Chapter C5

THE ROLE OF HUMORAL IMMUNITY IN MOUSE HEPATITIS VIRUS INDUCED DEMYELINATION

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Abstract: Pathogenesis induced by mouse hepatitis virus (MHV) infection of rodents is characterized by acute viral encephalomyelitis and demyelination which progresses to a persistent CNS infection associated with ongoing myelin loss, pathologically similar to multiple sclerosis (MS). Although humoral immunity appears redundant for the control of acute virus replication, it is vital in maintaining virus at levels detectable only by RNA analysis. T cell mediated control of acute infection cannot be sustained in antibody (Ab) deficient mice, resulting in virus reactivation. The protective role of Ab during persistence is strongly supported by detection of Ab in the cerebrospinal fluid of MHV infected rodents and maintenance of virus specific Ab secreting cells (ASC) in the CNS long after virus clearance. Ab mediated neutralization constitutes the major mechanism of protection, although fusion inhibition also plays a minor role. Delayed accumulation of ASC, concomitant with a decline in T cell function, assures control of residual virus while minimizing T cell mediated pathology. Although there is little evidence for a detrimental role of Ab in demyelination, an association between Ab mediated protection and remyelination is unclear.

Key words: CNS, demyelination, mouse hepatitis virus, B cells, Antibody.

HUMORAL IMMUNITY AND MULTIPLE SCLEROSIS

MS is a chronic demyelinating disease of the CNS characterized by inflammation and white matter destruction, but of unknown etiology (1). The chief pathological features are focal areas of myelin loss associated with an

inflammatory response consisting of B cells, T cells and macrophages (2). The relative contribution of each of these individual immune components remains poorly understood. A variety of cellular and humoral abnormalities have been observed in MS patients (2). The seminal findings of elevated Ig in CSF (3) as well as humoral involvement in demyelination during experimental allergic encephalomyelitis (EAE) (4) were the first hints implicating humoral components in the demyelinating disease. Increased B cell abundance as well as detection of myelin specific Ab in acute lesions with active ongoing demyelination compared to older, inactive lesions have supported their possible involvement in the disease pathogenesis (5,6). Although T cell studies have predominated this field of research based on the detection of activated T cells in MS plaques and involvement of T cells in EAE, current research in both MS and MOG protein induced EAE has re-ignited interest in the potential role of humoral immunity in the pathogenesis of demyelinating disease (2,7).

One hallmark of MS is that cerebral spinal fluid (CSF) in ~90% of patients is characterized by the presence of oligoclonal immunoglobulin (Ig) bands which exhibit restricted isoelectric focusing points (3,7). The CSF Ig response is sustained by resident B cells not represented in peripheral compartments (8). The IgG sequences feature extensive somatic mutations suggesting active antigen (Ag) driven B cell selection and clonal expansion (8). Oligoclonal bands are also encountered routinely following CNS infections with measles virus, Herpes simplex virus-1 and mumps virus; however, the majority of the Ig are specific for the causal agent (9). Moreover, detailed studies into the clonality of B cells in both CSF and MS plaques has revealed no single dominant Ag target. These Ab express varied Ag specificities from myelin components, oligodendrocyte protein, viruses, cell nuclei, endothelial cells, fatty acids, gangliosides, and axolemma (7,10). Auto Ab could potentially contribute to demyelination by various mechanisms including Ab dependent cell mediated cytotoxicity (ADCC), stimulation of Fc receptors expressed on NK cells or macrophages/microglia resulting in release of inflammatory molecules, opsonization of myelin resulting in phagocytosis by macrophages, or complement activation. Direct evidence of Ab mediated mechanisms in MS lesions was revealed with the detection of IgG deposition on the borders of the actively demyelinating plaques along with the presence of activated complement fragments and complexes (11). Ab eluted from MS plaques preferentially bind to CNS self Ag (7), although this reactivity to a variety of CNS Ag could also be due to liberation of Ag as a consequence of inflammatory tissue destruction. It thus appears that a concerted action of humoral and cellular factors could participate in the induction and/or maintenance of an inflammatory response specific for CNS Ag.

MHV PATHOGENESIS

Infection of the CNS with MHV produces an acute encephalomyelitis associated with a focal loss of myelin. Virus replicates in various CNS cell types including astrocytes, oligodendroglia and microglia / macrophages. Distinct mechanisms of CD8 T cell mediated control are required to clear virus from the CNS as described elsewhere in this book (12,13). As virus replication is controlled, focal myelin loss however, is increased, suggesting a temporal lag between the maximal number of virus infected cells and the loss of myelin. The inability of cell mediated immunity to completely eliminate virus results in a non-productive persistent infection confined to the CNS, predominantly in spinal cord oligodendroglia. Persistent infection is associated with ongoing myelin loss and repair. These traits make MHV infection of the CNS along with Theiler's murine encephalitis virus infection and EAE the three primary murine models to study mechanisms underlying both encephalomyelitis and demyelination (12,13).

HUMORAL IMMUNITY AND ACUTE INFECTION

Analysis of viral pathogenesis in mice depleted of T cell subsets, distinct T cell functions or humoral components demonstrated that acute infection by MHV-JHM strain (JHMV) is controlled by cellular immune responses (12,13). Humoral responses are redundant during acute infection as shown by initial virus clearance in the CNS of B cell deficient mice with similar kinetics to wt mice (14,15). Unlike the almost complete clearance of JHMV which expresses the immunodominant spike protein derived S510 epitope, infection with the MHV-A59 strain, which lacks this CTL epitope revealed minimal clearance in the B cell deficient mice, thus emphasizing the dominant role of cellular immunity (16). Similarly, mice deficient in T cells were unable to clear virus (12,13). Consistent with a redundant early role of humoral immunity, examination of serum anti-viral Ab titers showed that IgM and IgG are detectable only after the majority of the infectious virus has already been cleared from the CNS (12,17). Moreover, examination of the two biological activities of the Ab response directed at the spike protein, i.e., neutralization and fusion inhibition (17), determined that while neutralizing Ab was initially detectable by day 10 p.i., when most of infectious virus had

already been cleared from the CNS, fusion inhibiting Ab was not detected until much later (day 21 p.i.). Thus the delayed appearance of serum Ab relative to virus clearance further supported a minor, if any role of Ab early in the adaptive host response to acute infection.

Transfer of monoclonal Ab specific for different virus structural proteins including spike, nucleocapsid and matrix proteins prior to or concomitant with JHMV infection nevertheless resulted in protection from virus induced mortality, but was not always associated with reductions in virus replication (12,18-20). The mechanisms of protection by pre-established Ab may merely reside in neutralization of the bolus of infectious virus prior to establishing CNS infection. Alternatively, Ab may modify tropism by prevention of infection of critical cells specifically neurons, thereby increasing survival rates (18,19). In other models, suckling mice weaned on immunized dams or transgenic mice which express neutralizing IgA in the milk are protected from acute JHMV induced encephalomyelitis; however, the role of Ab in these models is unclear (21,22). A variable percentage of protected weanling mice exhibit a delayed onset of acute encephalitis associated with demyelination. However, delayed onset with progressive disease is associated with CTL escape variants rather than Ab escape mutants or the loss of Ab mediated protective mechanisms (21).

Irrespective of the apparent redundancy of Ab in controlling acute infection, flow cytometric analysis of CNS inflammatory cells from infected wt mice revealed that mature B cells ($CD19^+/sIg^+$) are rapidly recruited into the CNS along with T cells (12,17). Furthermore, following the peak of virus replication, a low but constant percentage of B cells (5-7%) is retained in the CNS during viral persistence (12,17). A potential Ab independent, innate anti-viral effector function of B cells *in vivo* was initially suggested by the observation that B cells from naïve mice exhibit the ability to lyse virus infected target cells *in vitro*. Lysis is mediated through an interaction between viral receptor expressed at high levels on B cells and the viral spike protein expressed on the surface of infected cells (23). This unique cytolysis is inhibited by Ab specific for the viral spike protein, which *in vivo* is detected only after CNS virus replication has been initially controlled. Hence the absence of Ab early during infection could allow B cells to interact with virus infected cells *in vivo*, thereby contributing to viral control. However, convincing evidence for this mechanism was negated by studies in transgenic mice containing B cells unable to secrete anti-viral Ab (15). These mice cleared virus with similar kinetics as B cell deficient mice, excluding an effect of innate B cell function *in vivo*.

B cells are recruited to and activated in secondary lymphoid organs following infection (24,25). It has been shown that Ag within the CNS drains to cervical lymph nodes (CLN), which are the major sites of B cell

activation following intrathecal administration of Ag (25). However, virus specific Ab secreting cells (ASC) appear in the spleen prior to detection in the CLN (17). While there was no major increase in virus specific IgM ASC, the frequencies of IgG ASC increases almost four fold in the CLN compared to spleen suggesting that the majority of initial activation occurs in the spleen, followed by transient CLN accumulation. No virus specific ASC were observed in blood, consistent with previous data suggesting that ASC do not migrate by this route (25). It is assumed that activated B cells/plasmablasts traffic to the target organ containing Ag where they differentiate to plasma cells and secrete Ab (25). Recruitment kinetics of anti-viral ASC into the CNS revealed that low frequencies detected prior to maximal viral replication increased only slightly as virus replication was controlled (17). Thus, based on: 1) the inability of B cells to express detectable anti-viral activity, 2) the paucity of plasma cells in the peripheral lymphoid compartments at any time post infection and 3) the appearance of serum Ab and virus specific ASC within the CNS following viral clearance, humoral immunity appears to have play a redundant role during acute MHV infection of the CNS.

HUMORAL IMMUNITY AND VIRAL PERSISTENCE

Humoral immunity protects the CNS from infection by prevention of virus dissemination from peripheral sites. However, if CNS infection is already well established prior to induction of Ab responses, Ab can help further reduce infectious virus (26) or maintain viral persistence at non-detrimental levels (17). The beneficial effect of an early Ab response was demonstrated in the rat model of JHMV infection. Resistant Norway rats exhibit a more rapid and robust neutralizing Ab response, determined by detection of serum and CSF Ab and ASC compared to susceptible Lewis rats (27). Incomplete virus clearance in B cell deficient mice at days 12-14 p.i. support that neutralizing Ab, which first appears by day 10 p.i., indeed may act in concert with T cells during the later stages of acute infection to completely eradicate infectious virus from CNS, particularly as resident CD8 T cells lose their CTL activity (15,28). This prompted detailed analysis of the emergence of ASC relative to the decline in T cell activity within the CNS. Virus specific ASC were barely detectable in the peripheral compartments following virus clearance in mice (17). Furthermore, despite early recruitment of ASC into the CNS, only a minority was virus specific (17). However, the limited recruitment of virus specific ASC into the CNS

towards the end of the acute phase, at days 10-12 p.i., was followed by a dramatic increase, which continued up to day 21 p.i. While there was a slight decrease in virus specific IgM ASC, frequencies of IgG ASC in the CNS remained constant. The majority of the ASC at this time point were of the IgG2b isotype indicating massive expansion of this subtype specific Ab, with fewer numbers of IgG2a and IgG1 ASC. Preferential expansion of ASC during this period of infection, concomitant with down regulation of CTL activity and inflammation, implies a major role for ASC in CNS during both the terminal phase of acute infection and during chronic infection.

Virus specific ASC are retained in the CNS, although there is a decline following the peak of expansion. The relatively long-term presence of ASC accompanied by the decline of other inflammatory cells, including T cells and macrophages, in addition to reactivation of virus in B cell deficient mice enforce the role of Ab in controlling viral persistence in the CNS. Following virus clearance from the CNS in wt mice, viral Ag can be detected beyond day 35 p.i. and viral RNA for up to a year (13). Virus specific ASC were found at higher frequencies in the CNS compared to bone marrow or any other peripheral compartment even at 90 days p.i. (Tschen & Stohlman, unpublished). This is in contrast to detection of plasma cells predominantly in the bone marrow following resolution of peripheral infections (24). The persistence of plasma cells within the CNS appears to be a common trait of many viral CNS infections (26,29). While the majority of Ag is cleared from the brain during persistence, viral Ag and RNA are detectable for longer periods of time in the spinal cord (13). Spinal cords also exhibited increased ASC by histology compared to brain, thereby signifying a causal relationship between prolonged retention of ASC and viral Ag / RNA (16). A variety of cellular and soluble factors could also contribute to the survival of these cells in the CNS: cytokines including TNF-α, interleukins 1, 2, 4, 6 and 10, nerve growth factor, or stimulation with CD40L on T cells and Fc receptors on microglia / macrophages. The possible presence of chemokines such as SDF-α, MIP-3β or BLC could also contribute towards long-term retention of plasma cells in the brain. Although trapping of B cells cannot be excluded, these data suggest that the CNS provides a nourishing microenvironment that facilitates retention and maintenance of ASC.

Neutralizing Ab is maintained at relatively high levels in the serum beyond 90 days p.i. (Tschen, unpublished). Given the short half life of Ab, maintenance of Ab secretion may be attributed to one or a combination of factors: (i) re-exposure to Ag due to persistent low grade chronic viral infection; (ii) structural homologies of viral and CNS Ag generating cross reactive responses; (iii) persistent Ag presentation by dendritic cells; (iv) presence of long lived plasma cells in either bone marrow or the CNS. Work

is ongoing to find a causal relationship between long-lived plasma cells and possible sustaining factors, viral control, and demyelination.

Virus recrudescence in the CNS of Ab deficient mice strongly implies a more crucial protective function of persisting ASC versus T cells during chronic infection (14-17). Virus reactivation is accompanied by sustained clinical disease and high mortality rates in contrast to recovery of wt mice that had cleared infectious virus (14-16). A similar picture was observed in transgenic mice, which contained B cells that could not secrete anti-viral Ab (15,16). Following initial virus clearance, virus reactivated in the CNS of the transgenic mice by day 14 p.i. albeit at a slower, but statistically insignificant rate compared to B cell deficient mice, thus ruling out a prominent role of innate B cell effector function (15,16). In addition to multiple Ag positive cells compared to wt mice, histological examination suggested differential Ag tropism in the transgenic mice containing B cells as compared to B cell deficient mice during reactivation (15). While Ag was detected in all CNS cell types in B cell deficient mice, similar to acute infection, the transgenic mice containing B cells harbored Ag predominantly in oligodendrocytes. Preferential elimination of virus from astrocytes and microglia, suggests either B cell lytic activity during persistence or increased class II mediated stimulation of virus specific CD4 T cells. Irrespective of potential B cell mediated lysis in transgenic mice, this mechanism is irrelevant in wt mice, which have neutralizing Ab in the CNS at this time (15). Oligodendroglial tropism in these mice is reminiscent of the resistance of these cells to perforin-mediated cytolysis (30). However, it appears that following initial virus clearance, the CNS environment preempts further T cell mediated antiviral effects. There is no evidence for either increased CD8 T cell recruitment or re-expression of CTL activity in response to reactivation in Ab deficient mice (15,28). Such an altered CNS environment, likely resulting from T cell mediated or virus induced pathology, emphasizes the necessity for alternative, less destructive modes of protection during recovery, such as provided by Ab.

These observations are reinforced by protection of B cell deficient mice treated with polyclonal neutralizing Ab at time points simulating the appearance of serum neutralizing Ab in wt mice following i.c. inoculation (14-16). Ab transfer reduced virus to almost undetectable levels in recrudescing recipients at 21 days p.i., compared to high titers in the CNS of control mice. These results in B cell deficient mice, in conjunction with the burst of virus specific ASC in wt mice between days 12 and 21 p.i., clearly suggested that control of viral persistence in glial cells is solely dependent on anti-viral Ab. Further investigation of the mechanisms involved in Ab mediated protection revealed that only neutralizing Ab and to a limited

extent, fusion inhibiting Ab could reduce virus reactivation (31). Monoclonal Ab specific for matrix, nucleocapsid protein or even spike protein with no neutralizing activities were unable to suppress virus reactivation (31). Suppression of virus reactivation appears to require the constant presence of Ab, as virus reemerged following discontinuation of Ab administration resulting in increased mortality rates. The transient nature of virus control is comparable to other infectious models wherein immunocompromised mice required continuous infusion of Ab to control virus replication (26,32).

Other humoral components do not appear to play a major role in virus clearance. Analysis of MHV infection in mice devoid of complement (C3) revealed no role for complement in virus clearance (16,18). Furthermore, complete clearance of virus in FcR deficient mice (16) and the ability of neutralizing F(ab)2 fragments to afford protection (33) indicate a minor role for ADCC mechanisms in virus clearance, although each mechanism may suffice to make individual contributions.

ANTIBODY AND DEMYELINATION

Despite the presence of oligoclonal Ig bands in CSF of MS patients, there is little evidence for the involvement of Ab responses during most experimental virus induced demyelinating diseases. Robust demyelination is induced by both JHMV and MHV-A59 strains in B cell deficient mice as well as transgenic mice containing B cells, but deficient in virus specific Ab (14-16). These data support multiple studies implicating T cells and macrophages/microglia as the prominent players in the initiation of demyelination during MHV infection (12,13). However demyelination in Ab deficient mice does not exclude other humoral mechanisms, including complement and FcR, as contributors to the demyelinating process. Furthermore, infection of nude mice demonstrated the presence of demyelinating plaques albeit to a lesser degree than wt mice, supporting a role for humoral immune responses in the demyelinating process (34). Infected SCID and RAG-/- mice, deficient in both B and T cells, by contrast, do not develop demyelination. Adoptive transfer of splenocytes from immunized nude mice into infected SCID mice however failed to induce demyelination (34), leaving a pathogenic role for B cells controversial. Nevertheless, a pathogenic role for humoral immunity was also suggested by the detection of IgG, complement (C9) deposits and plasma cells in areas of actively demyelinating plaques in Lewis rats (35). While an Ab-dependent role in initiation of demyelination is not established, the presence of ASC with viral as well as yet unidentified specificities in the CNS does not exclude a possible involvement in the demyelinating process. Following Ag

clearance in wt mice, demyelination is reduced compared to increasing levels of demyelination in B cell deficient mice (14,15). Whether this is due to higher viral load or absence of B cells or Ab is unclear. Although the effect of Ab on remyelination has not been studied in this model, Ab mediated control of viral recrudescence and thereby prevention of further virus induced cell death, results in less severe demyelination in wt mice as compared to infected B cell deficient mice. Furthermore, preliminary analysis of B cell deficient mice protected by the transfer of Ab, suggests an ameliorating, rather than detrimental effect on demyelination, similar to the TMEV model (31,36). These results support an overall beneficial role of Ab in MHV induced CNS pathogenesis, predominantly by enhancing elimination of infectious virus and controlling viral persistence. Further studies are required to assess potential effects of Ab and other components of humoral immunity on the dynamic processes of myelin loss and repair.

CONCLUSIONS

In summary, despite unequivocal evidence that acute CNS infection is controlled by T cells with minimal B cell involvement, B cells and Ab regulates viral persistence. Virus reactivation in the CNS in mice devoid of antiviral Ab results in increased demyelination. A protective role of B cells is supported by accumulation of virus specific ASC following T cell mediated clearance. The prominent mechanism of Ab mediated protection appears to reside in its neutralizing activity. Furthermore, humoral immune mediated control does not appear to initiate or enhance the severity of demyelination. Thus, an evolving model of biphasic immune control of viral infection of the CNS has developed with acute clearance and initiation of demyelination being controlled by T cells while viral persistence and possibly repair is regulated by humoral immunity.

REFERENCES:

1. Correale J., de Los Milagros Bassani Molinas M. Oligoclonal bands and antibody responses in Mulitple sclerosis. J Neurol. 2002. 249: 375-89.
2. Connor O., Kevin C., Bar-Or A., Hafler D. A. The Neuroimmunology of Multiple Sclerosis: Possible roles of T and B lymphocytes in Immunopathogenesis. J Clin Immunol. 2001. 21: 81-92.
3. Kabat E. A., Glusman M., Knaub V. Quantitative estimation of the albumin and gamma globulin in normal and pathologic cerebrospinal fluid by immunochemical methods. Am J Med. 1948. 4: 653-62.

4. Bornstein M. B., Appel S. H. The application of tissue culture to the study of experimental allergic encephalomyelitis. I. Patterns of demyelination. J Neuropathol Exp Neurol. 1961. 20: 141-7.
5. Esiri M. M., Immunoglobulin-containing cells in multiple sclerosis plaques. Lancet. 1977. ii: 478-80.
6. Genain C. P., Cannella B., Hauser S. L., Raine C. S. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat. Med. 1999. 5: 170-5.
7. Cross A. H., Trotter J. L., Lyons J-A. B cells and antibodies in CNS demyelinating disease. J NeuroImm. 2001. 112: 1-14.
8. Colombo M., Dono M., Gazzola P., Roncella S., Valetto A., Chiorazi N., Mancardi G. L., Ferrarini M. Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. 2001. J Immunol. 164: 2782-89.
9. Gilden D. H., Devlin M. E., Burgoon M. P., Owens G. P. The search for virus in multiple sclerosis brain. Mult Scler. 1996. 2: 179-83.
10. Archelos J. J., Storch M. K., Hartung H-P. The role of B cells and autoantibodies in Multiple sclerosis. Annals Neurol 2000. 47: 694-706.
11. Lumsden C. E. The immunopathogenesis of the multiple sclerosis plaque. Brain Res. 1971. 28: 365-90.
12. Stohlman, S. A., Bergmann C. C., Perlman S. "Mouse hepatitis virus." In *Persistent Viral Infections* R. Ahmed, I. Chen. ed. New York, NY: Wiley Publishers. 1999.
13. Marten N. W., Stohlman S. A., Bergmann C. C. MHV infection of the CNS: Mechanisms of immune-mediated control. Viral Immunol. 2001. 14: 1-18.
14. Lin M. T., Hinton D. R., Marten N. W., Bergmann C. C., Stohlman S. A. Antibody prevents virus reactivation within the central nervous system. J Immunol. 1999. 162: 7358-68.
15. Ramakrishna, C., Stohlman S. A.,. Atkinson R., Shlomchik M. J.,. Bergmann C. C. Mechanisms of central nervous system viral persistence: the critical role of antibody and B cells. J Immunol. 2002. 168: 1204-11.
16. Matthews A. E., Weiss S. R., Shlomchik M. J., Hannum L. G., Gombold J. L., Paterson Y. Antibody is required for clearance of infectious murine hepatitis virus A59 from the central nervous system, but not the liver. J Immunol. 2001. 167: 5254-63.
17. Tschen, S., Bergmann C. C., Ramakrishna C., Morales S., Atkinson R., Stohlman S. A. 2002. Recruitment kinetics and composition of antibody secreting cells within the central nervous system following viral encephalomyelitis. J. Immunol. 168: 2922-29.
18. Fleming J. O., Shubin R. A., Sussman M. A., Casteel N., Stohlman S. A. Monoclonal antibodies to the matrix (E1) glycoprotein of mouse hepatitis virus protect mice from encephalitis. Virology. 1989. 168: 162-7.
19. Buchmeier M. J., Lewicki H. A., Talbot P. J., Knobler R. L. Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated in vivo by monoclonal antibody. Virology. 1984. 132: 261-70.
20. Nakanaga K., Yamanouchi K., Fujiwara K. Protective effect of monoclonal antibodies on lethal mouse hepatitis virus infection in mice. J Virol. 1986. 59: 168-71.
21. Perlman S., Schelper R., Bolger E., Ries D. Late onset, symptomatic, demyelinating encephalomyelitis in mice infected with MHV-JHM in the presence of maternal antibody. Microb Pathog. 1987. 2: 185-94.
22. Kolb A. J., Pewe L., Webster J., Perlman S., Whitelaw C. B., Siddel S. G. Virus-neutralizing monoclonal antibody expressed in milk of transgenic mice provides full protection against virus induced encephalitis. J. Virol. 2001. 75:2803-9.
23. Morales S., Parra B., Ramakrishna C., Blau D. M., Stohlman S. A. B cell mediated lysis of cells infected with the neurotropic JHM strain of mouse hepatitis virus. Virol. 2001. 286: 160-7.
24. Slifka M. K., Ahmed R. Long lived plasma cells: a mechanism for maintaining persistent antibody production. Curr Opin Immunol. 1998. 10: 252-8.

25. Knopf P.M., Harling-Berg C. J., Cserr H. F., Basu B., Sirulnick E. J., Nolan S. C., Park J. T., Keir G., Thompson E. T., Hickey W. F. Antigen-Dependent intrathceal antibody synthesis in the normal rat brain: Tissue Entry and local retention of Antigen specific B cells. J Immunol. 1998. 161: 692-701.
26. Griffin D., Levine B., Tyor W., Ubol S., Despres P. The role of antibody in recovery from alphavirus encephalitis. Immunol Rev. 1997. 159: 155-61.
27. Schwender S., Imrich H., Dorries R. The pathogenic role of virus-specific antibody secreting cells in the central nervous system of rats with different susceptibility to coronavirus-induced demyelinating encephalitis. Immunol. 1991. 74: 533-8.
28. Bergmann C. C., Ramakrishna C., Kornacki M.,, Stohlman S. A. Impaired T cell immunity in B cell-deficient mice following viral central nervous system infection. J. Immunol. 2001. 167:1575-83.
29. Smith-Norowitz T. A., Sobel R. A., Mokhatarian F. B cells and antibodies in the pathogenesis of myelin injury in Semliki Forest Virus encephalomyelitis. Cell Immunol. 2000. 200: 27-35.
30. Lin M. T., Stohlman S. A., Hinton D. R. Mouse hepatitis virus is cleared from the central nervous systems of mice lacking perforin-mediated cytolysis. J Virol. 1997. 71:383-91.
31. Ramakrishna C., Bergmann C. C., Atkinson R., Stohlman S. A. Control of central nervous system persistence by neutralizing antibody. J. Virol. Submitted.
32. Furrer, E., T. Bilzer, L. Stitz, and O. Planz. Neutralizing antibodies in persistent Borna disease virus infection: prophylactic effect of gp94-specific monoclonal antibodies in preventing encephalitis. J Virol. 2001. 75: 943-51.
33. Lamarre A., Talbot P. J. Protection from lethal Coronavirus infection by immunoglobulin fragments. J Immunol. 1995. 154: 3975-84.
34. Houtman J. J., Fleming J. O. Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. J Neurovirol. 1996. 2: 101-10.
35. Zimprich F., Winter J., Wege H., Lassmann H. Coronavirus induced primary demyelination: indications for the involvement of a humoral immune response. Neuropath Applied Neurobiol. 1991. 17: 469-84.
36. Drescher K. M., Pease L. R., Rodriguez M. Antiviral immune responses modulate the nature of central nervous system (CNS) disease in a murine model of multiple sclerosis. Immunol Rev. 1997. 159:177-93.