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Monoclonal Anti-I-A Antibody Reverses Chronic Paralysis and Demyelination in Theiler's Virus-Infected Mice: Critical Importance of Timing of Treatment

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Susceptibility to demyelination caused by the WW isolate of Theiler's murine encephalomyelitis viruses is linked to class II genes of the major histocompatibility complex. SJL/J (*H-2*^{s}) mice, expressing only I-A^s class II gene products of the major histocompatibility complex, are highly susceptible to Theiler's murine encephalomyelitis virus infection with the WW virus isolate, with chronic paralysis and severe inflammation and demyelination in the central nervous system. The effect of in vivo administration of anti-I-A^s monoclonal antibodies on Theiler's murine encephalomyelitis virus infection was observed. SJL/J mice were treated in various protocols pre- or postinfection. Anti-I-A^s monoclonal antibody reversed chronic paralysis and reduced inflammation and demyelination when given after the establishment of persistent infection. The effect was long lasting, but clinical signs, inflammation, and demyelination recurred 2 months after treatment ceased. Anti-I-A^s antibodies had no effect on viral titers within the central nervous system. The timing of the administration of monoclonal antibodies was critical. Administration of anti-I-A^s before the establishment of the persistent infection resulted in fatal encephalitis.

Theiler's murine encephalomyelitis virus (TMEV) induces a persistent infection of the mouse central nervous system. Depending on the virulence of the TMEV strain used for inoculation, the resultant infection may be either monophasic with an initial fatal encephalitis, typical of the GDVII and FA strains, or biphasic with an initial poliomyelitis, occurring within a few days, followed by persistent infection with chronic paralysis and demyelination manifest after a latency of several weeks (5).

The histopathologic picture of chronic TMEV infection reveals lymphocytic leptomeningeal and perivascular infiltrates in the vicinity of demyelinated regions (5). The picture is altogether compatible with that evident in chronic, relapsing experimental allergic encephalomyclitis (CR-EAE) mediated by myelin basic protein-specific T-cell clones (17, 18). These T-cell clones bear the L3T4 surface marker, characteristic of T cells mediating delayed-type hypersensitivity (DTH), and are I-A restricted (10, 17, 18). In parallel with clone-mediated CR-EAE, susceptibility to TMEV-induced demyelination maps to genes within the major histocompatibility complex (*H-2*), and susceptible strains develop TMEV-specific, L3T4-positive, I-A-restricted T cells mediating a DTH response. These T cells may play a central role in TMEV-induced demyelination (3, 4).

We have demonstrated that both acute EAE and CR-EAE could be prevented and that ongoing clinical symptoms could be ameliorated with monoclonal antibodies to products of the *I-A* subregion of *H-2* (8, 9). Since I-A-restricted, L3T4-positive cells may play a critical role in demyelination caused by TMEV, we investigated whether we could prevent TMEV-induced disease by prior administration of anti-I-A or even reverse ongoing paralysis and demyelination by treatment after the establishment of persistent virus infection.

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MATERIALS AND METHODS

Virus. The WW isolate of TMEV, plaque purified in BHK-21 cells after isolation from morbid SJL mouse brains was used; 3×10^4 PFU in 20 μl was injected intracranially. Viral titers were measured by plaque assay (5).

Mice. SJL/J mice were bred in the Institute of Life Sciences, Hebrew University, Jerusalem. B10.S(7R), B10.S(9R), BALB/c, A.TL, and C3H mice were purchased from Olac Ltd. (Bicester, Oxon, England). Mice 3 to 4 weeks of age were used.

MAbs. Anti-I-A^s (10-3.6) was used (8–10, 17, 18). This antibody is a mouse immunoglobulin G2a (IgG2a) and recognizes Ia.17 (I-A^s) (8, 9). Monoclonal antibodies (MAbs) were purified with ammonium sulfate as described previously (6, 14). MAbs were administered intraperitoneally (i.p.) by following three protocols. Protocol 1 was one injection of 500 μg of anti-I-A^s MAb i.p. on the same day but before virus inoculation. Protocol 2 was one injection of 200 μg of anti-I-A^s MAb i.p. 2 weeks postinfection and then 100 μg of MAb i.p. once a week for 4 weeks. Protocol 3 was one injection of 500 μg of anti I-A^s MAb i.p. at the onset of the clinical signs of chronic disease.

Clinical examination. Poliomyelitis (flaccid paralysis) was scored by daily examination of mice during the first 14 days postinfection. Gait disorder is manifested by a wobbling or spastic gait. This was checked with at least four examinations per week postinfection to time of sacrifice.

Histopathology. Mice to be sacrificed were anesthetized with pentobarbital and then perfused with redistilled 10% glutaraldehyde in Sorensen buffer. Paraffin sections were used for light microscopy, and Epon (812)-embedded thin sections of spinal cord were used for electron microscopy. Sections were viewed in a JEOL 100CX electron microscope.

At least 10 spinal cord sections were examined from each animal. The following scoring system was used. All sections

TABLE 1. Influence of *H-2* genes on susceptibility to infection with TMEV WW strain

Mouse strain	*H-2* haplotypes					Incidence, no. of animals/total (%), of:		Virus titer (PFU per spinal cord) on day 97	Degree of demyelination
	K	*A*	*E*	*S*	*D*	Poliomyelitis	Chronic gait disorder		
SJL/J	*s*	*s*	*s*	*s*	*s*	16/20 (80)	15/15 (100)	1.5×10^2	+++
B10.S(7R)	*s*	*s*	*s*	*s*	*d*	8/10 (80)	8/10 (80)	3.3×10^2	+++
B10.S(9R)	*s*	*s*	*k*	*d*	*d*	9/15 (60)	9/15 (60)	5×10^2	++
C3H	*k*	*k*	*k*	*k*	*k*	11/15 (73)	7/14 (50)	5×10^2	++
A.TL	*s*	*k*	*d*	*d*	*d*	4/5 (80)	2/5 (40)	4.2×10^2	++
BALB/c	*d*	*d*	*d*	*d*	*d*	12/20 (60)	3/16 (19)	1.3×10^3	+

were read by an observer without knowledge of the treatment regimen. The degree of inflammation was scored as follows: +, slight inflammation of meninges; ++, inflammation of meninges and perivascular cuffs; +++, severe inflammation and infiltration of white matter. The degree of demyelination was scored as follows: +, sporadic; ++, involvement of greater than 50% of spinal cord white matter; +++, involvement of the entire spinal cord white matter.

RESULTS

Mapping of genetic susceptibility to demyelination. The incidence of poliomyelitis with flaccid paralysis varied between 60 and 80% in all strains tested. SJL/J and B10.S (7R) mice developed spasticity and severe, widespread demyelination; B10.S(9R), A.TL, and C3H mice developed intermediate degrees of spasticity and demyelination; whereas BALB/c mice had a low frequency of spasticity and only sporadic demyelination (Table 1). In the present experiments, of note is that *H-2D* class 1 major histocompatibility (MHC) genotypes did not influence susceptibility to demyelination caused by the WW virus. Both SJL/J and B10.S(7R) mice showed severe clinical disease and severe demyelination but differed at *H-2D*, with B10.S(7R) sharing the $H\text{-}2D^d$ allele with BALB/c mice, which showed the least degree of demyelination and gait disorder of all strains tested. The class 2 MHC genes, *I-A* and *I-E*, were critical for full expression of spasticity and demyelination, since B10.S(7R) and SJL/J mice bearing $I\text{-}A^s$ and $I\text{-}E^s$ had the most frequent incidence of spasticity and severe demyelination, whereas B10.S(9R) mice carrying $I\text{-}A^s$ $I\text{-}E^k$ and C3H mice bearing $I\text{-}A^k$ $I\text{-}E^k$ and A.TL mice bearing $I\text{-}A^k$ $I\text{-}E^d$ had intermediate degrees of clinical disease and demyelination, and BALB/c mice ($I\text{-}A^d$ $I\text{-}E^d$) had the least degree of spasticity and demyelination. Viral titers were highest in BALB/c mice and lowest in $I\text{-}A^s$ strains with A.TL, C3H, and B10.S(9R) mice showing intermediate titers. These data reveal a trend, namely, that chronic disease is influenced by the class 2 MHC genotype. There is a strong concordance when either viral titers, degree of demyelination, or incidence of gait disorder (3 of 16 BALB/c mice versus 23 of 25 SJL and B10.S mice; $\chi^2 = 18.1$, $P < 0.01$) is examined. In contrast the incidence of poliomyelitis does not seem to be associated with class 2 genotype. The somewhat lower incidence of chronic disease and lesser degree of demyelination in B10.S(9R) mice may indicate a role for *I-E* class 2 products as well. We thus chose to study the effect of monoclonal anti-$I\text{-}A^s$ in SJL/J mice, in which only $I\text{-}A^s$ products are expressed, not $I\text{-}E^s$ products. We employed monoclonal antibody 10-3.6 (anti-$I\text{-}A^s$), which has proven effective in preventing and reversing CR-EAE and acute EAE in SJL/J mice (8, 9).

Treatment with anti-$I\text{-}A^s$ before viral inoculation: protocol 1. The clinical, pathologic, and virologic status of control mice inoculated with WW virus and treated with phosphate-buffered saline (PBS) is shown in Table 2. The biphasic nature of TMEV disease is noted with acute poliomyelitis evident on days 1 through 3 followed by chronic paralysis beginning on days 21 through 33. The degree of histologic inflammation and demyelination is shown (Fig. 1B and C) with some mice sacrificed on day 40, 50, or 70. Virus titers varied from 2×10^2 to 5×10^2 PFU per spinal cord in control mice. The results in Table 2 serve as controls for Tables 3, 4, and 5. Tables 2 through 5 comprise two experiments with protocols 1, 2, and 3 plus PBS controls.

TABLE 2. Poliomyelitis in PBS-treated[a] SJL mice

Expt[b]	Mouse no.	Onset of poliomyelitis (day p.i.[c])	Onset of chronic disease (day p.i.)	Inflammation	Demyelination	Virus titer (PFU per spinal cord)
A	1		21	ND[d]	ND	3×10^2
	2	3	28	++	+	
	3	2	31	+++	++	
B	4	3	22	−	−	2×10^2
	5	3	26	+++	++	
	6	1	28	++	++	
C	7	2	30	ND	ND	5×10^2
	8	2	27	++	+++	
	9	3	33	+	++	

[a] PBS was given on day −1.
[b] Animals were sacrificed on days 40, 50, and 70 postinfection in experiments A, B, and C, respectively.
[c] p.i., Postinfection.
[d] ND, Not done.

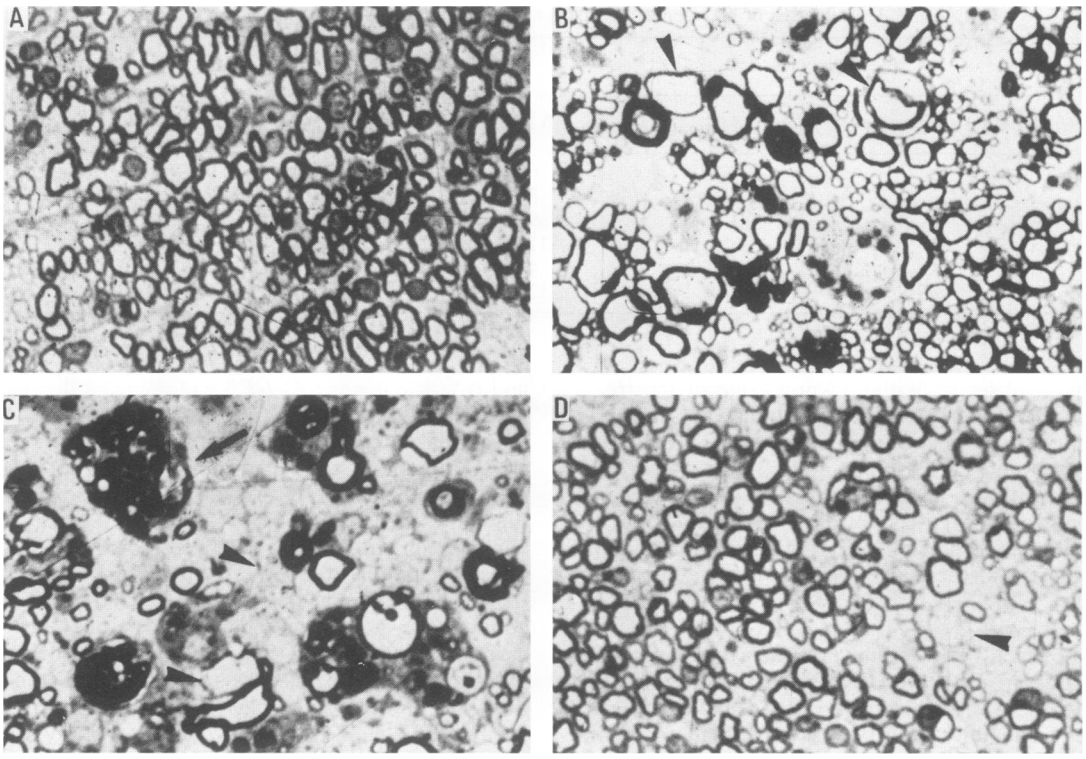

FIG. 1. Degree of demyelination in SJL mice infected with WW virus and treated with anti-I-A^s MAbs. Light microscopy of paraffin-embedded hematoxylin-eosin-stained spinal cord sections. (A) Mock-infected, PBS-treated mouse 35 days after PBS treatment. No demyelination was observed. (B) Medium degree of demyelination denoted (++) in a mouse infected with WW virus and treated with PBS 33 days after the onset of the chronic stage; the arrowhead indicates a demyelinated axon. (C) Severe demyelination and inflammation (+++) in a mouse infected with WW virus and treated with PBS 27 days after the onset of chronic disease. The arrowheads indicate demyelinating axons, and the arrow indicates a lymphocyte engaged in phagocytosis of myelin debris. (D) Slight demyelination (±) in an SJL mouse infected with WW virus and treated with anti I-A^s MAb according to protocol 3.

When mice were given 500 μg of anti-I-A^s MAb before viral inoculation, four of four died of overwhelming poliomyelitis on days 4 through 6, and a fifth mouse was sacrificed when moribund on day 5 (Table 3). All mice displayed severe encephalitis. The virus titer of 2×10^7 PFU/g in the anti-I-A-treated mouse on day 5 is typical when compared with titers from untreated mice during the first 7 days postinjection (5).

Chronic treatment with anti-I-A^s beginning 2 weeks after viral inoculation: protocol 2. Seventeen mice were given 200 μg of anti-I-A^s i.p. 2 weeks after inoculation with WW virus and were then given 100 μg of anti-I-A^s every week for the next 4 weeks. All mice (except mouse number 15) developed acute poliomyelitis, followed by the onset of chronic paralysis on days 26 to 35. Instead of persistent chronic paralysis, the chronic phase in these treated animals lasted only 6 to 19 days. One mouse recovered from acute poliomyelitis and did not develop chronic paralysis. This picture contrasts sharply with PBS-treated mice, which retained evidence of persistent infection with chronic paralysis. Histopathology revealed minimal (meninges only inflamed) or no inflammation in 11 of 12 mice checked on day 40, 50, or 70, compared with only 2 of 7 PBS-treated mice. Demyelination was sporadic in 10 of 12 mice observed on day 40, 50, or 70, whereas moderate to severe (++ to +++) demyelination was seen in 5 of 7 PBS-treated mice. Viral titers were in the same range in MAb- and PBS-treated mice.

TABLE 3. Poliomyelitis in mice pretreated with anti-I-A^s (protocol 1)

Mouse no.	Onset of poliomyelitis (day p.i.[a])	Death (day p.i.)	Cause of death or disease
1	2	5	Encephalitis
2	3	6	Encephalitis
3	2	5	Encephalitis
4	3	4	Encephalitis
5	4	Sacrificed day 5	Encephalitis[b]

[a] p.i., Postinfection.
[b] Viral titer of 2×10^7 PFU/g of central nervous system tissue.

Two mice were monitored beyond day 70 to see whether relapse might occur. After recovering from the initial acute poliomyelitis and then the chronic phase of paralysis, both mice relapsed on day 100, 60 to 65 days after recovering from clinical paralysis and 58 days after receiving their last dose of anti-I-A^s MAb. Histopathologic examination of these mice revealed greater degrees of inflammation and demyelination than that seen in treated mice examined at day 40, 50, or 70.

One-dose treatment with anti I-A^s at the onset of clinical signs of chronic disease: protocol 3. Mice given one dose of 500 μg of anti-I-A^s MAb at the onset of chronic disease showed complete recovery from symptoms in 6.9 ± 1.1 days. They showed no relapses when examined daily up to 70 days after viral inoculation. No mice had inflammation beyond a slight meningeal reaction. Only 2 of 13 mice had ++ demyelination, with 11 to 13 mice having only sporadic demyelination (Fig. 1D). Viral titers in the central nervous

TABLE 4. Poliomyelitis in mice treated continuously with anti-I-A^s (protocol 2)

Expt[a]	Mouse no.	Onset and duration of poliomyelitis (days p.i.[b])	Onset of chronic disease (days p.i.)	Recovery signs (days p.i.)	Recurrence of chronic disease (days p.i.)	Inflammation	Demyelination	Virus titer (PFU per spinal cord)
A	1	3–15	29	37		ND[c]	ND	8×10^2
	2	2–15	31	40		+	+	
	3	3–11	32	40		+ +	+	
	4	2–15	28	38		+	+ +	
	5	2–12	29	40		+	+	
B	6	3–10	32	40		ND	ND	2×10^2
	7	3–11	29	36		±	+ +	
	8	2–21	35	41		−	+	
	9	2–20	32	40		+	+	
	10	3–12	26	45		−	+	
C	11	3–16	29	35		ND	ND	3×10^2
	12	3–18				−	+	
	13	2–16	33	40		±	+	
	14	3–16	26	37		+	+	
	15		29	40		−	+	
D	16	3–16	31	40	100	+ +	+ +	
	17	2–18	26	35	100	+	+ +	

[a] Animals were sacrificed on days 40, 50, 70, and 100 postinfection in experiments A, B, C, and D, respectively.
[b] p.i., Postinfection.
[c] ND, Not done.

system were similar to those seen on day 40, 50 or 70 in PBS-treated mice.

DISCUSSION

Administration of I-A^s MAb successfully reversed chronic paralysis caused by the WW strain of TMEV. Either weekly treatment beginning 2 weeks postinfection or one injection beginning at the time of onset of chronic paralysis was successful in ameliorating clinical and histopathologic evidence of TMEV infection. Compared with those in PBS-treated mice, viral titers in the spinal cord were unaffected by anti-I-A^s treatment beginning 2 weeks postinfection or at the onset of chronic infection. This marks an example of the successful treatment with anti-I-A antibody of a virus-mediated disease.

TABLE 5. Poliomyelitis in mice treated with anti-I-A^s by protocol 3

Expt[a]	Mouse no.	Recovery from clinical symptom (days after administration of MAbs)	Inflammation	Demyelination	Virus titer (PFU per spinal cord)
A	1	5	ND[b]	ND	5×10^2
	2	5	±	+	
	3	7	+	+	
	4	8	−	+	
	5	13	+	+	
	6	7	+	+	
B	7	6	ND	ND	2×10^2
	8	8	+	+	
	9	6	−	+	
	10	8	−	+ +	
	11	5	±	+	
	12	7	−	+	
C	13	5	ND	ND	3×10^2
	14	6	+	+	
	15	7	−	+	
	16	7	+	+ +	

[a] Animals were sacrificed on days 40, 50, and 70 postinfection in experiments A, B, and C, respectively.
[b] ND, Not done.

Thus far anti-I-A antibodies have reversed several conditions triggered by intentional autoimmunization with self-antigens like EAE after immunization with myelin basic protein (8, 9), experimental autoimmune myasthenia gravis after acetylcholine receptor inoculation (15), experimental autoimmune thyroiditis after thyroglobulin immunization (11), and collagen arthritis after type II collagen injection (8). Anti-I-A antibodies have been used successfully to treat two spontaneously occurring autoimmune conditions, specifically the lupuslike disease in NZB/W mice (1) and the diabeteslike condition in BB rats (2).

A recent study by Rodriguez and colleagues also demonstrates the successful treatment of TMEV-induced demyelination with anti-I-A MAbs (6c). In the report by Rodriguez et al. (6c), demyelination was decreased by giving the anti-I-A MAb either 1 day before and 1 day after the time of virus inoculation or after inflammation in the spinal cord was established. Their results with the DA strain of TMEV stand in contrast to our findings with administration of anti-I-A^s MAb before inoculation with the WW strain of TMEV. In our experiments, mice died within 4 to 6 days of overwhelming encephalitis when pretreated with anti-I-A^s MAb. Our differing results are likely due to differences in virulence between the DA and WW strains of TMEV, especially in the initial polioencephalitic stage of disease.

Our results raise a cautionary note in the use of MAbs to suppress immune responses related to infectious agents. Neutralizing antibodies to TMEV are detectable by 1 week postinoculation (5), and these antibodies may play a role in the immune response to acute TMEV infection. We have demonstrated that treatment with the doses of anti-I-A MAb used in these experiments causes a profound depression of splenic and lymph node IgM-positive, IgD-positive B cells

within 48 hours of treatment (13). This B-cell depletion may permit overwhelming, unchecked TMEV proliferation. In addition to the depletion of B cells, treatment with anti-I-A MAb may interfere with macrophage-mediated viral clearance as well as with the activation of T cells mediating DTH responses. The latter influence, decrease in the activation of TMEV-specific DTH-responding cells, may be critical for the attenuation of inflammation and demyelination in the chronic, persistent phase of TMEV-mediated disease.

Anti-I-A treatment impairs antibody responses of the IgG1 isotype more so than IgG2a or IgG2b responses to common haptens like dinitrophenol and to a common carrier like keyhole limpet hemocyanin (13). Despite this impairment, no evidence of suprainfection has been noted in mice treated with anti-I-A antibodies for periods as long as 3 to 6 months (1, 8). It is noteworthy that recovery of B-cell numbers in lymphoid organs as well as restoration of normal $Ly1^+$ $Ly2^-/Ly1^+$ $Ly2^+$ ratios occurs 50 to 60 days after a single treatment with anti-I-A. The relapses seen in protocol 2 occur at the time of restoration of normal T-cell subset ratios and B-cell levels (13). This return of normal numbers of immune T and B cells may herald recurrent inflammation.

Recently we have demonstrated that depletion of I-A-restricted $L3T4^+$ cells with a MAb led to reactivation of chronic toxoplasmosis with resultant overwhelming infection and death (12). Unlike treatment with anti-L3T4 MAb, anti-I-A treatment does not impair anamnestic antibody responses, whereas it suppresses immune responses in an allele-specific manner in heterozygotes (7). Thus, in an F_1 ($H\text{-}2^k \times H\text{-}2^b$) mouse, anti-$I\text{-}A^k$ antibodies inhibited (H,G)-(A--L) responses linked to $I\text{-}A^k$ but did not reduce anti-(T,G)-(A--L) responses linked to $I\text{-}A^b$. Since the immune response to most infectious agents is not restricted to a limited number of class II alleles, the probability of suppressing critical immune responses to pathogens with anti-I-A antibodies in heterozygotes seems low. Although anti-I-A treatment has shown a great deal of promise in treating autoimmune conditions, careful attention must be given to the possibility of acute infection during periods when the side effects of anti-I-A therapy are maximal.

These experiments underscore the critical role of I-A-linked immune responses in TMEV-induced infection. Susceptibility to demyelination induced with the WW strain of TMEV mapped to the class II region of the MHC, encoding I-A molecules. This stands in contrast to susceptibility to TMEV-induced demyelination caused by the DA strain which maps to a gene locus within the 3′ end of the *H-2D* gene (6d). Since SJL/J mice express only $I\text{-}A^s$ class II MHC products, we chose to treat these WW-infected animals with anti-$I\text{-}A^s$. The probable sites of action of anti-I-A MAb in suppressing paralysis, inflammation, and demyelination include blockade of activation of L3T4-positive, I-A-positive restricted T cells mediating DTH. These T cells may react to TMEV antigens in infected cells in the central nervous system and then destroy neighboring tissue, the so-called "innocent bystander" effect. Alternatively the TMEV may share sequence homologies with myelin antigens. By mounting a T-cell response to certain TMEV epitopes shared with myelin, the DTH-responding T cells may then inadvertantly attack myelinated structures, injuring them as a consequence of molecular mimicry (8a). We have isolated peptide sequences in myelin basic protein that define the epitope recognized by T-cell clones that cause demyelination (8a, 16a). Whether these sequences are homologous to sequences in TMEV protein will become apparent when the full sequence of TMEV is known.

These experiments demonstrate the utility of anti-I-A antibodies for treatment of autoimmunelike conditions triggered by viruses. It has been demonstrated that immunosuppressive drugs like cyclophosphamide as well as antithymocyte serum were effective in treating TMEV-induced disease (6a, 6b, 6e). The side effects of antineoplastic drugs and antilymphocyte sera limit their usefulness.

In contrast, anti-I-A antibodies are effective in treating diseases where susceptibility is linked to class II MHC genes. These diseases include those triggered by autoimmunization like EAE (8, 9), EAMG (15), EAT (11), and collagen arthritis (16), those due to unknown etiologies like NZB/W lupuslike disease (1) or diabetes in BB rats (2), and now virus-mediated disease like the chronic demyelinating condition caused by TMEV. The similarities between TMEV and the human demyelinating disease multiple sclerosis suggest that treatment of multiple sclerosis may be practical with MAbs to class II MHC gene products associated with disease susceptibility.

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