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.

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Introduction

Systemic lupus erythematosus (SLE) and insulin-dependent diabetes mellitus (IDDM) are distinct autoimmune diseases 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 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 thy- mectomy 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 develop an anti-anti-autoantibody (anti-anti-Id; Ab3), that may have similar binding characteristics to Ab1 [13]. Such immunized mice can develop a clinical
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 mouse 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; LabiInstruments, 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 (FITC) 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.
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 become hyperglycaemic 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 ß-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...
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.

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

<table>
<thead>
<tr>
<th>Immunization with</th>
<th>MIV-7</th>
<th>HIgM</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/6 h)</td>
<td>13±3*</td>
<td>2±1</td>
<td>1±1</td>
</tr>
<tr>
<td>WBC (cells ×10^3 mm^-3)</td>
<td>2705±989*</td>
<td>7065±2004</td>
<td>5971±1428</td>
</tr>
<tr>
<td>Platelet count (×10^3 mm^-3)</td>
<td>840±77.4*</td>
<td>1.084±76.7</td>
<td>1.263±65.3</td>
</tr>
<tr>
<td>Proteinuria (mg/dl)</td>
<td>&gt;500</td>
<td>&lt;100</td>
<td>&lt;100</td>
</tr>
</tbody>
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ESR Erythrocyte sedimentation rate; WBC, white blood cell count.

*P<0.05–0.005 by ANOVA 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 unleashing 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 than 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
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 favours treatments aimed, not at inactivation of the autoimmune response, but rather at activation of natural regulation [28, 36].

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References

importance of the pathogenic 16/6 idiotype in the induction of SLE in naive mice. Scand. J. Immunol. 31: 45–52


