B Cell Mediated Lysis of JHMV Infected Targets

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1. INTRODUCTION

Humoral immunity has been implicated in various models of mouse hepatitis virus (MHV) central nervous system (CNS) infection (Stohlman, *et al.*, 1999). However, the lack of neutralizing Ab until the virus has been cleared suggested that humoral immunity played little or no role in clearance of JHMV from the CNS. Infection of mice lacking the ability to produce Ab showed that following acute clearance, infectious virus reactivates within the CNS (Lin *et al.*, 1999), demonstrating an essential role for humoral immunity in preventing CNS virus reactivation. The absence of both B cells and Ab in these mice leaves open the question of whether the B cells limit CNS virus reactivation, a role potentially masked by Ab mediated neutralization (Lin *et al.* 1999). B cells from naïve mice interact with MHV-A59 infected cells resulting in lysis (Holmes *et al.* 1986, Welsh *et al.* 1986). Lysis is blocked by neutralizing Ab (Wysocka *et al.* 1989), suggesting a role of the S protein. B cells, although resistant to MHV infection, express high levels of the MHV receptor (MHV-R) (Coutelier *et al.* 1994). Interactions between the S protein and MHV-R appear to result in cytolysis via cell-cell fusion (Wysocka *et al.* 1989) and is independent of Fas/FasL interactions and TNF-α (Nishioka *et al.* 1993). These studies were initiated to explore the potential role of B cell-MHV-R interactions in contributing to the suppression of infectious virus within the CNS.

The Nidoviruses (Coronaviruses and Arteriviruses).
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1.1 Materials and Methods

Mice: BALB/c and C57/BL6 mice were purchased from the Jackson Laboratory, Bar Harbor MA. SJL mice were purchased from the National Institutes of Health (Fredrick, MD).

Viruses: JMHV was propagated and assayed described (Bergmann *et al.* 1993). Recombinant vaccinia viruses (rVV) expressing the JHMV S protein (vJS), the MHV-A59 S protein (vAS) and beta galactosidase (vSC8) were propagated as described (Bergmann *et al.* 1996).

Preparation of Effector cells: Single spleen cell suspensions were incubated on plastic petri plates at 37 C for 1 hour to remove adherent cells. This preparation contained 50 to 60% $CD19^+$ B cells. HeLa cells expressing the MHV-R (MHVR-HeLa) and a control line (HeLa+TA) were provided by Thomas Gallagher, Loyola University Medical Center.

Flow Cytometry: B cell were stained with anti-CD19 (ID3; PharMingen, San Diego CA). Biotinylated MHV-R specific mab CC-1 was supplied by Katheryn Holmes, University of Colorado Health Science Center.

Target cells: J774.1 cells were infected with rVV at a multiplicity of infection of 5 and with MHV at a multiplicity of 1 for 90 min. at 37C and incubated for 10 h prior to use.

Cytolytic assays: Targets were labeled with $Na^{51}Cr$. Labeled cells (1×10^4) were added to round bottomed 96 well plates and mixed with effector cells to achieve desired Effector/Target (E:T) ratios. Release was determined after 4 h incubation at 37C. Data are presented as % specific ^{51}Cr release = 100 x (experimental cpm – spontaneous cpm)/ (maximum cpm – spontaneous cpm).

2. RESULTS

2.1 MHV-R Expression and B Cell Mediated Cytolysis

In contrast to $CD19^+$ B cells from C57BL/6 and BALB/c mice, which all express MHV-R, on only 30% of B cells from SJL mice express MHV-R (data not shown). B cells from BALB/c mice (Fig. 1) and C57BL/6 mice (data not shown) lyse targets infected with JHMV. These data confirm the lack of a requirement for MHC restriction in B cell mediated cytolysis (Holmes *et al.* 1986, Welsh *et al.* 1986). S protein-MHV-R interactions was confirmed by inhibition of lysis via anti-MHV-R mAb (Fig 1). B cells from SJL mice showed no demonstrable lysis of JHMV infected cells (Fig. 1).

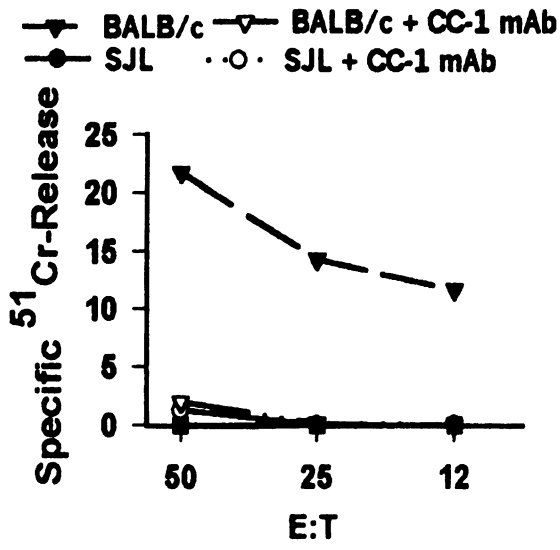

Figure 1. Receptor Dependence of B cells Mediated Cytolysis. B cells from BALB/c and SJL mice were tested for lyse of JHMV infected targets in the presence or absence of 1 ug/ml of anti-MHV-R CC-1 mAb.

2.1.1 S protein Fusion Dependent B Cell Cytolysis

To confirm that neutralizing Ab inhibits cytolysis and that lysis involves fusion, B cells were tested for lysis in the presence of anti-S protein mAb. J.2.6 (neutralization$^+$/fusion inhibition$^+$) and J.2.5 (neutralization$^+$/fusion inhibition$^-$) (Fleming *et al.* 1983), inhibit cytolysis (Fig. 2). M protein specific mAb J.3.9 did not inhibit cytolysis (Fig. 2), demonstrating that neutralizing anti-S protein mAb inhibit cytolysis. Cytolysis was also tested in the presence of the anti-S protein mAb J.2.5 (neutralization$^-$/fusion inhibition$^+$) (Fleming *et al.* 1983). J.2.5 inhibits B cell mediated cytolysis, consistent with a fusion dependent mechanism (Wysocka *et al.* 1989). In addition, cells infected with the nonfusogenic MHV-2 strain were also not susceptible to cytolysis, nor was cytolysis mediated by B cells from perforin deficient mice (data not shown). These data indicate that cytolysis requires expression of the S protein, involves cell-cell fusion, but is independent of other cytolytic pathways, i.e., Fas/FasL interactions, TNF-α or perforin mediated cytolysis.

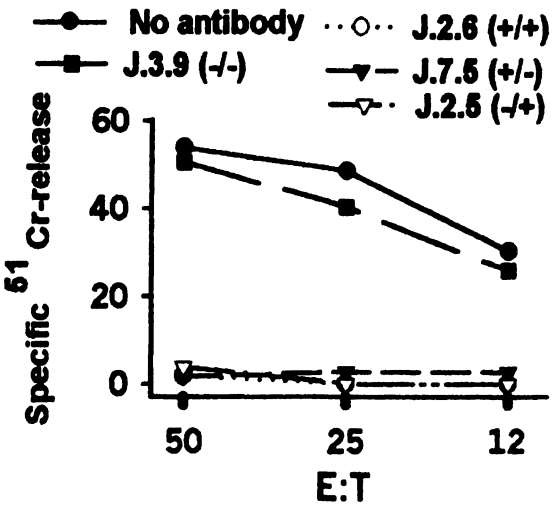

Figure 2. JHMV Neutralizing and Fusion Inhibiting mAb Inhibit Cytolysis. B cells from BALB/c mice were tested for lysis of JHMV infected targets in the presence of JHMV-specific mAb. The mAb were added at initiation at a final concentration of 1 ug/ml. Specific release was determined after 4 h incubation.

2.1.2 S Protein Expression is Sufficient for Cytolysis

To determine if cytolysis required other viral components, JHMV infected cells were compared to cells expressing only the MHV-A59 S protein (Vac-A59 S) or the JHMV S protein (Vac-JHMV S). Cells expressing the S proteins from MHV-A59 or JHMV were lysed at levels comparable to those found with JHMV infected cells (Fig. 3). No cytolysis was detected using cells infected with rVV vSC8 (Vac-Control). These data demonstrate that MHV infection is not required for B cell mediated cytolysis and that expression of the MHV S protein is sufficient to initiate cytolysis.

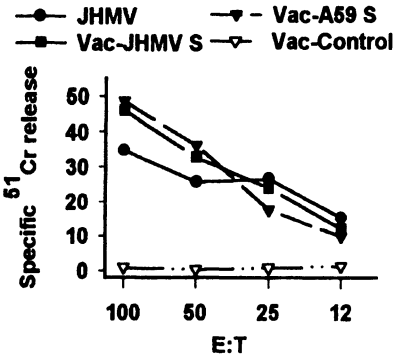

Figure 3. S Protein Expression is Sufficient for Cytolysis. Cells were infected with rVV expressing the MHV-A59 and JHMV S protein or control rVV. B cells were derived from BALB/c mice. Specific release was determined after 4 hours incubation.

2.1.2.1 MHV-R Expression is Sufficient for Cytolysis

To determine if cytolysis is dependent upon a unique B cell property, MHVR HeLa were examined for cytolysis. MHVR HeLa cells express similar levels of MHV-R compare to cytolytic $CD19^+$ B cells derived from naïve BALB/c mice (data not shown). JHMV infected cells were lysed by HeLa cells expressing MHV-R, but not control HeLa cells (HeLa+TA) (Fig. 4). Inhibiting S-MHV-R interactions via addition of 1 ug/ml anti-MHV-R mAb completely abolished cytolysis (data not shown). These data demonstrate that cytolysis resulting from interactions between S protein and cells expressing high levels of the MHV-R is not a unique to B cells.

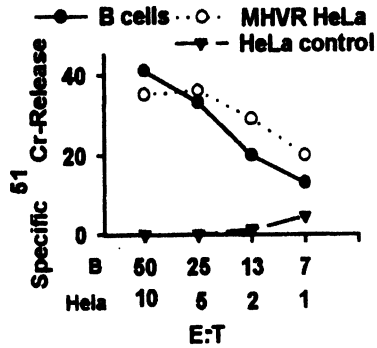

Figure 4. B Cells are not Required for Cytolysis. Cytolysis by B cells compared to HeLa cells expressing the MHV-R (MHVR-HeLa). B cells were added at E:T ratios beginning at 50:1. MHVR-HeLa and control HeLa+TA were added at E:T ratios beginning at 10:1. Specific release was determined after 4 h incubation.

3. DISCUSSION

Reactivation of JHMV in the absence of Ab suggests that either Ab or B cells contribute to viral persistence. In addition to Ab secretion B cells could inhibit viral reactivation either via direct cytolysis or, since B cells are not susceptible to infection, by sequestering virus. To understand the potential role of B cells in preventing JHMV reactivation. The present study explored the mechanism(s) of B cell mediated cytolysis. The data demonstrate that B cells lyse cells expressing the JHMV S protein via a fusion dependent process. Lysis does not require infection; therefore, other components of the viral infection are not required for cytolysis. In addition MHV-R expression is sufficient and cytolysis is not dependent upon a unique B cell property.

A conceptual difficulty with a role for B cell cytolysis in preventing viral recrudescence is inhibition by anti-S protein Ab with either neutralizing or anti-fusogenic activity. During JHMV infection Ab can first be detected in serum at d 5 p. i. Neutralizing Ab is detected at approx. 10 d p. i. It is

unclear how rapidly anti-fusogenic Ab are secreted or rapidly Ab infiltrates the CNS. It is also unclear how rapidly B cells secreting anti-S protein Ab are recruited into the CNS or if they express MHV-R. One possibility is that MHV-R+ B cells are lysed contributing a minimal amount to the reduction of infectious virus due to its intracellular site of replication but providing a plausible mechanism for the somewhat delayed appearance of the Ab response. Understanding the role of B cells during JHMV infection will await an examination of JHMV pathogenesis in mice which possess MHV-R^+ B cells, but are unable to secrete Ab.

REFERENCES

Bergmann, C., McMillan, M., and Stohlman, S.A., 1993, Characterization of the L^d restricted CTL epitope in the mouse hepatitis virus nucleocapsid protein. *J. Virol.* **67**:7041-7049.

Bergmann, C.C., Yao, Q., Lin, M., and Stohlman, S.A., 1996, The JHM strain of mouse hepatitis virus induces a spike protein-specific D^b-restricted cytotoxic T cell response. *J. Gen. Virol.* **77**:315-325.

Coutelier, J.-P., Godfraind, C., Dveksler, G.S., Wysocka, M., Cardellichio, C.B., Noel, H., and Holmes, K.V., 1994, B lymphocyte and macrophage expression of carcinoembryonic antigen-related adhesion molecules that serve as receptors for murine coronavirus. *Eur. J. Immunol.* **24**:1383-1390.

Fleming, J.O., Stohlman, S.A., Harmon, R.C., Lai, M.M.C., Frelinger, J.A., and Weiner, L.P., 1983, Antigenic relationship of murine coronaviruses: Analysis using monoclonal antibodies to JHM (MHV-4) virus. *Virology* **131**:296-307.

Holmes, K.V., and Welsh, R.M., 1986, Natural cytotoxicity against mouse hepatitis virus-infected target cells. I. Correlation of cytotoxicity with virus binding to leukocytes. *J. Immunol.* **136:**1446-1453.

Lin, M.T., Hinton, D.R., Marten, N.W., Bergmann, C.C., and Stohlman, S.A., 1999, Antibody prevents virus reactivation within the central nervous system. *J. Immunol.* **162**:7358-7368.

Nishioka, W.K., and Welsh, R.M., 1993, B cells induce apoptosis via a novel mechanism in fibroblasts infected with mouse hepatitis virus. *Nat. Immun.* **12**:113-127.

Stohlman, S.A., Bergmann, C.C., and Perlman, S., 1999, Mouse hepatitis virus. In *Persistent Viral Infections.* (R. Ahmed and I. Chen, eds.), John Wiley & Sons, p. 537-557.

Welsh, R.M., Haspel, M.V., Parker, D.C., and Holmes, K.V., 1986, Natural cytotoxicity against mouse hepatitis virus-infected cells. *J. Immunol.* **136**:1454-1460.

Williams, R.K., Jiang, G.-S., Snyder, S.W., Frana, M.F., and Holmes, K.V., 1990, Purification of the 110-kilodalton glycoprotein receptor for mouse hepatitis virus (MHV)-A59 from mouse liver and identification of a nonfunctional, homologous protein in MHV-resistant SJL/J mice. *J. Virol.* **64**:3817-3823.

Wysocka, M., Korngold, R., Yewdell, J. and Bennink, J., 1989, Target and effector cell fusion accounts for B lymphocyte-mediated lysis of mouse hepatitis virus-infected cells. *J. Gen. Virol.* 70:1465-1472.