AUTOANTIBODIES EXACERBATE THE SEVERITY OF MHV-INDUCED ENCEPHALITIS

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1. INTRODUCTION

The relationship between autoimmunity and infections by viruses and bacteria is complex. Infectious agents can initiate autoimmune responses by mechanisms such as molecular mimicry or bystander activation (reviewed in Ref.1). In addition, pathogens might also provoke relapses or worsen preexisting autoimmune pathologies. For example, common infections augment both the risk and the severity of relapses in multiple sclerosis (MS) patients.[2]

To study the effects of a viral infection on a preexisting autoimmune background, we have combined the neurotropic strain of mouse hepatitis virus (MHV) and mice that express auto-antibodies to a CNS antigen. MHV A59 provokes acute encephalitis, which is followed in a proportion of the surviving mice by a demyelinating disease that shares several features with MS.[3,4] Litzenburger and colleagues have generated transgenic mice (referred here as anti-MOG Ig mice) that constitutively express auto-antibodies specific of the myelin oligodendrocyte glycoprotein (MOG). These mice show an exacerbated version of experimental autoimmune encephalomyelitis (EAE), but do not develop any spontaneous disease.[5]

2. ANTIBODIES TO MOG AUGMENT THE SEVERITY OF A CNS INFECTION

Intracranial injection of a sublethal dose of MHV A59 resulted in an exacerbation of the clinical disease in mice with MOG-specific antibodies compared with controls (Figure 1, A). The mortality was increased in anti-MOG transgenic mice compared with controls, with 18% vs. 73% survival at day 21, respectively ($p<0.001$, Fisher's log-rank

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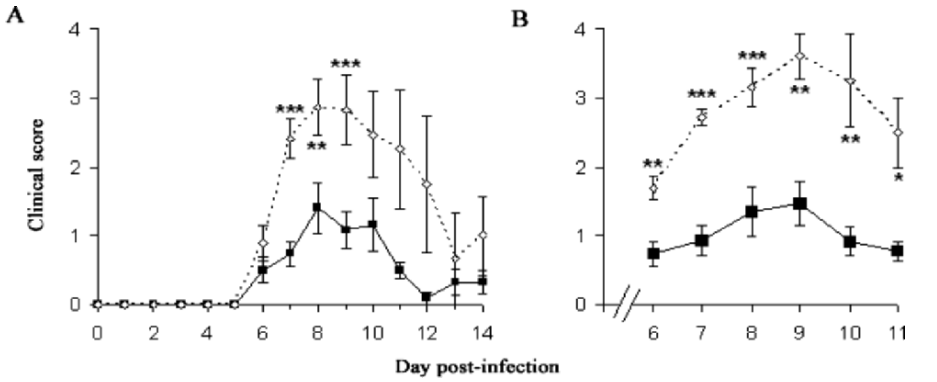

Figure 1. A. C57Bl/6 (solid line, n=15) or transgenic anti-MOG Ig knock-in mice (dashed lines, n=17) mice were infected 10 PFU MHV A59 I.C. Clinical signs of neurological disease were evaluated daily (0, no disease. 1, ruffled fur. 2, hunched posture. 3, lethargy. 4, moribund). B. C57Bl/6 mice received 500 µl of either normal mouse serum (solid line, n=13) or serum from anti-MOG Ig mice (dashed lines, n=16) at the time of infection with 10 pfu MHV A59 I.C. (Mann-Whitney test, **, $p<0.01$, ***, $p<0.001$).

survival test). In addition to the increased clinical scores and mortality, clinical signs were also detected 2 days earlier in the autoantibody transgenic mice than in the controls when a dose of 100 or 1000 pfu was used (data not shown). Injection of UV-inactivated virus (1000 pfu-equivalent) did not trigger any clinical signs in anti-MOG Ig mice, indicating that breaking the blood-brain barrier or the presence of viral antigens was not sufficient to trigger the pathogenicity of the autoantibodies, and that viral replication was required.

We found no significant difference between the viral titers in the CNS of control and MOG-Ig transgenic mice (Table 1), showing that the exacerbated disease could not be attributed to a difference in viral replication. Transfer of a single dose of serum from anti-MOG Ig mice to C57Bl/6 at the time of infection was sufficient to reproduce the clinical disease that was observed in the transgenic animals (Figure 1B), and resulted in a comparable increase of the mortality (13% survival at day 21 in recipients of anti-MOG Ig serum vs. 85% in controls, $p<0.01$). These results confirm that the anti-MOG auto-antibodies account for the exacerbation of the virally-induced CNS disease in our model.

Table 1. Viral titers in brains of infected controls and mice with autoantibodies, as determined by plaque assay.

	Day postinfection		
	3	5	6
C57Bl/6	$6.5*10^4 \pm 5.8*10^4$ [a]	$5.9*10^5 \pm 1.5*10^5$	$2.2*10^7 \pm 7.5*10^6$
Anti-MOG Ig	$1.1*10^5 \pm 4.3*10^4$	$6.6*10^5 \pm 1.7*10^5$	$2.3*10^7 \pm 9.6*10^6$

[a] PFU/g, average±SE, n=3 to 6.

Table 2. Demyelination score in lesions in the brains of anti-MOG Ig and WT mice at day 9 postinfection.

	n	Demyelination score	Range
C57Bl/6	5	1.30±0.53[a]	0-3
Anti-MOG Ig	4	1.25±0.72	0-3

[a] Slides were examined in a blinded fashion, and given a score on a 0 to 4 scale. Mean±SE.

3. MHV-INDUCED EARLY DEMYELINATION IS NOT INCREASED BY ANTI-MOG AUTO-ANTIBODIES

In the MHV A59 model, demyelination can be detected as early as day 7 post-infection in the brains of a subset of mice. As anti-MOG antibodies have been shown to exacerbate demyelination in EAE[5,6] or in MS,[6] we have examined luxol fast blue–stained brain sections obtained from anti-MOG Ig and control mice. Preliminary results do not show any significant difference between the number or importance of demyelinating lesions in the brains of transgenic and control animals (Table 2).

The mechanisms responsible for the augmented virus-induced pathology in mice with CNS auto-antibodies are currently under investigation. We are also trying to determine if the ability to trigger the auto-aggressivity of the auto-antibodies is limited to MHV, or a more general feature of viral infections of the CNS.

Our study illustrates the additive effects of the concomitant presence of autoantibodies to a CNS antigen and a viral CNS infection, each of which, by themselves, is relatively harmless or leads to milder disease (Figure 1), respectively. These findings support the concept that infectious and autoimmune components can act in synergy leading to enhanced disease.

4. ACKNOWLEDGMENTS

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5. REFERENCES

1. Von Herrath, M., Fujinami, R. S., and Whitton, J. L., 2003, Viruses as triggers of autoimmunity – making the barren field fertile, *Nat. Rev. Microbiol.* **1**:151-157.
2. Buljevac, D., Flach, H. Z., Hop, W. C., Hijdra, D., Laman, J. D., Savelkoul, H. F., van Der Meche, F. G., van Doorn, P. A., and Hintzen, R. Q., 2002, Prospective study on the relationship between infections and multiple sclerosis exacerbations, *Brain* **125**:952-960.
3. Lane, T. E. and Buchmeier, M. J., 1997, Murine coronavirus infection: a paradigm for virus-induced demyelinating disease, *Trends Microbiol*. **5**:9-14.
4. Matthews, A. E., Weiss, S. R., and Paterson, Y., 2002, Murine hepatitis virus–a model for virus-induced CNS demyelination, *J. Neurovirol.* **8**:76-85.

5. Litzenburger, T., Fassler, R., Bauer, J., Lassmann, H., Linington, C., Wekerle, H., and Iglesias, A., 1998, B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice, *J. Exp. Med.* **188**:169-180.
6. Genain, C. P., Cannella, B., Hauser, S. L., and Raine, C. S., 1999, Identification of autoantibodies associated with myelin damage in multiple sclerosis, *Nat. Med.* **5**:170-175.