Modulation of low-dose streptozotocin-induced diabetes in mice by administration of antibodies to I-A, I-E and I-J determinants

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Summary. In male mice of strains C3H and C57BL/6 an experimental immune-mediated diabetes can be induced by multiple low doses of streptozotocin. The delay and partial suppression of hyperglycaemia after anti-I-A monoclonal antibody administration was dose dependent. Even saturation levels of anti-I-A did not cause complete protection from diabetes development. Administration of anti-I-E monoclonal antibody also significantly delayed the onset of hyperglycaemia. Surprisingly, the combined treatment with anti-I-A and anti-I-E did not result in better protection from diabetes. Thus, there is an I-A and I-E independent component of the disease. Furthermore, there is no restriction to either I-A or I-E. Anti-I-A was only effective when given at the beginning

of the experiment, which implies that I-A molecules have a primary function during the induction of diabetes. The contribution of I-J to the disease process is different. Administration of a polyspecific alloantiserum to I-J almost completely prevented hyperglycaemia. Injections of monospecific antibodies to I-J determinants enhanced hyperglycaemia, especially when given after the induction of diabetes. This indicates that I-J is involved in initial as well as in later stages of the disease process.

Key words: Experimental diabetes, mice, streptozotocin, anti-Ia-antibodies, major histocompatibility complex, immunomodulation.

In the past few years *in vivo* therapy of experimental autoimmune diseases with (monoclonal) antibodies to immune response gene products has received some attention [1]. Particularly, antibodies to I-A gene products were shown to suppress the immune response to acetylcholine receptor [2, 3], thus preventing the development of myasthenia gravis. A similar effect of such antibodies was demonstrated in experimental autoimmune encephalomyelitis [4]. In the latter case, suppression even of the ongoing disease could be achieved [5]. Administration of Ia antibodies in (NZB × NZW)_{F1} with lupus-like nephritis resulted in an increased survival rate with no further deterioration of proteinuria [6]. Murine type II collagen induced arthritis as well as experimental autoimmune thyroiditis could also be suppressed by treatment with anti-Ia antibody [7, 8].

In a previous paper [9] we demonstrated that administration of monoclonal antibodies to I-A and of an alloantiserum to I-J partially suppressed the development of diabetes in mice treated with low doses of streptozotocin. Treatment with an H-2 alloantiserum (probably containing Ia antibodies) also partially preserved B-cell function and B-cell mass [10]. The B-cell toxin streptozotocin, when given in multiple low doses causes, in susceptible mice, a form of diabetes resembling human Type 1 (insulin-dependent) diabetes [11, 12]. The animal model is characterized by progressive B-cell loss with concomitant insulinitis, resulting in hyperglycaemia and hypoinsulinaemia. The disease in mice is controlled by genes both within and outside the major histocompatibility complex [13, 14]. Diabetes development seems

to be immune-mediated since anti-lymphocyte serum [15] as well as administration of monoclonal antibodies to T lymphocytes [16–18] prevents the disease.

In the present communication we have investigated the effect of time and dose dependency of the injection of monoclonal antibodies to I-A gene products, the complementary effect of antibodies to I-A and I-E gene products as well as the influence of monoclonal antibodies to I-J, on the course of low-dose streptozotocin induced diabetes.

Materials and methods

Mice

Male mice of strains C3H/HeHan (H-2^k) and C57BL/6J/Bom (H-2^b) were obtained from the Zentralinstitut für Versuchstierzucht (Hannover, FRG) and from Gl. Bomholtgard Ltd. (Ry, Denmark), respectively. The animals had free access to water and standard pellet diet ("ssniff M", Ssniff Co., Soest, FRG).

Streptozotocin treatment

Eight to 14-weeks-old mice were treated with five doses of 40 mg streptozotocin (Boehringer, Mannheim, FRG) per kg body weight on consecutive days [11].

Antibody treatment

The monoclonal antibody to I-A^k gene products (clone H 116-32.R5, IgG 2b; Camon, Wiesbaden, FRG) was specific for the determinant Ia.m6/Ia.19. In pilot experiments this antibody was selected as being the only one among three anti-I-A monoclonals to cause reproducible suppression of diabetes development in the low-dose streptozotocin

Fig. 1. **a** Dose response of anti-I-A^k monoclonal antibody on the development of hyperglycaemia (mean $\pm$ SEM) in C3H mice when injected at day 0. (●) 5 $\times$ 40 mg streptozotocin/kg ($n=42$); (■) streptozotocin plus 5 μ l ascites ($n=10$); (□) streptozotocin plus 20 μ l ascites ($n=10$); (▲) streptozotocin plus 80 μ l ascites ($n=10$). * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Hatched area = mean of untreated control mice + 3 SD. The arrows indicate the days of streptozotocin injections. **b** Suppression of hyperglycaemia by anti-I-A^k monoclonal antibody when injected at day 8 or in multiple doses of 20 μ l. (●) 5 $\times$ 40 mg streptozotocin/kg ($n=42$); (■) streptozotocin plus anti-I-A^k injected at days 0, 7, 14, and 21 ($n=10$); (▲) streptozotocin plus anti-I-A^k injected at days -2, 0 and 2 ($n=10$). For further information see **a**. **c** Dose response of anti-I-A^k monoclonal antibody on the course of hyperglycaemia (mean $\pm$ SEM) in C3H mice when injected at day 8 and effect of combined administration of anti-I-A^k with anti-I-E^k monoclonal antibodies injected at day 0. (●) 5 $\times$ 40 mg streptozotocin/kg ($n=42$); (■) streptozotocin plus 5 μ l ascites of anti-I-A at day 8 ($n=10$); (□) streptozotocin plus 20 μ l ascites of anti-I-A at day 8 ($n=10$); (▲) streptozotocin plus 80 μ l ascites of anti-I-A at day 8 ($n=10$); (○) streptozotocin plus 20 μ l ascites of anti-I-A at day 0 ($n=10$); (◆) streptozotocin plus 20 μ l ascites of anti-I-E at day 0 ($n=10$); (◇) streptozotocin plus combined administration of 20 μ l of each anti-I-A and anti-I-E at day 0 ($n=10$). For further information see **a**

model. The monoclonal IgG 2a antibody against I-E^k gene products was specific for the determinant Ia.m7 (clone 13/4.R5, Camon). Three monoclonal antibodies against I-J^b determinants (clones E10 (IgG 2a), F4 (IgG 2b) and H6 (IgG 2a)) were prepared as described [19]. All monoclonal antibodies were used as ascites fluid.

An alloantiserum was prepared by immunization of mice against I-J^k (lot J002, Camon).

In all experiments ascites fluid or antisera were diluted with distilled water to a final volume of 200 μ l and injected into the retro-orbital vein plexus. In those cases where antibody and streptozotocin should be administered the same day, the antibody was injected two h before the drug to avoid any interference.

Blood glucose determination

Blood glucose values were determined from unfasted mice between 09.00 and 10.00 hours. Mice were bled from the tail and glucose concentrations measured by the hexokinase method (Gluco-quant, Boehringer Mannheim). All values were expressed in mmol/l.

Statistical analysis

The arithmetic means of blood glucose concentrations $\pm$ S.E.M. were calculated on all experimental groups. Statistical differences were evaluated by Mann-Whitney rank test.

Results

Treatment of male C3H (H-2^k) mice with multiple low-dose streptozotocin caused the development of hyperglycaemia around day 14, and a continuous rise in blood glucose levels until day 60 (Fig. 1 a). The administration of 20 μ l of anti-I-A antibody at day 0 did not cause complete protection from diabetes development, but resulted in a delay of hyperglycaemia and lower mean blood glucose levels at all times ($p < 0.05$ or $p < 0.001$ for days 14–60). Injection of 5 μ l of antibody did not significantly retard diabetes development, demonstrating dose dependency (Fig. 1 a). The later occurrence of (milder) hyperglycaemia after anti-I-A antibody administration could not be suppressed by increasing antibody dose (80 μ l, Fig. 1 a), by multiple early injections (at days -2, 0, 2) nor by weekly injections during the first month (Fig. 1 b).

Administration of anti-I-A antibody was not effective when given after induction of the disease process, i.e. after completion of low-dose streptozotocin treatment. At this point, (day 8) neither low nor high amounts of anti-I-A antibody altered the course of hyperglycaemia development (Fig. 1 c).

C3H mice express gene loci coding for different class II major histocompatibility antigens I-A and I-E. The administration of monoclonal antibodies to I-E delayed the onset of hyperglycaemia with similar kinetics as for anti-I-A (Fig. 1 c). Combination of the two antibodies showed neither an additive nor a synergistic effect (Fig. 1 c).

A third phenotype, of which the expression is controlled by the class II major histocompatibility complex region, is I-J. In contrast to anti-I-A or anti-I-E antibodies, administration of an allo-antiserum to I-J^k almost completely protected from hyperglycaemia (Table 1 A). Again, antiserum injected after the start of streptozotocin treatment did not prevent diabetes development. Injections of anti-I-J at days 5 or 8 even slightly enhanced hyperglycaemia ($p < 0.05$ at days 14, 30 or 60 respectively).

Enhancement, in most cases, rather than suppression of diabetes development, was noted when three different monoclonal antibodies to I-J^b were tested ac-

Table 1. Effect of treatment with antibodies to I-J on the development of hyperglycaemia (mmol/l, mean $\pm$ SEM) when injected on different days: **A** Effects of alloantiserum to I-J^k (40 μ l) in male C3H mice; **B** Effects of 3 different monoclonal antibodies to I-J^b (25 μ l ascites) in C57BL/6 mice

		Day 14	Day 30	Day 60
A				
SZ ^d	42	12.9 $\pm$ 0.8	16.9 $\pm$ 1.1	21.9 $\pm$ 1.3
SZ ^d + I-J ^e				
day -2	10	8.9 $\pm$ 0.3 ^b	10.9 $\pm$ 1.0 ^a	8.8 $\pm$ 0.6 ^c
SZ ^d + I-J ^e				
day 0	20	9.2 $\pm$ 0.2 ^c	9.9 $\pm$ 0.8 ^b	10.5 $\pm$ 1.2 ^c
SZ ^d + I-J ^e				
day 2	10	12.3 $\pm$ 0.9	14.5 $\pm$ 1.3	19.1 $\pm$ 1.8
SZ ^d + I-J ^e				
day 5	30	14.8 $\pm$ 0.7 ^a	18.4 $\pm$ 1.2	26.6 $\pm$ 1.6 ^a
SZ ^d + I-J ^e				
day 8	20	15.1 $\pm$ 1.1	20.0 $\pm$ 2.4 ^a	25.2 $\pm$ 2.9
B				
SZ ^d	21	12.4 $\pm$ 0.8	16.1 $\pm$ 1.1	17.0 $\pm$ 1.3
SZ ^d + E10 ^f				
day 0	10	17.1 $\pm$ 1.1 ^b	21.4 $\pm$ 2.0 ^b	19.6 $\pm$ 2.2
SZ ^d + E10 ^f				
day 8	9	15.8 $\pm$ 0.8 ^b	21.0 $\pm$ 0.8 ^b	26.4 $\pm$ 1.4 ^c
SZ ^d + F4 ^g				
day 0	10	15.6 $\pm$ 1.1 ^a	20.2 $\pm$ 1.1 ^a	24.6 $\pm$ 2.0 ^b
SZ ^d + F4 ^g				
day 8	9	15.2 $\pm$ 1.0 ^a	20.8 $\pm$ 1.3 ^b	20.0 $\pm$ 1.6
SZ ^d + H6 ^h				
day 0	10	13.8 $\pm$ 0.8	14.2 $\pm$ 1.3	17.6 $\pm$ 2.0
SZ ^d + H6 ^h				
day 8	9	15.5 $\pm$ 1.1	19.4 $\pm$ 0.8	23.8 $\pm$ 1.2 ^b

^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$: significance refers to values obtained in the streptozotocin treated group (unmarked data are non significant); ^d SZ = 5 $\times$ 40 mg streptozotocin per kg body weight; ^e I-J = alloantiserum to I-J^k; ^f E10 = anti-I-J^b (clone E10); ^g F4 = anti-I-J^b (clone F4); ^h H6 = anti-I-J^b (clone H6)

cording to the same protocol in C57BL/6J (H-2^b) mice (Table 1B). Enhancement was more pronounced when antibodies were given 4 days after, rather than shortly before, streptozotocin injections (Table 1B).

Discussion

The use of different antibodies to products controlled by the I-region of the major histocompatibility complex, and the variation of dose and treatment schedule in the present experiments, has led to further insight into the mechanisms involved in the development of immune diabetes.

Firstly, there is a dose dependence of the protective effect of anti-I-A monoclonal antibody. Maximum effect is reached with 20 μ l of the antibody, doses 2 or 4 times higher do not lead to a significantly higher outcome. This observation indicates the saturation of relevant binding site or transgression of a threshold binding level for anti-I-A antibodies.

It is of interest that "saturation" levels of anti-I-A antibodies, even when given repeatedly over one month

did not provide complete protection from hyperglycaemia, but only induced a delay of diabetes development with subsequent milder hyperglycaemia. We would argue that part of the disease process is non-I-A dependent. This is in accord with previous observations that beside I-region genes, loci outside the major histocompatibility gene complex also contribute to disease susceptibility [13, 14]. In this context it should be noted that the depletion of T cells in the same model not only failed to completely prevent diabetes development, but resulted in a delayed onset of milder hyperglycaemia [17, 20]. Thus, there are I-A-independent and T cell-independent components to the disease. Such components may be the T cell independent activity of macrophages [17, 21] or the late death of streptozotocin damaged B-cells [22].

The administration of anti-I-A was effective only when given early in the diabetes induction period. A primary function of I-A molecules, therefore, is during disease induction. It is improbable that anti-I-A acts on the level of islet B-cells. Although islet cells may express I-A antigens some time after streptozotocin injection [23], antibodies to I-A are effective when given before streptozotocin, i.e. at a time when islet cells are I-A negative.

I-A and I-E are separate molecular entities and it is surprising that anti-I-A gives similar protection from diabetes as anti-I-E, and that the two do not add up to better protection. In another model of insulin-dependent diabetes – the spontaneous autoimmune diabetic BB rat – it was found that repeated administration of an anti-I-E equivalent antibody but not of an anti-I-A antibody decreased the incidence rate of diabetes plus thyroiditis [24]. I-A and I-E generally act as restriction elements for different proteins, probably because the two receptors recognize different peptide motifs [25, 26]. If I-A and I-E both act as restriction elements in the low-dose streptozotocin model one would expect more than one antigen (or peptide sequence) to be involved in the disease process. A contribution of both I-A and I-E to T cell autoimmunity was also found in experimental autoimmune encephalomyelitis [27].

Our results with anti-I-J antibodies show that highly effective immunomodulation can be reached by this approach. Results vary from complete protection with a conventional antiserum to I-J, to substantial enhancement by some monoclonal antibodies. Suppression was only seen when anti-I-J was given prior to diabetes induction. Enhancement was most marked when anti-I-J was administered after diabetes induction. I-J determinants are expressed on immunoregulatory T cells and some macrophages, are linked in their genetic polymorphism to the major histocompatibility complex and have been implicated in the generation of suppressor, contrasuppressor and helper amplifier activities [28].

As described [29], there are two types of I-J: one is expressed on some T helper cells (Th₂) the other on suppressor T cells. Therefore, it is quite possible that the conventional antiserum contains two types of anti-I-J antibodies, whereas our monoclonal anti-I-J^b antibodies recognize the idiotypic structure on suppressor

T cells and are specific for them but not for Th₂ cells [30]. Thus, treatment with these anti-I-J antibodies eliminates or suppresses suppressor T cell activity and results in the enhancement of the disease.

Our results do not help to identify the nature of I-J antigens, except that by judging the contrasting effects of monoclonal antibodies to I-A, I-E versus I-J suggested that these molecules are not functionally similar.

In summary, the data indicate an important function of I-A, I-E and some I-J molecules early during diabetes development and the involvement of I-J also, at a later stage.

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