Gamma interferon (IFN-γ) is deeply involved in the pathogenesis triggered indirectly by lactate dehydrogenase-elevating virus (LDV) in mice (Coutelier & Brinton, 2007; Musaji et al., 2004, 2006). For example, we showed that LDV exacerbates the susceptibility of mice to shock induced by lipopolysaccharide (LPS). This effect was partly mediated by the secretion of IFN-γ. Interestingly, mice deficient for type I IFN receptor showed an increased susceptibility to septic shock after LDV infection, which correlated with high IFN-γ titres (Le Thi Phuong et al., 2006). This prompted us to examine whether type I IFNs could regulate LDV-induced IFN-γ production.

After infection of 129/Sv and IFN-α/βR−/− (IFNAR KO) mice [originally derived by M. Aguet (Müller et al., 1994) and bred by G. Warnier at the Ludwig Institute for Cancer Research, Brussels, Belgium] with LDV (2 × 10^7 ID_{50} Riley strain; ATCC), IFN-γ was measured in plasma by ELISA using a commercial kit (Biosource). Although the kinetics of production were similar, IFN-γ reached higher levels in mice deficient for the type I IFN receptor than in their normal counterparts (Fig. 1a; significant difference at day 1 after infection, P=0.0079). The difference varied from one experiment to another, probably due to the sharp kinetics of IFN-γ production, with rapid increase and decrease of cytokine levels. However, in all 14 experiments performed, IFN-γ levels were higher in IFNAR KO mice than in 129/Sv animals, with increases ranging from 1.8- to 21-fold (mean difference of 4.96-fold). This result indicated that, after LDV infection, IFN-γ secretion is controlled negatively by type I IFNs.

The cell populations that are responsible for IFN-γ production following LDV infection were analysed by double labelling in flow cytometry. Cell populations were determined by using a fluoresceinated anti-mouse DX5 monoclonal antibody (mAb) (BD Biosciences) as described previously (Markine-Goriaynoff et al., 2002). Production of IFN-γ by these cells was analysed by using a mouse IFN-γ secretion assay detection kit following the manufacturer’s instructions (Miltenyi Biotec). For each sample, 10 000 cells were analysed. Although the proportion of natural killer (NK) cells in the spleen of 129/Sv animals was low, a large proportion of those cells secreted IFN-γ after LDV infection (Table 1; a typical experiment among four performed is shown). In contrast, few DX5-negative cells produced this cytokine. Similar results were obtained with cells derived from the liver of such animals, where the proportion of NK cells was much higher (Table 1; independent experiments among three performed). Interestingly, no major modification in the cellular origin of IFN-γ was found in animals deficient for type I IFN receptor (Table 1). The preponderant role of NK cells in IFN-γ production following LDV infection fits well with previous observations suggesting the involvement of this cell population in this process (Markine-Goriaynoff et al., 2002).

Because type I IFNs may modulate the replication of viruses dramatically, with possible consequences on the subsequent immune response, LDV viraemia was measured in 129/Sv and IFNAR KO mice by a sensitive particle-counting immunoassay based on the agglutination of latex beads coated with two different anti-LDV mAbs by viral particles, as described previously (Markine-Goriaynoff et al., 2002). A mere 2-fold increase in LDV titres, which was, however, significant (P=0.0022 by non-parametric Mann–Whitney test), was observed in mice unresponsive to type I IFNs at 16 h post-infection, which corresponds to the highest level of viraemia (Coutelier & Brinton, 2007), as shown in Fig. 1(b). At 40 h after infection, when viraemia had started to decrease, a similar enhancement of LDV titres was also found in animals deficient for type I IFN receptor. This modest increase in LDV replication, found previously (Markine-Goriaynoff et al., 2002). Production of IFN-γ by these cells was analysed by using a mouse IFN-γ secretion assay detection kit following the manufacturer’s instructions (Miltenyi Biotec). For each sample, 10 000 cells were analysed. Although the proportion of natural killer (NK) cells in the spleen of 129/Sv animals was low, a large proportion of those cells secreted IFN-γ after LDV infection (Table 1; a typical experiment among four performed is shown). In contrast, few DX5-negative cells produced this cytokine. Similar results were obtained with cells derived from the liver of such animals, where the proportion of NK cells was much higher (Table 1; independent experiments among three performed). Interestingly, no major modification in the cellular origin of IFN-γ was found in animals deficient for type I IFN receptor (Table 1). The preponderant role of NK cells in IFN-γ production following LDV infection fits well with previous observations suggesting the involvement of this cell population in this process (Markine-Goriaynoff et al., 2002).

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Fig. 1. Cytokine production and LDV replication in type I IFN receptor-deficient mice. (a) IFN-γ was measured by ELISA in the plasma of groups of four 129/Sv (○) and IFNAR KO (●) mice at different times after infection with LDV. Results are shown as means ± sd. (b) LDV titres were measured by particle-counting immunoassay in groups of six 129/Sv (empty bars) and IFNAR KO (filled bars) mice at different times after infection and are expressed in arbitrary units (AU; mean ± sd). (c) IL-12 levels were measured in the plasma of groups of five 129/Sv (empty bars) and IFNAR KO (filled bars) mice 26 h after infection with LDV. Results are shown as means ± sd. (d) IFN-γ was measured by ELISA in the serum of groups of five 129/Sv (empty bars) and IFNAR KO (filled bars) mice 18 h after infection with LDV. Anti-IL-12 C17.8 mAb or control LO-DNP-16 mAb was administered 1 day before infection (500 μg per mouse). Results are shown as means ± sd.

Table 1. IFN-γ expression by spleen- and liver-cell populations

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>LDV infection†</th>
<th>Organ</th>
<th>Percentage of total cells‡</th>
<th>Percentage of IFN-γ-producing cells‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DX5⁻</td>
<td>DX5⁺</td>
</tr>
<tr>
<td>129/Sv</td>
<td>−</td>
<td>Spleen</td>
<td>96.4</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Spleen</td>
<td>97.8</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>Liver</td>
<td>83.0</td>
<td>17.1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>Liver</td>
<td>89.5</td>
<td>10.5</td>
</tr>
<tr>
<td>IFNAR KO</td>
<td>−</td>
<td>Spleen</td>
<td>97.0</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
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<td>Spleen</td>
<td>98.1</td>
<td>1.9</td>
</tr>
<tr>
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<td>−</td>
<td>Liver</td>
<td>81.1</td>
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</tr>
<tr>
<td></td>
<td>+</td>
<td>Liver</td>
<td>52.2</td>
<td>47.8</td>
</tr>
</tbody>
</table>

*Infection 18 h before analysis.
†Cell populations were determined by flow cytometry in pooled cells from groups of eight mice. Analysis of spleen and liver cells was performed in independent experiments.
‡Percentage of cells producing IFN-γ in each cell population.
in two experiments and which fits well with previous data reported after administration of an IFN inducer (Crispens, 1970), together with the tightly restricted tropism of LDV, suggest that other causes should explain IFN-γ modulation after infection of IFNAR KO mice.

As interleukin-12 (IL-12) is known to promote IFN-γ production, the secretion of this cytokine was measured after LDV infection. No difference in IL-12 production, measured by ELISA with a Quantikine kit (R&D Systems), was observed between 129/Sv and IFNAR KO animals (Fig. 1c; one experiment among four is shown). Involvement of this cytokine in IFN-γ production after LDV infection was analysed by treating mice with 500 μg anti-IL-12 p40 C17.8 neutralizing mAb (derived by G. Trinchieri, Wistar Institute, Philadelphia, PA, USA) or with a control antibody (LO-DNP-16, rat IgG2a mAb). In three of four experiments, anti-IL-12 treatment had little effect on IFN-γ secretion in normal 129/Sv mice (Fig. 1d), whilst in one experiment, it resulted in some decrease of this cytokine. In sharp contrast, in all experiments, injection of anti-IL-12 antibody abrogated IFN-γ production completely in mice deficient for type I IFN receptor (Fig. 1d). As IFN-γ production has been shown to be IL-23-independent (Khader et al., 2005), this effect of the anti-p40 mAb may be ascribed to effective IL-12 neutralization.

Therefore, our results demonstrate that two distinct pathways control IFN-γ production in the course of LDV infection. In normal mice, the major pathway leads to an IL-12-independent secretion of this cytokine by NK cells. Moreover, a second IFN-γ-production pathway by the same cell population is controlled tightly by type I IFNs and requires the presence of IL-12. IL-12 production itself does not appear to increase in LDV-infected type I IFN-unresponsive animals, suggesting that, after infection, type I IFNs control the NK-cell response to IL-12, rather than the production of this cytokine. However, when LDV-infected IFNAR KO mice are exposed to LPS, a strong increase of IL-12 production is observed (T. Le-Thi-Phuong, unpublished data), indicating that, under some circumstances, the secretion of this cytokine may also be regulated by type I IFNs. Although IFN-α/β can also promote IFN-γ secretion, such an inhibitory effect of type I IFNs has also been reported in response to Staphylococcus aureus, mouse cytomegalovirus (MCMV) and lymphocytic choriomeningitis virus (LCMV) (Cousens et al., 1997).

Previous reports have explained a dual role of type I IFNs on IFN-γ production by distinct STAT activation (Nguyen et al., 2000, 2002); whilst activation of STAT4 is required for an enhancement of IFN-γ induction by type I IFNs, activated STAT1 plays a preponderant role in the down-regulation of IFN-γ by type I IFNs. STAT expression was therefore analysed by Western blot as described previously (Demoulin et al., 2003) and following the manufacturers’ instructions, using anti-β-actin (Sigma), anti-STAT1, anti-pSTAT1 (Tyr701) (Cell Signaling Technology), anti-STAT4 (Transduction Laboratories) and anti-pSTAT4 (Zymed) antibodies. Our results indicated that the induction of activated STAT4 by LDV infection in the spleen of LDV-infected IFNAR KO mice was much lower than in normal counterparts, whilst induction of activated STAT1 was depressed only slightly (Fig. 2; one experiment among two). This suggested that the dual role of STATs reported in other models cannot explain by itself the regulation of IFN-γ production by type I IFNs in LDV-infected animals.

In contrast to LCMV infection (Cousens et al., 1999), the IL-12-independent pathway of IFN-γ production after LDV infection involves NK rather than CD8 T cells. The immune response elicited by LDV therefore appears closer to that following infection with MCMV, with possibly distinct roles of STAT molecules. Although the cells responsible for IFN-γ production and the regulatory mechanisms that control this cytokine may be different after infection with diverse viruses, IFN-γ suppression by type I IFNs probably corresponds to common ways of limiting the severe pathology that might be induced by the former molecule (Meite et al., 2000; Musaji et al., 2004, 2006).

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