Rapid Publications

Suppressive Effect of Antibodies to Immune Response Gene Products on the Development of Low-Dose Streptozotocin-induced Diabetes

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SUMMARY

Low-dose streptozotocin-induced diabetes in mice serves as a model of type I diabetes. Suppression of the development of diabetes (hyperglycemia) in C3H/He mice was achieved with in vivo administration of antibody reactive to *Ir*-gene products before streptozotocin treatment. A persistent effect was reached with two monoclonal antibodies directed against *I-A*^{k} gene products and, surprisingly, by an allo-antiserum to *I-J* determinants. These results suggest a role for *I-A* and *I-J* positive T-lymphocytes and/or macrophages in B-islet cell autoimmunity. DIABETES *32*:869–871, September 1983.

Low-dose streptozotocin-induced diabetes serves as a model of type I diabetes.[1] It shares with the human disease (1) delayed onset after partial destruction of B-islet cells by an exogenous factor, (2) lymphocytic infiltration of islets (insulitis), (3) presence of islet cell antibodies (Kolb-Bachofen and Kolb, to be published), and (4) genetic control by genes within and outside the major histocompatibility complex.[1-6] The latter observation suggested that in vivo manipulation of the *Ir* gene function might influence the expression of the disease. Based on the same reasoning McDevitt recently has studied the effect of anti-Ia-antibodies on the development of experimental autoimmune encephalomyelitis. Administration of anti-Ia-antibodies was found to prevent the disease.[7]

In the present communication conventional and monoclonal antibodies against *I*-region gene products have been studied in their effect on the development of low-dose streptozotocin-induced diabetes.

MATERIALS AND METHODS

Male mice of strain C3H/HeHan were obtained from Zentralinstitut für Versuchstiere, Hannover, Federal Republic of Germany; mice of strains C57BL/10ScSn/Ola, B10.BR/Ola were from Olac, Oxon, United Kingdom. Mice received standard diet ("ssniff M," Ssniff, Soest, Federal Republic of Germany) and water ad libitum. At the age of 10–14 wk animals were treated with five doses of streptozotocin (40 mg/kg body wt, Boehringer Mannheim, Federal Republic of Germany) on consecutive days as described previously.[3]

The following anti-Ia-antibodies were used: alloantiserum to *I-J*^{k}, alloantiserum to I-a^k, monoclonal IgG2a anti-*I-A*^{k} (against determinant Ia.2) as Ig fraction from ascites (all from Cedarlane, Hornby, Canada), and monoclonal IgG2b anti-*I-A*^{k} (H 116.32, ascites, against determinant Ia.m6/Ia.19, a gift from Dr. H. Lemke, University of Kiel). Antibodies were injected intravenously 2 h before the first streptozotocin dose. Of alloantisera and monoclonal anti-*I-A*^{k}(Ia.2) 40 μl were given, and 20 μl of monoclonal anti-*I-A*^{k}(Ia.m6). Azathioprine (Wellcome, Beckenham, United Kingdom) was given intraperitoneally (75 mg/kg body wt) 2 h before and daily on days 1–10 after the first streptozotocin injection. Blood of nonfasted animals was collected between 0900 and 1100 h. Glucose was determined by the hexokinase method using an autoanalyzer (Technicon Instruments Corp., Tarrytown, New York).

Statistical significance of differences in blood glucose values between experimental groups were analyzed using Student's *t* test.

RESULTS

Mice of strain C3H develop hyperglycemia about 2 wk after the start of streptozotocin injections. The delayed and progredient rise in blood glucose levels could be substantially inhibited by treatment of mice with the immunosuppressive drug azathioprine (Figure 1A). This demonstrates the autoimmune nature of low-dose streptozotocin-induced diabetes in C3H mice. The effect of anti-Ia-antibodies on diabetes development is shown in Figure 1B. Of the two alloantisera, anti-Ia^k shows a weak ($P < 0.1$) and anti-*I-J*^{k} a

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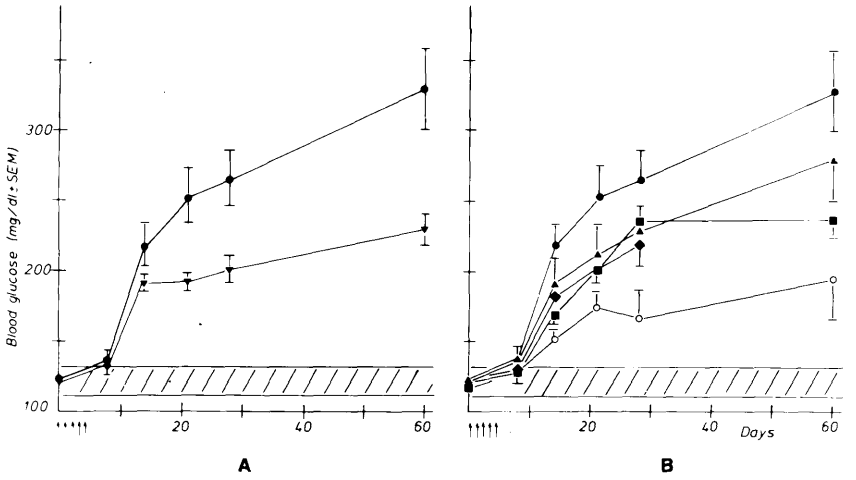

FIGURE 1. Suppressive effect of azathioprine and Ia-antibodies on the development of low-dose streptozotocin-induced diabetes in C3H mice. (A) Streptozotocin-treated mice (——●——) (N = 32); streptozotocin + azathioprine (——▼——) (N = 8). (B) Streptozotocin-treated mice (——●——) (N = 32); streptozotocin + anti-Ia^k-antiserum (——▲——) (N = 14); streptozotocin + anti-*I-A*^{k} (Ia.2) (——■——) (N = 10); streptozotocin + anti-*I-A*^{k} (Ia.m6) (——♦——) (N = 20); streptozotocin + anti-*I-J*-antiserum (——○——) (N = 29). Hatched areas: mean of untreated control mice ± 2 SD.

strong ($P < 0.001$) suppressive effect. Significant inhibition of diabetes development is also seen with two different monoclonal anti-*I-A*^{k}-antibodies ($P < 0.05$).

The strong inhibitory effect of anti-*I-J*^{k}-antibodies was unexpected since these antibodies are thought to enhance rather than suppress an immune response. We therefore tested another mouse strain sharing with C3H the *H-2* haplotype *k*, B10.BR, and found a similar impairment ($P < 0.001$) of diabetes development (Figure 2A). As further control we tested mice from a congenic resistant strain with the *H-2* haplotype *b*, B10. In this strain, treatment with anti-*I-J*^{k}-serum did not significantly affect the development of hyperglycemia (Figure 2B).

DISCUSSION

The experiments described here show that in vivo administration of antibodies reactive with *I*-region gene products at least partially suppress the development of low-dose streptozotocin-induced diabetes. The inhibitory effect is in most cases of the same order as seen with a general immunosuppressive drug, azathioprine.[8] Although anti-Ia-antibodies were only given once at the start of experiments, suppression was still noted 2 mo later.

The possible mechanisms responsible for the suppressive effects are not yet known. *I-A* determinants are present on B-lymphocytes, activated T-lymphocytes, and on some antigen-presenting cells. Furthermore, it has been speculated that islet cells after streptozotocin treatment are induced to express Ia-antigens on the cell surface.[9] Ia-antibodies thus may directly interact with the target organ. A major effect of Ia (including *I-A*) antibodies, however, is a blockade of the process of immune induction by interfering with antigen presentation by macrophages and lymphocyte-macrophage interactions.[10] In addition, animals treated with anti-*I-A*-sera also develop a population of suppressor T-cells capable of inhibiting T helper-cell responses.[11] Thus, it seems possible that anti-*I-A*-antibodies inhibit the induction of islet cell autoimmunity after low-dose streptozotocin treatment rather than block the effector phase. The suppressive effect of antibodies to *I-A* gene products provides direct evidence for the role of *Ir* genes in susceptibility to low-dose streptozotocin-induced diabetes. We have reported previously that probably two genes within the *H-2* region control susceptibility, one at or near the *I-A* subregion, the other at or near the *I-E* subregion.[3,4] In these studies *H-2*^{k} (B10.BR) was found associated with high susceptibility and *H-2*^{b} (B10) associated with intermediate susceptibility. This observation is repeated here. It is also of interest that non-*Ir* genes contribute substantially to disease susceptibility.[2,4] Nevertheless, anti-*Ia*-antibodies have turned out to be potent effectors in this animal model.

An intriguing finding in the experiments reported here is the strong suppressive effect of an anti-*I-J*^{k}-serum on disease

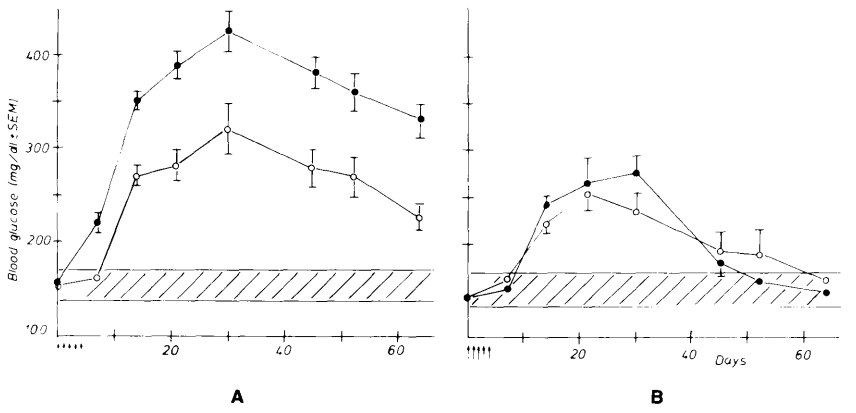

FIGURE 2. Suppressive effect of anti-*I-J* antiserum is restricted to *H-2*^{k} mice. (A) Studies with B10.BR (*H-2*^{k}) mice: streptozotocin treated mice (——●——) (N = 10); streptozotocin + anti-*I-J*-antiserum (——○——) (N = 10). (B) Studies with B10 (*H-2*^{b}) mice: streptozotocin-treated mice (——●——) (N = 10); streptozotocin + anti-*I-J*-antiserum (——○——) (N = 10). Hatched areas: mean of untreated control mice + 2 SD.

development. This antiserum and other *J*-specific antibodies have been shown repeatedly to have potent biologic effects; in general, an enhancement of humoral and cellular immunity is seen.[12-14] In experimental autoimmune encephalomyelitis, however, an effect of *I-J* antibodies was not noted.[7] J determinants have been demonstrated on T suppressor cells and factors in a variety of systems. In addition, J determinants were found on a subfraction of T helper-cells and macrophages.[12-15] Although even monoclonal anti-*I-J*-antibodies have been generated, the nature of J determinants and corresponding gene(s) is still a matter of controversy.[15-17] The suppressive effect of anti-*I-J*-antibodies leaves the interesting possibility that T suppressor lymphocytes or *I-J* positive T helper lymphocytes are involved in the process of insulitis and B-islet cell destruction in low-dose streptozotocin-induced diabetes.

T suppressor cells recently have been shown in acute graft-versus-host disease (GVHD) to infiltrate host tissue and mediate the lethal effect.[18] Acute GVHD is associated with spontaneous and strong insulitis and it has been suggested that the majority of islet-infiltrating lymphocytes are T suppressor cells.[9,19]

A final point is the possible relevance of our finding to the corresponding human disease. Susceptibility to type I diabetes is linked to *HLA-D*.[5,6] In type I diabetes of recent onset, Ia-positive T lymphocytes were found to be increased in number by one group[20] but not in another study (Bertrams and Lander, personal communication). However, it is too early to consider anti-*HLA-D* monoclonal antibodies for immunotherapy. It has to be shown first that in vivo administration of antibodies to *Ir*-gene products not only prevents the development of diabetes (as reported here) but also cures the ongoing disease, before or after the onset of overt diabetes.

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