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In vivo treatment with anti-α_4 integrin suppresses clinical and pathological evidence of Borna disease virus infection

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Abstract

Borna disease virus (BDV) infection of the rat brain induces a severe T-lymphocyte mediated inflammatory response that parallels the course of clinical Borna disease. In other models of CNS inflammation, the recruitment of T-lymphocytes from the circulation to sites of inflammation is believed to be directed, in part, by the cellular adhesion molecules α_4 β_1 integrin (expressed on T-lymphocytes) and its ligand VCAM-1 (expressed on blood brain barrier endothelium). Since BDV-specific T-lymphocytes are known to express the α_4 β_1 integrin, we examined the effect of in vivo treatment with an anti-α_4 integrin monoclonal antibody (GG5/3) on the development of BDV-specific encephalitis and Borna disease. Here, we report that the inhibition of α_4 integrin provided significant clinical benefit in slowing the progression of Borna disease. Antibody treatment greatly reduced the immune cell infiltrates in the CNS of BDV-infected animals, but we found that this inhibition of the immune response did not result in enhanced viral levels. © 1998 Elsevier Science B.V.

Keywords: α_4 Integrin; Disease; Inflammation; Virus

1. Introduction

The migration of lymphocytes from the peripheral blood across the blood brain barrier to the site of encephalitis is prerequisite to the development of several central nervous system (CNS) inflammatory diseases. Studies using experimental allergic encephalomyelitis (EAE), an experimentally induced demyelinating disease of the CNS (O'Neill et al., 1991; Raine et al., 1990; Yednock et al., 1992; Baron et al., 1993; Steffen et al., 1994) and lymphocytic choriomeningitis virus (LCMV) infection (Christensen et al., 1995) models demonstrate that T-lymphocyte entry into the CNS is mediated by cell adhesion molecules.

Cell adhesion molecules are cell surface receptors involved in the direct binding of one cell to another (Long, 1992). The integrin and the immunoglobulin super gene families of adhesion molecules have been shown to be key in CNS lymphocyte trafficking (Hemler, 1990; Springer, 1994; Issekutz, 1992). The integrin group of adhesion molecules are heterodimers composed of non-covalently linked α and β chains (Hemler, 1990). There are multiple families of integrins, members of which share a common β chain. Two prominent adhesion molecules present on the surface of most circulating T-lymphocytes are α_4 β_1 integrin (VLA-4) and α_L β_2 integrin (LFA-1) (Shimizu et al., 1990; Shimizu and Shaw, 1991), their respective ligands are vascular cell adhesion molecule (VCAM-1) and intercellular cell adhesion molecule (ICAM-1), members of the immunoglobulin supergene family present on the surface of endothelial cells (Elices et al., 1990; Carlos et al., 1990; Shimizu et al., 1992).

The upregulation of cell adhesion molecule expression on endothelium during EAE or LCMV infection in vivo (Osborn, 1990; Cannella et al., 1991; Christensen et al., 1995) and the ability of anti-α_4 integrin antibodies to prevent the development of inflammation in these models (Yednock et al., 1992; Baron et al., 1993; Christensen et al., 1995) has led to the following proposed model: antigen-primed T-lymphocytes randomly leave the circulation and enter the CNS, where, by chance, they encounter their specific antigen. This interaction leads to a release of cytokines from T-lymphocytes resulting in the upregula-

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tion of appropriate adhesion molecules, thereby recruiting more effector cells and lymphocytes to the local area (Christensen et al., 1995; Baron et al., 1993). Although the majority of recruited cells are nonspecific, a percentage of the cells will be responsive to antigens presented at the inflammatory site. Thus, non-activated T-lymphocytes can be recruited to the site of inflammation in an antigen-independent manner, consistent with the observation that most T-lymphocyte infiltrates in CNS tissue are naive cells (Wekerle et al., 1986, 1987; Cross et al., 1990; Hickey et al., 1991).

Like EAE and LCMV, Borna disease virus (BDV) causes a severe T-lymphocyte mediated meningoencephalitic response in the brain (Stitz et al., 1995). BDV, an 8.9 kb negative strand RNA virus, produces sporadic but fatal neurological disease in horses and sheep (Rott and Becht, 1995). Experimentally, BDV persistently infects a broad spectrum of species ranging from chickens to primates, and, possibly, humans (Waltrip et al., 1995; Bode et al., 1995; Kishi et al., 1995). BDV is a non-lytic virus, and, Borna disease, for the most part, is due to the immune response to BDV antigens, rather than direct effects of BDV damage to the brain.

Because of the immunopathogenic similarity of BDV encephalitis to EAE, along with a recent report demonstrating that activated, BDV-specific T-lymphocytes express the α_4 integrin (Planz et al., 1995), we sought to investigate the contribution of the α_4 integrin to Borna disease and BDV-specific encephalitis in vivo. In addition, these studies were designed to test the potential usefulness of in vivo therapy with anti-α_4 integrin antibody in preventing immune mediated CNS damage following viral encephalitis.

2. Materials and methods

2.1. Inoculation of rats

On day 0, four-week old inbred male Lewis rats (Harlan, Indianapolis, IN) ($n = 33$) were inoculated with 2×10^4 $TCID_{50}$ of BDV stock (strain CRP_3), or sham inoculated ($n = 8$) with an equal volume of uninfected material intracranially. On days 14 and 18 post infection (p.i.), one group of BDV infected rats ($n = 15$) received an injection (intraperitoneally) of 1.0 mg of the anti-α_4 integrin MAb GG5/3 prepared as previously described (Keszthelyi et al., 1996).

All rat experimentation conformed to the National Resource Council's Guide for the care and use of laboratory animals.

2.2. Borna disease

On days 26 and 30 post BDV-inoculation, BDV-infected and BDV-infected/MAb-treated rats were weighed and examined for incidence and severity of Borna disease. Severity of disease was assessed in a blinded fashion and ranked on a 0 to 4 scale as follows: (0) no disease, (1 +) early evidence of disease (e.g., lack of grooming, increased activity), (2 +) definite hyperactivity, (3 +) signs of neurologic disease (e.g., ataxia, paresis, but mobile, eating and hydrated), (4 +) severe disease (e.g., paralysis, immobile, unable to eat or drink, moribund).

2.3. Borna disease virus encephalitis and viral distribution

On days 26 and 30 post BDV-inoculation, a representative set of three BDV-infected, five BDV-infected/MAb-treated, and two sham-infected rats were deeply anesthetized. The brain was removed aseptically and sagittally divided. One half of the brain was saved for viral titer and the other half was fixed in 4% paraformaldehyde, paraffin embedded and cut sagittally into 8-μm thick sections. Following haematoxylin and eosin staining, the severity of the encephalitic response was scored in a blinded fashion as follows: (0) normal, (1 +) one to two layers of inflammatory infiltrates per perivascular cuff, focal; (2 +) one to two layers of inflammatory infiltrates per perivascular cuff, widely distributed; (3 +) three or more layers of inflammatory infiltrates per perivascular cuff, focal; (4 +) three or more layers of inflammatory infiltrates per perivascular cuff, widely distributed throughout brain.

To examine viral distribution in the brain, sections were stained by avidin–biotin immunohistochemistry (Vector, Burlingame CA) using a polyclonal mouse anti-BDV antibody followed by biotinylated anti-mouse IgG (Vector) as described previously (Carbone et al., 1987).

2.4. Infectivity assay

Infectivity in brain homogenates was determined by immunofluorescence of BDV-specific antigens in inoculated cultures of C6 cells as described earlier (Carbone et al., 1987).

3. Results

3.1. Reduction in prevalence of clinical Borna disease following anti-α_4 integrin monoclonal antibody treatment

By day 26 p.i., anti-α_4 integrin MAb treatment was associated with a reduction in clinical Borna disease. Borna disease was present in 72% (13/18) of the BDV-infected rats and only 33% (5/15) of the BDV-infected/MAb-treated rats. By day 30 p.i., 80% of the BDV-infected rats (12/15) and 50% of the BDV-infected/MAb treated rats (5/10) displayed signs of Borna disease. None of the uninfected control rats showed signs of disease.

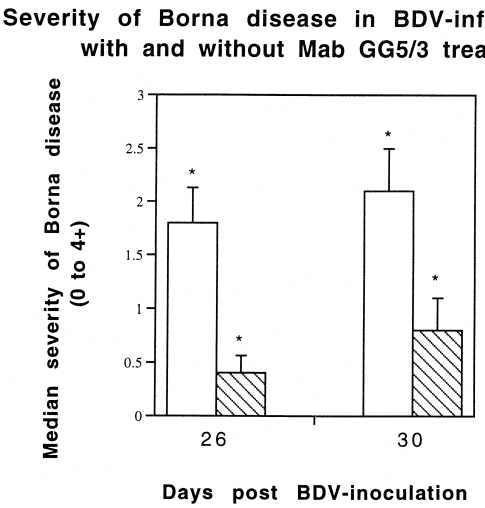

Fig. 1. Reduction in the severity of Borna disease (rated on a 0 to 4+ scale) in BDV-infected rats treated anti-α_4 integrin MAb (hatched bars) compared to untreated BDV-infected rats (open bars) on days 26 and 30 post BDV-inoculation. $^{*}P < 0.05$.

3.2. Reduction in severity of Borna disease following anti-α_4 integrin monoclonal antibody treatment (Fig. 1)

By day 26 p.i., anti-α_4 integrin MAb treatment was associated with a reduction in the severity of Borna disease. The severity of disease decreased from 1.8 + (range: 0 to 4 + ; SEM 0.329; $n = 18$) in the BDV-infected rats to 0.4 + (range: 0 to 2 + ; SEM 0.163; $n = 15$) in the BDV-infected/MAb treated group ($P < 0.05$). By day 30 p.i., anti-α_4 integrin MAb treatment continued to protect BDV-infected rats from developing severe Borna disease. The severity of disease decreased from 2.1 + (range: 1 + to 4 + ; SEM 0.4; $n = 14$) in the BDV-infected group compared to 0.8 + (range: 0 to 2 + ; SEM 0.3; $n = 10$) in the BDV-infected/MAb treated group ($P < 0.05$).

3.3. Protection from body weight gain inhibition following anti-α_4 integrin monoclonal antibody treatment (Fig. 2)

Body weight gain inhibition in BDV-infected rats has been reported as a measure of disease progression (Richt et al., 1991; Bautista et al., 1995). On day 26 p.i., there was no significant difference between the BDV-infected rat's mean weight of 151 g (range: 114 g to 180 g; SEM 20; $n = 3$) and BDV-infected/MAb-treated rat's mean weight of 183 g (range: 136 g to 211 g; SEM 14; $n = 5$) ($P = 0.2$). However, by day 30 p.i., a significant effect of anti-α_4 integrin MAb treatment in limiting BDV-induced weight gain inhibition was observed. The BDV-infected group had a mean weight of 122 g (range: 96 g to 155 g; SEM 17; $n = 3$) compared to a mean weight of 194 g (range: 164 g to 222 g; SEM 10; $n = 5$) in the BDV-infected/MAb-treated group ($P < 0.05$). During these time points the uninfected control rats continued to gain weight with mean weights of 214 g ($n = 2$) on day 26 p.i. and 229 g ($n = 2$) on day 30 p.i.

3.4. Reduction in the severity of encephalitis following anti-α_4 integrin monoclonal antibody treatment (Fig. 3)

The degree of encephalitis was rated by microscopic examination of haematoxylin and eosin stained sections of paraffin embedded brain tissue. On day 26 p.i., the BDV-infected rats had a mean encephalitis score of 2.7 + (range: 2 + to 3 + ; SEM 0.33; $n = 5$) compared to a much reduced rating of 1.2 + (range: 1 + to 2 + ; SEM 0.2; $n = 3$) in the BDV-infected/MAb treated group ($P < 0.05$). By day 30, the mean severity of encephalitis in the BDV-infected rats increased to 3.3 + (range: 3 + to 4 + ; SEM 0.33; $n = 3$) (Fig. 3A), whereas the mean severity of encephalitis in the BDV-infected/MAb treated rats remained unchanged at 1.2 + (range: 1 + to 2 + ; SEM 0.2; $n = 5$) (Fig. 3B) ($P < 0.05$). None of the uninfected control rats showed evidence of encephalitis (Fig. 3C).

3.5. Viral titer and BDV protein distribution

BDV protein expression was detected in brain sections of all rats in the BDV-infected and BDV-infected/MAb treated groups by immunohistochemistry. BDV proteins were not detected in any of the uninfected rats (data not shown).

A comparison of viral titers with and without anti-α_4 integrin MAb treatment showed that the reduction in encephalitis did not enhance production of infectious BDV, as no statistically significant differences in viral titer were

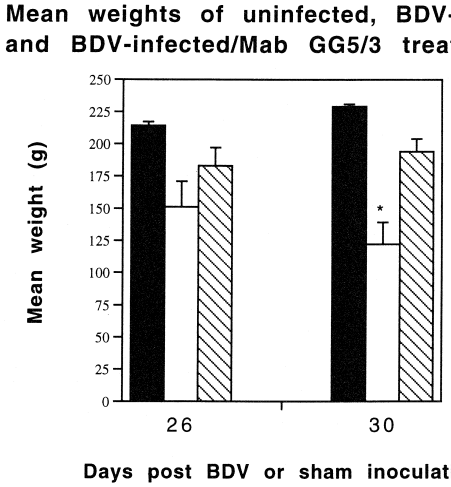

Fig. 2. Mean weights (g) of uninfected (solid bars), BDV-infected (open bars) and BDV-infected/MAb treated (hatched bars) rats on days 26 and 30 post BDV-inoculation. Note that on day 26, weights were similar among the three groups of rats. By day 30, however, the BDV-infected rats suffered significant weight gain inhibition while the BDV-infected/MAb treated rats showed no significant change from normal control rats. $^{*}P < 0.05$.

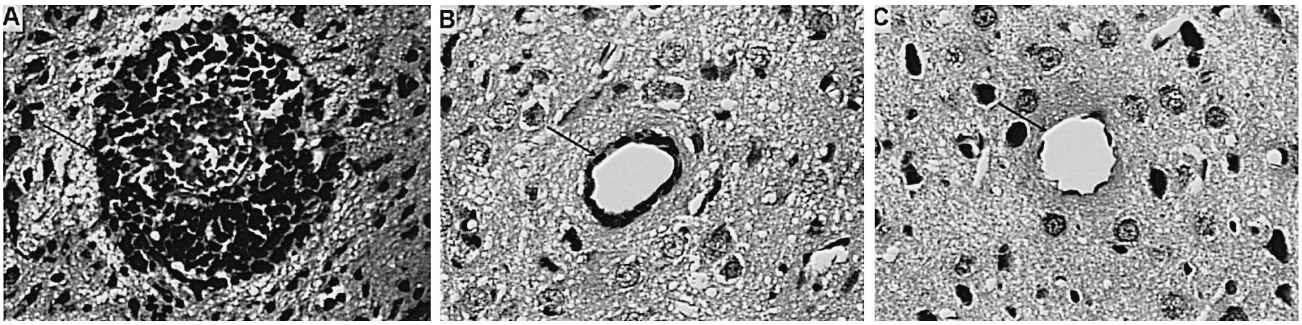

Fig. 3. Reduction in brain inflammatory response to BDV in rats treated with an anti-α_4 integrin monoclonal antibody (day 30 post BDV-inoculation). (A) BDV-infected rat brain showing extensive encephalitis; (B) BDV-infected rat brain showing a reduction in encephalitis following anti-α_4 integrin MAb treatment; (C) uninfected rat brain control without encephalitis. Arrows show blood vessel. Hematoxylin and eosin stain; magnification, $\times 200$.

seen between the two groups of rats. On day 26 p.i., a mean of 3.8×10^4 tissue culture infectious dose 50 ($TCID_{50}$) of BDV was detected in the brains of the BDV-infected rats, as compared to a mean titer of 1.2×10^4 $TCID_{50}$ in the BDV-infected/MAb-treated rats, ($P = 0.32$). Likewise, on day 30 p.i., a mean of 1.5×10^4 $TCID_{50}$ of BDV was detected in the brain of the BDV-infected rats as compared to a mean of 1.3×10^4 $TCID_{50}$ in the BDV-infected/MAb treated rats, ($P = 0.83$)

Finally, no qualitative differences in viral antigen distribution were observed in the brains of BDV-infected rats as compared to BDV-infected/MAb treated rats (data not shown).

4. Discussion

Several studies have shown that α_4 integrin plays a prominent role during inflammation of the CNS (Yednock et al., 1992; Baron et al., 1993; Steffen et al., 1994; Christensen et al., 1995). Although many reports have investigated the role of α_4 β_1 integrin in endothelial extravasation of T-lymphocytes to sites of inflammation (Shimizu et al., 1992; Meerschaert and Furie, 1995; Faull and Ginsberg, 1994; Sato et al., 1995; Issekutz, 1991, 1992; Alon et al., 1995), very few have examined the in vivo contribution of α_4 integrin to inflammation during active virus infection. Because of these findings and the detection of α_4 integrin expression on BDV-specific inflammatory T-lymphocytes (Planz et al., 1995), we examined and report here the effect of anti-α_4 integrin monoclonal antibody treatment on BDV-induced encephalitis and Borna disease in the infected rat in vivo.

Using the Lewis rat model of BDV, we found that the intraperitoneal administration of the anti-α_4 integrin MAb (GG5/3) after infection with BDV, and prior to onset of Borna disease, reduced clinical signs of Borna disease, encephalitis and weight gain inhibition. On day 26 p.i., the majority of BDV-infected rats showed signs of Borna disease while the majority of the infected rats treated with MAb GG5/3 were symptom-free. Not only was the overall incidence of Borna disease reduced in association with anti-α_4 integrin MAb treatment, but there was also a reduction in disease severity. By day 30, rats in the BDV-infected/MAb treated group still showed only mild signs of disease (mean disease rating of $0.8+$, and a BDV-induced weight gain inhibition of only 15% compared to uninfected rats), whereas signs of disease continued to progress in the BDV-infected untreated group (mean disease rating of $2.1+$ and a BDV-induced weight gain inhibition of 47%). In EAE, the protective effects of anti-α_4 integrin subside in the absence of continued administration (Keszthelyi et al., 1996). We found the same to be true in the current studies with Borna disease; both clinical and pathological signs of disease eventually returned without repeated antibody injection (data not shown). However, while disease returns rapidly in EAE animals as antibody levels fall, Borna disease returned at a much slower pace with significant benefit still measured 12 days after the final antibody dose. Independent measurements of antibody levels following intravenous administration indicate that the antibody would have been cleared from the circulation within 3 days (Yednock, unpublished data). Thus, as with EAE, it is possible that additional doses of antibody would have afforded an even greater level of protection in Borna disease.

Since Borna disease is largely caused by the inflammatory response to BDV and not by the direct effects of the virus itself, it is reasonable that we could protect rats from immune mediated Borna disease by blocking the α_4 integrin molecules on the surface of T-lymphocytes. We found that this treatment, which inhibits lymphocyte trafficking across the blood brain barrier, resulted in decreased BDV-induced inflammation, and, thus, protection from Borna disease.

Many CNS viral infections are cleared from the brain by the immune system response. In the case of BDV, complete clearance of virus from the CNS is rare and persistent infection is the rule. However, there are reports that protective immunity to BDV that may impose some hindrance to viral replication (Richt et al., 1994). Therefore, it was possible that the use of anti-α_4 integrin

antibodies to suppress the development of an encephalitic reaction in BDV infection might result in enhanced virus replication and increase direct virus-induced damage in the CNS. Our data show that despite the remarkable difference in the degree of encephalitis between BDV-infected and BDV-infected/MAb treated rats, viral distribution and infectious virus titers were equivalent in the brains of both groups of rats. Thus, the lack of a destructive encephalitic response did not result in elevated BDV replication in the brain. These data suggest that, in specific cases of viral encephalitis, treatments designed to block immunopathological immune responses do not necessarily result in enhanced virus replication.

In a medical setting, blocking of α_4 integrins (e.g., by anti-α_4 integrin antibody administration) may be of therapeutic value in preventing immunopathological responses due to viral encephalitis either in conjunction with antiviral drugs in the case of treatable viral encephalitis (e.g., herpes simplex virus induced encephalitis) or in cases where no specific antiviral treatments exists. Thus, the use of this antibody or other anti-α_4 integrin treatments may alleviate adverse symptoms of viral CNS inflammation while pharmacologically treating the infectious agent with anti-viral medication. Moreover, our results suggest a role for the development of small, non-immunogenic molecules as α_4 integrin antagonists for effective long-term treatment of the immunopathogenic response to viral encephalitis.

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