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Short communication

Polyclonal B lymphocyte activation induced by mouse hepatitis virus A59 infection

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Mouse hepatitis viruses (MHV) diversely affect immune responses, depending on the viral strain and the mouse genetic background. Here, we studied the effect of MHV-A59 infection on B cell responses of 129/Sv and CBA mice. Our results indicate that in these strains, MHV-A59 induces spleen cell activation that leads to enlargement of the spleen without structural alteration. Infection triggers production by B lymphocytes of large

amounts of immunoglobulin G2a, mostly without viral specificity. This polyclonal immunoglobulin production is dependent on the presence of functional T helper cells. This polyclonal B lymphocyte activation induced by MHV-A59 infection can have pathological implications, such as the enhancement of concomitant autoimmune reactions.

In vivo B lymphocyte polyclonal activation has been reported after infection with several viruses such as adenovirus or lactate dehydrogenase-elevating virus (LDV) (Coutelier & Van Snick, 1985; Coutelier et al., 1988, 1990). Mouse hepatitis viruses (MHV) are known to be lymphotropic (Lamontagne et al., 1989) and to induce diverse alterations of immune responses that depend on the viral strain and on the mouse genetic background. MHV effects on B cell functions are quite variable. MHV-3 infection of C57BL/6 or (C57BL/6 X A/J)F₁ B lymphocytes at different stages of differentiation leads to atrophy of the spleen (Jolicoeur & Lamontagne, 1990; Lamontagne et al., 1989) and depletion of cells of the B lineage (Jolicoeur & Lamontagne, 1994). Mice chronically infected with MHV-3 display depressed immunoglobulin levels as well as decreased antibody responses following simultaneous immunization with sheep red blood cells (Leray et al., 1982; Virelizier et al., 1976). In contrast, MHV-A59 infection of CBA/Rij mice causes IgG2a-restricted hypergammaglobulinaemia (Coutelier et al., 1988). MHV-A59 infection of 129/Sv mice at the time of

To determine whether MHV could also induce spleen cell activation, female 129/Sv, CBA/Rij and CBA/Ht mice bred at the Ludwig Institute for Cancer Research by G. Warnier were infected by intraperitoneal (i.p.) injection of approximately 50 TCID₅₀ of the A59 strain of MHV, grown in NCTC1469 cells. Efficiency of infection was checked by examination of the development of liver lesions. Spleens obtained at different times post-infection (p.i.) were fixed in Bouin solution and embedded in paraffin wax. Sections (5 µm) were stained with haemalum-eosin or Giemsa. A strong enlargement of spleen size was induced by the virus and reached a maximum at 7 days p.i. (Fig. 1a). Spleen sections showed that the overall structure of this organ was preserved. However, germinal centres were enlarged after infection and more cells were found in the red pulp of infected mice, when compared to controls (Fig. 1c). Numerous large blastic lymphocytes were observed both in the red and the white pulp of the spleen from infected

immunization with a soluble protein antigen results in immunostimulation with complete modification of the isotypic distribution of the anti-protein antibodies towards the IgG2a subclass (Coutelier et al., 1988, 1991). Enhancement of antibody production which could be related to variations of interferon- γ (IFN- γ) levels has also been reported in BALB/c and C57BL mice acutely infected with the MHV-JHM and MHV-3, respectively (Smith et al., 1991; Virelizier et al., 1976).

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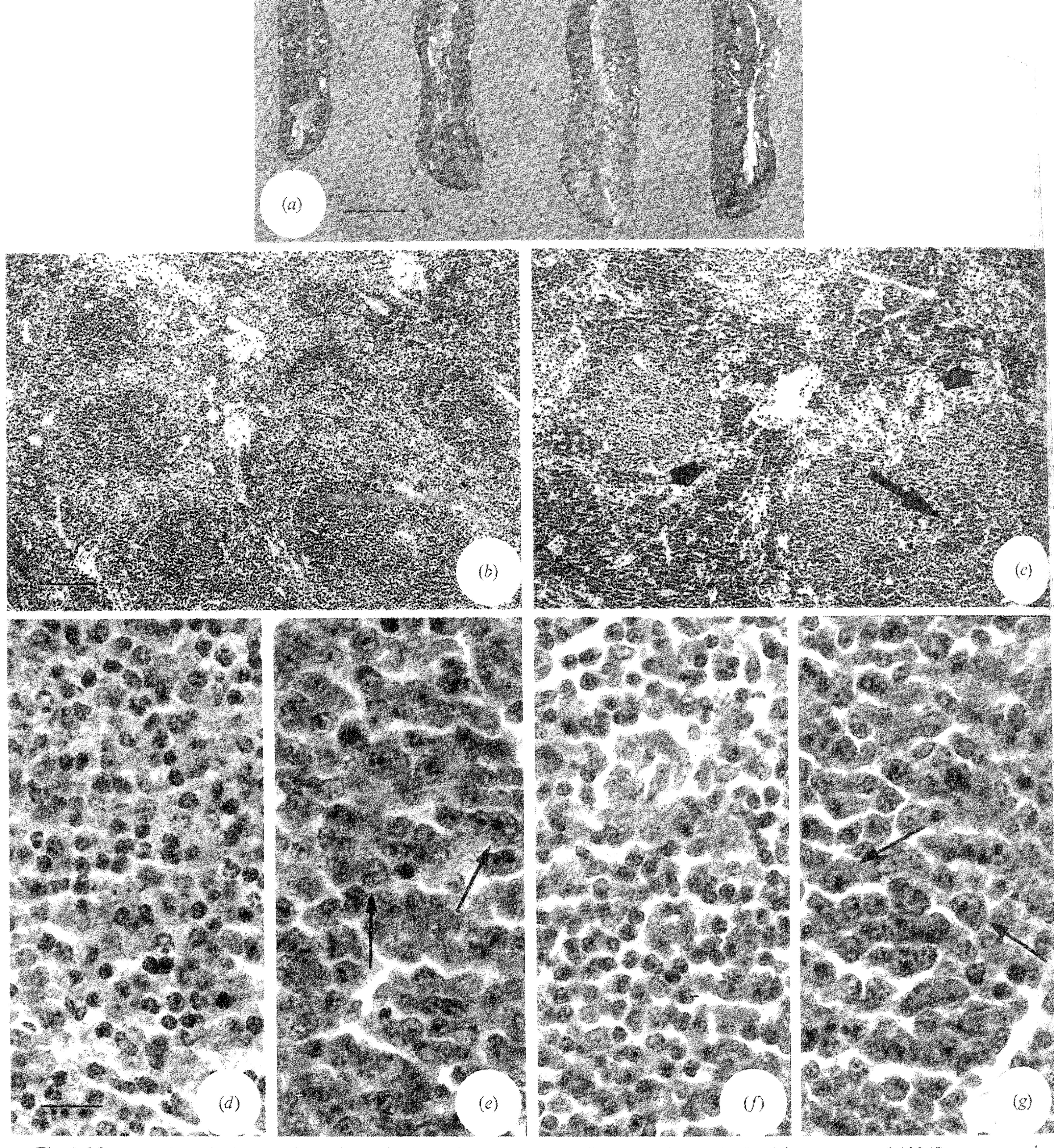


Fig. 1. Macroscopic and microscopic analysis of spleen after MHV-A59 infection. Spleens obtained from a control 129/Sv mouse and from animals infected for 4, 7 and 10 days, respectively from left to right, are shown (a). Microscopic analysis of control 129/Sv (b, d, f), and of mice at 7 days after infection (c, e, g) shows a conserved structure, but enlarged germinal centres (c, arrow) and an increased cellularity of the red pulp (c, arrowheads). Blastic cells were observed in the white (e) and red (g) pulp of infected mice (arrows), but not in the similar structures (d and f, respectively) of control animals. Scale bar in (a) represents 4 mm, that in (b) represents 0.4 mm and applies also to (c) and that in (d) represents 33 μ m and applies also to (e), (f) and (g).

animals (Fig. 1e, g) and the mitotic index was enhanced. This observation correlated with flow cytometry analysis of spleen cell size that showed enlargement of a significant proportion of cells and by an increase in

[methyl-³H]thymidine incorporation that reached a maximum on day 7 p.i. (data not shown).

Immunoglobulin production by spleen cells from control 129/Sv mice or from animals at different times

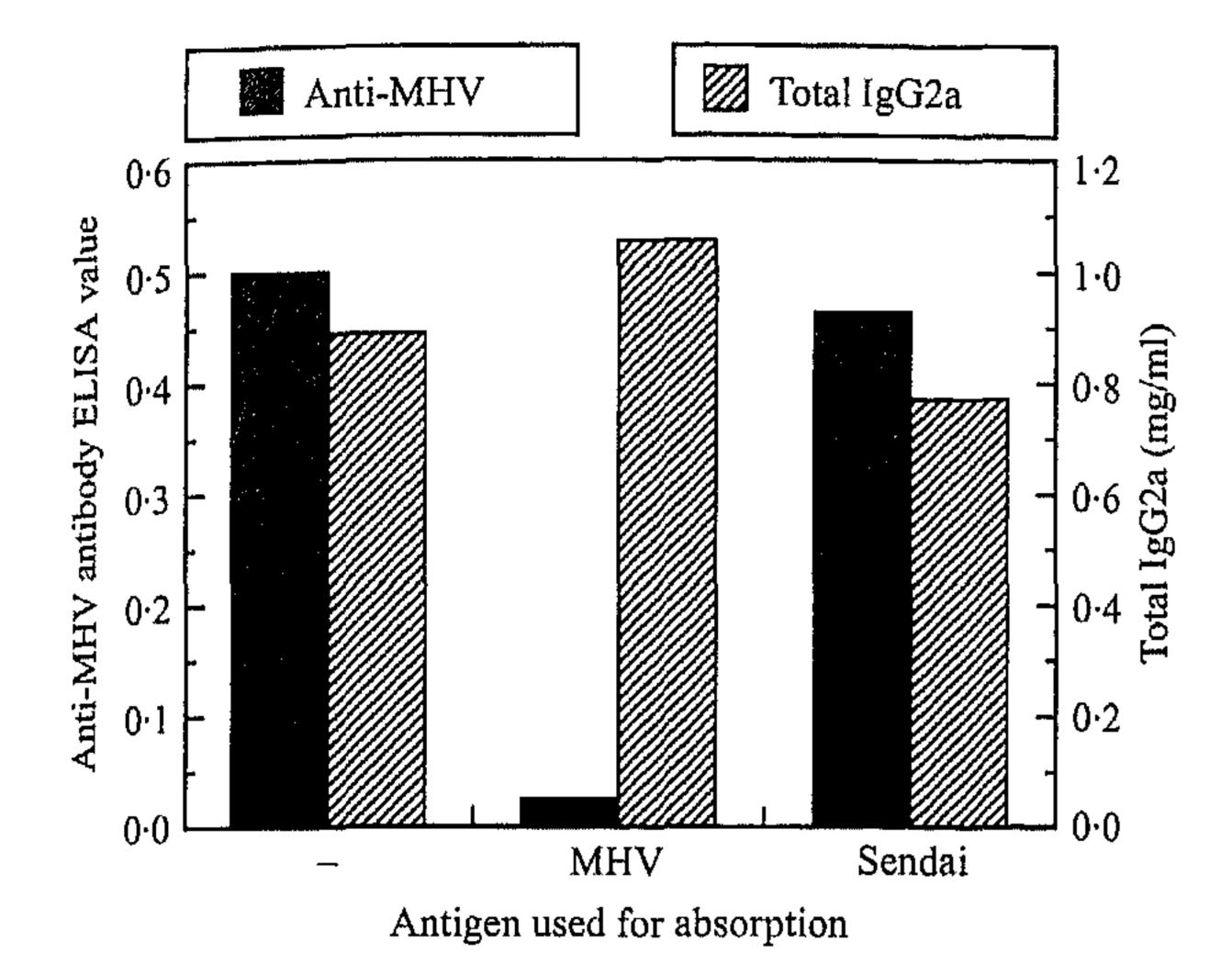


Fig. 2. Absorption of anti-MHV antibodies. Pooled serum from 5 CBA/Rij mice obtained 3 weeks after MHV-A59 infection was absorbed with buffer, with purified MHV-A59 or with purified Sendai virus particles. After removal of viral particles by centrifugation, remaining anti-MHV-A59 IgG antibody (black columns) and total IgG2a (hatched columns) were measured in supernatants by ELISA.

after infection with MHV-A59 was assayed by ELISA as described previously (Coutelier et al., 1990). A strong enhancement of the secretion of immunoglobulins that were mostly of the G2a isotype was observed with a maximum 7 days p.i. (data not shown). The specificity of IgG2a produced after infection was assessed by absorption experiments. Pooled sera from infected mice were diluted 100-fold in PBS (120 mm-NaCl, 5 mm-Na₂HPO₄, 3 mm-KH₂PO₄ pH 7·2) containing 2 % FCS and incubated for 2 h at 4 °C with serial doses of virus purified by centrifugation for 16 h at 20000 r.p.m. on a 20-50% sucrose gradient in a SW25 rotor or with buffer only. Viral particles were then removed from sera by centrifugation through a 15% sucrose cushion (35000 r.p.m. for 90 min in a SW41 rotor; Beckman Instruments) and remaining total immunoglobulins as well as antiviral antibodies in the supernatant were measured by ELISA. This treatment efficiently removed anti-MHV antibodies, as shown for a typical experiment (Fig. 2). However, total IgG2a levels were only marginally affected by this absorption, indicating that most immunoglobulins produced after MHV-A59 infection were not antiviral antibodies. As a control, Sendai virus particles absorbed neither anti-MHV-A59 antibodies nor total IgG2a, although they fully removed anti-Sendai antibodies from sera obtained from mice infected with this virus. The preponderance of B lymphocytes secreting non-antiviral IgG2a after MHV-A59 infection was confirmed by somatic hybridization performed as reported previously (Coutelier & Van Snick, 1985). Of 137 wells containing immunoglobulin-secreting hybridomas derived from pooled spleen cells of four 129/Sv

mice obtained 7 days after MHV-A59 infection, 92 (67%) contained IgG2a and 45 (33%) IgM. In contrast, IgG1, IgG2b and IgG3 were detected in 4, 11 and 2 wells, respectively. Interestingly, anti-MHV antibodies were found in only 3 wells, therefore confirming that the bulk of IgG2a produced early after MHV-A59 infection was not reacting with viral antigens.

The role of T helper lymphocytes in the polyclonal B lymphocyte activation induced by MHV-A59 was investigated by the injection of an anti-CD4 monoclonal antibody capable of eliminating in vivo functional T helper lymphocytes (GK 1.5, made available by F. W. Fitch, and obtained through the courtesy of H. R. MacDonald and P. G. Coulie; Dialynas et al., 1983). In our mice, GK1.5 antibody injection resulted in a drop in spleen CD4+ cell proportion, from 18% in control animals to less than 1% in treated mice. This treatment completely inhibited the IgG2a production by spleen cells from infected animals (Fig. 3a). No compensative increase of other isotypes was observed (data not shown).

Little is known about the mechanisms leading to virally induced B lymphocyte activation. The role of T helper cells, together with the production of IFN-y (Smith et al., 1991; Virelizier et al., 1976; J.-P. Coutelier, unpublished data) points to a selective differentiation of T cells towards the Th1 phenotype. This hypothesis is confirmed by the observation that interleukin-12 (IL-12) message expression is transiently increased soon after MHV-A59 infection (Coutelier et al., 1995). It is quite possible that production of such lymphokines by macrophages stimulated after infection results in an indirect modulation of non-antiviral responses leading to secretion of large amounts of IgG2a, in addition to an isotypic bias of antiviral antibodies (Coutelier et al., 1988). Other mechanisms might be involved in the proliferation of B lymphocytes. Some years ago, it was demonstrated that influenza virus can directly activate murine B lymphocytes in vitro by binding a receptor on the surface of these cells (Scalzo & Anders, 1985). It has recently been observed that glycoproteins in the carcinoembryonic antigen family which serve as receptors for MHV-A59 (Dveksler et al., 1993; Williams et al., 1991) are expressed on B lymphocytes (Coutelier et al., 1994a). Therefore, one may postulate that in vivo activation of B lymphocytes can result, at least partly, from direct interaction between these cells and viral particles.

Because it has been previously reported that MHV-A59 infection may result in an enhancement of antibody responses elicited at the time of virus inoculation (Coutelier et al., 1988, 1991), it was examined whether such an effect could also increase autoimmune responses. Thus, mice were infected with MHV-A59 at the time of immunization with rat red blood cells following a classical protocol of anti-erythrocyte autoantibody in-

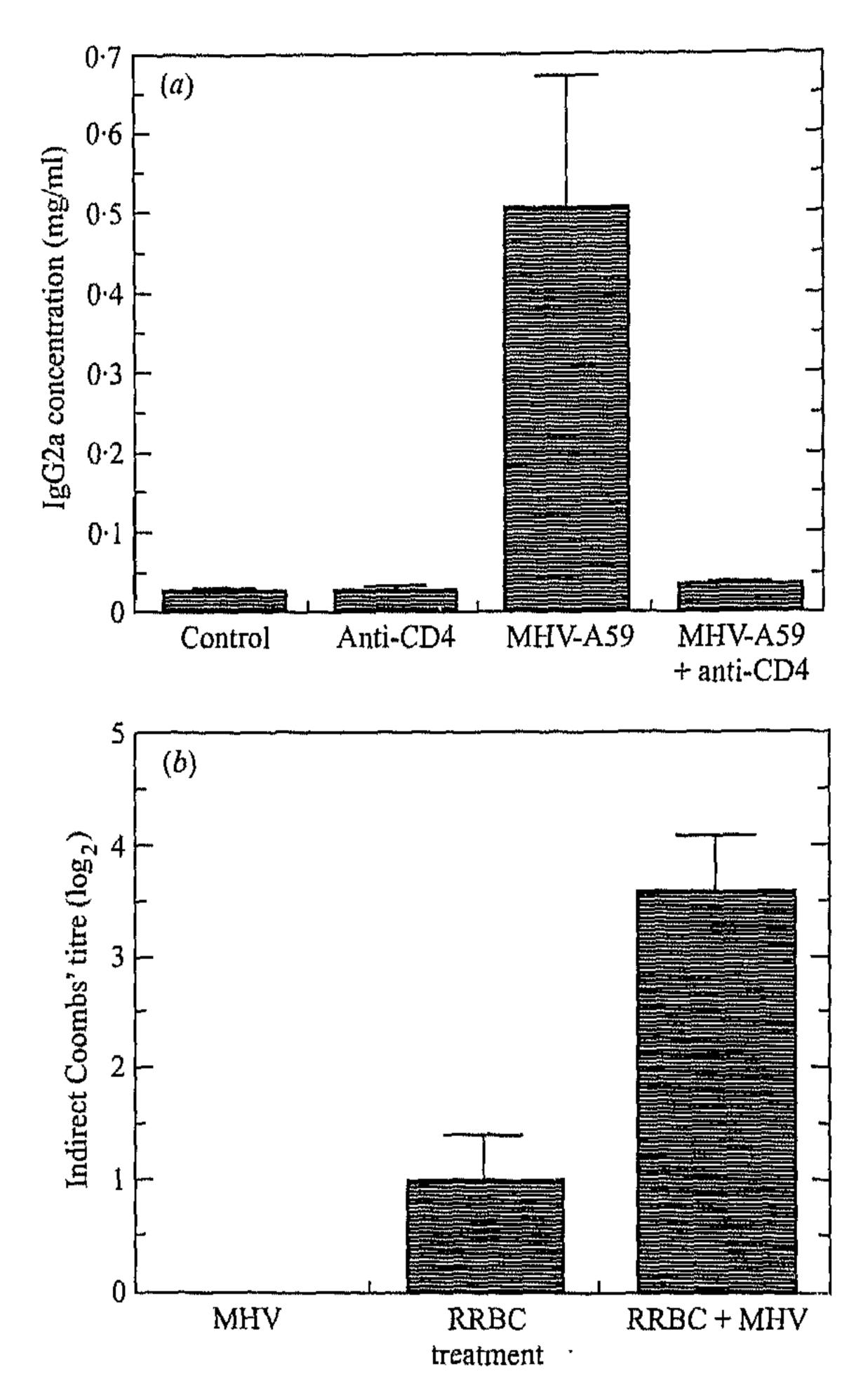


Fig. 3. (a) Effect of anti-CD4 treatment on immunoglobulin G2a production after MHV-A59 infection. Immunoglobulin G2a was assayed by ELISA in supernatants from spleen cells obtained from control 129/Sv mice and from animals infected with MHV-A59 for 7 days. Animals treated with anti-CD4 antibody received 1 ml of ascitic fluid containing GK1.5 monoclonal antibody at the time of infection. Results are shown for groups of 4-7 mice (mean \pm sE). (b) Effect of MHV-A59 infection on autoimmune response. Anti-erythrocyte auto-antibody response was measured by indirect Coombs' test in sera of CBA/Ht mice that received either virus alone (MHV), weekly injection of 2×10^8 -4 $\times 10^8$ rat red blood cells (RRBC) or both virus and RRBC. MHV was inoculated at the time of the first RRBC immunization and results are shown after 7 weeks (mean \pm sE).

duction (Cox & Howles, 1981; Naysmith et al., 1981; Verdonck et al., 1994). Anti-erythrocyte autoantibodies were titrated by indirect Coombs' test in sera obtained 7 weeks after the first immunization. As shown in Fig. 3(b), mice immunized only with rat erythrocytes developed a moderate autoantibody production. In contrast, high levels of autoantibodies were found in animals that received MHV-A59 with their first rat red cell administration. No anti-erythrocyte antibodies were detected in mice that were infected without erythrocyte administration. These results indicated that MHV-A59 infection may strongly enhance concomitant autoimmune responses.

It remains to be demonstrated whether such virally induced modulations of immune responses have implica. tions for the health of the infected host. It is quite possible that the pathogenicity of some autoimmune responses may be enhanced, in addition to the increase of the total amount of autoantibodies, by a preferential differentiation of autoreactive T helper lymphocytes towards the Th1 subtype and by a restriction of autoantibodies to the IgG2a isotype. The observation that lymphocytic choriomeningitis virus triggers T celldependent autoimmune haemolytic anaemia, linked to B lymphocyte polyclonal activation and secretion of IgG2a anti-erythrocyte autoantibodies that do not react with viral antigens (Coutelier et al., 1994b; Stellrecht & Vella.) 1992; Vella & Pfau, 1991) may well fit with this hypothesis.

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