Lymphocytic Choriomeningitis Virus-Induced Alterations of T Helper-Mediated Responses in Mice Developing Autoimmune Hemolytic Anemia During the Course of Infection

(44303)

M. EL AZAMI EL IDRISSI,* G. MAZZA,† P. MONTEYNE,* C. J. ELSON,† M. J. DAY,† C. J. PFAU,‡ AND J.-P. COUTELIER*,[1]

Unit of Experimental Medicine, International Institute for Cellular Pathology, Université Catholique de Louvain, 1200 Bruxelles, Belgium; Department of Pathology and Microbiology,† School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK; Department of Biology,‡ Rensselaer Polytechnic Institute, Troy, New York 12180-3590*

Abstract. **The effect of LCMV on CD4+ T lymphocytes was analyzed in C3HeB/FeJ mice after infection with the Docile strain of this virus. Our results indicated that LCMV triggers: i) an inhibition of Th2 lymphocyte differentiation induced by concomitant immunization with a nonviral protein antigen; ii) a depression of T helper–dependent antibody responses elicited by such an immunization; and iii) a CD4+ cell-mediated proliferation of spleen cells leading to increased interleukin-4 and interferon-γ message expression and IgG2a-restricted total immunoglobulin secretion. Taken together, these results indicate that LCMV profoundly affects CD4+ cell-mediated immune responses in infected animals. Such modulations of T-helper functions may explain the preponderance of IgG2a in the antierythrocyte autoimmune response induced by the virus in C3HeB/FeJ mice.** [P.S.E.B.M. 1998, Vol. 218]

Multiple and diverse modulations of immune responses have been reported after infection of mice with several strains of lymphocytic choriomeningitis virus (LCMV). On the one hand, stimulation of the immune system that results from infection with this virus may lead to polyclonal activation of both B lymphocytes (1–4) and CD8+ T lymphocytes (5, 6). Although the role of CD4+ cells has been demonstrated in the B cell activation (4), but not in the CD8+ lymphocyte activation (7), little is known about a possible stimulation of T helper cells by this virus. Several reports have already pointed to an increased cytokine secretion in lymphocytes triggered by this virus. Both CD4+ and CD8+ T cells have been shown to produce high levels of interferon-gamma (IFN-γ) (8–10). Increase of interleukin-2 (IL-2) transcription and secretion in these two cell types has been reported (7, 11). At low levels, IL-4 and IL-5 were also detected in the serum of mice acutely infected with LCMV (12). Together, all of these observations suggest that LCMV triggers a general nonspecific T helper lymphocyte stimulation in infected animals.

On the other hand, depression of immune responses is frequently triggered by LCMV (reviewed in Ref. 13). Responses to mitogens, evaluated by their IL-2 production or proliferation, are decreased in T lymphocytes derived from LCMV-infected animals (14, 15). Cytolytic as well as B-cell responses against various antigens, including other pathogens, are also depressed (16, 17). Various mechanisms

[1] To whom requests for reprints should be addressed at Unit of Experimental Medicine, UCL MEXP 7430, Av. Hippocrate 74, B-1200 Bruxelles, Belgium. E-mail: coutelier@mexp.ucl.ac.be

This work was supported by the Fonds National de la Recherche Scientifique (FNRS), Fonds de la Recherche Scientifique Médicale (FRSM), Loterie Nationale, Fonds de Développement Scientifique (UCL), NATO (grant CRG 920169), and the State-Prime Minister's Office - S.S.T.C. (interuniversity attraction poles, grant n°44) and the French Community (concerted actions, grant n° 88/93-122), Belgium. The study was also funded by a project grant from the Wellcome Trust and travel grants from the British Council and C.G.R.I. (Belgian French Community). J.-P.C. is a senior research associate with the FNRS.

Received September 24, 1997. [P.S.E.B.M. 1998, Vol 218]
Accepted April 24, 1998.

0037-9727/98/2184-0349$10.50/0

that are not mutually exclusive have been proposed to explain these alterations of immune responses elicited by LCMV. These include functional impairment or destruction of antigen-presenting cells (18–20) and increased susceptibility of activated T lymphocytes to apoptosis (21). Since it is well known that the outcome of LCMV infection can vary significantly depending on the combination of mouse and virus strain (13), this could be the reason for the seemingly contradictory results described above.

It has been shown previously that several viruses, including lactate dehydrogenase–elevating virus, mouse hepatitis virus, and murine adenovirus, could modulate B- and T-helper-lymphocyte responses, leading to a restricted secretion of IgG2a antibodies toward nonviral antigens and preferential differentiation of T helper cells toward the Th1 pathway (2, 22–25). Whether such an effect may be induced by LCMV has not been reported so far. However, it has been reported that infection of C3HeB/FeJ mice by the Docile strain of LCMV triggers a hemolytic anemia with production of antierythrocyte autoantibodies that belong mainly to the IgG2a subclass (4, 26, 27). Quite possibly, this type of virally induced autoimmune disease may result, at least partially, from alterations in the immune system triggered by the infection. As it has been shown that LCMV-induced autoimmune anemia was dependent on the presence of functional T helper cells, it may be postulated that this disease results from some interaction of the virus with this cell subpopulation. Therefore, we examined whether or not LCMV infection of C3HeB/FeJ mice induced modulation of T-helper subset differentiation, immunostimulation of T-helper–dependent responses and CD4-mediated lymphocyte polyclonal activation.

Materials and Methods

Virus. A triple plaque purified "docile" substrain of LMCV was used (28). Virus stocks were grown in a canine kidney cell line (MDCK) for no more than three passages after plaque purification. Mice were injected intravenously (i.v.) with 300 plaque-forming units (PFU) of virus.

Animals. Six-to-eight-week-old female C3HeB/FeJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and held for at least 1 week before use. Mice were kept in micro-isolators and given an unlimited supply of sterilized food and water. Mice were lightly etherized prior to LCMV injections. Specific pathogen-free female CBA/Ht mice were bred by Dr. G. Warnier at the Ludwig Institute for Cancer Research (Bruxelles, Belgium) and used at the age of 6–8 weeks. Experiments were carried out in compliance to standard and local guidelines and regulations.

Immunization. Mice were immunized by subcutaneous injection at the tail base of 100 μg keyhole limpet hemocyanin (KLH; Calbiochem, San Diego, CA) in 100 μl saline (NaCl, 9 g/l) or i.p. in 500 μl saline. When indicated, 50% Complete Freund's Adjuvant (CFA) was added. Immunization with rat red blood cells (RRBC) was performed as described previously (29). Mice were injected i.p. weekly with 2–4 × 10^8 RRBC.

Antibody Treatment. Anti-CD4 monoclonal antibody GK1.5 was made available by F. W. Fitch (University of Chicago, IL) and obtained through the courtesy of H. R. MacDonald (Ludwig Institute for Cancer Research Ejalinges sur Lausanne, Switzerland) (30). It was used as ammonium sulfate–precipitated antibody and injected i.p. at the dose of 1 mg/mouse. Anti-CD8 monoclonal antibody (53/6.72, rat IgG2a, from ATCC; 31) was injected i.p. at the dose of 2.5 mg/mouse. Both anti-CD4 and anti-CD8 antibodies were administered 5 hr before and 5 days after infection. As reported previously (4) anti-CD4 treatment resulted in almost complete ablation of splenic CD4+ cells. Although not as effective, administration of anti-CD8 antibody performed in different mouse strains usually decreased the splenic CD8+ cell count from 11%–18% in control animals to 0.1%–5% in treated mice.

Spleen Cell Cultures. As described previously (32), 25 × 10^6 spleen cells were cultured in 5 ml Iscove's medium containing 10% FCS and supplemented with 0.24 m*M* L-asparagine, 0.55 m*M* L-arginine, 1.5 m*M* L-glutamine, and 0.05 m*M* 2-mercaptoethanol. Supernatants were collected 24 hr after initiation of cultures.

Thymidine Incorporation. Spleen cells (250,000) were incubated for 24 hr at 37°C in 200 μl of Iscove medium supplemented as described above and containing 0.5 μCi of (methyl-^{3}H thymidine) (specific activity, 25 Ci/mmol; Amersham International, Amersham, U.K.) (32). The cells were counted for radioactivity in a Microplate Scintillation Counter (Packard, A Canberra Company, Meridien, CT).

Antibody Determination. Specific antibody IgG subclasses were assayed by ELISA as previously described (33). Polystyrene plates precoated with antigen (10 μg/ml) were incubated with serial dilutions of sera, followed by rabbit antibodies specific for each mouse IgG subclass and by peroxidase-conjugated donkey anti-rabbit IgG. Calibration was obtained in reference to the binding of anti-DNP IgG1, IgG2a, IgG2b, and IgG3 mAb of equivalent affinity to DNP-BSA-coated plates. Total IgG subclasses were assayed in spleen cell cultures by ELISA as described previously (22). The binding of antibodies to dinitrophenylated bovine serum albumin (DNP-BSA) was measured by ELISA as described (34).

RBC Collection. Blood was collected *via* the retrobulbar plexus into 3.8% sodium citrate solution and the RBC washed six times in phosphate-buffered saline pH 7.4 (PBS). A suspension of washed RBC in PBS/0.2% bovine serum albumin (BSA) was fixed for 30 min at room temperature with 0.15% glutaraldehyde (Sigma, Poole, UK). Fixed RBC were washed three times in PBS and stored as a 2% suspension in PBS at −70°C until used in the direct DELAT (see below).

Direct Enzyme-Linked Antiglobulin Test (Direct DELAT). To quantitate the molecules of IgG of each sub-

class bound to the surface of RBC, a cellular ELISA (DELAT) was used (35). Briefly, 50 μl of a 2% suspension of fixed RBC were added to duplicate wells of round-bottom microtiter plates (Life Technologies, Scotland) and incubated with subclass-specific sheep anti-mouse IgG antibody diluted in PBS/0.2% BSA (anti-IgG1: 1/250, anti-IgG2a: 1/300, anti-IgG2b: 1/300, anti-IgG3: 1/400, The Binding Site, Birmingham, UK) for 1 hr at 37°C. RBC were washed three times and incubated with alkaline phosphatase-conjugated donkey anti-sheep IgG (Sigma) diluted 1/3000 in PBS/BSA, washed a further three times, and allowed to react with phosphatase substrate solution (P-nitrophenyl phosphate, Sigma) for 1 hr at 37°C. The supernatants were transferred into flat-bottom microtiter plates (Dynatech, Sussex, UK) and the absorbance measured at 405 nm (Titertek multiscan II, Labsystems, Hants, UK). The number of molecules of each murine IgG subclass bound to the RBC was calculated by interpolation from a standard curve generated by tanning normal RBC with a known concentration of each purified IgG subclass (Sigma) (35).

RNA Extraction and PCR Amplification. Cells freshly obtained from animals were lysed in TRIzol reagent (BRL, Gaithersburg, MD). Total RNA was first extracted with chloroform, then precipitated with isopropanol, washed in ethanol, and finally resuspended in 50 μl water. Oligo-dT primed cDNA was prepared from approximately 5 μg RNA using 200 U M-MLV reverse transcriptase (BRL, Gaithersburg, MD) according to the manufacturer's instructions. cDNA was amplified by PCR with a Gene Amp kit (Perkin Elmer Cetus, Norwalk, CT) in a Thermal Reactor (Hybaid, Middlesex, U.K.). The primers were as follows:

actin: ATGGATGACGATATCGCTGC and GCTGGAAGGTGGACAGTGAG; IFN-γ. GACAATCAGGCCATCAGCAAC and CGCAATCACAGTCTTGGCTAA; IL-4: ATGGGTCTCAACCCCCAGCTA and GCATGGTGGCTCAGTACTACG.

The post-PCR products were analysed in 1% agarose gels containing ethidium bromide. Semiquantitative results were obtained after blotting of the PCR products on Zeta-Probe membranes (Biorad), and hybridization overnight at 42°C in Denhardt's solution with internal probes labelled with ^{32}P. The radioactivity was quantitated with a Phosphor Imager (Molecular Dynamics), and the ratios between IL-4 or IFN-γ and actin messages were calculated after substraction of nonspecific background and shown as arbitrary units (defined as: value of message of interest/value of actin message × 10,000). The sequence of the probes was: actin: CGTACCACAGGCATTGTGATGG; IFN-γ. TCGCCTTGCTGTTGCTGA; IL-4: GATGATCTCTCTCAAGTG.

Results

Effect of LCMV Infection on a Concomitant Immunization with a Nonviral Antigen. It has been reported previously that infection with LDV or MHV could both increase concomitant antibody responses against nonviral antigens and modify their isotypic distribution in favor of the IgG2a subclass (2, 23). This effect of viral infection may be explained by a specific inhibition of Th2 responses, including a strong depression of IL-4 message expression (24). To examine whether LCMV can trigger a similar effect in C3HeB/FeJ mice, we simultaneously infected animals and immunized them with KLH. The effect of infection on T-helper-lymphocyte differentiation was analyzed by RT-PCR for IL-4 and IFN-γ messages in cells obtained from inguinal and periaortic lymph nodes. Day four after KLH injection was chosen since this is the time of maximal IL-4 expression elicited by this type of immunization (24). As expected, immunization alone induced a strong IL-4 message, but no detectable IFN-γ expression (Fig. 1). This pattern was completely reversed by a concomitant LCMV

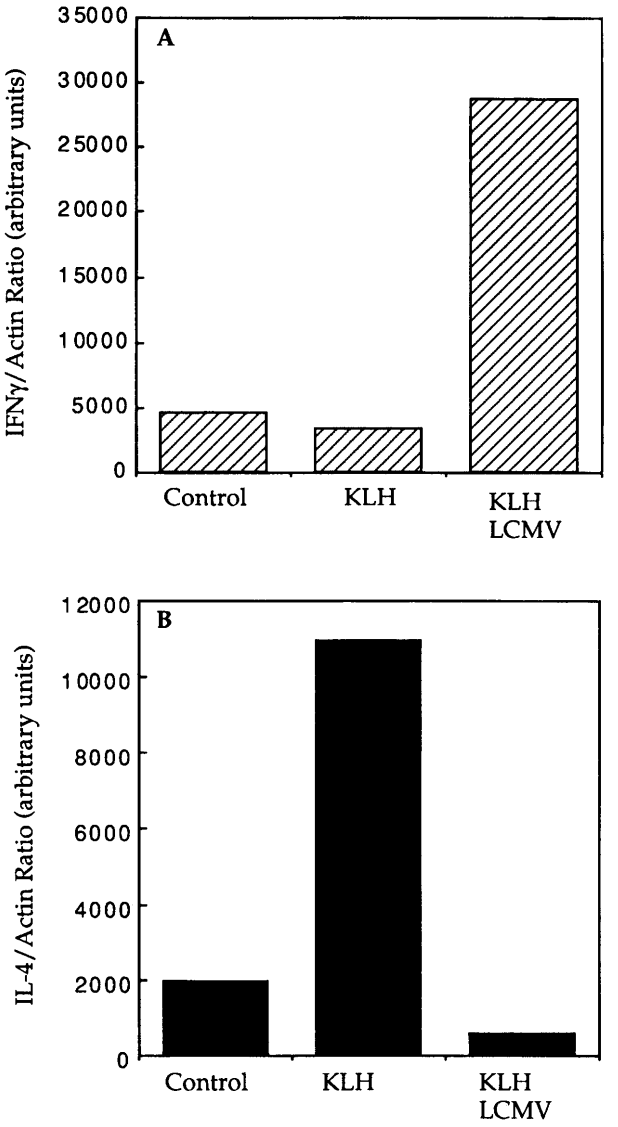

Figure 1. Effect of LCMV infection on IL-4 and IFN-γ messages induced by KLH immunization. Expression of (A) IFN-γ and (B) IL-4 was analyzed by RT-PCR in periaortic and inguinal lymph node cells obtained from groups of four control C3HeB/FeJ mice and from animals at 4 days after immunization with KLH. When indicated, LCMV infection was performed 1 day before immunization.

infection: the virus abrogated the KLH-induced IL-4 gene expression and induced a strong IFN-γ message (Fig. 1). Interestingly, a similar IFN-γ message was observed in mice that were infected with LCMV alone, and this cytokine expression could therefore not be related exclusively to KLH-specific T cells (data not shown). In addition, anti-KLH antibodies were assayed in the sera of C3HeB/FeJ mice 3 weeks after i.p. immunization with KLH alone, or with LCMV. As shown in Table I, LCMV infection led to an overall decrease of the anti-KLH response. This inhibition was relatively more evident for IgG1 than for IgG2a and IgG2b antibodies and suggested a Th1-dependent redistribution of anti-KLH isotype. This result confirmed that LCMV could decrease Th2 responses directed against non-viral antigens.

Analysis of the isotypic distribution of antierythrocyte autoantibodies produced in the course of LCMV infection of C3HeB/FeJ mice has indicated a preponderance of the IgG2a subclass (27, 36). Here, the effect of infection with the Docile strain of LCMV on antierythrocyte autoantibody isotypic distribution was also tested in CBA/Ht mice that produce such autoantibodies after immunization with rat red blood cells (RRBC) (29, 37, 38). As found for anti-KLH responses in C3HeB/FeJ mice, LCMV infection resulted in a depression of antierythrocyte response that was more marked for the IgG1 than for the IgG2a isotype. Indeed, in CBA/Ht mice injected with RRBC alone, IgG1 was the dominant isotype in the autoantibody response. In contrast, a concomitant infection with LCMV triggered modulation of the antierythrocyte response leading to an IgG2a-skewed distribution similar to that of the antired cell autoimmune response spontaneously induced by the virus in C3HeB/FeJ mice (Fig. 2).

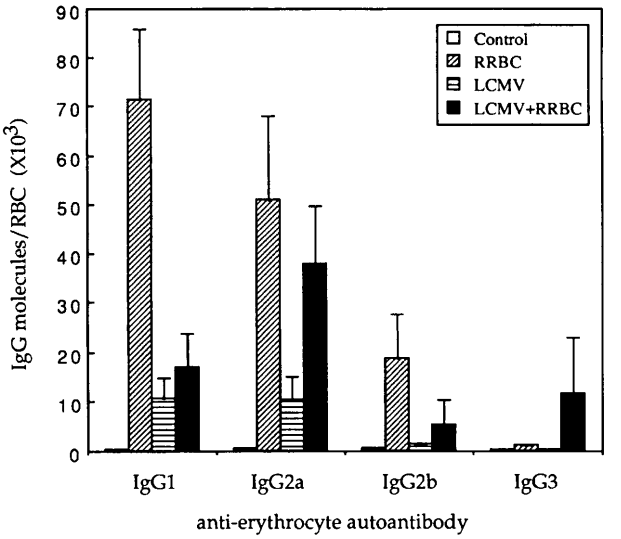

Figure 2. Effect of LCMV infection on the antierythrocyte autoantibody response induced in CBA/Ht mice by immunization with RRBC. Antierythrocyte autoantibody subclasses were assayed by DELAT in groups of four to six CBA/Ht mice 28 days after the first of a series of immunizations with RRBC. When indicated, LCMV infection was performed on the day of the first immunization with RRBC. Results are shown as number of IgG molecules fixed per red blood cell (mean ± SE).

General Lymphocyte Activation After LCMV Infection of C3HeB/FeJ Mice. To determine the effect of LCMV on total CD4+ T cell population, expression of cytokine mRNA by spleen cells was analyzed by RT-PCR at different times after infection. Both IL-4 and IFN-γ mRNA were enhanced after LCMV infection (Fig. 3), but with different kinetics: IFN-γ expression was maximal 4 days after virus inoculation, whereas strong IL-4 message was detected only at Day 7. The cellular origin of this cytokine gene expression was analyzed by depletion of CD4+ and CD8+ T cells by *in vivo* antibody treatment. Elimination of CD4+ T cells almost completely inhibited LCMV-induced IL-4 and IFN-γ mRNA production (Fig. 4). In contrast, anti-CD8 treatment did not induce any decrease of IL-4 expression and had only variable effect on IFN-γ message (data not shown). These data suggest that LCMV strongly activates CD4+ cells, independent of their Th1 or Th2 subpopulation, resulting in increased cytokine message expression.

This LCMV-induced activation of CD4+ T lymphocytes was confirmed by analysis of the role of these cells in spleen cell proliferation and total immunoglobulin secretion observed in the course of infection. Typically, spleen cell thymidine incorporation gradually increased between 7 and 11 days after LCMV infection (data not shown). This spleen cell proliferation was strongly, although not completely, reduced by anti-CD4 treatment, whereas it remained unchanged after administration of anti-CD8 antibody (Table II). Assays of immunoglobulin production in supernatants of spleen cells revealed a dramatic increase of IgG2a secretion at Days 7 to 11 (data not shown), in agreement with the previously reported isotypically restricted hypergammaglobulinemia triggered by LCMV (2, 4). The polyclonal nature of these immunoglobulins was indicated by a strong increase of antibodies reacting with DNP-BSA in the sera of infected animals (not shown). The role of T lymphocytes in this polyclonal B lymphocyte activation was also analyzed

Table I. Effect of LCMV Infection on Anti-KLH Antibody Response

LCMV infection[a]	Anti-KLH antibody (ng/ml)[b]			
	IgG1	IgG2a	IgG2b	IgG3
–	1550 ± 448	45000 ± 22060	26210 ± 5520	<1.4
+	26 ± 25	14850 ± 3230	6380 ± 278	<1.4

[a] Performed concomitantly with i.p. immunization with KLH
[b] Determined by ELISA 3 weeks after immunization for groups of three to four C3HeB/FeJ mice (mean ± SE).

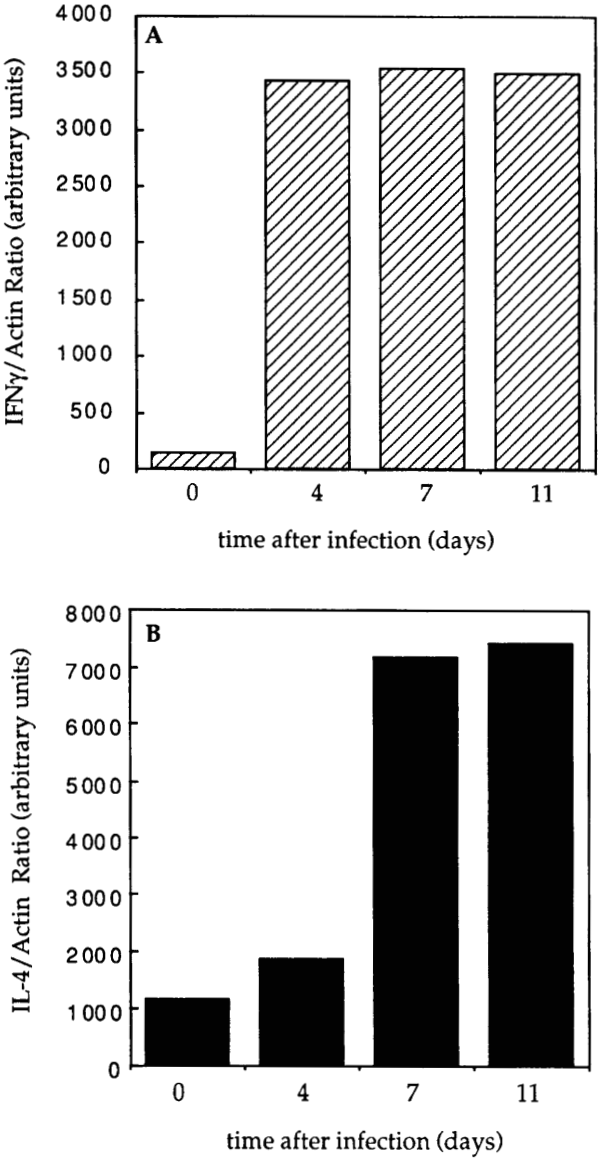

Figure 3. Spleen cell expression of cytokine message after LCMV infection of C3HeB/FeJ mice. Actin, (A) IFN-γ and (B) IL-4 messages were determined by RT-PCR in pooled spleen cells obtained at different times after LCMV infection from groups of four C3HeB/FeJ animals. The post-PCR products were semiquantitatively analyzed with a phosphor imager.

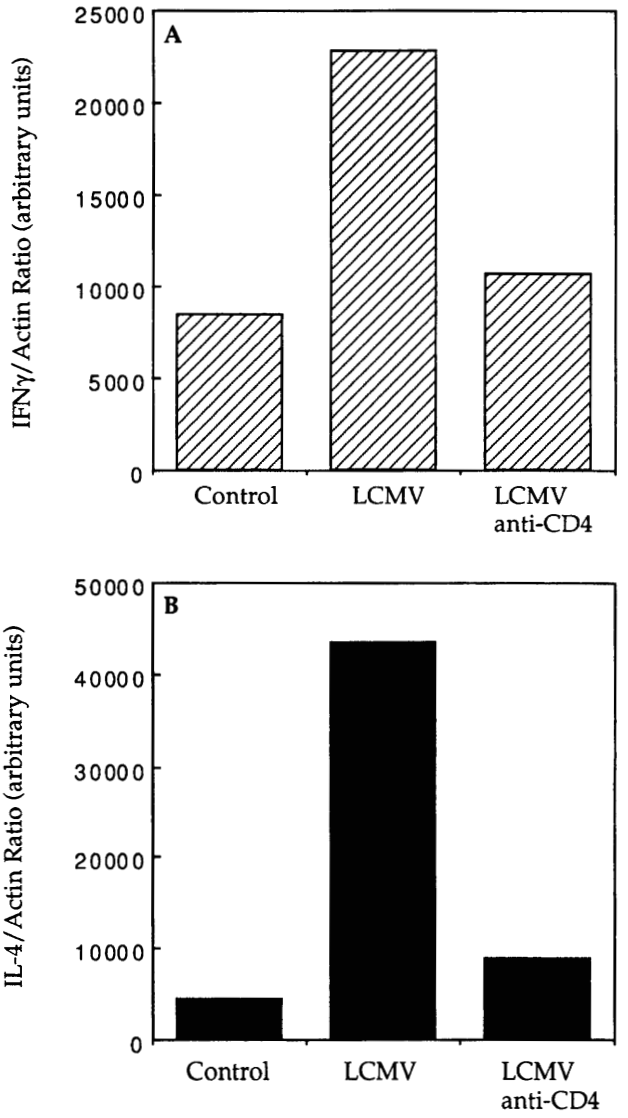

Figure 4. Effect of anti-CD4 treatment on cytokine messages induced by LCMV. (A) IFN-γ and (B) IL-4 messages were analyzed by RT-PCR in pooled spleen cells obtained from groups of four control C3HeB/FeJ mice or from animals 10 days after LCMV infection with or without anti-CD4 treatment.

by CD4+ cell depletion which almost completely inhibited immunoglobulin secretion by cultured spleen cells (Fig. 5). These data suggest that LCMV strongly activates CD4+ cells, resulting in increased cytokine message expression and leading to spleen cell proliferation and IgG2a production by B lymphocytes.

Discussion

In this report, we analyzed the effect of infection with the Docile strain of LCMV on CD4+ T-cell responses in C3HeB/FeJ mice. Strikingly different results were obtained, depending on the type of response analyzed. Indeed, on the one hand, the virus induced a strong depression of CD4+ T-lymphocyte functions elicited by a concomitant immunization with a nonviral protein antigen, like KLH, and especially an inhibition of Th2 subpopulation differentiation. This correlated with a decrease of anti-KLH antibody production that was most marked for the IgG1 isotype and therefore led to a relative preponderance of the IgG2a sub-

Table II. Effect of Anti-CD4 and Anti-CD8 Treatment on LCMV-Induced Spleen Cell Proliferation

LCMV infection	Anti-CD4 treatment	Anti-CD8 treatment	Thymidine incorporation (CPM)[a]
–	–	–	3124 ± 507
+	–	–	24029 ± 2591
+	+	–	10426 ± 982
+	–	+	20230 ± 2725

[a] Measured 10 days p.i. in groups of four to five C3HeB/FeJ mice (mean ± SE).

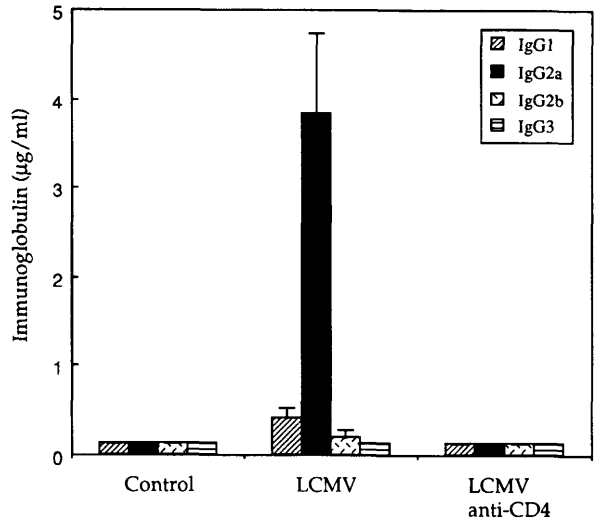

Figure 5. Effect of anti-CD4 treatment on LCMV-induced immunoglobulin secretion. IgG subclass levels were determined by ELISA in the supernatants of spleen cells of control C3HeB/FeJ mice (four animals per group) or from mice infected for 10 days with LCMV, with or without anti-CD4 treatment (mean ± SE).

class. In contrast, LCMV triggered a general stimulation of the CD4+ cell-dependent immune response, leading to an increase of cytokine gene expression, spleen cell proliferation, and total immunoglobulin production. Thus immunostimulation and immunodepression may be induced simultaneously by this viral infection.

Our observation of decreased anti-KLH responses in infected C3HeB/FeJ mice fits well with previously described immunosuppressive effects of LCMV (16, 17) and contrasts sharply with the enhancement of concomitant antibody responses directed against nonviral antigens, reported after infection with LDV, adenovirus, and MHV (2, 23). However, despite this different effect on the level of antibody secretion, LCMV like these other viruses triggers a shift both in the differentiation of T helper cells, and, as a probable consequence of this, in the isotypic distribution of secreted immunoglobulins. In LCMV-infected animals, as well as in LDV-, or MHV-infected mice, this results in a relative preponderance of the Th1 lymphocyte subpopulation and IgG2a antibodies in responses initiated concomitantly with the onset of infection, regardless of their specificity. A similar LCMV-induced restricted depression of Th2 cytokines, as well as increased Th1-secreted IFN-γ, has been observed after T-lymphocyte activation with mitogens (14, 15). The strong IFN-γ response observed in LCMV-infected mice might account partly for the immunosuppressive effect that the virus induces on antibody responses, through a depression of Th2-dependent B-cell activation, although impairment of antigen presentation is most probably also involved (18–20). The ability of LCMV, as well as of other viruses, to trigger such potent IFN-γ production should be related to the production of IL-12 by macrophages reported after infection (39–41). Other cytokines such as IL-18 might as well be involved (42). Whatever the mechanisms leading to this immunomodulation, the bias in T-helper-cell differentiation induced by LCMV may be reflected in the IgG2a restriction of antierythrocyte autoantibodies spontaneously produced in infected C3HeB/FeJ mice (27, 36). This isotypic restriction to the IgG2a subclass may increase the pathogenicity of these autoantibodies, for instance, by allowing easier phagocytosis by macrophages that express receptors for this isotype (43). Interestingly, pathogenic antierythrocyte autoimmune response in NZB mice are also dominated by Th1 cells (44), whereas IgG1 is the predominant isotype in nonpathogenic autoantibody production elicited by immunization of mice with rat red blood cells (35).

The depression by LCMV of specific antibody responses elicited by immunization concomitant with infection contrasts sharply with the general stimulation of CD4-dependent immune functions also triggered at the same time. With regard to polyclonal B lymphocyte activation, infection with LCMV does not differ from infection with other viruses, like LDV, MHV, and adenovirus, that also induce an IgG2a-restricted production of antibodies with a broad specificity (2, 22, 32). In these models it has also been reported that viruses could trigger proliferation of B lymphocytes in a T-independent fashion (22, 25, 32). Here, the strong reduction of LCMV-induced spleen cell proliferation after treatment with an anti-CD4 antibody suggests that LCMV stimulates both B and CD4+ T lymphocytes, although it is possible that part of the B cell proliferation depends on the presence of functional T-helper lymphocytes. Several previous papers report a strong LCMV-induced activation of CD8+ T lymphocytes (7, 45, 46), including proliferation of these cells, a finding that we could not confirm in this study. This discrepancy may be explained by the use of different virus and mouse strains. LCMV-triggered activation of C3HeB/FeJ CD4+ T lymphocytes was indicated here by the proliferative response, but also by the enhancement of IL-4 and IFN-γ messages in spleen cells that could be inhibited by anti-CD4 treatment. This confirms and extends several reports pointing to an increased cytokine secretion triggered by this virus in lymphocytes (8, 9, 11, 12). The sharply different effect of infection on IL-4 message expression, which is increased in total CD4+ splenic cells but suppressed in T helper cells stimulated in response to KLH immunization, clearly points to different mechanisms elicited by the virus. These mechanisms lead to a simultaneous general activation of T-helper lymphocytes and suppression of specific Th2 responses, probably through a defect of antigen presentation. How may the general stimulation of the C3HeB/FeJ immune system by LCMV relate to the production of antierythrocyte autoantibodies? We previously reported that B-lymphocyte, polyclonal activation is not sufficient to explain the development of hemolytic anemia (4), although it might be involved in an increase of red cell autoantibody secretion if these antibodies are present in the normal B repertoire of C3HeB/FeJ mice. Since LCMV-induced immunosuppression is also evident in the antierythrocyte responses elicited

by concomitant immunization with RRBC, it seems unlikely that the associated autoimmune reaction observed in C3HeB/FeJ mice is actually merely related to the enhancement of a response that would be induced by a classical presentation of an autoantigen released in the course of the infection. Therefore, other mechanisms, such as antigenic mimicry at the level of B- or T-cell epitope may be postulated (36). The activation of general CD4+-dependent responses induced by the virus could then increase an autoantibody production that, unlike classical antibody responses elicited by immunization unrelated to the infectious process, would not be impaired by the immunosuppressive effect of LCMV.

The authors are indebted to Drs. R. H. vahns'Yqule and P. L. Masson for critical reading of this manuscript, to T. Briet, M.-D. Gonzales, and J. Van Broeck for expert technical assistance.

1. Ahmed R, Oldstone MBA. Mechanisms and biological implications of virus-induced polyclonal B-cell activation. In: Notkins AL, Oldstone MBA, Eds. Concepts in Viral Pathogenesis. New York: Springer-Verlag, pp 231–238, 1984.
2. Coutelier JP, van der Logt JTM, Heessen FWA, Vink A, Van Snick J. Virally induced modulation of murine IgG antibody subclasses. J Exp Med **168:**2373–2378, 1988.
3. Stellrecht KA, Vella AT. Evidence for polyclonal B-cell activation as the mechanism for LCMV-induced autoimmune hemolytic anemia. Immunol Lett **31:**273–278, 1992.
4. Coutelier JP, Johnston SJ, El Azami El Idrissi M, Pfau CJ. Involvement of CD4+ cells in lymphocytic choriomeningitis virus-induced autoimmune anaemia and hypergammaglobulinaemia. J Autoimmun **7:**589–599, 1994.
5. Yang H, Dundon PL, Nahill SR, Welsh RM. Virus-induced polyclonal cytotoxic T lymphocyte stimulation. J Immunol **142:**1710–1718, 1989.
6. Nahill SR, Welsh RM. High frequency of cross-reactive cytotoxic T lymphocytes elicited during the virus-induced polyclonal cytotoxic T lymphocyte response. J Exp Med **177:**317–327, 1993.
7. Kasaian MT, Leite-Morris KA, Biron CA. The role of CD4+ cells in sustaining lymphocyte proliferation during lymphocytic choriomeningitis virus infection. J Immunol **146:**1955–1963, 1991.
8. Gessner A, Moskophidis D, Lehmann-Grube F. Enumeration of single INF-γ-producing cells in mice during viral and bacterial infection. J Immunol **142:**1293–1298, 1989.
9. Gessner A, Drjupin R, Löhler J, Lother H, Lehmann-Grube F. IFN-γ production in tissues of mice during acute infection with lymphocytic choriomeningitis virus. J Immunol **144:**3160–3165, 1990.
10. Colle JH, Saron MF, Shidani B, Lembezat MP, Truffa-Bachi P. High frequency of T lymphocytes committed to interferon-γ transcription upon polyclonal activation in spleen from lymphocytic choriomeningitis virus-infected mice. Intern Immunol **5:**435–441, 1993.
11. Kasaian MT, Biron CA. The activation of IL-2 transcription in L3T4+ and Lyt-2+ lymphocytes during virus infection *in vivo*. J Immunol **142:**1287–1292, 1989.
12. Moskophidis D, Frei K, Löhler J, Fontana A, Zinkernagel RM. Production of random classes of immunoglobulins in brain tissue during persistent viral infection paralleled by secretion of interleukin-6 (IL-6) but not IL-4, IL-5, and gamma interferon. J Virol **65:**1364–1369, 1991.
13. Borrow P, Oldstone MBA. Lymphocytic choriomeningitis virus. In: Nathanson N, Ed. Viral Pathogenesis. Philadelphia: Lippincott-Raven, pp 593–627, 1997.
14. Colle JH, Saron MF, Truffa-Bachi P. Altered cytokine genes expression by conA-activated spleen cells from mice infected by lymphocytic choriomeningitis virus. Immunol Lett **35:**247–253, 1993.
15. Butz EA, Southern PJ. Lymphocytic choriomeningitis virus-induced immune dysfunction: Induction of and recovery from T-cell anergy in acutely infected mice. J Virol **68:**8477–8480, 1994.
16. Roost H, Charan S, Gobet R, Rüedi E, Hengartner H, Althage A, Zinkernagel RM. An acquired immune suppression in mice caused by infection with lymphocytic choriomeningitis virus. Eur J Immunol **18:**511–518, 1988.
17. Tishon A, Borrow P, Evans C, Oldstone MBA. Virus-induced immunosuppression. 1. Age at infection relates to a selective or generalized defect. Virology **195:**397–405, 1993.
18. Odermatt B, Eppler M, Leist TP, Hengartner H, Zinkernagel RM. Virus-triggered acquired immunodeficiency by cytotoxic T-cell-dependent destruction of antigen-presenting cells and lymph follicle structure. Proc Natl Acad Sci USA **88:**8252–8256, 1991.
19. Althage A, Odermatt B, Moskophidis D, Kündig T, Hoffman-Rohrer U, Hengartner H, Zinkernagel RM. Immunosuppression by lymphocytic choriomeningitis virus infection: Competent effector T and B cells but impaired antigen presentation. Eur J Immunol **22:**1803–1812, 1992.
20. Borrow P, Evans CF, Oldstone MBA. Virus-induced immunosuppression: Immune system-mediated destruction of virus-infected dendritic cells results in generalized immune suppression. J Virol **69:**1059–1070, 1995.
21. Razvi ES, Welsh RM. Programmed cell death of T lymphocytes during acute viral infection: A mechanism for virus-induced immune deficiency. J Virol **67:**5754–5765, 1993.
22. Coutelier JP, Coulie PG, Wauters P, Heremans H, van der Logt JTM. *In vivo* polyclonal B-lymphocyte activation elicited by murine viruses. J Virol **64:**5383–5388, 1990.
23. Coutelier JP, van der Logt JTM, Heessen FWA. IgG subclass distribution of primary and secondary immune responses concomitant with viral infection. J Immunol **147:**1383–1386, 1991.
24. Monteyne P, Van Broeck J, Van Snick J, Coutelier JP. Inhibition by lactate dehydrogenase-elevating virus of *in vivo* interleukin 4 production during immunization with keyhole limpet haemocyanin. Cytokine **5:**394–397, 1993.
25. Lardans V, Godfraind C, van der Logt JTM, Heessen FWA, Gonzalez MD, Coutelier JP. Polyclonal B lymphocyte activation induced by mouse hepatitis virus A59 infection. J Gen Virol **77:**1005–1009, 1996.
26. Broomhall KS, Morin M, Pevear DC, Pfau CJ. Severe and transient pancytopenia associated with a chronic arenavirus infection. J Exp Pathol **3:**259–269, 1987.
27. Vella AT, Pfau CJ. The presence of an anti-erythrocyte autoantibody in C3HeB/FeJ mice after lymphocytic choriomeningitis virus infection. Autoimmunity **9:**319–329, 1991.
28. Jacobson S, Pfau CJ. Viral pathogenesis and resistance to defective interfering particles. Nature (London) **283:**311–313, 1980.
29. Verdonck E, Pfau CJ, Gonzalez MD, Masson PL, Coutelier JP. Influence of viral infection on anti-erythrocyte autoantibody response after immunization of mice with rat red blood cells. Autoimmunity **17:**73–81, 1994.
30. Dialynas DP, Wilde DB, Marrack P, Pierres A, Wall KA, Havran W, Otten G, Loken MR, Pierres M, Kappler J, Fitch FW. Characterization of the murine antigenic determinant, designated L3T4a, recognized by monoclonal antibody GK1.5: Expression of L3T4a by functional T-cell clones appears to correlate primarily with class II antigen-reactivity. Immunol Rev **74:**29–56, 1983.
31. Ledbetter JA, Herzenberg LA. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol Rev **47:**63–90, 1979.
32. Coutelier JP, Van Snick J. Isotypically restricted activation by B lymphocytes by lactic dehydrogenase virus. Eur J Immunol **15:**250–255, 1985.

33. Coutelier JP, Van Roost E, Lambotte P, Van Snick J. The murine antibody response to lactate dehydrogenase elevating virus. J Gen Virol **67:**1099–1108, 1986.
34. Coutelier JP, van der Logt JTM, Heessen FWA. Rheumatoid factor production and polyclonal non-antiviral antibody responses elicited by infection with common mouse viruses. J Autoimmun **3:**681–686, 1990.
35. Mazza G, Day MJ, Barker RN, Corato A, Elson CJ. Quantitation of erythrocyte bound IgG subclass autoantibodies in murine autoimmune haemolytic anaemia. Autoimmunity **23:**245–255, 1996.
36. Mazza G, El Azami El Idrissi M, Coutelier JP, Corato A, Elson CJ, Pfau CJ, Day MJ. Infection of C3HeB/FeJ mice with the docile strain of lymphocytic choriomeningitis virus induces autoantibodies specific for erythrocyte band 3. Immunology **91:**239–245, 1997.
37. Cox KO, Howles A. Induction and regulation of autoimmune hemolytic anemia in mice. Immunol Rev **55:**31–53, 1981.
38. Naysmith JD, Ortega-Pierres MG, Elson CJ. Rat erythrocyte-induced anti-erythrocyte autoantibody production and control in normal mice. Immunol Rev **55:**55–87, 1981.
39. Coutelier JP, Van Broeck J, Wolf SF. Interleukin-12 gene expression after viral infection in the mouse. J Virol **69:**1955–1958, 1995.
40. Orange JS, Biron CA. An absolute and restricted requirement for IL-12 in natural killer cell IFN-γ production and antiviral defense: Studies of natural killer and T-cell responses in contrasting viral infections. J Immunol **156:**1138–1142, 1996.
41. Kanangat S, Thomas J, Gangappa S, Babu JS, Rouse BT. Herpes simplex virus type 1-mediated upregulation of IL-12 (p40) mRNA expression: Implications in immunopathogenesis and protection. J Immunol **156:**1110–1116, 1996.
42. Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M. Cloning of a new cytokine that induces IFN-γ production by T cells. Nature (London) **378:**88–91, 1995.
43. Stellrecht-Broomhall KA. Evidence for immune-mediated destruction as mechanism for LCMV-induced anemia in persistently infected mice. Viral Immunol **4:**269–280, 1991.
44. Shen CR, Mazza G, Perry FE, Beech JT, Thompson SJ, Corato A, Newton S, Barker RN, Elson CJ. T-helper 1 dominated responses to erythrocyte band 3 in NZB mice. Immunology **89:**195–199, 1996.
45. Christensen JP, Röpke C, Thomsen AR. Virus-induced polyclonal T-cell activation is followed by apoptosis: Partitioning of CD8+ T cells based on α4 integrin expression. Intern Immunol **8:**707–715, 1996.
46. Tough DF, Borrow P, Sprent J. Induction of bystander T-cell proliferation by viruses and type I interferon *in vivo*. Science **272:**1947–1950, 1996.