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Interleukin-12 Gene Expression after Viral Infection in the Mouse

JEAN-PAUL COUTELIER,[1]* JOHAN VAN BROECK,[1] AND STANLEY F. WOLF[2]

Unit of Experimental Medicine, Catholic University of Louvain and International Institute for Cellular and Molecular Pathology, B-1200 Brussels, Belgium,[1] and Genetics Institute, Inc., Cambridge, Massachusetts 02140[2]

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Interleukin-12 is a lymphokine that triggers gamma interferon secretion by various cells and differentiation of T-helper lymphocytes towards the Th1 subtype. Since viruses are potent inducers of gamma interferon production and elicit immune responses most probably mediated by Th1 cells, like B-cell immunoglobulin G2a secretion, we analyzed interleukin-12 message expression after infection of mice with lactate dehydrogenase-elevating virus, mouse hepatitis virus, and mouse adenovirus. Our results indicated that the message for the p40 component of interleukin-12 was transiently increased shortly after infection. Interleukin-12 was expressed mainly by macrophages. Therefore, production of interleukin-12 might constitute the initial event that would determine the subsequent characteristics of the immune response elicited by viral infections.

Interleukin-12 (IL-12) is a heterodimeric lymphokine composed of two subunits, p35 and p40, expressed either constitutively by a large variety of cells or, after activation, by cells from the immune system (28). Both human activated cells of the B-lymphocyte lineage and human and mouse macrophages have been shown to secrete the functional molecule in response to a variety of stimuli, including bacteria and parasites (10, 12, 16, 18, 31, 33). IL-12 is a potent inducer of gamma interferon production by natural killer (NK) cells and T-helper lymphocytes (12, 13, 29, 34). It plays a crucial role in the early induction of immune responses by triggering the differentiation of T-helper lymphocytes towards the Th1 subtype (13, 16, 21, 22, 27, 29). Through its gamma interferon-inducing effect on T-helper cells, IL-12 increases immunoglobulin G2a production by B lymphocytes in naive and stimulated mice (24). In addition, IL-12 enhances NK cell and T-cell cytotoxicities against virus-infected and transformed cells (2).

In view of these properties, it is not surprising that IL-12 may protect mice against infection with parasites such as *Leishmania major* and *Toxoplasma gondii* (12, 14, 32) or that its use as an adjuvant for efficient antiparasite vaccination has been considered (1). Similarly, low doses of IL-12 have been shown to increase antiviral immunity and reduce lymphocytic choriomeningitis virus replication, whereas high doses of the same lymphokine were detrimental for the infected mice (25). However, IL-12 production in the course of viral infection has not been reported so far. The aim of this study was therefore to analyze by reverse transcriptase-PCR (RT-PCR) whether viruses induced IL-12 gene expression and to determine the cellular origin of such a message.

Lactate dehydrogenase-elevating virus (LDV) induces strong activation of B lymphocytes, resulting in a level of immunoglobulin G2a production that might be linked with selective activation of Th1-type lymphocytes (4, 9, 20). It can therefore be postulated that LDV infection results in IL-12 production. To determine whether LDV triggered an increase in IL-12 gene expression, 8- to 14-week-old specific-pathogen-free female CBA/Ht mice bred at the Ludwig Institute for Cancer Research by G. Warnier were infected by intraperitoneal injection of approximately 2×10^7 50% infective doses of LDV (Riley strain; from the American Type Culture Collection [ATCC], Rockville, Md.). RNA was extracted from spleen cells obtained at different times after infection, either by homogenization in guanidine thiocyanate and centrifugation on cesium chloride (11) or after cell lysis and homogenization in TRIzol (Gibco BRL), separation with chloroform, and precipitation with isopropanol as recommended by the manufacturer. cDNA was prepared with Moloney murine leukemia virus reverse transcriptase (Gibco BRL) and amplified by PCR with a Gene Amp kit (Perkin-Elmer Cetus) in a Thermal Reactor (Hybaid, Middlesex, United Kingdom) as described previously (23). The sequences of primers and the experimental conditions used for the analysis of the expression of IL-12 p35 and p40 and of actin as a control are indicated in Table 1. The 0.85- and 0.55-kb bands obtained by RT-PCR were further identified as corresponding to the IL-12 p35 and p40 messages by hybridization with ^{32}P-labeled oligonucleotides specific for the p35 and p40 genes (5′-CTTGAGAATTCAGGCGGAGCT CAG-3′ and 5′-TGGAATGGCGTCTCTGTCTGC-3′, respectively) (data not shown). As shown in Fig. 1, a strong enhancement of the message corresponding to the p40 component of IL-12 was detected between 12 and 24 h after virus inoculation. IL-12 p35 message was expressed equally in control and infected animals. Similar kinetics of IL-12 p40 message enhancement were observed after LDV infection of 129/Sv and BALB/c animals (data not shown). At later times after infection, no further increase of IL-12 expression was detected (data not shown).

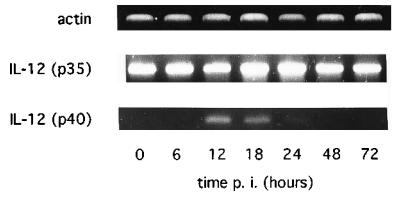

FIG. 1. Spleen cell expression of IL-12 message after infection with LDV. Actin and IL-12 p35 and p40 messages in pooled spleen cells from groups of four CBA/Ht mice at different times after LDV inoculation were analyzed by RT-PCR. p.i., postinoculation.

* Corresponding author. Mailing address: Unit of Experimental Medicine, ICP, UCL, Av. Hippocrate 7430, B-1200 Brussels, Belgium. Phone: 32 2 764 7437. Fax: 32 2 764 7430.

TABLE 1. Primers and experimental conditions used for PCR in this study

Message	Primer sequences	Annealing temp (°C)	No. of cycles
Actin	5′-ATG GAT GAC GAT ATC GCT GC-3′ 5′-GCT GGA AGG TGG ACA GTG AG-3′	55	25
IL-12 p35	5′-TTG CCC TCC TAA ACC ACC TCA-3′ 5′-CTT GCT CTT CTG CTA ACA CAT-3′	55	35
IL-12 p40	5′-CTC ACA TCT GCT GCT CCA CAA-3′ 5′-CTC CTT CAT CTT TTC TTT CTT-3′	55	35–40

IL-12 transcripts in peritoneal cells were also analyzed (Fig. 2). Increased expression of IL-12 p40 message was observed 12 h after LDV infection. Because peritoneal cells contain large proportions of macrophages and of B lymphocytes that have both been shown, in rodents or in humans, to be capable of secreting IL-12, the cellular origin of the increased IL-12 message observed after LDV infection was further investigated. Adherent cells separated from spleen cells of LDV-infected mice by plating on tissue culture dishes expressed more IL-12 (p40) message than nonadherent cells (Fig. 3a). This observation supported the hypothesis that macrophages were at least partly responsible for IL-12 production, since adherent cells were strongly enriched and the nonadherent fraction was depleted in macrophages. However, the proportion of B lymphocytes was also increased in adherent cells versus that in nonadherent cells and further cell purification was therefore required.

Thus, spleen cell subpopulations were purified by magnetic cell sorting (MACS; Miltenyi Biotec, Bergisch-Gladbach, Germany) as described previously (5). Briefly, cells were incubated with biotinylated monoclonal antibodies specific for subpopulation surface markers before further incubation with fluoresceinated streptavidin and MACS biotin microbeads. Cells were loaded into a MACS on a cooled B2 column through a 22-gauge needle, were washed with phosphate-buffered saline (PBS) containing 1% bovine serum albumin through first a 21-gauge needle and then a 19-gauge needle, and were eluted with 40 ml of PBS with bovine serum albumin. The purity of the cell preparations was analyzed after labeling with biotinylated antibodies followed by alkaline phosphatase streptavidin and naphthol-AS-MX-phosphate or by flow cytometry analysis.

An enriched macrophage population was prepared by MACS with M1/70 anti-Mac-1 antibody (ATCC) (30) from spleen cells obtained 15 h after LDV infection and analyzed by RT-PCR for IL-12 expression. As shown in Fig. 3b, a strong IL-12 p40 message was found in this enriched macrophage fraction which contained 27% Mac-1$^+$ cells, 6% T lymphocytes, and 7% B lymphocytes. Similarly, purified Ly-6C$^+$ cells, that contain some T cells as well as macrophages (17, 26), but not B lymphocytes, also expressed IL-12 p40 message (data not shown). On the other hand (Fig. 4a), virtually no IL-12 p40 transcripts were detected in a T-cell fraction (<1% macrophages, 87% T lymphocytes, and 3% B lymphocytes) purified with A6703A8 anti-Thy-1 antibody (gift of P. Coulie) and in a B-lymphocyte fraction (<1% macrophages, 2% T lymphocytes, and 90% B lymphocytes) isolated with RA3-3A1 anti-B220 antibody (ATCC) (3). Since B-lymphocyte activation reaches a maximum 4 days after LDV infection (9), mRNA was also prepared from purified B cells obtained at this time and analyzed by RT-PCR but no increase of IL-12 p40 expression was observed (data not shown). Because Mac-1-selected cells also contain NK cells and granulocytes, these subpopulations were purified by MACS with anti-NK1.1 PK136 (19) and anti-Gr-1 RB6-8C5 antibodies (15) (PharMingen, San Diego, Calif.) after the removal of macrophages by incubation on tissue culture dishes. Expression of p40 message was considerably reduced in these enriched subpopulations (containing

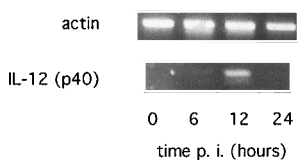

FIG. 2. Expression of IL-12 p40 message in peritoneal cells from mice infected with LDV. Expression of actin and IL-12 p40 messages in pooled peritoneal cells from groups of three or four BALB/c mice obtained at different times after LDV infection was analyzed by RT-PCR. p.i., postinoculation.

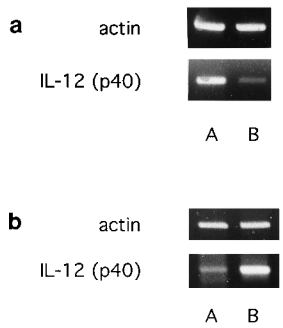

FIG. 3. Macrophage IL-12 expression after LDV infection. Actin and IL-12 p40 messages in purified spleen cell subpopulations prepared 15 h after LDV infection were measured by RT-PCR. (a) Adherent (lane A) and nonadherent (lane B) fractions after plating on tissue culture dishes. (b) Total spleen cells (lane A) and macrophages (lane B) purified by MACS with an anti-Mac-1 antibody.

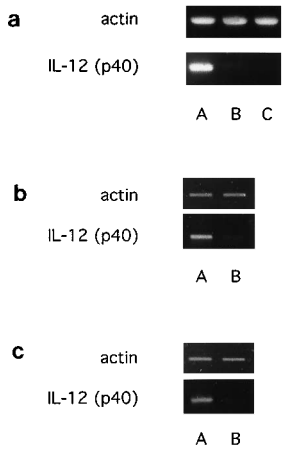

FIG. 4. IL-12 expression in B and T lymphocytes, NK cells, and granulocytes. Actin and IL-12 p40 messages in purified spleen cell subpopulations prepared 15 h after LDV infection were measured by RT-PCR. (a) Total spleen cells (lane A) and B (lane B) and T (lane C) lymphocytes purified by MACS with anti-B220 and anti-Thy-1 antibodies, respectively. (b) Total spleen cells (lane A) and NK cells (lane B) purified with anti-NK1.1 antibody. (c) Total spleen cells (lane A) and granulocytes (lane B) purified with anti-Gr-1 antibody.

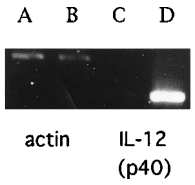

FIG. 5. IL-12 message in SCID mice infected with LDV. Actin and IL-12 p40 transcripts in pooled spleen cells obtained from groups of three control BALB/cBy-SCID mice (lanes A and C) or from infected mice 15 h after LDV infection (lanes B and D) were analyzed by RT-PCR.

65% NK cells and 40% granulocytes, respectively) when compared with that in total spleen cells (Fig. 4b and c).

Finally, IL-12 message in BALB/cBy-SCID mice infected with LDV was analyzed. An increase in IL-12 p40 expression similar to that observed in immunocompetent animals was found in spleen cells from these mice lacking B and T lymphocytes (Fig. 5). Together, these results indicate that macrophages rather than B lymphocytes are responsible for the enhancement of IL-12 p40 message triggered by LDV infection.

To determine whether an increase of IL-12 message was a peculiarity of LDV or was a rather general phenomenon after viral infection, we analyzed by PCR spleen cell mRNA from mice infected with 10^4 tissue culture infectious doses of the A59 strain of mouse hepatitis virus and 10^7 50% infective doses of the FL strain of adenovirus (gift of J. T. M. van der Logt) that also elicit strong T-helper-dependent immunoglobulin G2a responses (4, 6–8). Both viruses induced a transient increase in IL-12 p40 gene expression which was slightly delayed when compared with that induced by LDV infection (Fig. 6). Similar IL-12 message induction in peritoneal cells was observed (data not shown). Therefore, our results indicate that transient IL-12 expression is a common feature of viral infections inducing strong immunoglobulin G2a-restricted antibody production. It is further postulated that IL-12 production by activated macrophages constitutes an early event triggered by viruses that influences the characteristics, including the antiviral efficiency and the possible pathogenicity, of subsequent immune responses.

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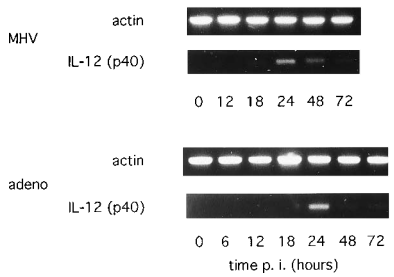

FIG. 6. IL-12 gene expression after mouse hepatitis virus (MHV) and adenovirus (adeno) infection. Actin and IL-12 p40 messages in pooled spleen cells obtained at different times after MHV and adenovirus infection of groups of three 129/Sv and CBA/Ht mice, respectively, were analyzed by RT-PCR. p.i., postinoculation.

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