Antiidiotype Modulation of Herpes Simplex Virus Infection Leading to Increased Pathogenicity

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Antiidiotype reagents that recognize idiotypic determinants associated with the combining site of monoclonal antibodies to herpes simplex virus type 2 (HSV2) were used to manipulate the immune response to HSV2 in BALB/c mice. The injection of antiidiotype antibodies into mice before challenge with a 50% lethal dose of HSV2 resulted in a shorter survival time than that of mice receiving either preimmune rabbit immunoglobulin G or antiidiotype reagents against hepatitis B surface antigen before HSV2 challenge. These findings indicate that the immune response to HSV2 in mice can be modulated through idiotype-antiidiotype networks, thereby increasing the pathogenicity of HSV2 infections.

Manipulation of the immune response by injection of antiidiotype (anti-ID) antibodies has been documented in numerous antibody systems (1, 2, 4, 6-8, 10, 18, 20-23). The *in vivo* administration of anti-ID reagents followed by antigen exposure often has resulted in suppression of idiotype (ID)-positive, antigen-binding molecules (4, 6, 8). However, enhanced ID expression and antigen-binding activity have also been reported (1, 2, 10, 20, 22, 23). Recently, anti-ID antibodies have been used to enhance the antibody response to infectious agents (12, 21) and transplantation antigens (1, 18). Extension of these findings to the immune response to herpes simplex virus (HSV) is of both practical and theoretical importance. Intraperitoneal infection of mice with either HSV type 1 (HSV1) or type 2 (HSV2) can result in ascending neurological illness and death (3, 14). In this study we found that injection of rabbit anti-ID reagents decreased the survival time of mice challenged with HSV2. Thus, treatment of mice with anti-ID appears to suppress the natural defense mechanisms or the specific immune response to HSV2 or both, leading to increased pathogenic activity of the virus.

We have previously characterized rabbit anti-ID reagents to two immunoglobulin M (IgM) monoclonal antibodies (McAb) against HSV2 (11). These anti-ID antibodies were made specific for the unique (IdI) determinants by adsorption to and elution from Sepharose columns bearing the appropriate ID (13). Other anti-ID reagents that recognized IdI determinants associated with a third IgM anti-HSV2 McAb (HS2) (11) and an IgG1 McAb to hepatitis B surface antigen (HBsAg) were also generated in rabbits and characterized by similar methods. The antibodies used in this experiment represented a pool of the three (equal proportions on a weight basis) affinity-purified rabbit anti-ID antibodies to the IgM anti-HSV2 McAb. A pool of IgG molecules obtained from the preimmune serum of the rabbits before injection of the ID and affinity-purified rabbit anti-ID to the HBsAg McAb (anti-HBs) were used as controls. The antibody concentration of the three preparations was determined spectrophotometrically at 280 nm with an extinction coefficient of 15 for a 1% preparation.

In experiment 1 (Table 1), groups of 8-week-old female BALB/c mice were given intraperitoneal injections of (i)

anti-ID antibodies generated to anti-HSV2 McAb, (ii) preimmune rabbit IgG (pre-IgG), (iii) anti-ID to anti-HBs McAb, or (iv) saline. After day 14, the animals were given intraperitoneally a 50% lethal dose (LD₅₀) of HSV2 strain M186 (5). Mice were observed daily for 21 days for signs of neurological illness and for death. None of the 14 mice that received the anti-ID pool specific for anti-HSV2 McAb survived the 21-day study period, whereas 57% (8 of 14) of the pre-IgG-treated mice survived. In addition, 62% (five of eight) of the mice receiving either saline or the anti-HBs anti-ID survived after an LD₅₀ injection of HSV2. A significant difference in the mean survival times was observed between mice receiving the anti-HSV2 anti-ID and all other treatment groups ($P < 0.005$) (Table 1). These data suggested that prior injection of anti-ID that is specific for anti-HSV2 McAb enhanced the pathogenicity of HSV2 in mice. The effect did not result from modification of rabbit antibodies produced by adsorption to and elution from affinity columns since the mean survival times were similar in mice injected with anti-ID antibodies to an anti-HBs McAb, pre-IgG, or saline. Because the methods used to specifically purify anti-ID antibodies for all anti-ID reagents used in this study were similar, any modification of rabbit antibodies which might result in nonspecific killing of BALB/c mice would also be expected to occur in both the anti-HBs treatment group and the anti-HSV anti-ID treatment groups. Mice receiving multiple injections of anti-HSV2 anti-ID alone did not develop any adverse symptoms which resulted in death.

In experiment 2 (Table 2), we analyzed the effects of different doses of anti-ID antibodies on the decreased survival of mice subsequently challenged with HSV2. Previous reports have demonstrated that the concentration of anti-ID injected before antigenic stimulation determined whether ID expression would be enhanced or suppressed. In particular, high and intermediate concentrations of an IgG2 subclass of guinea pig anti-ID completely suppressed the expression of a streptococcal carbohydrate ID in mice, whereas high doses of an IgG1 anti-ID enhanced the production of antibodies expressing this idiotype (6). Alternatively, low doses of anti-ID have not been found to mediate anti-ID suppression of the immune response (6, 17). It was apparent from the dose-response study (Table 2) that the decreased survival time of mice challenged with HSV2 was dependent on the concentrations of anti-ID. In this regard, injection of either 5 or 50

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TABLE 1. Survival time of BALB/c mice treated with anti-ID antibodies and challenged with HSV2

Treatment ^a	No. of mice	No. of survivors	Mean survival time (days) ^b	SD
Anti-ID (anti-HSV2)	14	0	8.5 ^c	1.8
Pre-IgG	14	8	18.4	5.4
Anti-ID (anti-HBs)	8	5	17.4	5.1
Saline	8	5	18.1	4.8

^a Mice received saline, alum-precipitated pre-IgG, or anti-ID (50 µg per mouse) on day 0 followed by an LD₅₀ of HSV2 on day 14, all by the intraperitoneal route. Mice were observed daily for neurological illness and death for 21 days after HSV2 infection.

^b To aid calculations, those mice that were alive on day 21 were arbitrarily given a survival time of 21 days.

^c Based on the two-tailed Student's *t* test; *P* < 0.005.

µg of anti-ID into mice resulted in a shorter survival time than that of mice given similar concentrations of pre-IgG. No effect was observed in groups of mice receiving 10-fold-less anti-ID (0.05 or 0.5 µg of anti-ID). It was noteworthy that lower concentrations of anti-ID did not result in a more significant difference in response to HSV2 than that observed with mice treated with pre-IgG, saline, or anti-HBs anti-ID antibodies (*P* < 0.2).

Alum-precipitated antibodies were used to modulate the immune response of mice to HSV2 based on our previous investigation (R. C. Kennedy, K. Adler-Storthz, Y. Sanchez, R. D. Henkel, J. L. Melnick, and G. R. Dreesman, Fed. Proc. 42:1378, 1983), which indicated that the immune response to HBsAg was enhanced by prior injection of anti-ID. In this system, higher anti-HBs titers were obtained in mice receiving alum-precipitated versus soluble anti-ID. In the next experiment, we examined the effect of the individual anti-ID antibodies on the apparent increased pathogenicity by HSV2. Based on the data in Table 3, the anti-ID pool had the lowest mean survival time when compared with each of the individual anti-ID-treated groups. Only in the anti-ID

TABLE 2. Effect of dose of anti-HSV2 anti-ID antibody on survival time of BALB/c mice challenged with HSV2

Treatment ^a (µg)	No. of survivors in groups of six mice each	Mean survival time (days)	SD
Anti-ID (50)	0	8.8 ^b	2.4
Pre-IgG (50)	4	19.7	2.1
Anti-ID (5)	0	8.5 ^c	1.3
Pre-IgG (5)	3	16.0	4.5
Anti-ID (0.5)	4	19.7	2.1
Pre-IgG (0.5)	4	20.0	3.0
Anti-ID (0.05)	4	18.7	4.8
Pre-IgG (0.05)	4	18.8	4.8

^a Each group of six mice received different doses of either alum-precipitated anti-ID or pre-IgG on day 0 followed by an LD₅₀ of HSV2 on day 14, all by the intraperitoneal route. The day of death for each mouse was determined. Calculations of mean survival time were similar to those described in Table 1.

^b Based on two-tailed Student's *t* test; *P* < 0.005.

^c *P* < 0.05.

TABLE 3. Effect of individual anti-ID antibodies on survival time of BALB/c mice challenged with HSV2

Treatment ^a	No. of survivors in groups of six mice each	Mean survival time (days)	SD
Anti-ID (pool)	0	7.33	1.45
Anti-ID (HS2)	0	8.66	2.90
Anti-ID (18B)	0	10.0	2.39
Anti-ID (1B)	1	12.66	4.42
Saline	2	16.66	5.23
Pre-IgG	3	18.33	4.22

^a Each group of six mice received 5 µg of alum-precipitated anti-ID, pre-IgG, or saline on day 0 followed by an LD₅₀ of HSV2 on day 14, all by the intraperitoneal route. The day of death was determined for each mouse. Calculations of mean survival times were similar to those described in Table 1, footnotes *b* and *c*.

preparation from McAb 1B did a single mouse survive the challenge with HSV2. However, due to the large standard deviation, this treatment group did not differ from the other groups of mice receiving anti-ID. Of the individual anti-ID reagents, the HS2-treated group most closely resembled the mice receiving the anti-ID pool (mean survival time of 8.66 versus 7.33). Previously, we demonstrated that the 18B and 1B idiotypes were expressed on only a small population of the total anti-HSV2 produced when immunizing BALB/c mice with HSV2 (11). The possibility exists that the HS2 idiomorph may represent a major family of cross-reactive idiotypes that are induced in BALB/c mice upon infection with HSV2. This could explain why pretreatment of mice with the anti-ID preparation to HS2 more dramatically affected the rate of increase in the apparent pathogenicity of HSV2 when compared with the effect of pretreatment with two other anti-ID preparations. The results indicate that, although the individual anti-ID preparations can mediate the decreased survival time of mice infected with HSV2, the greatest effect was seen when the three anti-ID preparations were pooled. The lower mean survival times seen with the anti-ID pool and saline-treated groups of mice in Table 3 when compared with the mean survival times of groups in Tables 1 and 2 may reflect the small sample size used (*n* = 6). It was apparent that the mean survival times along with the standard deviations obtained in the experiments were consistent.

The mechanism of the immune defense to HSV infections is complex. We show here that anti-ID treatment before HSV2 infection increases the apparent pathogenicity of the virus in mice. This effect does not appear to be anti-HSV antibody mediated because groups of mice receiving several injections of anti-ID alone failed to produce detectable anti-HSV titers as measured by either radioimmunoassay or virus neutralization. Recently, it has been shown that anti-ID antibodies generated to antibodies against viral agents may be important in protective immunity. We have demonstrated that immunizing mice with anti-ID antibodies that recognize a common human anti-HBs ID will induce an anti-HBs response without subsequent HBsAg stimulation (12; R. C. Kennedy and G. R. Dreesman, J. Exp. Med., in press). In addition, pretreatment of mice with anti-ID before HBsAg injection enhances the anti-HBs response. Similar results have been reported with rabies virus, in which anti-ID reagents to McAb that recognize a viral surface glycoprotein induce low levels of neutralizing antibody to the virus (19).

Alternatively, anti-ID antibodies have been produced that bind to reovirus-specific hemagglutinin receptors on neural and lymphoid cells and block reovirus attachment (9, 15, 16). The reason that anti-ID treatment has deleterious effects on the outcome of HSV2 infections is not known.

The use of anti-ID antibodies to manipulate the immune response to HSV2 represents a potential model to study the effects of different lymphocyte populations involved in disease states leading to fatal HSV encephalitis or possibly to a state of latency. Experiments are in progress to more clearly define the modulating events that occur during HSV infection with prior anti-ID injection. These studies may provide insight into early immune events and resulting pathology that occur during HSV infection.

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