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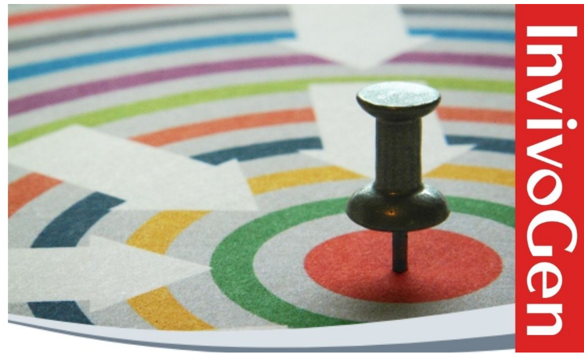

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An Absolute and Restricted Requirement for IL-12 in Natural Killer Cell IFN-γ Production and Antiviral Defense

Studies of Natural Killer and T Cell Responses in Contrasting Viral Infections[1]

Jordan S. Orange and Christine A. Biron[2]

IL-12 is a potent inducer of IFN-γ and is associated with a wide variety of immunoregulatory activities. Using murine CMV (MCMV) and lymphocytic choriomeningitis virus (LCMV) models of acute viral infection, we show here that IL-12 is integrally involved in certain aspects of antiviral immunity. Experiments evaluating the production and function of IL-12 demonstrated the induction of biologically active factor early during Smith strain MCMV, but not Armstrong strain LCMV, infection. The MCMV-induced IL-12 was responsible for early NK cell IFN-γ production and viral control, as in vivo IL-12 neutralization by Ab treatment blocked both of these. In contrast, NK cell IFN-γ production could not be detected during LCMV infection, and IL-12 neutralization had no effect on early LCMV replication. In both infections, treatments neutralizing IL-12 failed to alter either early NK cell cytotoxicity or later T cell responses, including IFN-γ production and viral clearance on days 7 and 9 after infection. These data demonstrate the induction of IL-12 production during certain viral infections and the function of IL-12 for eliciting NK cell IFN-γ production and antiviral defense. Furthermore, they suggest the existence of IL-12-independent mechanisms for IFN-γ induction and viral control by T cells. *The Journal of Immunology,* **1996, 156: 1138–1142.**

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Acute infection of mice with murine CMV (MCMV) or lymphocytic choriomeningitis virus (LCMV) leads to early activation of NK cells, peaking on days 2 to 5 after infection, and later activation of T cells, peaking on days 7 to 10. Immune responses to these agents (1–14) are distinct from those against most bacteria and parasites, as virus-induced IFN-αβ contributes to the activation of NK cell-mediated lysis (10, 11). Moreover, protective responses can require $CD8^+$ T cells (3–6) in addition to T cell production of IFN-γ (12–14). IL-12 is a heterodimeric cytokine made up of constituitively expressed p35 chain and inducible p40 chain. These are covalently linked to form the biologically active p70 protein that potently induces IFN-γ production and activates NK and T cells (15). In models of parasitic and bacterial infections, endogenous IL-12 has been shown to be central to NK and T cell IFN-γ responses and control of the infectious agents (16–20). Although limited PCR analysis has suggested that p40 mRNA expression can be induced by viral infections (21), expression of IL-12 protein has not been documented. Furthermore, the biologic consequences of endogenous IL-12 during viral infection have not been defined. The studies presented here were undertaken to evaluate the induction of IL-12 expression and IL-12 function in eliciting protective NK and T cell responses to acute viral infections with MCMV or LCMV. The results demonstrate that significant levels of IL-12 protein are induced during certain viral infections, and that the factor plays an important regulatory role in endogenous immune responses.

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[3] Abbreviations used in this paper: MCMV, murine cytomegalovirus; LCMV, lymphocytic choriomeningitis virus; AGM1, asialo ganglio-*N*-tetraosylceramide.

Materials and Methods

Animals and treatments

C57BL/6 male mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) and were 5 to 10 wk old when used in the experiments. Mice were uninfected or infected i.p. with either 2×10^5 plaque-forming units of Smith strain MCMV V70 salivary gland extract or 2.5×10^4 plaque-forming units of Armstrong strain LCMV clone E350 for 1 to 9 days. Under these conditions, peak MCMV liver titers were detected on day 2, and peak LCMV spleen titers were detected on days 3 to 5 after infection. Where specified, mice were infected with MCMV stocks reduced in virulence by growth in tissue culture. Blood was drawn retro-orbitally, and mice were killed by cervical dislocation. NK cells were depleted in vivo by i.p. administration of 0.25 mg of purified anti-AGM1 IgG (Wako Chemicals, Dallas TX) or 1 mg of 0.2-μm filter-sterilized PK136 ascites, specific for NK1.1 (hybridoma provided by Dr. Vinay Kumar, Southwestern Medical School, Dallas, TX) on the day before and on the day after infection. Control treatments for anti-AGM1 and anti-NK1.1 consisted of purified rabbit IgG (Sigma Chemical Co., St. Louis, MO) and 0.2-μm filter-sterilized P3NS1 ascitic fluid (hybridoma obtained from American Type Culture Collection, Rockville, MD), respectively. IL-12 was neutralized in vivo by the administration of 1 mg of C17.8 neutralizing rat anti-mouse IL-12 (hybridoma provided by Drs. M. Wysocka and G. Trinchieri, Wistar Institute, Philadelphia, PA) prepared as an ammonium sulfate-precipitated ascites. Control treatment consisted of 1 mg of rat IgG (Sigma Chemical Co.) diluted in ammonium sulfate-precipitated P3NS1 ascites. In day 2 and 3 experiments, one injection of anti-IL-12 was given 14 h before infection with MCMV or LCMV, and in day 7 and 9 experiments, a second injection was given on day 4 after infection.

Cytokine assays

IL-12 p40 sandwich ELISA was performed by coating Immulon 4 microtiter plates (Dynatech, Chantilly, VA) with 20 μg/ml of ammonium sulfate-precipitated ascites from the C15.1 monoclonal rat-anti-mouse IL-12 hybridoma (provided by Drs. M. Wysocka and G. Trinchieri). Plates were blocked with 5% fetal bovine serum-PBS, and biologic samples were incubated overnight at 4°C. Polyclonal sheep anti-IL-12 (Genetics Institute, Cambridge, MA) at 10 μg/ml and peroxidase-conjugated donkey anti-sheep (Jackson ImmunoResearch, West Grove, PA) at 2 μg/ml were used to detect bound IL-12. The limit of detection for the conditions used was 100 pg/ml serum. The IL-12 biologic assay used nonneutralizing C15.1 to

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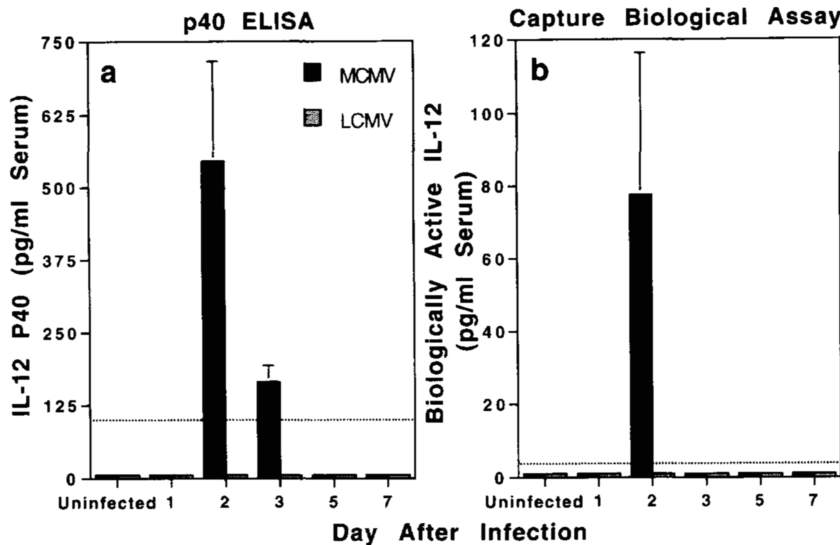

FIGURE 1. IL-12 production during acute viral infection. IL-12 production was measured by p40 ELISA (*a*) and capture biological assay (*b*). Serum samples from C57BL/6 mice infected with MCMV (solid bars) or LCMV (stippled bars) were examined. IL-12 was significantly induced on day 2 after infection with MCMV in either assay ($p < 0.05$). For all time points measured, $n = 3$ mice; error bars show the SE. The dotted line shows the limit of detection for each assay. Conditioned media were prepared with splenic leukocytes isolated from uninfected mice or from mice on days 1, 2, 3, 5, and 7 after infection and similarly demonstrated significant production of IL-12 only on day 2 after infection with MCMV.

capture IL-12, followed by the addition of 1 × 10^6 normal C57BL/6 splenocytes and 50 U/ml IL-2 to each microtiter well (22). IFN-γ production in cell supernatants was measured by ELISA and compared with levels obtained from an IL-12 standard curve (Genetics Institute) to determine the quantity of biologically active IL-12 heterodimer in each sample. The specificity of IL-12 in the assay was determined by using neutralizing C17.15 rat anti-mouse IL-12 to prevent IFN-γ induction (hybridoma provided by Drs. M. Wysocka and G. Trinchieri). The limit of detection for the conditions employed was 4.5 pg/ml serum. The procedure used for IFN-γ ELISA was similar to that used for IL-12 p40 ELISA, except XMG1.2 monoclonal rat anti-mouse IFN-γ (DNAX, Palo Alto, CA) was used as capture Ab, and polyclonal rabbit anti-IFN-γ (provided by Dr. P. Scott, University of Pennsylvania) followed by peroxidase-conjugated donkey anti-rabbit were used for detection. The limit of detection was 40 pg/ml for IL-12 biologic assay samples, 160 pg/ml for serum, and 1 pg/million cells for conditioned medium. Splenic leukocyte-conditioned medium was prepared by incubating 5 × 10^6 cells/ml in RPMI medium containing 1% fetal bovine serum for 24 h at 37°C and then concentrating supernatants approximately 10-fold using Centricon-10 devices (Amicon Corp., Beverly, MA). Both concentrated and unconcentrated samples were evaluated by ELISA.

Cytotoxicity assays, flow cytometry, and virus measurement

The NK cell cytotoxic activity of splenic leukocyte and bone marrow cell suspensions was evaluated, as previously described (23), by a ^{51}Cr release assay using YAC-1 lymphoma cells as target cells. Virus-specific CTL activity in the spleen was measured in a ^{51}Cr release assay against infected and uninfected histocompatible MC57G target cells as previously described (24). Flow cytometric analysis of $CD8^+$ and $CD4^+$ T cell subsets was also performed as previously described (24). LCMV replication was quantitated by plaque assay on confluent monolayers of Vero cells (24), and MCMV replication was determined by plaque assay on confluent monolayers of mouse embryo fibroblasts (25). The left lateral lobe of liver was weighed before homogenization. Viral titers are expressed in plaque-forming units per gram of liver, and the renal viral titer is expressed per set of two kidneys. Hepatic necrotic foci are grossly demonstrable individual areas of liver necrosis approximately ≥1 mm^2 that appear histologically as a broad area of eosinophilic necrotic hepatocytes (25).

Results and Discussion

To measure the induction of IL-12 during viral infection, both serum and splenic leukocyte-conditioned media from infected animals were examined for factor by ELISA and biologic assay. The conditions were selected based on challenge virus dose titrations establishing peak NK cell cytotoxic responses on days 2 and 3, and protective T cell responses on days 7 to 10 after infection. MCMV infection induced systemic IL-12 primarily on day 2, as detected by p40 subunit ELISA (Fig. 1*a*) and by biologic assay of serum

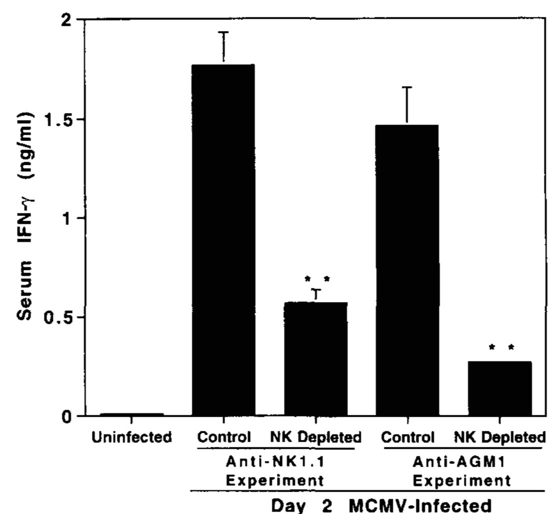

FIGURE 2. Effects of NK cell depletion on systemic induction of IFN-γ during MCMV infection. Induction of IFN-γ in serum was evaluated on day 2 after infection with MCMV by ELISA. NK cells were depleted in vivo by Ab treatment as described in *Materials and Methods*. Values shown are the averages of four animals, and error bars show the SE. The reduction in serum IFN-γ mediated by NK cell depletion was significant (**, $p < 0.0005$), as determined by Student's *t* test.

(Fig. 1*b*). IL-12 production by ex vivo splenic leukocytes also demonstrated the induction of factor only on day 2 after infection with MCMV (data not shown). In contrast, during LCMV infection, systemic (Fig. 1) or splenic cell production of IL-12 protein was not found on day 1, 2, 3, 5, or 7 after infection. These data show that IL-12 production is detectable at early times after MCMV infection. The results demonstrate, for the first time, the production of biologically active IL-12 p70 heterodimer during viral infection. In addition, they show that all viral infections are not the same with regard to IL-12 induction.

We have previously shown that NK cells activated during MCMV infection produce early IFN-γ, and that the production of this factor contributes to NK cell-mediated antiviral defense (25). The studies presented here showed that significant levels of IFN-γ were present in serum on day 2 after infection (Fig. 2). As depletion of NK cells by in vivo Ab treatment reduced serum levels by up to >80%, NK cells were responsible for the systemic IFN-γ

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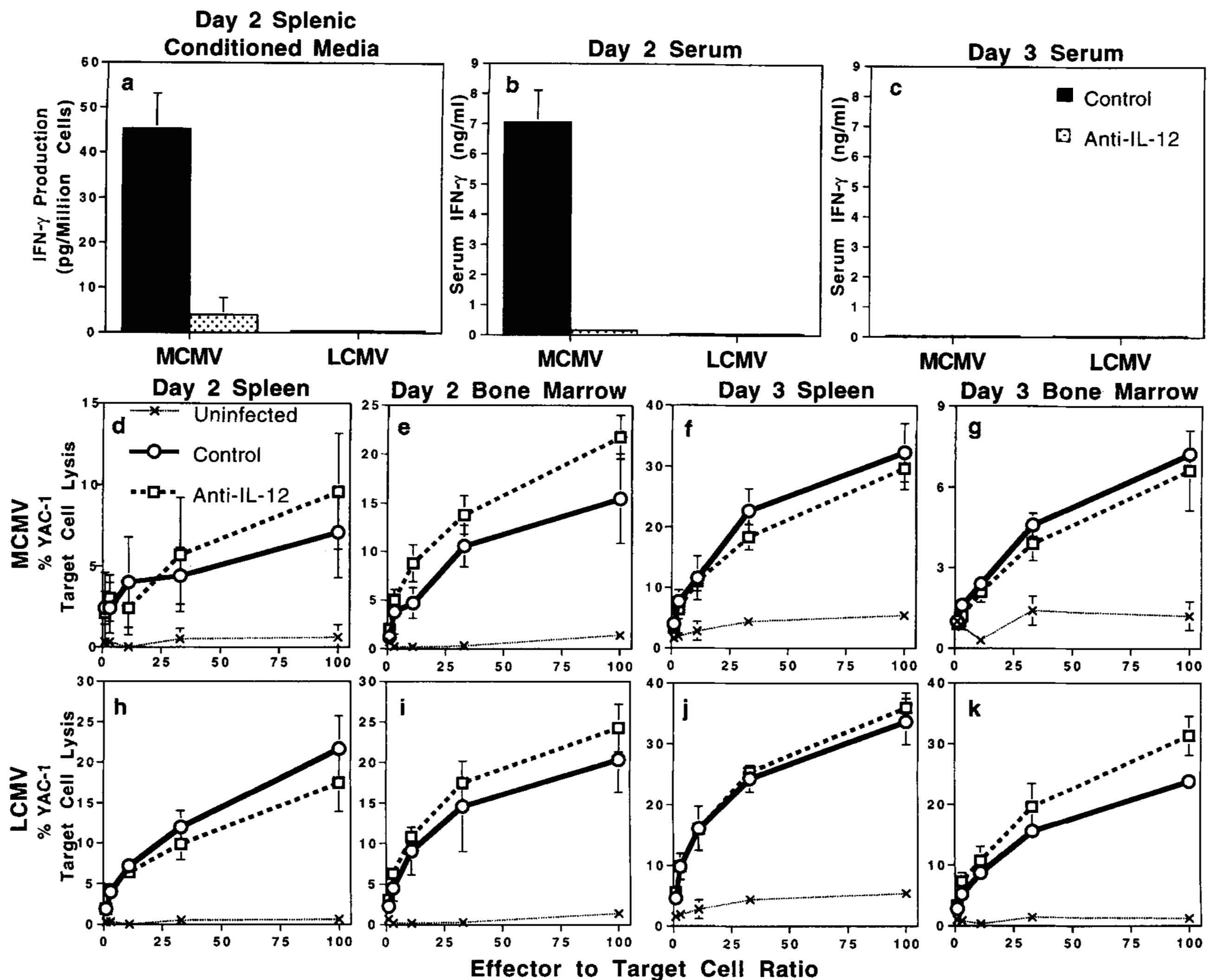

FIGURE 3. Effects of in vivo IL-12 neutralization on NK cell IFN-γ production and cytotoxicity during MCMV or LCMV infection. IFN-γ induction was evaluated in splenic leukocyte-conditioned medium on day 2 after infection (*a*), and in serum on day 2 (*b*) and day 3 (*c*) after infection with MCMV or LCMV by ELISA. The reduction of IFN-γ levels by anti-IL-12 treatment (stippled bars) on day 2 after infection with MCMV was significantly different from that in controls (solid bars; **, $p < 0.05$), as determined by Student's *t* test. NK cell cytotoxic activity against YAC-1 target cells was measured by a ^{51}Cr release assay on day 2 after infection with MCMV in the spleen (*d*) or bone marrow (*e*) and on day 3 after infection with MCMV in the spleen (*f*) or bone marrow (*g*). NK cell cytotoxicity was also evaluated on day 2 after infection with LCMV in the spleen (*h*) or bone marrow (*i*) and on day 3 after infection with LCMV in the spleen (*j*) or bone marrow (*k*). In all cases, there was no significant difference between control treatment (squares) and anti-IL-12 treatment (circles). In all panels, $n = 3$ mice; error bars show the SE.

(Fig. 2). MCMV induction of IFN-γ could be demonstrated in the serum on day 2 even after infection with 10-fold less MCMV or virus rendered less virulent by tissue culture passage. To determine the role of IL-12 in NK cell production of IFN-γ, Abs neutralizing IL-12 were administered in vivo. Anti-IL-12 treatment prevented NK cell IFN-γ production on day 2 after infection with MCMV, as measured in culture (Fig. 3*a*) and in serum (Fig. 3*b*). The early IFN-γ response to MCMV infection was no longer apparent by day 3 after infection (Fig. 3*c*). There was no detectable production of IFN-γ during the NK cell response to LCMV infection (Fig. 3, *a–c*). Interestingly, NK cell cytotoxicity induced by MCMV (Fig. 3, *d–g*) or LCMV (Fig. 3, *h–k*), in the spleen or bone marrow on days 2 and 3 after infection, was not affected by IL-12 neutralization. Thus, IL-12 is required for IFN-γ production by NK cells, but not NK cell cytotoxicity, during viral infection.

In vivo neutralization of IL-12 had no demonstrable effect on late T cell responses to MCMV and LCMV. Although there were only modest levels of serum IFN-γ at late times after infection with LCMV or MCMV (Fig. 4, *a* and *b*), there was a dramatic IFN-γ response, as measured by splenic cell production during LCMV infection, and a smaller, but significant, splenic IFN-γ response during MCMV (Fig. 4, *c* and *d*). The late production of IFN-γ occurred in the absence of detectable IL-12 production (Fig. 1). Unlike the NK cell IFN-γ response, IL-12 neutralization had no effect on late IFN-γ production (Fig. 4, *a–d*). Furthermore, IL-12 neutralization did not alter the generation of LCMV-specific CTL (Fig. 4, *e* and *f*) or the expansion of $CD8^+$ T cell populations during either infection (Fig. 4, *g* and *h*). $CD4^+$ T cell numbers were also not significantly affected by IL-12 neutralization (Fig. 4, *i* and *j*). Therefore, at late times after viral infection, when IL-12

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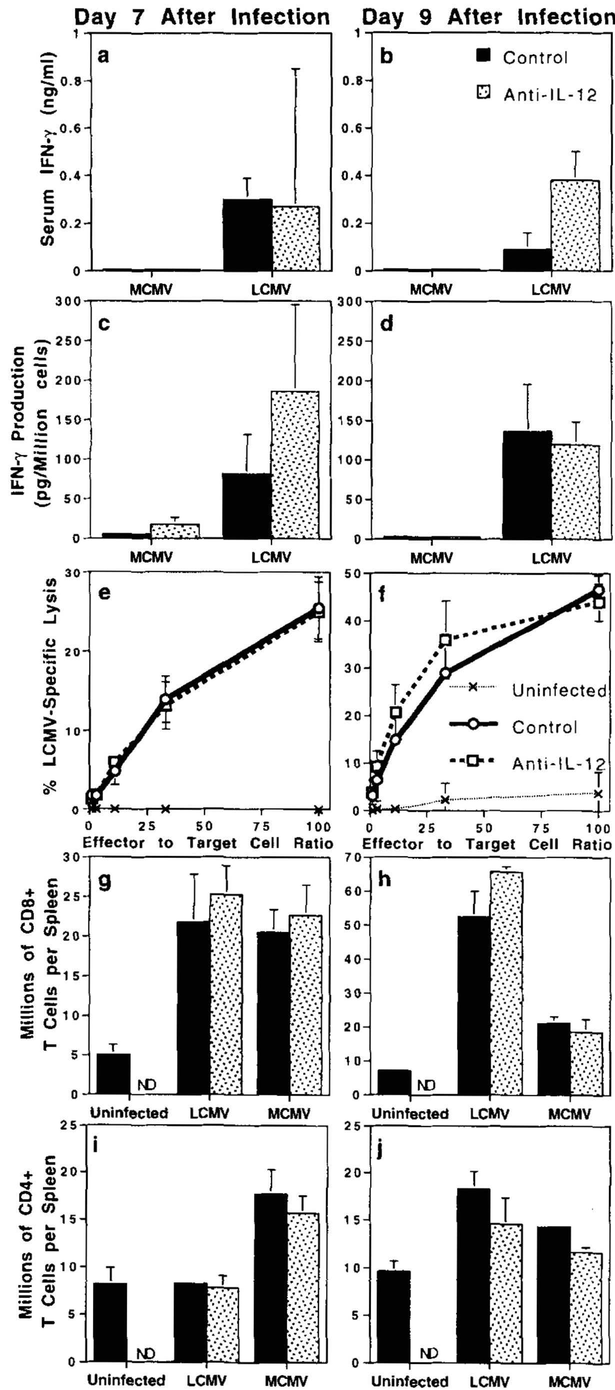

FIGURE 4. Effects of IL-12 neutralization on T cell responses to infection with MCMV or LCMV. Induction of IFN-γ was evaluated in serum (*a* and *b*) or splenic leukocyte-conditioned medium (*c* and *d*) on day 7 (*a* and *c*) or day 9 (*b* and *d*) after infection with MCMV or LCMV. There were no significant differences between anti-IL-12-treated (stippled bars) or control groups (solid bars). Ex vivo LCMV-specific lytic activity was measured on day 7 (*e*) and day 9 (*f*) after infection, and cells from uninfected (x), control (circles), or anti-IL-12-treated (squares) cultures were tested. Expansion of $CD8^+$ (*g* and *h*) and $CD4^+$ (*i* and *j*) T cell subsets was quantified on day 7 (*g* and *i*) and day 9 (*h* and *j*) after infection with MCMV or LCMV. In all cases, anti-IL-12 treatment did not cause a significant change compared with the control. All data points represent the means of three to six mice, and error bars show the SE. ND, not done.

Table I. *Role of IL-12 in antiviral defense*[a]

Virus	Day Post-Infection	Antibody Treatment	Log PFU per g Liver	Log PFU per Spleen	Log PFU per 2 Kidneys
LCMV	3	Control	3.30 ± 0.19	5.67 ± 0.05	3.07 ± 0.30
LCMV	3	Anti-IL-12	3.05 ± 0.18	5.63 ± 0.17	3.44 ± 0.25
LCMV	7	Control	2.50 ± 0.10	2.59 ± 0.21	2.00 ± 0.00
LCMV	7	Anti-IL-12	2.64 ± 0.29	2.61 ± 0.29	2.52 ± 0.30
MCMV	3	Control	4.22 ± 0.19	3.85 ± 0.19	1.60 ± 0.73
MCMV	3	Anti-IL-12	5.19 ± 0.14[b]	4.45 ± 0.28[b]	2.13 ± 0.08[b]
MCMV	7	Control	<2.00	<1.00	<2.00
MCMV	7	Anti-IL-12	<2.00	<1.00	<2.00

[a] Viral replication and hepatic pathology were assessed, and animals were treated as described in *Materials and Methods*. Three to six mice were used for all data points, and values shown are ± SE.

[b] Anti-IL-12 treatment mediated significant increase relative to control treatment $p < 0.05$ determined by Student's *t* test.

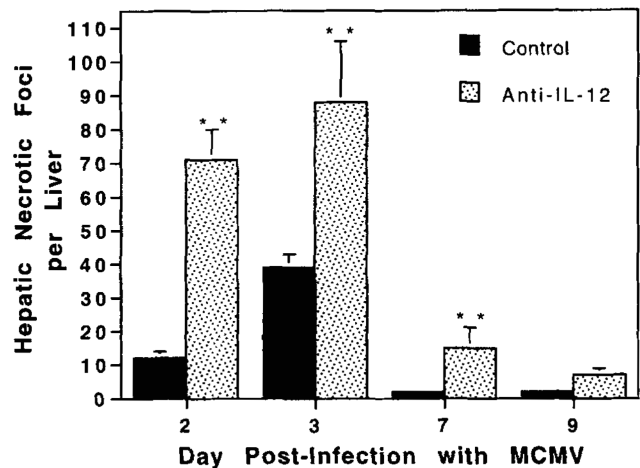

FIGURE 5. Effects of in vivo IL-12 neutralization on development of hepatic pathology during MCMV infection. Mice were MCMV infected for 2, 3, 7, or 9 days and evaluated for the presence of hepatic necrotic foci as described in *Materials and Methods*. Animals were either anti-IL-12-treated (stippled bars) or controls (solid bars). Anti-IL-12 treatment mediated increases in pathology, as determined by Student's *t* test ($p < 0.05$) where indicated (**). All data points represent the means of three to six mice, and error bars show the SE.

production is not detectable, T cell responses appear to be regulated in an IL-12-independent manner.

To determine the role of IL-12 in antiviral defense, viral replication and virus-induced pathology were examined in mice treated with anti-IL-12. The immune response to LCMV functioned independently of IL-12; viral replication in liver, spleen, and kidneys was unaffected by IL-12 neutralization at both early and late times after infection (Table I). The immune response to MCMV infection, however, required the factor for early control of infection. IL-12 neutralization resulted in significantly greater MCMV replication in liver, spleen, and kidneys, and substantially more liver pathology at early times after infection (Table I and Fig. 5). Once an effective T cell response was established, the increased viral replication by IL-12 neutralization was no longer present (Table I, day 7 after infection). In addition, increases in MCMV-induced pathology in anti-IL-12-treated animals decreased by day 7 after infection and were not present by day 9 after infection (Fig. 5). Thus, IL-12 is a significant component of early NK cell-mediated defense against MCMV, but is not essential for T cell-mediated viral clearance of either MCMV or LCMV.

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The studies presented here demonstrate the expression and function of IL-12 during viral infection. Although this laboratory has previously shown that LCMV-induced NK cells express detectable levels of IFN-γ mRNA (26), it has not been possible to demonstrate IFN-γ protein production by these populations (C. A. Biron, unpublished observations). This report conclusively shows that the conditions required for detectable NK cell IFN-γ production are present during MCMV, but not LCMV, infection. NK cells clearly play an important role in early MCMV infection by producing IFN-γ (25); however, they have no demonstrable role in early LCMV infections (2). The experiments presented here prove that IL-12 is essential for induction of the early NK cell-mediated and IFN-γ-dependent mechanism of antiviral defense. They suggest that the ability of a virus to induce significant IL-12 levels will determine whether NK cells contribute to antiviral defense.

Surprisingly, T cell responses to either virus produced IFN-γ and functioned independently of IL-12. These results are distinct from those examining immune responses to certain intracellular bacterial and parasitic pathogens (17–19, 27, 28). During infections with those pathogens, IL-12 is responsible for NK cell production of IFN-γ (18, 20, 27) and is necessary for the development of the Th1 lymphocyte responses associated with IFN-γ (17–19, 29, 30). Although the mechanisms by which T cells are activated during viral infections are poorly understood, the virus-induced IFN-$\alpha\beta$ (11, 31) and $CD8^+$ T cell responses (3–8) demonstrate that regulation proceeds through pathways not readily accessed by other infectious agents. Thus, when $CD8^+$ T cells are a major component of protection, initiation by IL-12 might be helpful, but is not necessary. Taken together, the results demonstrate that IL-12 is required for induction of protective NK cell responses to viral infections and suggest that there are IL-12-independent pathways for induction of T cell antiviral defenses.

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References

1. Bukowski, J. F., B. A. Woda, and R. M. Welsh. 1984. Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. *J. Virol. 52:119.*
2. Welsh, R. M., C. A. Biron, J. F. Bukowski, K. McIntyre, and H. Yang. 1984. Role of natural killer cells in virus infections of mice. *Surv. Synth. Pathol. Res. 3:409.*
3. Moskophidis, D., S. P. Cobbold, H. Waldmann, and F. Lehmann-Grube. 1987. Mechanism of recovery from acute virus infection: treatment of lymphocytic choriomeningitis virus-infected mice with monoclonal antibodies reveals that Lyt-2^+ T lymphocytes mediate clearance of virus and regulate the antiviral antibody response. *J. Virol. 61:1867.*
4. Kasaian, M. T., K. A. Leite-Morris, and C. A. Biron. 1991. The role of $CD4^+$ cells in sustaining lymphocyte proliferation during lymphocytic choriomeningitis virus infection. *J. Immunol. 146:1955.*
5. Reddehase, M. J., W. Mutter, K. Munch, H.-J. Buhring, and U. H. Koszinowski. 1987. CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. *J. Virol. 61:3102.*
6. Ahmed, R., L. D. Butler, and L. Bhatti. 1988. $T4^+$ T helper cell function in vivo: differential requirement for induction of antiviral cytotoxic T-cell and antibody responses. *J. Virol. 62:2102.*
7. Kasaian, M. T., and C. A. Biron. 1989. The activation of IL-2 transcription in $L3T4^+$ and LYT-2^+ lymphocytes during virus infection in vivo. *J. Immunol. 142:1287.*
8. Rahemtulla, A., W. P. Fung-Leung, M. W. Schilham, T. M. Kundig, S. F. Sambhara, A. Narendran, A. Arabian, A. Wakeham, C. J. Paige, R. M. Zinkernagel, R. G. Miller, and T. W. Mak. 1991. Normal development and function of $CD8^+$ cells but markedly decreased helper cell activity in mice lacking CD4. *Nature 353:180.*
9. Jonjic, S., W. Mutter, F. Wieland, M. J. Reddehase, and U. H. Koszinowski. 1989. Site-restricted persistent cytomegalovirus infection after selective long-term depletion of $CD4^+$ T lymphocytes. *J. Exp. Med. 169:1199.*
10. Welsh, R. M. 1978. Cytotoxic cells induced during lymphocytic choriomeningitis virus infection of mice. I. Characterization of natural killer cell induction. *J. Exp. Med. 148:161.*
11. Grundy (Chalmer), J. E., J. Trapman, J. E. Allan, G. R. Shellam, and C. J. M. Melier. 1982. Evidence for a protective role of interferon in resistance to murine cytomegalovirus and its control by non-H-2-linked genes. *Infect. Immun. 37:143.*
12. Leist, T. P., M. Eppler, and R. M. Zinkernagel. 1989. Enhanced virus replication and inhibition of lymphocytic choriomeningitis virus disease in anti-gamma interferon-treated mice. *J. Virol. 63:2813.*
13. Gessner, A., D. Moskophidis, and F. Lehmann-Grube. 1989. Enumeration of single IFN-γ-producing cells in mice during viral and bacterial infection. *J. Immunol. 142:1293.*
14. Lucin, P., I. Pavic, B. Polic, S. Jonjic, and U. H. Koszinowski. 1992. Gamma interferon-dependent clearance of cytomegalovirus infection in salivary glands. *J. Virol. 66:1977.*
15. Trinchieri, G. 1994. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory function in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood 84:4008.*
16. Tripp, C. S., M. K. Gately, J. Hakimi, P. Ling, and E. R. Unanue. 1994. Neutralization of IL-12 decreases resistance to *Listeria* in SCID and C. B-17 mice. *J. Immunol. 152:1883.*
17. Hsieh, C.-S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of Th1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science 260:547.*
18. Gazzinelli, R. T., M. Wysocka, S. Hayashi, E. Y. Denkers, S. Hieny, P. Caspar, G. Trinchieri, and A. Sher. 1994. Parasite-induced IL-12 stimulates early IFN-γ synthesis and resistance during acute infection *with Toxoplasma gondii. J. Immunol. 153:2533.*
19. Sypek, J. P., C. L. Chung, S. E. H. Mayor, J. M. Subramanyam, S. J. Goldman, D. S. Sieburth, S. F. Wolf, and R. G. Schaub. 1993. Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J. Exp. Med. 177:1797.*
20. Scharton-Kersten, T., L. C. C. Afonso, M. Wysocka, G. Trinchieri, and P. Scott. 1995. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development in experimental leishmaniasis. *J. Immunol. 154:5320.*
21. Coutelier, J.-P., J. Van Broeck, and S. F. Wolf. 1995. Interleukin-12 gene expression after viral infection in the mouse. *J. Virol. 69:1955.*
22. Martinotti, A., A. Stoppacciaro, M. Vagliani, C. Melani, F. Spreafico, M. Wysocka, G. Parmiani, G. Trinchieri, and M. P. Colombo. 1995. CD4 T cells inhibit in vivo the CD8-mediated immune response against murine colon carcinoma cells transduced with interleukin-12 genes. *Eur. J. Immunol. 25:137.*
23. Su, H. C., J. S. Orange, L. D. Fast, A. T. Chan, S. J. Simpson, C. Terhorst, and C. A. Biron. 1994. IL-2-dependent NK cell responses discovered in virus-infected β_2-microglobulin-deficient mice. *J. Immunol. 155:5674.*
24. Orange, J. S., S. F. Wolf, and C. A. Biron. 1994. Effects of IL-12 on the response and susceptibility to experimental viral infections. *J. Immunol. 152:1253.*
25. Orange, J. S., B. Wang, C. Terhorst, and C. A. Biron. 1995. Requirement for natural killer cell-produced interferon γ in defense against murine cytomegalovirus infection and enhancement of this defense pathway by interleukin 12 administration. *J. Exp. Med. 182:1045.*
26. Biron, C. A., H. A. Young, M. T. Kasaian. 1990. Interleukin 2-induced proliferation of murine natural killer cells in vivo. *J. Exp. Med. 171:173.*
27. Tripp, C. S., S. F. Wolf, and E. R. Unanue. 1993. Interleukin 12 and tumor necrosis factor α are costimulators of interferon γ production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc. Natl. Acad. Sci. USA 90:3725.*
28. Scharton-Kersten, T. M., L. C. C. Afonso, M. Wysocka, G. Trinchieri, and P. Scott. 1995. IL-12 is required for natural killer cell activation and subsequent T helper 1 cell development experimental leishmanasis. *J. Immunol. 154: 5320.*
29. Seder, R. A., R. T. Gazzinelli, A. Sher, and W. E. Paul. 1993. IL-12 acts directly on $CD4^+$ T cells to enhance priming for IFN-γ production and diminishes IL-4 inhibition of such priming. *Proc. Natl. Acad. Sci. USA 90:10188.*
30. Scott, P. 1993. IL-12: initiation cytokine for cell-mediated immunity. *Science 260:496.*
31. Muller, U., U. Steinhof, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science 264:1918.*

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