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Lethal Hepatitis After Gene Transfer of IL-4 in the Liver Is Independent of Immune Responses and Dependent on Apoptosis of Hepatocytes: A Rodent Model of IL-4-Induced Hepatitis

Cécile Guillot,²* Hélène Coathalem,²* Jérôme Chretitt, Anne David, Pedro Lowenstein, Emmanuelle Gilbert, Laurent Tesson, Nico van Rooijen, Maria Cristina Cuturì, Jean-Paul Soulillou,* and Ignacio Anegon³*

The putative role of IL-4 in human and animal models of hepatitis has not yet been directly determined. We now report that direct expression of IL-4 in the liver of rats or mice using recombinant adenoviruses coding for rat or mouse IL-4 (AdrIL-4 and AdmIL-4, respectively) results in a lethal, dose-dependent hepatitis. The hepatitis induced by IL-4 was characterized by hepatocyte apoptosis and a massive monocyte/macrophage infiltrate. IL-4-induced hepatitis was independent of T cell-mediated immune responses. Hepatitis occurred even after gene transfer of IL-4 into nude rats, CD8-depleted rats, cyclosporine A-treated rats, or recombinase-activating gene 2−/− immunodeficient mice. Peripheral depletion of leukocytes using high doses of cyclophosphamide, and/or the specific depletion of liver macrophages with liposome-encapsulated dichloromethylene diphosphonate in rats did not block lethal IL-4-induced hepatitis. Direct transduction of hepatocytes with adenoviruses was not essential, since injection of AdrIL-4 into the hind limb induced an identical hepatitis. Finally, primary rat hepatocytes in culture also showed apoptosis when cultured in the presence of rIL-4. IL-4-dependent hepatitis was associated with increases in the intrahepatic levels of IFN-γ, TNF-α, and Fas ligand. Administration of AdmIL-4 to IFN-γ, TNF-α receptor type I, or TNF-α receptor type II knockout mice also resulted in lethal hepatitis, whereas a moderate protection was observed in Fas-deficient lpr mice. IL-4-dependent hepatocyte apoptosis could be abolished by treatment with caspase inhibitory peptides. Our results thus demonstrate that IL-4 causes hepatocyte apoptosis, which is only partially dependent on the activation of Apo-1-Fas signaling and is largely independent of any immune cells in the liver. The Journal of Immunology, 2001, 166: 5225–5235.

The attempt to immunomodulate human immune responses using IL-4 has been hampered by the development of clinically relevant hepatic injury in patients and primates (1–4). Furthermore, various other liver diseases, such as autoimmune hepatitis (5), chronic hepatitis (5), and primary biliary cirrhosis (6) have all been associated with increased IL-4 production. Hepatocytes express IL-4R (7), and IL-4 has been shown to modulate the function of hepatocytes in vitro (8, 9) and in vivo (10). Also, IL-4 is essential for the development of hepatic lesions in the widely used model of Con A-induced hepatitis (11). However, the direct role of IL-4 and the mechanisms of IL-4-mediated hepatic injury have not yet been determined.

IL-4 is a major cytokine released during T cell responses polarized toward a Th2 phenotype. IL-4 itself displays a variety of either anti- or proinflammatory actions, mainly on cells of various hemopoietic lineages (8). Its actions include antiapoptotic actions on B cells (12) and proapoptotic effects on macrophages (13), mast cells (14), or eosinophils (15). Although IL-4 has also been described as modulating functions of nonhemopoietic cells, such as endothelial cells, fibroblasts, and hepatocytes (8), much less is known about its actions on these cells compared with those on hemopoietic cells.

We recently showed that adenovirus-mediated IL-4 expression in the liver by intraportal administration of 2.5 × 10⁸ PFU of adenovirus coding for rat IL-4 (AdrIL-4)⁴ results in nonlethal, reversible hepatitis in rats (16). At this dose of AdrIL-4 the hepatitis is partially due to an increase in cellular and humoral anti-adenovirus immune responses (17). In the present study, we show that higher doses of AdrIL-4 (10¹⁰ PFU) induce lethal hepatitis in rats, we reproduced this lethal hepatitis model in mice using an adenovirus coding for mouse IL-4 (AdmIL-4) and investigated the inflammatory and immune mechanisms as well as the role of apoptosis underlying acute and lethal IL-4-mediated hepatitis.

Our present results show that expression of IL-4 in the liver results in extensive hepatocyte apoptosis and mortality, which are

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⁴ Abbreviations used in this paper: AdrIL-4, adenovirus coding for rat IL-4; AdmIL-4, adenovirus coding for mouse IL-4; FasL, Fas ligand; RAG, recombinase-activating gene; TNF-αRI, TNF-α receptor type I; ALAT, alanine transaminase; ASAT, aspartate transaminase; CL2-MDP, liposome-encapsulated dichloromethylene diphosphonate; CsA, cyclosporine A; Cp: cyclophosphamide; HES, hematoyxin-ösün-saf-iron; HPRT, hypoxanthine phosphoribosyltransferase; ICE, IL-1β-converting enzyme; mox, multiplicity of infection; X-Gal, X-galactosidase.
prevented by treatment with the caspase inhibitory YVADcmk peptide and was statistically significantly reduced in lpr mice. Le- thal hepatitis was associated with production of IFN-γ, TNF-α, and Fas ligand (FasL). In mice, IL-4–dependent lethal hepatitis was also not reduced in recombinase-activating gene-2 (RAG2)−/−, IFN-γ, TNF-α receptor type 1 (TNF-αRI), or TNF-αRII knockout mice. Although the livers of rats or mice expressing IL-4 were heavily infiltrated with monocytes/macrophages, prevention of he- patic monocyte infiltration, depletion of resident macrophages, and abolition of T cell immune responses in rats failed to protect against hepatic lesions and death. Hepatitis was independent of anti-adenovirus immune responses, since systemic IL-4 production as a result of administration of AdrIL-4 into the hind limb of rats also resulted in hepatitis. Finally, rIL-4 or transduction with Ad- rIL-4 induced apoptosis of primary cultures of rat hepatocytes, which was prevented by caspase inhibitors.

In summary, our results indicate that besides the already known chemotactic and immune activator actions of IL-4, lethal hepatitis induced by this cytokine is mediated through apoptosis of hepato- cytes by mechanisms independent of immune response activa- tion, proinflammatory effects on leukocytes, or TNF-α or IFN-γ production. Therefore, IL-4 secretion could play an important role in various types of hepatitis by different mechanisms. Treatment with IL-4-blocking agents or antiapoptotic caspase peptides could prevent IL-4–mediated liver injury in these situations.

Materials and Methods

Animals and in vivo transduction

Male Wistar rats (8 wk old; CERJ, Le Genest St. Isle, France) and nude rats (Rowett Hooded nu/nu, 8 wk old; Charles River, St. Aubin les Elbeuf, France) were injected via the portal vein with the indicated numbers of PFUs of recombinant adenovirus as previously described (16). Gene transfer to the hind limb was performed by i.m. injection of 2 × 10^10 PFU (in a total volume of 100 μl at four injection points).

C57BL/6 mice were purchased from Charles River, and RAG2−/− and lpr mice (Fas−/−) were obtained from Centre National de la Recherche Scientifique-Centre de Developpement des Techniques Arducede (Orlés, France). Mice deficient in IFN-γ, TNF-αRI, or TNF-αRII were purchased from The Jackson Laboratory (Bar Harbor, ME). All mutated mice were of the C57BL/6 background. Male mice (6 wk old) received the indicated doses of recombinant adenovirus by i.v. injection.

Recombinant adenovirus

AdrIL-4 was constructed, propagated, and titrated as previously described (16). Briefly, AdrIL-4 was constructed by homologous recombination in 293 cells using DNA from Addl324 (an Ad5-derived, E1- and E3-deleted adenovirus). Rat IL-4 cDNA sequences were placed under transcriptional control of the human elongation factor-1 α promoter. AdmIL-4 was pro- duced by F. Graham (McMaster University, Hamilton, Canada). AdlAcZ has been previously described (18). Adenoviruses were purified using two consecutive CsCl gradient ultracentrifugations and then aliquoted and stored at −80°C until used. Adenovirus preparations were tittered on 293 cells using PFU assays. Analysis of adenoviral particle concentration, as determined by A260 absorbancy, allowed us to calculate the ratio of PFUs to total physical adenovirus particles (PFU/particle ratio). The PFU/particle ratio varied from 1/20 to 1/40 for all of the recombinant adenovirus stocks used in this work. Endotoxin levels in purified recombinant adenovirus preparations were <0.1 endotoxin units/10^9 PFU as detected by the Limu- lus assay (Sigma, St. Louis, MO). AdrIL-4 and AdlAcZ preparations con- tained <10 PFU replication-competent adenovirus/10^9 PFU recombinant adenovirus, as determined using adenovirus replication-competent permissive A549 cells and Addl7001 (replication-competent adenovirus) as a positive con- trol (provided by P. Moullier, Nantes, France).

Depletion of leukocyte subpopulations and immunosuppression

On day 0, animals were injected with recombinant adenoviruses. To evaluate the role of CD8+ cells in vivo, rats received i.p. injections of anti-CD8 α- or β-Ab (200 μg/ml; IgG1; European, Wilts- hire, U.K.) or an irrelevant control, anti-human CD16 mAb (3G clone, IgG1; American Type Culture Collection, Manassas, VA) on days −1, 2, 5, and 8. CD8+ lymphocyte depletion in peripheral blood (>98%) was confirmed using the OX8 mAb and anti-β TCR R.7.3 mAb between days 0 and 8 in both naïve control rats as well as in the experimental animals (up to day 2, since death by IL-4–mediated hepatitis occurred between days 3 and 5). Cyclosporine A (CSA) was administered orally at daily doses of 10 mg/kg from day −1 for the duration of the experiment (CSA serum levels were confirmed at >960 μg/ml). Cyclophosphamide (CyP) was adminis- tered to rats and mice i.p. at 70 mg/kg on day −5 and then at 35 mg/kg every 3 days. Peripheral leukocytes in rats from day 0 onward were be- tween 0.5 and 2 × 10^10/liter (normal values, 12–17 × 10^10/liter). Prolonged treatment with CyP (>10 days) was associated with some toxicity, since Addl324-infused animals showed 20% mortality. CyP was not hepatotoxic, since this group showed normal hepatic biochemical values and had no hepatic lesions (data not shown). Depletion of Kupffer cells was obtained by i.v. injection of 1 ml of liposome-encapsulated dichloromethylene diphosphonate (liposomes-Cl2MDP; a gift from Roche, Mannheim, Germany) (17) on day −1 and confirmed on day 0 by immunostaining using the ED2 anti-macrophage mAb (liver ED2+ cells from 8.3 ± 1 n in non- treated controls to 0.4 ± 0.2% in liposomes-Cl2MDP-treated ones). Lipo- somes-Cl2MDP have been previously shown to deplete the liver from ED2+–resident macrophages, as well as Kupffer cells, rather than ED1+ peripheral blood monocytes or newly arrived tissue macrophages (19).

Treatment with anti-apoptotic YVADcmk peptide

The synthetic peptide (Ac-Tyr-Val-Ala-Asp-chloromethylketone (YVAD- cmk), Bachem, Bubendorf, Switzerland) is an irreversible inhibitor of the IL-1β-converting enzyme (ICE)-like family of caspases, previously used in vivo (20–23). Rats infused intraperitoneally with AdrIL-4 (day 0) received i.p. injections of 2 mg of YVADcmk daily from days 0 to 6. Analysis of liver histopathology and liver function

Liver samples were fixed in 4% formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin-eosin-saffron (HES). Alanine and as- partate transaminases (ALT and ASAT), bilirubin levels, and alkaline phosphatase activity were measured in serum as indicators of hepatocellular damage.

Immunohistochemistry

Immunohistochemistry on frozen sections was performed as previously described in detail (17). Briefly, tissue sections were incubated for 60 min with rabbit anti-Fasl (N-20; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-rat TNF-α (Serotec, Oxford, U.K.), or the following mouse mAb: anti-leukocyte CD45 (OX1 and OX30), anti-mono-morphic MHC class II Ags (ED1), anti-macrophage (ED2); a large proportion of tissue macrophages coexpress the ED1 and ED2 Ags, but at early and late time points of macrophage differentiation there is a preferential expression of ED1 and ED2 Ags, respectively (22), anti-αβ TCR (R.7.3), anti-CD4 (W3/25), anti-CD8 α-chain (OX8), anti-monomorphic MHC class II Ags (OX6) (all of these mAb were obtained from European Cell Culture Collection), and anti-NKRP1 (3.2.3; Serotec). After washing the sections exhaustively, they were incubated for 45 min with biotin-conjugated anti-rabbit or anti-mouse Ig Abs (Vector Laboratories, Burlingame, CA) and then with HRP-conju- gated streptavidin (Vector Laboratories; 45 min) and revealed using the VIP substrate (Vector Laboratories). Quantification of labeled cellular infiltrates was performed using the point-counting technique and expressed as the percent area of biopsies occupied by positive cells as previously described (17). The numbers of cells immunoreactive for either TNF-α or Fasl were determined using a microscope grid (0.0625 mm²; ×400 magnification).

Histological determination of hepatitis apoptosis

Apoptosis in liver sections was investigated using three different methods. First, hepatocyte apoptotic bodies were detected in HES-stained liver sections, and their numbers were determined using a microscope grid (×400 magnification).

Secondly, apoptosis was also analyzed using the TUNEL technique. Paraffin sections were deparaffinized, washed twice in PBS, digested with 20 μg/ml proteinase K (Sigma; 15 min at room temperature), and incubated with hydrogen peroxide (10%) in methanol (1/5, v/v, 30 min). The TUNEL ApopTag kit (Oncor, Gaithersburg, MD; for detection by immunoperoxidase using diaminobenzidine as the substrate) or the ApopTag kit (Oncor, Gaithersburg, MD; for detection by immunoperoxidase using FITC-labeled Abs) were used according to the manufacturers’ instructions.

Finally, nuclear condensation and fragmentation typical of apoptotic cells were assessed morphologically by qualified liver histopathologists using Hoechst 33258 staining. Liver cryosections or hepatocyte cytopsin
were incubated (10 min, room temperature) in an acetic acid/methanol (1/3, v/v) solution, washed twice in HBSS without red phenol (Life Technologies, Gaithersburg, MD), and incubated (for 30 min, at room temperature, protected from light) with 0.1 μg/ml of Hoechst 33258 (diluted in HBSS). DNA staining was visualized using an epifluorescence microscope with a 365-nm filter, and the percentage of apoptotic cells was determined by counting at least 500 cells from each experimental condition.

Quantitative competitive RT-PCR

RNA isolation, RT, PCR conditions, oligonucleotides, and standard DNA constructs used in this study have been described previously (24, 25).

The quantitative RT-PCR technique used allows precise quantification of transcripts using PCR amplification run to saturation. The basis of this technique is to coamplify the cDNA to be quantified with known amounts of an internal standard DNA whose sequence is identical with the sequence to be assayed except for a four-base deletion. Quantification is performed after an additional nested PCR using a fluorescent dye-labeled oligonucleotide. This PCR method allows the two amplified species (i.e., the experimental cDNA sample and the standard DNA) to be visualized and quantified on an electrophoresis gel using a DNA sequencer (Applied Biosystems, Courtaboeuf, France) equipped with Immunoscope software (Pasteur Institute, Paris, France).

The number of copies of the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) was quantified using the same technique, and results were expressed as the ratio between the number of copies of the mRNA analyzed and the number of copies of HPRT in the same sample.

Hepatocyte culture and adenovirus-mediated in vitro transduction

Rat hepatocytes were isolated by collagenase digestion as described previously (26) and were provided by C. Guguen-Guillouzo (Institut National de la Santé et de la Recherche Médicale, Unité 49, Rennes, France). Hepatocytes (>98% pure) were transduced with recombinant adenovirus at the indicated number of PFU per cell and were cultured in Williams medium supplemented with 1 g/L albumin, 5 μg/ml insulin, 7 × 10⁻³ M dexamethasone, 1 mM glutamine, 100 U/ml penicillin, and 1 mg/ml streptomycin (all of these reagents were from Sigma) for 72 h at 37°C in 5% CO₂. Hepatocytes were also cultured under the same conditions with rat IL-4 supplement (Pasteur Institute, Paris, France).

Biosystems, Courtaboeuf, France) equipped with Immunoscope software (Pasteur Institute, Paris, France).

The number of copies of the housekeeping gene hypoxanthine phosphoribosyltransferase (HPRT) was quantified using the same technique, and results were expressed as the ratio between the number of copies of the mRNA analyzed and the number of copies of HPRT in the same sample.

Transduction of rat livers with AdrIL-4 induces massive apoptosis and acute hepatitis

Intraportal infusion of AdrIL-4 in rats resulted in lethal, dose-dependent hepatitis (Fig. 1). Animals injected with 10⁵, 2.5 × 10⁶, 5 × 10⁶, or 10¹⁰ PFU of AdrIL-4 showed 0, 6.6, 70, or 85% mortality, respectively (Fig. 1). The time of death after administration of 10¹⁰ PFU of AdrIL-4 was 4 ± 1 days after gene transfer and was progressively delayed (up to day 14) with decreasing doses of AdrIL-4. Rats infused with 10¹⁰ PFU of Addl324 and with no further treatment showed no mortality. Animals injected with 10⁵, 2.5 × 10⁶, 5 × 10⁶, and 10¹⁰ PFU of AdrIL-4 showed a dose-dependent increase (mean ± SEM; n = 6–11) in serum bilirubin (5 ± 1.5, 27 ± 10, 32 ± 3.8, and 51 ± 3.5 μmol/L, respectively) and ALAT (42 ± 4, 68 ± 9, 81 ± 14, and 237 ± 22 IU, respectively). Rats infused with 10¹⁰ PFU of Addl324 showed values of bilirubin (4 ± 0.2 μmol/L) and ALAT (34 ± 4.5 IU) in serum comparable to those in nontreated rats (bilirubin, 5 ± 1 μmol/L; ALAT, 33 ± 3.5 IU).

Histological analysis of livers from Addl324-treated animals did not show major pathological lesions compared with untreated livers (Fig. 2, A and B). By contrast, livers treated with AdrIL-4 revealed confluent hepatocyte lesions predominantly in periportal...
areas associated with a leukocyte infiltrate composed of mononuclear cells (Fig. 2C). This leukocyte infiltrate also extended to centrilobular areas. AdrIL-4-infused livers contained a large number of hepatocytes showing the typical features of apoptotic cells: cell shrinkage, intensely acidophilic cytoplasm with condensed and fragmented chromatin, or even an absence of nuclei.

Livers transduced with Addl324 showed an absence of TUNEL-positive cells (Fig. 2D). By contrast, after administration of AdrIL-4 large numbers of apoptotic TUNEL-positive cells were detected (Fig. 2E). Most of these cells were hepatocytes, while a few were leukocytes. TUNEL signals were detected in apoptotic bodies and also in cells with condensed chromatin in the periphery of their nuclei. Apoptosis was confirmed by two-color immunofluorescence showing colocalization on the same cell of chromatin condensation (F) using Hoechst 33258 (arrow; as opposed to the diffuse and uniform staining in viable hepatocytes) and DNA fragmentation (G) using TUNEL assay (arrow) with different filters (magnification, ×1000). Photomicrographs are representative of five animals from each group.

Animals injected with ADRIL-4 showed no overt tissue histopathologic lesions in heart, lung, kidney, or brain, whereas thymus and spleen showed areas of atrophy (thymic cortex and spleen white pulp) or necrosis (thymic medulla and spleen red pulp; data not shown).

**TNF-α, FasL, and IFN-γ expression is increased in livers infused with AdrIL-4**

The expression of TNF-α, FasL, and IFN-γ has been implicated in the induction of apoptosis of hepatocytes in different liver pathologic processes (25, 27–29). Immunohistology using Abs directed against TNF-α or FasL showed no reactivity in livers infused with Addl324 (Fig. 3, A and C). In contrast, livers treated with AdrIL-4 contained numerous positive cells recognized by anti-TNF-α and FasL Abs (Fig. 3, B and D, respectively). Positive cells were mainly situated in areas infiltrated by leukocytes, but TNF-α reactivity showed a more diffuse pattern, with weak reactions in certain hepatocyte areas. Morphometric quantification of anti-TNF-α- and FasL-labeled cells showed a significant increase in livers infused with AdrIL-4 compared with untreated or Addl324-treated livers (Fig. 3E).

Analysis of TNF-α and FasL mRNA levels using a quantitative RT-PCR method showed significantly increased accumulation of specific mRNAs in livers infused with AdrIL-4 compared with untreated or Addl324-treated livers (Fig. 3F). Production of IFN-γ following administration of low doses of AdrIL-4 was previously described (16), and high levels of IFN-γ-positive cells were also observed by immunohistology and mRNA after gene transfer with 10¹⁰ PFU in the present study (data not shown). These results show that levels of liver TNF-α, FasL, and IFN-γ and their corresponding mRNAs are increased after adenovirus-mediated IL-4 expression and suggest that they could mediate IL-4-dependent hepatocyte apoptosis.

**Lethal hepatitis induced by adenovirus-derived IL-4 is largely independent of T cell-mediated anti-adenovirus immune responses**

To study the role of CTLs in mortality induced by Ad-derived IL-4 we pretreated rats with a depleting anti-CD8 mAb before injecting them with AdrIL-4. AdrIL-4-induced lethal hepatitis was not significantly reduced (57%, Fig. 1) in animals depleted of CD8⁺ T cells and subsequently infused with 10¹⁰ PFU. Liver histology in animals infused with 10¹⁰ PFU of AdrIL-4 in the absence of CD8⁺ cells showed hepatitis with the same features (apoptotic hepatocytes, mixed leukocyte infiltration, and reticulofibrosis) as that in animals with intact CD8⁺ cells, although increases in serum markers were of lower magnitude (data not shown). These results indicate that depletion of CD8⁺ cells had a modest protective effect against AdrIL-4-induced lethal hepatitis. To further investigate the role of T cells in AdrIL-4-induced hepatitis, we injected AdrIL-4 in T cell-deficient nude rats. This resulted in a high mortality (67%; Fig. 1), an intense leukocyte infiltrate composed of ED1⁺/
ED2⁺ monocytes/macrophages (Table I), and hepatic histopathology displaying apoptotic hepatocytes, leukocyte infiltration, and reticulofibrosis (Fig. 4). Hepatic biochemical values in CD8-depleted and nude rats were significantly raised (in both nonsurvivors and survivors; data not shown) compared with those in Addl324-infused or untreated rats.

Finally, immunosuppression with CsA did not prevent AdrIL-4-dependent mortality (100%, Fig. 1), massive leukocyte infiltration, or increased serum hepatic biochemistry values (data not shown). Injection of Addl324 in CsA-treated or nude animals was perfectly tolerated (0% mortality; Fig. 1). Altogether, these results indicate that lethal hepatitis induced by high doses of AdrIL-4 is largely independent of T cells.

**IL-4-dependent lethal hepatitis is independent of Kupffer cells or infiltrating leukocytes**

Since Kupffer cells comprise the predominant type of leukocyte present in normal liver and IL-4 induced a massive increase in infiltrating monocytes/macrophages, we asked whether depletion of Kupffer cells or prevention of liver infiltration by monocytes would protect against the development of hepatitis.

Animals depleted of Kupffer cells by injection of liposomes containing CI₂MDP prior to infusion of AdrIL-4 showed no protection against mortality (Fig. 1) compared with animals treated with AdrIL-4 alone despite greatly reduced macrophage counts at the moment of death (9 ± 1.1 vs 36.3 ± 2%, respectively; Table I).

### Table I. Quantification of liver mononuclear cell subsets after gene transfer with AdrIL-4 under different experimental conditions

<table>
<thead>
<tr>
<th>Specificity (mAb)</th>
<th>Control (n = 5)</th>
<th>Addl324⁺ (n = 4)</th>
<th>Untreated (n = 3)</th>
<th>Nude (n = 3)</th>
<th>Lip⁻ (n = 4)</th>
<th>CyP⁻ (n = 3)</th>
<th>CyP + Lip⁻ (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45/leukocytes (OX1 + OX30)</td>
<td>4 ± 0.7f</td>
<td>4.7 ± 1.5</td>
<td>32.4 ± 4.6f</td>
<td>25.2 ± 3.1f</td>
<td>27.6 ± 2.2f</td>
<td>6.5 ± 1.5f</td>
<td>1.2 ± 0.4f</td>
</tr>
<tr>
<td>Monocytes/macrophages (ED1)</td>
<td>3.7 ± 0.7</td>
<td>9.1 ± 1.3</td>
<td>32 ± 1f</td>
<td>32.3 ± 8f</td>
<td>24.6 ± 2f</td>
<td>8.5 ± 2.3f</td>
<td>0.9 ± 0.5f</td>
</tr>
<tr>
<td>Macrophages (ED2)</td>
<td>8.3 ± 1</td>
<td>12.7 ± 1.4</td>
<td>36.3 ± 2f</td>
<td>39.7 ± 7.5f</td>
<td>9 ± 1.1f</td>
<td>6.6 ± 0.8f</td>
<td>1.5 ± 0.1f</td>
</tr>
<tr>
<td>Anti-MHC II (OX6)</td>
<td>5.3 ± 1.3</td>
<td>8.9 ± 1.7</td>
<td>41.1 ± 1f</td>
<td>32.4 ± 4.1f</td>
<td>38.3 ± 4f</td>
<td>10.1 ± 4.1f</td>
<td>1.5 ± 0.6f</td>
</tr>
<tr>
<td>Anti-αβTCR (R.7.3)</td>
<td>0.3 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>6.6 ± 0.7f</td>
<td>0.2 ± 0.1f</td>
<td>2.8 ± 2.1f</td>
<td>0.2 ± 0.04f</td>
<td>0.1 ± 0.1f</td>
</tr>
<tr>
<td>Anti-NKR-P1 (3.2,3)</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>3 ± 1.5f</td>
<td>0.3 ± 0.05f</td>
<td>1.6 ± 1</td>
<td>0.2 ± 0.02f</td>
<td>0.4 ± 0.1f</td>
</tr>
</tbody>
</table>

* Addl324 or AdrIL-4 (10¹⁰ PFU) were delivered intraperitoneally at day 0 and livers were harvested 3 days later for immunohistological analysis.

* Liposomes-CI₂MDP (Lip) administered i.v. at day -1.

* CyP was administered i.p. at day -5 (70 mg/kg) and every 3 days (35 mg/kg).

* Percentage area of biopsies occupied by labeled cells.

* p < 0.016 as compared to control or Addl324-infused animals.

* At least p < 0.001 as compared to animals infused only with AdrIL-4 (untreated) and nonsignificant as compared to control or Addl324-infused animals.

* p < 0.0001 as compared to animals infused only with AdrIL-4 (untreated) and p < 0.03 as compared to control or Addl324-infused animals.
Nevertheless, numbers of MHC class II and ED1⁺ cells, probably newly arrived monocytes, were significantly increased in animals depleted of Kupffer cells and injected with AdrIL-4 (ED1⁺, 24.6 ± 2 vs 3.7 ± 0.7% in controls; Table I). Hepatic histology showed the typical morphology of IL4-induced acute hepatitis, e.g., ballooned hepatocytes, apoptotic cells, and leukocyte infiltration (Fig. 4).

To further completely prevent infiltration of the liver by circulating blood monocytes, leukocytes were depleted by a partially myeloablative treatment with CyP before the infusion of AdrIL-4. Animals treated with CyP and infused with AdrIL-4 were not protected against acute lethal hepatitis (Fig. 1). CyP effectively prevented infiltration of the liver by monocytes (ED1⁺ cells, 8.5 ± 2.3 vs 32 ± 1% in AdrIL-4-treated animals), macrophages (ED2⁺ cells), and T cells (Table I). Liver lesions included apoptotic hepatocytes, reticulofibrosis, and abnormal liver architecture (Fig. 4). Liver enzymes showed raised hepatic biochemistry values (data not shown).

**FIGURE 4.** Hepatic lesions and leukocyte infiltration in livers infused with AdrIL-4 under different experimental conditions. Addl324 or AdrIL-4 was delivered intraportally to normal animals, nude rats, or rats treated with either liposomes-Cl₂MDP (Lip) or CyP alone or in combination (CyP/Lip). Paraffin sections were stained with HES; arrows show apoptotic bodies (magnification, ×200). Cryosections were developed with the anti-macrophage ED2 mAb (magnification, ×100). Photomicrographs are representative of four animals from each group.
Despite the prevention of liver infiltration by leukocytes, CyP treatment did not deplete all Kupffer cells from the liver (ED2<sup>+</sup> cells, 6.6 ± 0.8 vs 8.3 ± 1 in controls; Table I). To simultaneously eliminate both Kupffer cells and infiltrating monocytes, animals were treated with liposomes-Cl<sub>3</sub>MDP and CyP before being infused with AdrIL-4. Immunohistology of leukocytes now demonstrated an efficient depletion of resident Kupffer cells by >98% (ED2<sup>+</sup> cells, 1.5 ± 0.1%), and the simultaneous inhibition by >99% of monocyte infiltration (ED1<sup>+</sup> cells, 0.9 ± 0.5%; Table I). However, such drastic elimination of monocytes and macrophages was unable to protect animals from AdrIL-4-dependent lethal acute hepatitis (Fig. 1), with blood biochemistry values being grossly abnormal (data not shown) and apoptotic bodies detected throughout the liver (Fig. 4). These results indicate that lethal hepatitis induced by IL-4 was totally independent of Kupffer cells or infiltrating monocytes.

### Induction of lethal hepatitis in mice following administration of AdmIL-4 and analysis of the molecular mechanisms using mutated or knockout mice

Injection of increasing doses of AdmIL-4 resulted in a dose-dependent mortality, with 100% of DBA/2 and C57BL/6 mice dying after administration of 4 × 10<sup>9</sup> or 8 × 10<sup>9</sup> PFU and 0% or 17% after administration of 10<sup>8</sup> or 2 × 10<sup>8</sup> PFU, respectively (Table II). In contrast, administration of 4 × 10<sup>9</sup> PFU to BALB/c, 129/Sv, and SJL/J resulted in 0, 0, and 20% mortality, respectively (Table II). Administration to DBA/2 and C57BL/6 mice of up to 8 × 10<sup>9</sup> PFU of the noncoding Addl324 adenovirus was nonlethal. Livers from DBA/2 and C57BL/6 mice injected with 4 × 10<sup>9</sup> PFU of AdmIL-4 showed a large proportion of apoptotic hepatocytes as well as an intense leukocyte infiltrate mainly composed of F4/80<sup>+</sup> macrophages (data not shown). Values of bilirubin, ALT, and ASAT were significantly elevated 6–9 days after injection of 4 × 10<sup>9</sup> PFU of AdmIL-4 (12.5 μmol/L, 96 IU, and 324 IU, respectively) compared with those in animals injected with Addl324 (3.7 μmol/L, 12 IU, and 72 IU) or not treated (3.1 μmol/L, 12 IU, and 78 IU; p < 0.05 for bilirubin, ALT, and ASAT).

Injection of 4 × 10<sup>9</sup> PFU of AdmIL-4 in mice undergoing a partially myeloablative regimen with CyP as well as in immunodeficient RAG2<sup>−/−</sup> animals were not protected against lethal hepatitis, but showed a small, but statistically significant, acceleration of the time to death (5.6 ± 1 and 6 ± 0.5, respectively, vs 7.5 ± 1.5 days in controls; Table II). Mice lacking IFN-γ, TNF-αRI, or TNF-αRII showed kinetics and percentages of dead animals identical with those of control mice (Table II). Fas-deficient mice (lpr) showed a moderate, but statistically significant, prolongation of survival compared with controls (10 ± 3 vs 7.5 ± 1.5, respectively; p = 0.004; Table II).

These results indicate that the induction of lethal hepatitis by IL-4 is not restricted to rats, but can also be induced in mice. In both species, the histopathologic characteristics and liver apoptosis are very similar. As in rats, IL-4-induced hepatitis was not prevented by elimination of T cell-dependent immune responses or liver infiltration by peripheral leukocytes. Furthermore, IL-4-induced hepatitis was independent of IFN-γ, TNF-αRI, or TNF-αRII expression and was only partially dependent on the presence of an active Fas pathway.

### Systemic high level expression of IL-4 from AdrIL-4 induces acute lethal hepatitis

The development of hepatic lesions independently of immune responses or leukocyte infiltration strongly suggested that IL-4 could be directly responsible for hepatocyte lesions in the absence of direct hepatocyte transduction by adenovirus. To explore this possibility, we injected AdrIL-4 in the hind limb of rats to obtain high levels of circulating IL-4 (n = 2). Rats injected with AdlacZ as controls (n = 2) showed undetectable levels of IL-4 in serum (detection limit, 10 pg/ml), large numbers of X-galactosidase (X-Gal)-positive cells in the injected muscle, but virtual absence of X-Gal-positive cells in the liver (four X-Gal positive cells in 10 liver sections from two different hepatic lobes at ×100 magnification) and normal liver histology (Fig. 5A). Rats injected with AdrIL-4 in the hind limb showed circulating IL-4 (49 and 36 pg/ml) for at least 3 days following gene transfer. These values were in the lower range of values observed in animals injected with AdrIL-4 into the portal vein (43–190 pg/ml). Hepatic histology on day 7 after injection showed apoptotic hepatocytes (Fig. 5B). Bilirubin and ALT levels were increased on day 3 and increased further on day 7 after gene transfer in the hind limb in the two animals receiving AdrIL-4 (54 and 145 μmol/L of bilirubin; 96 and 109.2 IU of ALAT) compared with those in animals receiving AdlacZ (3 and 4 μmol/L of bilirubin; 59.4 and 58.2 IU of ALAT). These results indicate that sustained levels of circulating IL-4 do

### Table II. Lethal hepatitis in mice after AdmIL-4 administration

<table>
<thead>
<tr>
<th>Strain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adenovirus</th>
<th>Dose (PFU)</th>
<th>Mortality (%)</th>
<th>Time to Death ± SE (days)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0</td>
<td>Indefinite</td>
<td>5</td>
</tr>
<tr>
<td>129/Sv</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0</td>
<td>Indefinite</td>
<td>6</td>
</tr>
<tr>
<td>SJL/J</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>20</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>DBA/2</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>7 ± 1</td>
<td>9</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>AdmIL-4</td>
<td>10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0</td>
<td>Indefinite</td>
<td>6</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>AdmIL-4</td>
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<td>17</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>7.5 ± 1.5</td>
<td>48</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>AdmIL-4</td>
<td>8 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>6</td>
<td>1 ± 1</td>
<td>5</td>
</tr>
<tr>
<td>C57BL/6 + Addl324</td>
<td>AdmIL-4</td>
<td>8 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0</td>
<td>Indefinite</td>
<td>5</td>
</tr>
<tr>
<td>RAG2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>5.6 ± 1&lt;sup&gt;f&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>IFN-γ KO</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>6 ± 0.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10</td>
</tr>
<tr>
<td>TNF-αRI KO</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>7 ± 3</td>
<td>5</td>
</tr>
<tr>
<td>TNF-αRII KO</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>8 ± 1.5</td>
<td>6</td>
</tr>
<tr>
<td>lpr</td>
<td>AdmIL-4</td>
<td>4 × 10&lt;sup&gt;9&lt;/sup&gt;</td>
<td>100</td>
<td>8 ± 1.5</td>
<td>6</td>
</tr>
</tbody>
</table>

<sup>a</sup> All knockout (KO) and mutated mice of C57BL/6 background.

<sup>b</sup> CyP, i.p. 100 mg/kg at day −5 and 50 mg/kg every 4 days.

<sup>c</sup> p = 0.01 as compared to C57BL/6 injected with 4 × 10<sup>9</sup> PFU of AdmIL-4.

<sup>d</sup> p = 0.004 as compared to C57BL/6 injected with 4 × 10<sup>9</sup> PFU of AdmIL-4.
induce direct hepatotoxicity, which is completely independent of hepatocyte transduction by adenoviruses.

**IL-4 induces apoptosis of primary hepatocytes**

To further identify the mechanisms underlying hepatocyte apoptosis observed in vivo, we analyzed whether apoptosis could be induced in purified hepatocytes by exposure to IL-4. Rat hepatocytes cultured for 48–72 h in the presence of rIL-4, AdrIL-4, or TNF-α showed higher numbers of apoptotic hepatocytes compared with untreated cells or hepatocytes transduced with AdlacZ at the same multiplicity of infection (moi; Fig. 6A). Transduction of hepatocytes with 2.5, 25, or 100 PFU of AdrIL-4 or AdlacZ resulted in 5, 50, and 90% of transduced cells as evaluated by X-Gal staining of AdlacZ-transduced cells. Hepatocytes incubated with heat-inactivated AdrIL-4 remained viable (data not shown). Cultures in the presence of rat rIL-4 also resulted in increased numbers of apoptotic cells, although lower numbers than those obtained with saturating concentrations of TNF-α (Fig. 6A). Control hepatocytes incubated with Hoechst 33258 showed uniform chromatin staining (Fig. 6B), whereas hepatocytes transduced with AdrIL-4 or cultured with recombinant rat IL-4 showed chromatin condensation and fragmentation typical of apoptosis (Fig. 6C).

Measurement of ALAT released into the culture supernatants from hepatocytes incubated with IL-4 (54.1 IU) or transduced with AdrIL-4 at an moi of 25 (46.8 IU) or 100 PFU (61.2 IU) also showed increases compared with those from hepatocytes transduced with AdlacZ at an moi of 25 (6 IU) or 100 PFU (16.2 IU) or to control cells (4.2 IU). These results indicate that IL-4 has a direct proapoptotic effect on highly purified hepatocytes, which is likely to explain the direct apoptosis of hepatocytes observed in vivo following gene transfer of IL-4.

**Prevention of AdrIL-4-induced hepatitis and in vitro hepatocyte apoptosis by treatment with caspase inhibitors**

Caspases play a central role in the execution phase of apoptosis (20). Inhibition of ICE-like caspases by the decoy peptide YVAD-cmk results in protection against Fas and TNF-α-mediated apoptosis of hepatocytes in vivo (21–23). To confirm the role of apoptosis in AdrIL-4 induced hepatitis and to explore potential therapeutic strategies, rats infused with AdrIL-4 were treated with YVAD-cmk. All animals treated with YVAD-cmk and infused with 5 × 10⁶ PFU of AdrIL-4 had survived by day 14 after gene transfer, whereas animals receiving AdrIL-4 alone showed 70% mortality (Fig. 7A). Bilirubin, but not ALAT, levels in rats receiving YVAD-cmk and AdrIL-4 were significantly lower (11.5 ± 4.9 μmol/L and 72.5 ± 12 IU; n = 6; p < 0.006 and p > 0.05, respectively) than those in animals receiving AdrIL-4 alone (32 ± 3.8 μmol/L and 81 ± 14 IU; n = 6). Compared with those in Addl324-treated animals or untreated controls, hepatic biochemistry values in rats receiving YVAD-cmk and AdrIL-4 were slightly higher without reaching statistical significance. Hepatic histology in animals treated with YVAD-cmk revealed a large reduction in the number of apoptotic bodies and leukocyte infiltration comparable to that in livers receiving AdrIL-4 alone (Fig. 7B and C).

Morphometric quantification of apoptotic bodies revealed significantly lower numbers in animals treated with AdrIL-4 and YVAD-cmk than in animals receiving AdrIL-4 alone (Fig. 7D). This protection against AdrIL-4-mediated lethal hepatitis conveyed by YVAD-cmk demonstrates the key biological relevance of IL-4 induction of hepatocyte apoptosis in this new model of hepatitis.

To further confirm these results and explore the effects of other caspase inhibitors, we treated hepatocytes in vitro with the caspase...
inhibitors YVADcmk, z-DEVDfmk, and z-VADfmk or the cathepsin inhibitor I z-FAfmk, as a negative control, before expression of IL-4. The results in Fig. 8 show that hepatocyte death induced by expression of IL-4 was prevented by preincubation with YVADcmk (60% inhibition), z-DEVDfmk, or z-VADfmk (both ~90% inhibition), whereas z-FAfmk did not protect hepatocytes.

These in vitro results show that other caspases are also involved in IL-4-induced hepatocyte apoptosis and that other caspase inhibitors may be even more efficient than YVADcmk in preventing this form of hepatitis.

Discussion
Liver expression of IL-4 in rats using recombinant adenoviruses resulted in a lethal, dose-dependent hepatitis with numerous apoptotic hepatocytes, a strong tissue infiltration by monocytes/macrophages, and activation of inflammatory and apoptotic mechanisms. Our work shows that lethal hepatitis was independent of liver Kupffer cells, circulating leukocytes, or T cells. Furthermore, an identical pathology could be induced in mice after in vivo expression of mouse IL-4. Using knockout mice we furthermore demonstrate that IL-4 induced lethal hepatitis in IFN-γ-, TNF-αRI-, TNF-αRII-, and Fas-deficient (lpr) mice. Death was only delayed by a few days in lpr mice, indicating that Fas-FasL interactions play only a partial role in IL-4-induced apoptosis.

Production of type 1 cytokines (mainly IFN-γ and IL-2) is associated with hepatic inflammatory damage in humans (30). Production of IL-4, a typical type 2 cytokine, is also elevated in various human liver diseases, such as autoimmune hepatitis (5), chronic hepatitis (5, 30, 31), and primary biliary cirrhosis (6). Furthermore, administration of IL-4 in primate preclinical studies (4) resulted in a lethal, dose-dependent hepatitis with numerous apoptotic hepatocytes, a strong tissue infiltration by monocytes/macrophages, and activation of inflammatory and apoptotic mechanisms in all reports. Finally, IL-4 production is increased, and depletion of CD8+ cells reduced the infiltration of the liver by leukocytes (17). The high rates of mortality after infusion of higher doses of AdrIL-4 (1010 PFU) in CD8-depleted, nude, and CsA- or CyP-treated rats clearly indicate that anti-adenovirus immune responses do not play an essential role in the lethal effects induced by AdrIL-4. Nevertheless, CD8-depleted rats showed a partial protection against lethality, and this may depend on the depletion of leukocytes other than T cells expressing CD8α, such as NK and NK1+ T cells. Therefore, although part of the hepatic lesions induced at lower and higher doses of AdrIL-4 are dependent on the recruitment of leukocytes and the increase in anti-adenovirus immune responses, at higher doses of AdrIL-4, leukocyte-independent pathological mechanisms predominate. These results also indicate that lethal hepatitis only develops when a relatively high production of IL-4 is obtained, which should be taken into consideration when the role of IL-4 is evaluated in other hepatitis models. Finally, the doses of AdrIL-4 and AdmIL-4 needed to obtain lethal hepatitis are comparable to those used in most gene transfer protocols in the liver and correspond to an moi of 10 PFU/hepatocyte in the rat model (32, 33).

The mouse IL-4R α-chain (IL-4Rα) shows a polymorphism among different mice strains that results in two groups of strains that express receptors with either higher or lower affinity for IL-4 (34). Administration of AdmIL-4 resulted in lethal hepatitis in strains with higher affinity (C57BL/6 and DBA/2), but not in those with lower affinity (BALB/C, SJL/J; no data are available for the IL-4R affinity in 129/Sv mice). The variability in strain susceptibility to the IL-4-induced hepatitis may explain why in previous studies systemic administration of high doses of rIL-4 or high IL-4-expressing transgenic animals showed adverse effects when the genetic background corresponded to the group with higher affinity IL-4R (35), but not when the mice were from the lower affinity group (36, 37). Furthermore, since polymorphisms in the IL-4Rα chain in humans also exist (38), IL-4 may play a different role in hepatocellular damage in individuals of different genetic backgrounds.

The fact that RAG2−/− immunodeficient and mice depleted of leukocytes by CyP treatment were not protected against lethal hepatitis induced by AdmIL-4 (but, in fact, mortality was slightly
accelerated) further supports the idea that this hepatitis is independent of immune cells and that the pathophysiology of IL-4-induced hepatitis in rats and mice is identical.

Finally, the development of hepatic lesions following injection of AdrIL-4 in the hind limb and the induction of high levels of circulating IL-4 clearly indicate that hepatitis is independent of the presence of adenovirus particles in the liver undergoing hepatitis. Furthermore, the fact that IL-4 mediates apoptosis of purified hepatocytes in vitro further strengthens the evidence that apoptosis of hepatocytes in vivo is independent of inflammatory and immune responses, but is mediated by direct actions of IL-4 on hepatocytes.

IL-4Rα is associated with either IL-2Rγ in hemopoietic cells or IL-13Rα in endothelial cells (39, 40). Although hepatocytes express receptors for IL-4 (7), the type of second chain expressed in this cell type has not been reported. The IL-4Rα intracellular segment contains distinct domains responsible for cell proliferation, regulation of apoptosis, and gene expression (39). Since the IL-4Rα does not contain death domains present in receptors capable of directly transducing apoptotic signals (such as Fas, TNF-α-R1, death receptor 3, and TNF-related apoptosis-inducing ligand-R3), IL-4 may induce hepatocytes to produce proapoptotic molecules. These molecules could be TNF-α, Fas, FasL, and reactive oxygen species acting in an autocrine manner, as has been shown in several hepatic pathological conditions (25, 27, 28, 41). Alternatively, the IL-4R may directly induce hepatocyte apoptosis, as has been described for members of the TNF receptor superfamily (TNF-αRII, CD40, CD30, and CD27) without death domains. Nevertheless, apoptosis mediated by TNF-αRII depends on intracellular recruitment of adapter proteins (Fas-associated death domain, TNFR-associated factor-2, and receptor-interacting protein) (42), and these protein interactions are unlikely to be possible with receptors other than those of the TNF receptor superfamily. Despite the association of increased liver synthesis of TNF-α and IFN-γ during IL-4-mediated apoptosis of hepatocytes, the fact that TNF-αRI-, TNF-αRII-, or IFN-γ-deficient mice developed hepatitis indicates that these cytokines do not mediate IL-4-induced hepatocyte cell death. Hepatocytes are more sensitive to certain types of apoptosis, probably due to the absence of Bcl-2 expression (43). Hepatocyte apoptosis may depend on the production of other proapoptotic molecules, such as TNF-related apoptosis-inducing ligand, death receptor 3, or reactive oxygen species, and the roles of these molecules need to be evaluated. In this regard, livers from animals expressing IL-4 show reactive oxygen-mediated lesions, and rats and mice treated with anti-oxidants such as melatonin or cobalt protoporphyrin are protected from IL-4-induced lethal hepatitis (I. Anegon, manuscript in preparation). The detailed molecular mechanisms underlying the hepatocellular damage induced by IL-4 remain to be determined.

Treatment with YVADcmk, a preferential, but not exclusive, inhibitor of caspase-1 (ICE) (44), improved hepatic lesions and survival after liver expression of IL-4. The protective effect of YVADcmk in animals expressing IL-4 could be due to several nonexclusive mechanisms. First, since caspase-1 cleaves and activates IL-1β (20) as well as IL-18 (45), a potent inducer of IFN-γ production, the protection conferred by YVADcmk from IL-4-induced hepatitis points to possible mechanisms involving IL-1β and/or IL-18. However, IFN-γ is not involved in IL-4-induced lethal hepatitis, since hepatitis is unaffected in IFN-γ-knockout mice. It should be noted that IL-18 participates in other models of hepatitis, such as that induced by endotoxin, which depend on mutual interactions and a positive loop consisting of IL-18, the Fas-FasL system, and IFN-γ and TNF-α production (46). In this regard, lpr mice show some protection against IL-4-induced hepatitis. Second, it has been demonstrated that caspase-1 processes and activates precaspase 3 (47) and that inhibition of caspase-1 could inhibit certain steps of the caspase cascade leading to apoptosis (20). Therefore, caspase-1 inhibition may indirectly inhibit caspase-3 activation. The key role of caspase 1 in apoptosis of hepatocytes is shown by the resistance of caspase-1-deficient mice to Con A-induced hepatitis (48). Finally, caspase 1 may not be the only caspase involved in IL-4-induced hepatocyte apoptosis. The in vitro results showing higher protection of hepatocytes from apoptosis induced by IL-4 with z-DEVDrFMk (preferential inhibitor for the effector caspase-3) and z-VADfmk (inhibitor for all caspasess) compared with YVADcmk, indicate that other proapoptotic mechanisms activating other caspases, such as oxidative stress (49), play a role. It should be stressed that YVADcmk has been previously shown to protect hepatocytes from apoptosis in other models of hepatitis (21–23) as well as neurons from oxidative stress (50), in which IL-1β and IL-18 are not involved.

IL-4 has been shown to regulate apoptosis in different cell types, being proapoptotic in endothelial cells (51), in mast cells from cord blood (14), and in eosinophils (15) and enhancing apoptosis in stimulated monocytes (13), while being antiapoptotic in B cells (12) and myeloid cells (8). The effects of IL-4 on the regulation of hepcyte function (8–10) have been previously described in the literature, but the capacity of IL-4 to induce hepatocyte apoptosis has not been previously described.

Our results suggest that IL-4-mediated hepatitis will prove a valuable tool to evaluate new treatments for hepatic disorders in which IL-4 production has been reported or is suspected. In addition, this new model of hepatitis makes it possible to investigate the mechanisms by which cytokines, endothelial cells, monocyte/macrophages, T cells, and hepatocytes interact to generate liver inflammation and apoptosis.

**Acknowledgments**

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References


