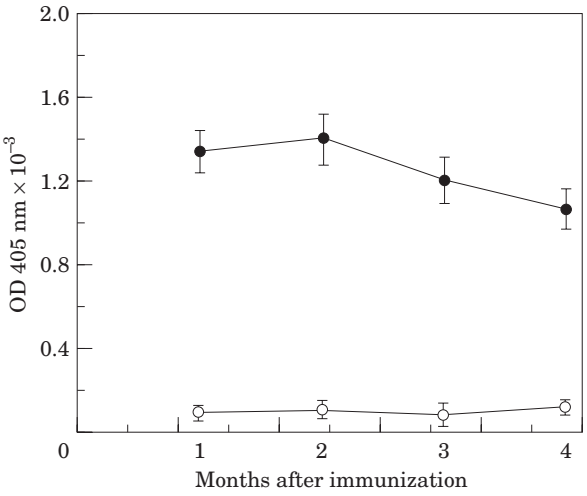

Figure 1. Kinetics of anti-dsDNA autoantibodies in the sera of NOD mice following immunization with anti-DNA (MIV-7) mAb (●) or non-anti-DNA (○).

against dsDNA, ssDNA, cardiolipin and histones. The untreated NOD mice and those immunized with normal human IgM or RIBI did not produce these autoantibodies. The NOD mice immunized with MIV-7 also manifested an elevated erythrocyte sedimentation rate, leukopenia, thrombocytopenia and significant proteinuria (Table 1), as well as deposits of Ig in their kidney glomeruli (Figure 3). Thus, NOD mice immunized with MIV-7 developed both the autoantibodies and the clinical features of mouse SLE.

Inhibition of IDDM

Figure 4 shows the cumulative incidence of IDDM in the mice. It can be seen that the untreated group of mice and the group treated with normal human IgM developed the incidence of IDDM expected for NOD mice: 90% or greater by 7 months of age. The MIV-7-treated mice, however, showed a significantly lower incidence of IDDM (25%; $P<0.003$).

Histological examination of the islets demonstrated that the inhibition of clinical IDDM was accompanied by amelioration of the insulitis (Figure 5). The islets of untreated or control IgM-treated NOD mice showed an intraislet infiltrate in most of the islets, and no islets were insulitis-free. In contrast, the MIV-7-treated mice showed significantly less insulitis: in the normoglycaemic mice, approximately 50% of the islets were free of any insulitis, and most of the remaining islets exhibited only mild peri-islet infiltrates ($P<0.0001$). Also, even those mice that did became hyperglyoaemic despite MIV-7 treatment, had a marked reduction in intra-islet insulitis ($P<0.001$). Thus, immunization with MIV-7, which induced SLE, led to decreased destructive insulitis and a lower incidence of IDDM.

Antibodies to hsp60

One of our laboratories has found that the autoimmune destruction of β-cells and subsequent IDDM can be arrested by the administration to NOD mice of a peptide, p277, comprising residues 437–460 of the 60 kDa heat shock protein [18, 19]. Successful treatment of IDDM with peptide p277 was associated with the production of antibodies to p277 [20]. To see if the inhibitory effect of MIV-7 immunization might be associated with the induction of such antibodies, we tested the sera of the NOD mice for antibodies to hsp60 and to p277. Figure 6A shows that the untreated NOD mice and the mice treated with normal human IgM started to develop antibodies to hsp60 only 3–4 months following immunization, late in the development of IDDM, and for the most part when

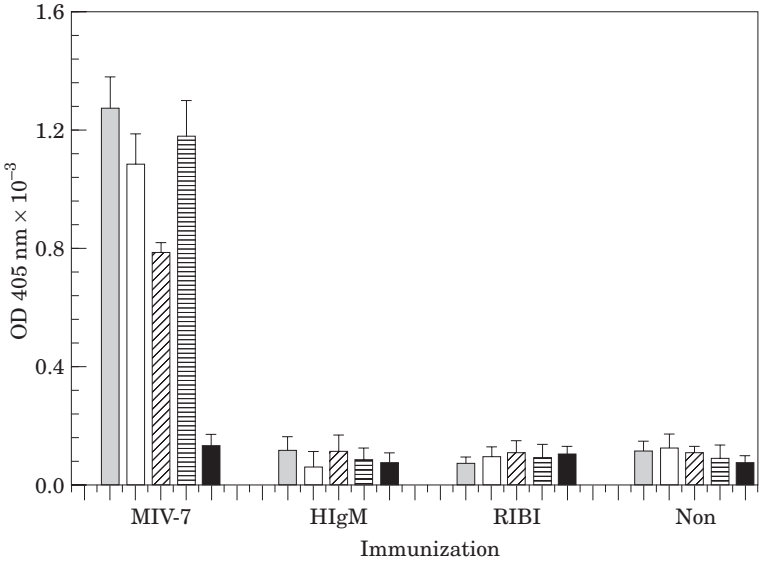

Figure 2. SLE-associated autoantibodies in the sera of NOD mice 3 months after immunization with MIV-7 mAb. Results show antibodies to ssDNA (▭), dsDNA (□), histones (▨) cardiolipin (▤) and PDH (■).

Table 1. *SLE findings in NOD mice 5 months after immunization with anti-DNA (MIV-7) antibodies (mean±SEM)*

	Immunization with		
	MIV-7	HIgM	None
ESR (mm/6 h)	13±3*	2±1	1±1
WBC (cells $\times 10^3$ mm^3)	2705±989*	7065±2004	5971±1428
Platelet count ($\times 10^3$ mm^3	840±77.4*	1.084±76.7	1.263±65.3
Proteinuria (mg/dl)	>500	<100	<100

ESR Erythrocyte sedimentation rate; WBC, white blood cell count.
*P<0.05–0.005 by ANOVA test.

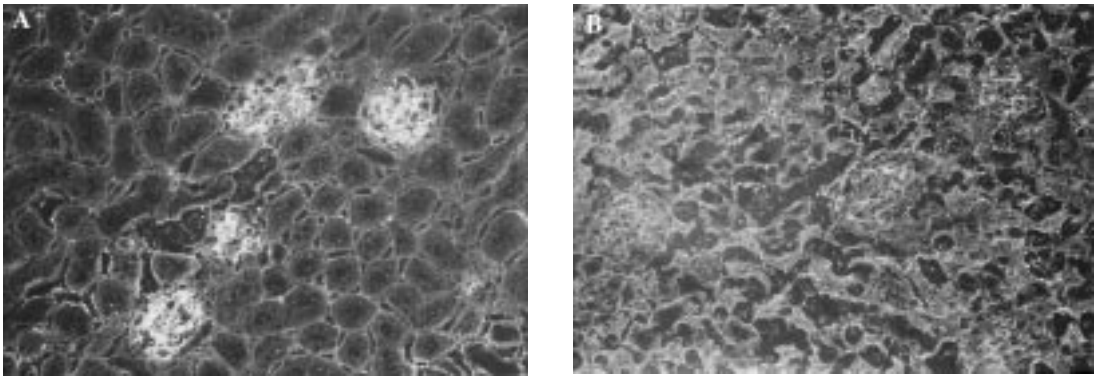

Figure 3. Immunofluorescent staining of kidney sections (×200) from NOD mice immunized with MIV-7 (A) or HIgM (B). Kidney sections from mice immunized with MIV-7 demonstrate granular IgG deposits in the glomeruli, while the control mice immunized with HIgM do not show IgG deposits. Frozen sections (5 μm) were stained with FITC-labelled anti-mouse IgG.

the mice were already suffering from IDDM (6 months after treatment). There were little or no anti-hsp60 antibodies detectable in these mice in the first 3 months of age. In contrast, mice immunized with MIV-7, which were protected from IDDM, manifested anti-hsp60 antibodies within 1 month after immunization (at 3 months of age), and these antibodies persisted.

Figure 6B shows that immunization with MIV-7 was also associated with low, but significantly elevated titers of antibodies to the p277 peptide, compared to the untreated or normal IgM-treated mice. Thus, the protection from IDDM afforded by the development of SLE was associated with the appearance of antibodies to hsp60 and to p277.

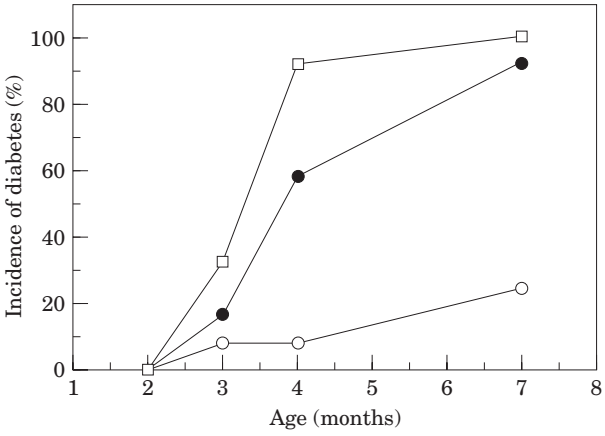

Figure 4. Prevention of NOD diabetes by MIV-7 immunization. Female NOD/Lt mice were allocated to two groups of 12 mice each, and were immunized with either MIV-7 (○) or control human IgM (●). The third group was left untreated (□). Mice were tested monthly for the development of diabetes, determined by blood glucose concentrations greater than 15 mM. The MIV-7-immunized group presented significantly lower diabetes (P<0.03), by the Wilcoxon non-parametric test.

Discussion

The origin of autoimmune disease, an attack by the immune system against otherwise healthy components of the body, is a critical question both medically and theoretically. Classically, it has been thought that autoimmune diseases could only arise by the accidental escape from self-tolerance of 'forbidden' clones of lymphocytes [21], thus, except for chance, there should be little immunological similarity between spontaneous autoimmune disease in different individuals [22]. Classical teaching notwithstanding, the immunological expressions of the major autoimmune diseases seem not to be haphazard. For example, both SLE and IDDM appear to involve autoimmunity to a predictable collective of

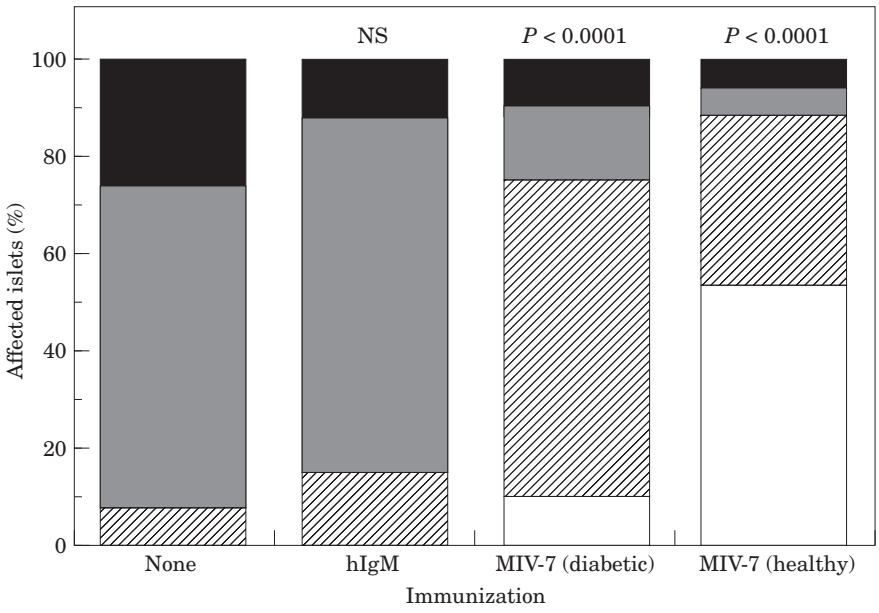

Figure 5. Prevention of destructive insulinitis by MIV-7 immunization. NOD/Lt female mice were left untreated or were immunized with either MIV-7 or control human IgM. After 6 months of follow-up, the mice were killed and the degree of insulinitis determined. At least 10 islets were scored from each pancreas. In the MIV-7 treated group, the majority of the mice were free from diabetes, but the three mice that developed hyperglycemia are shown as a separate subgroup. The affected islets are depicted as clear (open bars), peri-islet infiltrate (hatched bars), intra-islet infiltrate (grey bars) or atrophic islets (solid bars). The statistical difference between the groups was analysed using the Wilcoxon non-parametric test.

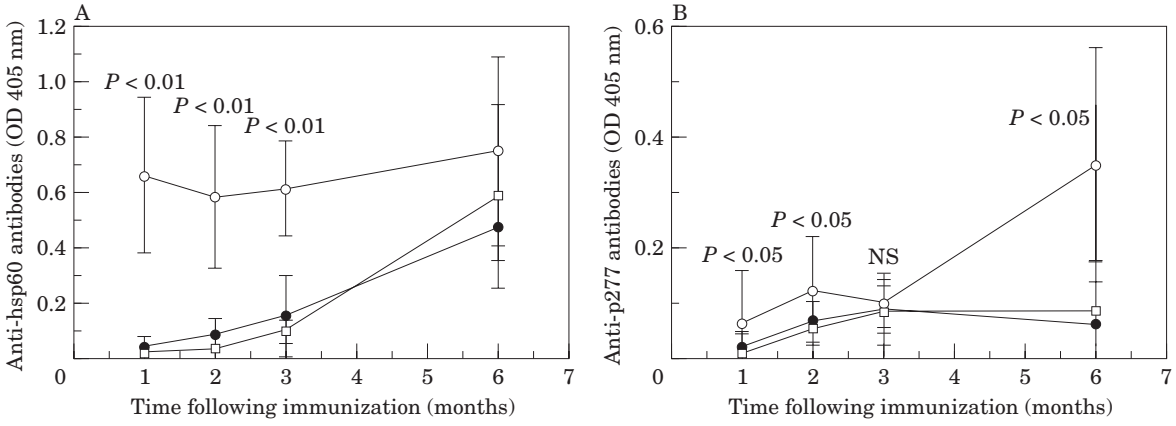

Figure 6. Induction of hsp60 and p277 specific antibodies by MIV-7 immunization. Groups of 12 NOD mice were tested for serum antibodies to hsp60 (upper panel, A) and p277 (lower panel, B), at various times after treatment. The mice were treated with MIV-7 (○), or control human IgM (●), or were untreated (□). Individual sera were tested at a 1:50 dilution, and the results are depicted as the mean±SD. Statistical differences between the groups were analysed by the Wilcoxon non-parametric test.

different antigens [23–25]. Moreover, the antigens in this collective may be functionally interrelated; down regulation or manipulation of the autoimmune T cell response to one of the antigens in IDDM, for example, can affect the nature of the autoimmune response to other target antigens in the collective [20, 26, 27]. The finding that the varied manifestations of SLE can be induced in mice by immunization with an anti-DNA antibody bearing a specific idiotype, 16/6, also suggests that the development of SLE is not the result of a random mutation in some T or B cell antigen receptor. The unlashing of the full SLE syndrome by anti-idiotypic immunization to the 16/6 idiotype is compatible with the notion that SLE is encoded in some way within the organization of the susceptible immune system [28]. Indeed, the uniformity of the immunology of SLE observable between individuals, and even between species, also supports the conclusion that the potential to develop SLE is intrinsic rather then random. The induction of SLE by immunization with the 16/6 idiotype raises the possibility that idiotypic connections take part in the pathogenesis of the disease [29].

The switch from an organ-specific autoimmune disease (IDDM), to a systemic autoimmune disease (SLE) demonstrated in this study, is an example of the

'kaleidoscope phenomenon', pointing to the importance of immune dysregulation in shifting from one autoimmune disease to another [4–6]. The immunomodulation, leading to a shift of autoimmunity, may be achieved by a variety of means including thymectomy [6, 7], splenectomy [9], or idiotypic immunization [13], as in our study. A similar turn from IDDM to an SLE-like disease was achieved by Horsfall *et al.* [2], using intravenous administration of mycobacteria to NOD/Lt mice, which prevented the onset of IDDM while precipitating the appearance of anti-dsDNA and anti-Sm autoantibodies accompanied by hemolytic anaemia and glomerular immune complex deposition [1, 2]. Mycobacteria might serve as a potent immunomodulator, owing to their antigenic similarity (molecular mimicry) with tissue antigens at both humoral and cellular levels [30]. Indeed, we have previously found that the 16/6 Id is extremely common in patients with untreated tuberculosis [31], and that monoclonal anti-micobacterium tuberculosis antibodies reacted with DNA, with some of them carrying the 16/6 Id [32].

The present study indicates that NOD mice are among the strains susceptible to induction of SLE by pathogenic idiotypic immunization. Although this study does not elucidate the mechanisms leading to SLE, the results demonstrate that inducing SLE in NOD mice can protect them from IDDM. It may be argued that the process leading to spontaneous IDDM in NOD mice is basically fragile: IDDM has been shown to be inhibited by various specific and 'non-specific' immunizations [33]. Thus, the induction of SLE in the present study may have merely derailed the IDDM process from its course for no more specific reasons than do any other 'strong' immunization. 'Non-specificity', however, would not readily account for the spontaneous appearance of antibodies to hsp60 and to its p277 peptide epitope in the mice with induced SLE. Specific antibodies to p277 have been found to mark the arrest of spontaneous IDDM induced by treatment of NOD mice with peptide p277 itself [18, 20]. Antibodies to peptide p277 are also associated with the treatment by p277 of the IDDM inducible in C57BL/KsJ mice by a very low dose of the toxin streptozocin [34]. Although the antibodies to p277 in the present study were not of high titer, it is intriguing that such antibodies appeared at all, in view of the fact that the mice had not been immunized with peptide p277. It is also worth noting that the mice induced by MIV-7 to develop SLE also showed a modification in their antibodies to intact hsp60. Such antibodies appeared in the control mice only very late in the course of IDDM. T cell autoimmunity to hsp60 is associated with the development of IDDM both in the spontaneous NOD model [20, 27] and in the toxin-induced C57BL/KsJ model [35]. Indeed, the switch from Th1-like effector T cells to antibodies to hsp60 epitopes appears to be involved in arresting IDDM [20]. Thus, the inhibition of IDDM associated with the induction of SLE was associated with the development of antibodies that mark the specific immune modulation of the IDDM process.

The present results support the idea that the immunology of IDDM and SLE, at least in mice, is not accidental, but dependent on specific regulation. This idea favuors treatments aimed, not at inactivation of the autoimmune response, but rather at activation of natural regulation [28, 36].

Acknowledgements

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