

Enhanced Expression of CD80 (B7-1), CD86 (B7-2), and CD40 and Their Ligands CD28 and CD154 in Fulminant Hepatic Failure

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To define a possible role for changes in the regulation of antigen presentation in fulminant hepatic failure (FHF), we studied the expression of co-stimulatory molecules CD80 (B7-1), CD86 (B7-2), and CD40 along with their ligands CD28 and CD154. We analyzed the liver tissue from patients with FHF ($n = 18$), chronic liver disease ($n = 30$), and acute hepatitis ($n = 3$) and from normal controls ($n = 9$) by immunohistochemistry and examined the temporal relationship between CD80/CD86 and CD40 expression and disease in the mouse models of galactosamine-lipopolysaccharide and galactosamine-tumor-necrosis-factor-induced FHF. In human controls, faint CD80/CD86 immunoreactivity was restricted to Kupffer cells, and CD40 expression was expressed on bile ducts, macrophages, and sinusoidal endothelial cells (SECs). In FHF, immunoreactivity for CD80 and CD86 was observed on significantly higher numbers of cells, including SECs. Increased CD80/CD86 expression corresponded to increased numbers of CD28-positive lymphocytes. The expression of CD40 was also clearly elevated on virtually all cell types in FHF. In both murine models, CD40 and CD80/CD86 expression was up-regulated before tissue damage could be detected. Our data suggest that up-regulated expression of co-stimulatory molecules might lead to an excessive antigen presentation in FHF as an early step in the pathogenesis before the onset of tissue damage. (*Am J Pathol* 1999, 154:1711–1720)

Hepatitis B virus infection or drug toxicity can initiate a sequence of severe inflammatory reactions that may lead to rapid organ failure and death. Although the precise pathogenesis is poorly understood, enhanced immune activation, including aberrant antigen presentation and T-cell activation, may essentially contribute to hepatocyte destruction in fulminant hepatic failure (FHF). A balance in the interaction between antigen-presenting cells

(APCs) and lymphocytes is of special importance to the liver, as the hepatic immune system is confronted with a high number of antigens that reach the liver via the portal tract and that normally do not elicit immune responses.

Recently, it has become evident that a sufficient immune response depends on an efficient T cell activation via co-stimulatory molecules.^{1–4} The interaction of CD80 (B7-1) and CD86 (B7-2) with their counter-receptors on T lymphocytes (CD28 and CD152) provides a particularly potent co-stimulatory signal, which amplifies the response of T cells. Although antigen presentation followed by co-stimulation induces full T-cell activation, such antigen presentation that lacks co-stimulatory signals results in tolerance or anergy for the specific antigen.^{5–7} CD80 is a 44- to 60-kd member of the immunoglobulin superfamily with a limited expression on professional APCs such as macrophages, dendritic cells, and activated B cells. CD86 is a 75- to 115-kd cell surface glycoprotein with 25% amino acid homology to CD80. It also has a restricted expression on APCs.

Regulation of co-stimulation may also involve the CD40 molecule. CD40 is a 45- to 48-kd glycoprotein, which interacts with its natural ligand CD154 (gp39) on lymphocytes and which can be expressed on a great variety of different cells.^{8–10} A favored hypothesis suggests that recognition of the MHC-antigen complex by T cells leads to the induction of CD154 in these T cells. Subsequently, the simultaneous engagement of the MHC-antigen complex by the T-cell receptor and CD40 by CD154 up-regulates the expression of the co-stimulatory molecules CD80 and CD86 in the APCs.^{11–14}

Following the hypothesis that the delicate balance between hepatic immune tolerance and immune stimulation could be deranged in FHF, we studied the expression of the co-stimulatory and immunoactivating molecules CD80, CD86, and CD40 as well as their ligands in patients with FHF. Furthermore, the time cascade of CD80/CD86 and CD40 expression was examined after inducing of experimental FHF in BALB/c mice by exposure to either galactosamine and lipopolysaccharide (GalN/LPS) or galactosamine and tumor necrosis factor (GalN/TNF).

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Materials and Methods

Patients

Explant tissue from 18 patients with FHF was included in the study. All patients fulfilled the King's College criteria¹⁵ for urgent transplantation. The etiology of hepatic failure was hepatitis B ($n = 10$), cryptogenic ($n = 5$), 5-methoxy-3,4-methylene-dioxymethamphetamine (MDMA) toxicity ($n = 1$), and autoimmune hepatitis ($n = 2$). The liver tissue from 30 patients with chronic liver disease (CLD);—nine cases of chronic persistent hepatitis B, five of chronic active hepatitis B, six of chronic hepatitis C, five of primary biliary cirrhosis, three of primary sclerosing cholangitis, and two of autoimmune chronic active hepatitis) as well as from two patients with fulminant Wilson's disease and from three patients with acute hepatitis (HAV, $n = 2$; HCV, $n = 1$)—were included as disease controls. Non-tumor-bearing liver tissue obtained during the resection of hepatic malignancies in four patients and wedge biopsies from five donor livers at the time of hepatectomy served as normal controls (NCs).

Immunostaining Procedures

Liver tissue was immediately embedded in Tissue Tek OCT compound (Miles Laboratories, Naperville, IL) and snap-frozen in liquid nitrogen. Frozen tissue was kept at -80°C until examined. Sections of 5 to 7 μm were stained by an indirect immunoperoxidase technique. In brief, endogenous peroxidase activity was blocked by 0.03% $\text{H}_2\text{O}_2/\text{NaNO}_3$ (peroxidase blocking reagent, Dako, Carpinteria, CA). The sections were incubated with mouse monoclonal primary antibodies in phosphate-buffered saline (PBS)/1% fetal calf serum (FCS) in a moist chamber at room temperature for 90 minutes. After washing in PBS, peroxidase-coupled goat anti-mouse antibody (Dianova, Hamburg, Germany) was applied for 30 minutes. Any bound antibody was detected with 3-amino-9-ethylcarbazole (Sigma Chemicals, Munich, Germany). All sections were then counterstained with Meyer's hematoxylin.

The results of the indirect immunoperoxidase technique were confirmed by a double-step immunoenzymatic labeling using immune complexes of alkaline phosphatase and monoclonal anti-phosphatase (APAAP). After incubating sections with the primary antibody, rabbit anti-mouse antibodies were applied to the slides for 25 minutes (Dako). Thereafter, APAAP complexes were detected with naphthol-AS biphosphate/neufuchsin (Sigma Chemicals).¹⁶

Monoclonal Antibodies

To ensure the specificity of monoclonal antibodies (MAbs) we stained two different antibodies for the detection of CD40, CD86, and CD28 expression. Both 5C3 (Dianova) and anti-CD40 (Genzyme, Cambridge, MA) react with the 45- to 48-kd type 1 integral membrane glycoprotein CD40 found on peripheral blood and tonsil-

lar B cells. Both Fun-1 (Dianova) and IT2.2 (Dianova) recognize different epitopes on the 75-kd cell surface protein CD86 (B7-2) expressed primarily on monocytes and activated B cells. Both Leu 28 (Becton Dickinson, Heidelberg, Germany) and anti-CD28 (Pharmingen, San Diego, CA) detect the disulfide-linked 44-kd CD28 antigen. CD8^+ lymphocytes were stained with Leu 2a (Becton Dickinson). MAb BB1 (CD80/B7-1) (Becton Dickinson) reacts with a 44- to 60-kd protein on B7-transfected mouse cells identical to CD80. MAb TRAP1 (Pharmingen) reacts with CD154 (CD40 ligand, gp39), a 39-kd type II membrane glycoprotein expressed on activated T cells.

We performed immunohistochemistry on mice with rat anti-mouse CD80 (1G10, Pharmingen, San Diego, CA), rat anti-mouse CD86 (GL1, Pharmingen), rat anti-mouse CD40 (3/23, Pharmingen), and rat anti-mouse CD8 (53-6.7, Pharmingen).

As isotype controls, irrelevant mouse monoclonal antibodies (IgG1 and IgG2; Becton Dickinson) were used in human and rat MAbs (Pharmingen) were used in the mouse models.

Serial Sections and Double Stainings

To identify different parenchymal and nonparenchymal cell types, we carried out serial liver slides and double stainings. We used anti-cytokeratin (KL-1; Immunotech, Hamburg, Germany; positive reaction to hepatocytes and bile ducts), anti-CD31 (JC/70A; Dako; positive reaction to endothelial cells) and anti-liver sinusoidal endothelium (HM15/3; Monosan, Uden, The Netherlands; positive reaction to SECs), CD68 (PGM1; Dako; positive reaction to macrophages), and anti-smooth muscle actin (1A4; Dako; positive reaction to Ito cells). We performed double stainings with FITC-conjugated anti-CD31 and anti-CD68 antibodies. After performing the immunoperoxidase reaction, we incubated the sections for 20 minutes with irrelevant IgG1 and IgG2 antibodies to block any possible free binding site of the secondary antibody before the FITC-conjugated antibody was applied for 30 minutes. Analysis of double-labeling experiments was done by bright-field and fluorescent photomicrographs on a Leica DMLB fluorescence microscope with a MPS60 photo camera.

D-Galactosamine-LPS and *D*-Galactosamine-TNF Mouse Model

Six- to eight-week-old pathogen-free male BALB/c mice were obtained from the Animal Research Institute of the Medizinische Hochschule Hannover. All of the experiments were started between 8 and 10 a.m. and were performed in agreement with the German Legal requirements. Either 10 $\mu\text{g}/\text{kg}$ LPS or 10 $\mu\text{g}/\text{kg}$ TNF- α were injected intravenously with 700 mg/kg *D*-galactosamine. The livers of the animals were removed 4, 8, and 12 hours after treatment and frozen in liquid nitrogen for further analysis. To confirm lethality of the chosen GalN/LPS and GalN/TNF doses, one mouse each was treated without further intervention and died between 12 and 24 hours.

Statistical Analysis

CD80-, CD86-, CD28-, CD40-, and CD8-positive cells were counted in at least six randomly selected fields at 400-fold magnification. CD154-positive cells were counted in 100 visual fields at 400-fold magnification. The results for each patient are given as the mean of all evaluated visual fields. The results of each patient group are given as mean \pm SD. Using the SPSS PC+ software package, we analyzed the differences between the groups for statistical significance by the nonparametric Mann-Whitney U test. Pearson's correlation coefficients were also calculated.

Results

Histology

In patients with FHF, several patterns of hepatocyte damage could be discerned. Six patients (1, 2, 3, 7, 9, and 18) had a complete loss of hepatocytes in their specimens, whereas CD31-positive, CD68-negative SECs were preserved. In 11 patients with FHF, 75% to 95% of hepatocytes were destroyed. Single surviving hepatocytes were found in 5 of these 11 patients (4, 8, 12, 14, and 16). In the six other patients (5, 6, 10, 11, 13, and 15), surviving hepatocytes formed islets of intact hepatic structure, which were sharply limited by dense infiltrates of macrophages (Figure 1C). No inflammatory cells were seen within these islets. One patient (17) had an intact hepatic structure with dense mononuclear infiltrates. Two patients with fulminant hepatitis from Wilson's disease showed complete cirrhosis with minimal necrosis and only very few inflammatory infiltrates. These two patients were studied as a separate group.

Hepatic Expression of CD28, CD80, and CD86 in Human Specimens

In all examined patients with FHF, a markedly increased number of both CD80- and CD86-positive cells was noted (data are given as number of cells per visual field (VF) at 400-fold magnification: CD80, 108 ± 37 cells/VF; CD86, 112 ± 36 cells/VF) as compared with patients with CLD (CD80, 23 ± 9 cells/VF, $P < 0.0001$; CD86, 22 ± 9 cells/VF, $P < 0.0001$) and NCs (CD80, 16 ± 7 cells/VF; CD86, 14 ± 6 cells/VF, $P < 0.0001$) (Figures 1, A–C and 3, A and B; Table 1). CD80/CD86 expression of patients with fulminant Wilson's disease, acute hepatitis, or chronic active hepatitis was not different from CLD. In FHF, most of these cells were CD68-positive macrophages, which constituted the predominant cell type in the liver infiltrates of FHF. In contrast, infiltrates in chronic liver disease were dominated by lymphocytes with only few interposed CD80- and CD86-positive macrophages. FHF was unique in that there was strong immunoreactivity of CD80/CD86 that was also found on a part of the SECs, as identified by morphological characteristics and positive reaction to the monoclonal antibody CD31 in serial sections (Figure 2, A–C) and double-staining experi-

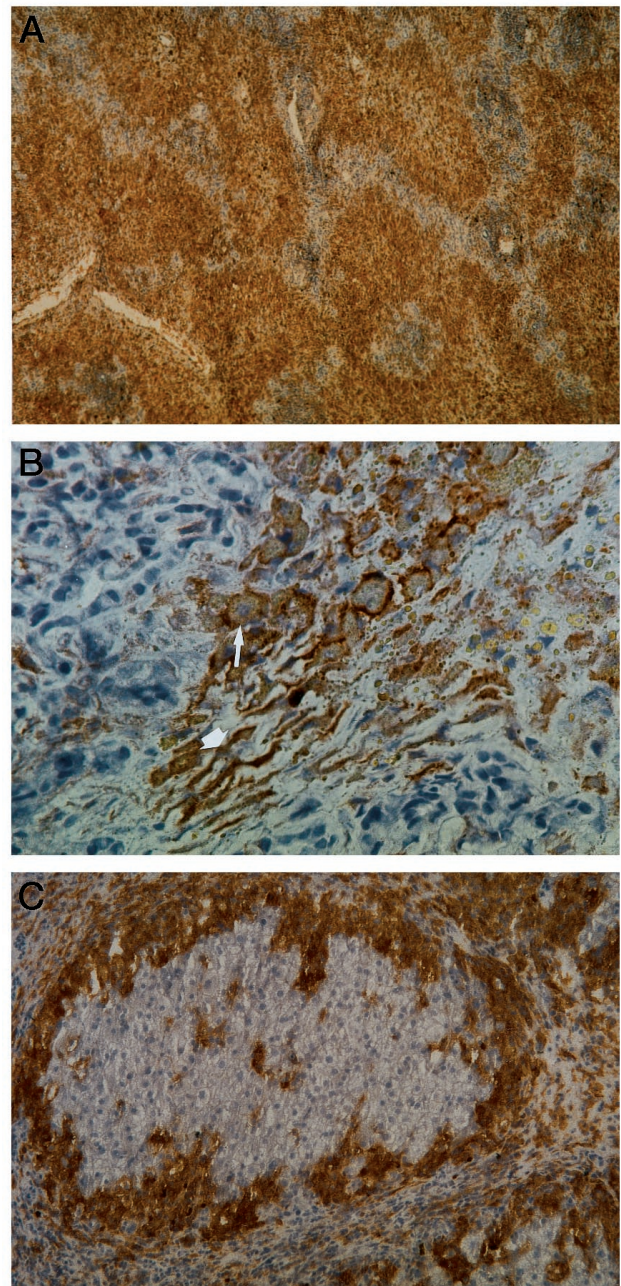


Figure 1. A and B: Hepatic CD86 expression in a patient with fulminant HBV (patient 2, Mab Fun-1). Cell types with typical morphological characteristics of macrophages (B, thin arrows) and of sinusoidal endothelial cells (thick arrows) are positive for CD86. Magnification, $\times 40$ (A) and $\times 400$ (B). C: Hepatic CD86 expression in a patient with toxic FHF (patient 9, Mab Fun-1). Islets of surviving hepatocytes showed absence of infiltrating cells and a normal expression of co-stimulatory molecules, exclusively on Kupffer cells, within the islets. The islets are surrounded by a dense wall of CD86-positive macrophages. In areas of liver necrosis outside the islets, macrophages and SECs, are CD86 positive. An identical pattern could be demonstrated for CD80 expression (not shown). Magnification, $\times 200$.

ments (Figure 2, D and E). No CD80 or CD86 immunoreactivity could be observed on hepatocytes, bile ducts, or Ito cells (data now shown). In FHF with islets of surviving hepatocytes, SECs within the islets were consistently negative for CD80 and CD86, as were all the SECs in the specimens from CLD and NCs (Figures 1C and 3, A and B).

Table 1. CD80, CD86, CD28, CD40, CD154, and CD8 Staining

Disease	n	Number of positive stained cells per high-power field					
		CD80 macrophages and SECs	CD86 macrophages and SECs	CD28 lymphocytes	CD40 total number of cells	CD154 lymphocytes	CD8 lymphocytes
Fulminant hepatic failure							
1 HBV	1	175	180	66	181	0.96	66
2 HBV	1	129	139	46	196	0.71	46
3 HBV	1	117	124	50	210	—*	50
4 HBV	1	115	109	62	241	0.46	62
5 HBV	1	173	166	64	233	0.09	64
6 HBV	1	117	126	65	180	0.47	65
7 HBV	1	109	122	55	159	0.56	55
8 HBV	1	95	99	36	147	0.45	41
9 HBV	1	146	153	26	77	0.6	43
10 HBV	1	138	143	52	87	0.32	40
11 toxic (MDMA)	1	132	125	38	211	0.51	36
12 autoimmune hepatitis	1	75	77	64	204	0.28	77
13 autoimmune hepatitis	1	52	73	28	100	0.05	57
14 cryptogenic	1	103	101	44	125	0.15	53
15 cryptogenic	1	85	91	67	278	0.21	67
16 cryptogenic	1	85	81	25	68	0.46	32
17 cryptogenic	1	29	31	16	20	0.06	8
18 cryptogenic	1	82	80	45	93	0.2	31
Total (mean ± SD)	18	108 ± 37 [†]	112 ± 36 [†]	48 ± 15 [†]	156 ± 68 [†]	0.39 ± 0.23 [†]	57 ± 12 [†]
Fulminant Wilson's disease							
19	1	26	28	13	55	0.08	24
20	1	37	37	26	45	0.1	52
Total (mean ± SD)	2	32 ± 6	33 ± 5	20 ± 7	50 ± 5	0.09 ± 0.01	38 ± 14
Acute hepatitis							
21 (HAV)	1	34	39	16	116	—*	27
22 (HCV)	1	39	46	15	38	—*	35
23 (HAV)	1	15	24	19	95	—*	35
Total (mean ± SD)	3	29 ± 10	36 ± 9	17 ± 2	83 ± 33	—*	32 ± 4
Chronic liver disease							
24–37 HBV	14	29 ± 9	28 ± 9	11 ± 8	66 ± 24	0.05 ± 0.05	18 ± 11
38–43 HCV	6	18 ± 6	18 ± 6	12 ± 6	51 ± 11	0.05 ± 0.07	16 ± 7
44–53 autoimmune (5 PBC, 3 PSC, 2 AIH)	10	18 ± 6	18 ± 6	10 ± 10	50 ± 19	0.05 ± 0.05	10 ± 7
Total (mean ± SD)	30	23 ± 9	22 ± 9	12 ± 8	57 ± 22	0.05 ± 0.05	15 ± 8
Normal controls							
54–62—total (mean ± SD)	9	16 ± 7	14 ± 6	1.4 ± 1	28 ± 17	0.003 ± 0.004	1.8 ± 1.4

Results are given as the mean of at least six high-power fields for CD80, CD86, CD28, CD40, and CD8. CD154-positive cells were counted in 100 high-power fields.

*As only needle biopsies were available, only an inadequate number of high-power fields could be evaluated.

[†]The number of CD80-, CD86-, CD28-, CD40-, CD154-, and CD8-positive cells is significantly enhanced in FHF compared with chronic liver disease and compared with normal controls ($P < 0.001$; CD154, $P < 0.005$).

A close correspondence was found between the numbers of CD80/CD86-positive cells in the liver tissue and the number of infiltrating CD28-positive lymphocytes ($r = 0.83$, $P < 0.001$), which was higher in FHF (48 ± 15 cells/VF) than in CLD (12 ± 8 cells/VF, $P < 0.0001$) or NCs (1.4 ± 1 cells/VF, $P < 0.001$; see Figure 6). Interestingly, in the islets of surviving hepatocytes, no infiltration of CD28-positive lymphocytes was noted.

Hepatic Expression of CD40, CD154, and CD8

Significantly higher numbers of CD40-positive cells were found in FHF than in control groups (FHF, 156 ± 68 cells/VF; CLD, 50 ± 5 cells/VF, $P < 0.001$; NC, 28 ± 17 cells/VF, $P < 0.001$), comprising macrophages, lymphocytes, Kupffer cells, hepatocytes, and bile duct epithelia. CD40 immunoreactivity was not found on hepatocytes in NCs, whereas $32 \pm 38\%$ of hepatocytes were

positive in CLD, and $63 \pm 25\%$ of hepatocytes were positive in FHF. Immunoreactivity for CD40 was detected on bile ducts and bile duct proliferates in FHF ($100 \pm 0\%$), in CLD ($89 \pm 23\%$), and in NCs ($48 \pm 18\%$). Sinusoidal CD40 immunoreactivity was observed in eight of nine NCs as well as in all specimens from patients with FHF and CLD. Infiltrating macrophages and Kupffer cells were consistently CD40 positive in all groups (Figure 4). In contrast to abundant hepatic CD40 immunoreactivity, numbers of CD154-positive lymphocytes were comparatively low (Table 1). However, still significantly more CD154-positive lymphocytes were detected in FHF (0.53 ± 0.32 cells/VF) than in CLD (0.05 ± 0.05 cells/VF, $P < 0.005$) and NCs (0.003 ± 0.004 cells/VF, $P < 0.005$).

Overall, the number of lymphocytes with cytotoxic/suppressor (CD8⁺) phenotype were higher in FHF (57 ± 12 cells/VF) than in CLD (15 ± 8 cells/VF, $P < 0.001$) or NCs (1.8 ± 1.4 cells/VF, $P < 0.001$). We found a close

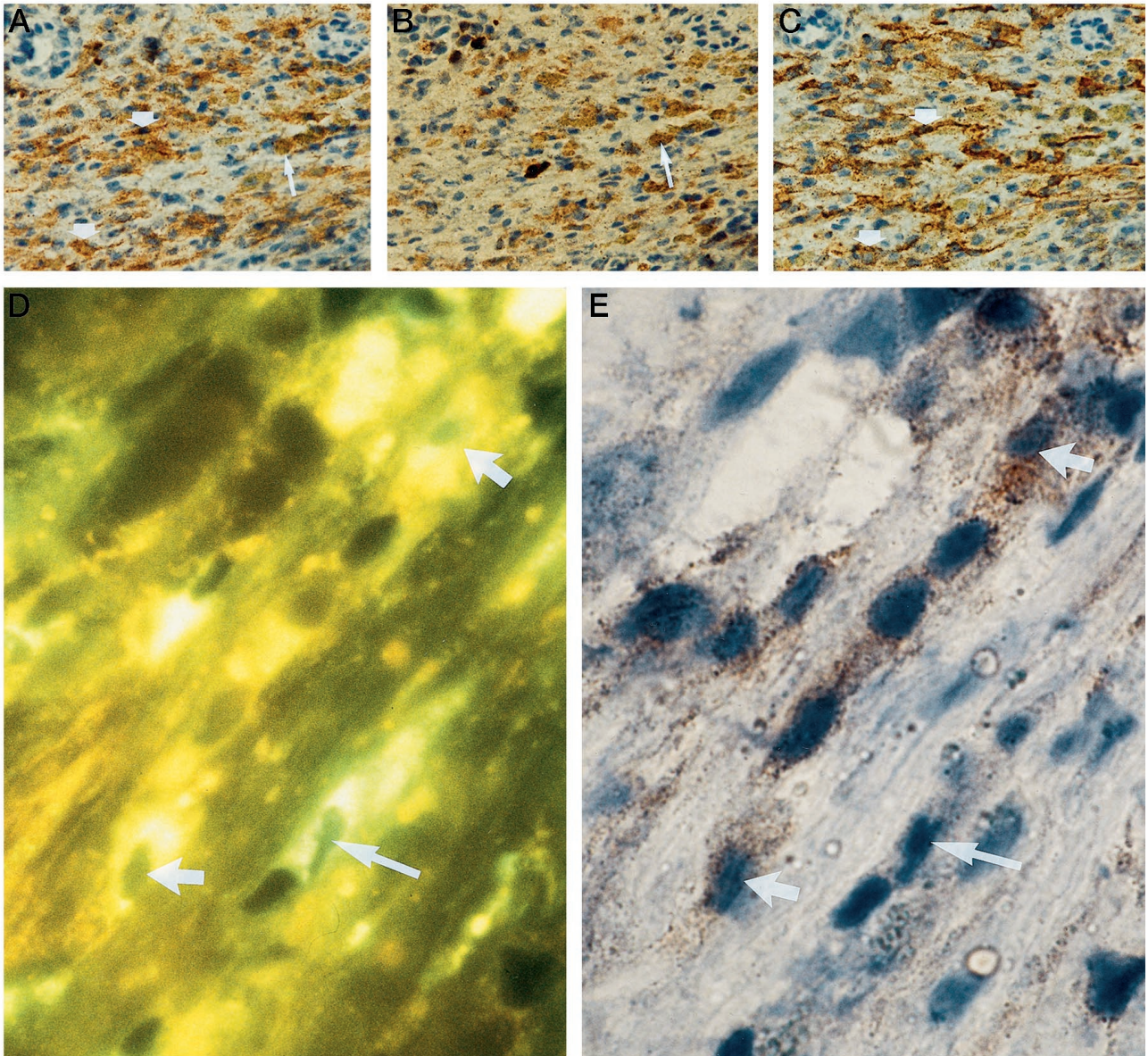


Figure 2. Assignment of CD86 expression to macrophages and SEC (patient 1). Serial sections were stained for CD86 (A, MAb Fun-1), CD68 (B, MAb JC/70A), and CD31 (C, MAb PGM1). As shown by the (thin arrows), expression of CD86 can be allocated to macrophages (CD86⁺, CD68⁺, CD31⁻) and as shown by the thick arrows also to SECs (CD86⁺, CD68⁻, CD31⁺). Double stainings with CD31-FITC (D) and CD86 (E) demonstrate CD86-positive (thick arrows) and CD86-negative (thin arrows) SECs. Magnification, $\times 400$ (A to C) and $\times 1000$ (D and E).

correlation between the number of total CD40-positive cells in the liver specimens and the counts of CD8- as well as CD28-positive lymphocytes, respectively ($r = 0.84$, $P < 0.001$; $r = 0.84$, $P < 0.001$; see Figure 6).

Animal Models

In the animal models, mice were killed 4, 8, and 12 hours after injection of GalN/LPS or GalN/TNF, respectively. Microscopic examination showed preserved liver architecture throughout the whole study period. However, 12 hours after treatment, focal edema and hepatocyte swelling was observed. The numbers of infiltrating macrophages and lymphocytes did not reach the extent seen in

human fulminant hepatic failure. With respect to CD80, CD86, and CD40 expression, untreated mice matched the physiological findings in healthy man; a faint CD80, CD86, and CD40 expression was exclusively restricted to some Kupffer cells. SECs, bile ducts, and hepatocytes were negative for CD80, CD86, and CD40. Only a low number of CD8-positive lymphocytes was observed in the control livers (0.4 per visual field).

In both murine models, a time-dependent up-regulation of CD80 and CD86 expression was noted (Figure 5, A-D). The numbers of CD80- and CD86-positive Kupffer cells increased 4 hours after induction of liver failure in each model. At 8 hours, additional expression of CD80 and CD86 was noted on SECs in focal areas. At 12 hours,

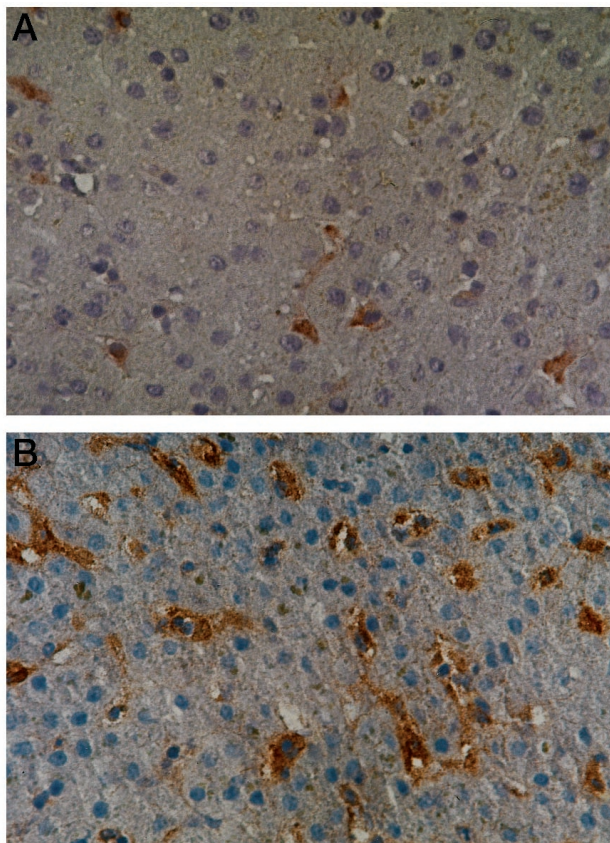


Figure 3. CD86 expression in a normal control (A, MAb Fun-1) and in a patient with chronic HBV infection (B). Only macrophages are CD86 positive in both normal controls and chronic liver disease. The number of positive Kupffer cells is higher and staining is stronger in chronic HBV compared with normal controls. Magnification, $\times 400$.

the numbers of CD80- and CD86-positive cells had further increased, and focal edema and swelling of hepatocytes became apparent particularly in areas with CD80/86-positive SECs. However, CD86 staining proved stronger than CD80 staining in each cell type throughout the study. In parallel to CD80/CD86 expression, the CD40 immunoreactivity increased on Kupffer cells, and an increase in immunoreactivity also became detectable 8 hours after induction of liver failure (Figure 5, E–H).

The induction of CD80, CD86, and CD40 was accompanied by a rising number of CD8-positive lymphocytes (NCs, 0.4 cells/VF; GalN/LPS at 4 hours, 0.4 cells/VF; 8 hours, 0.7 cells/VF; 12 hours, 1 cell/VF; GalN/TNF at 4 hours, 0.5 cells/VF; 8 hours, 0.7 cells/VF; 12 hours, 1.1 cell/VF).

Discussion

Our data lend strength to the hypothesis that insufficient co-stimulation by liver-specific APCs may be the basis for the physiological down-regulation of the hepatic immune response. We have demonstrated that only few Kupffer

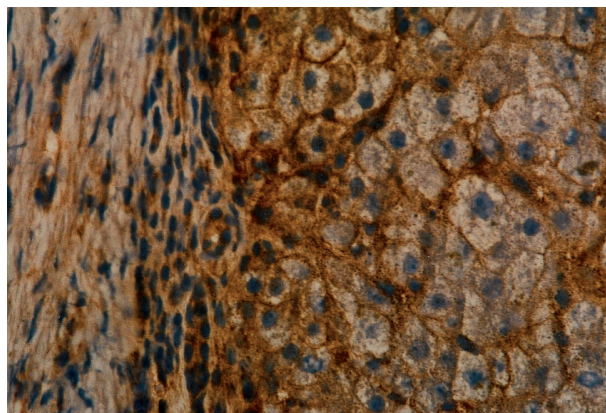


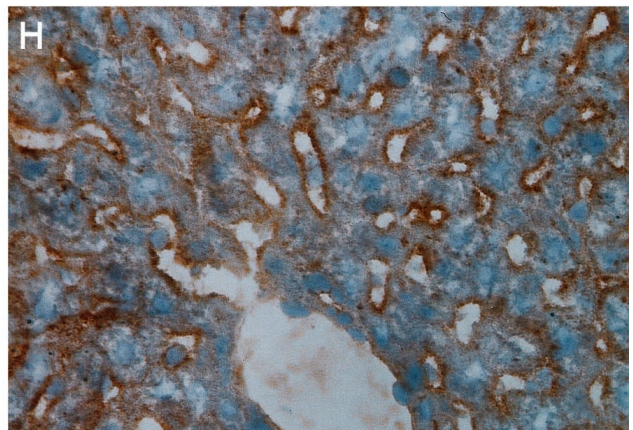
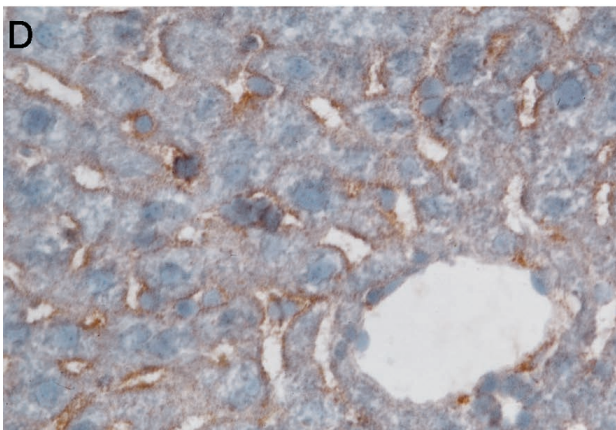
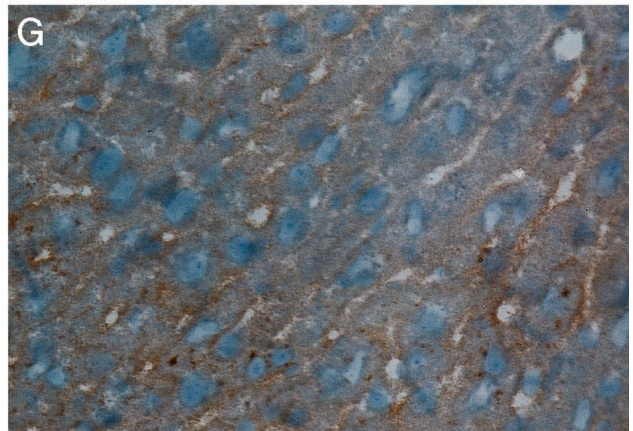
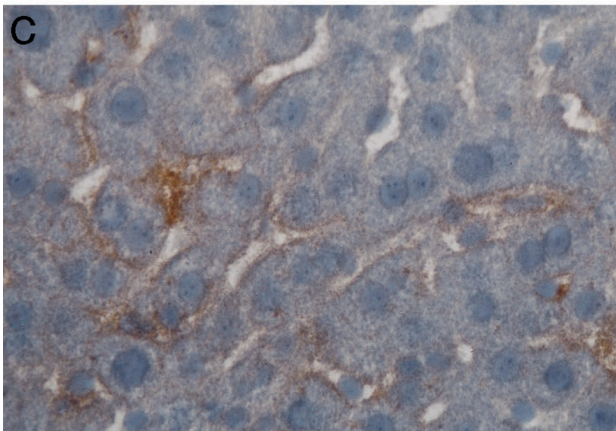
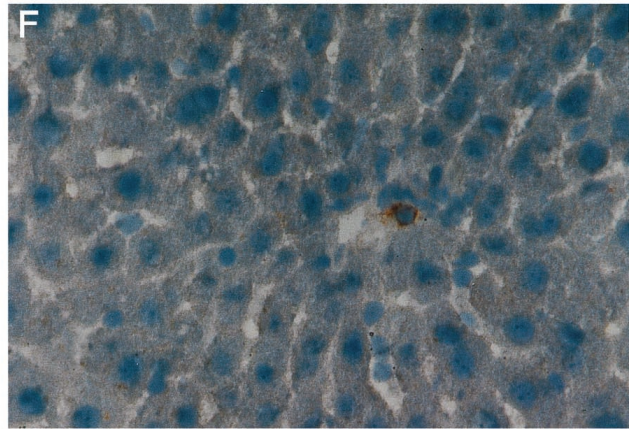
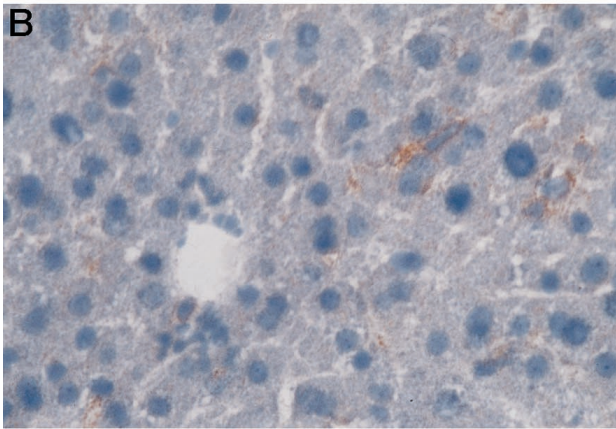
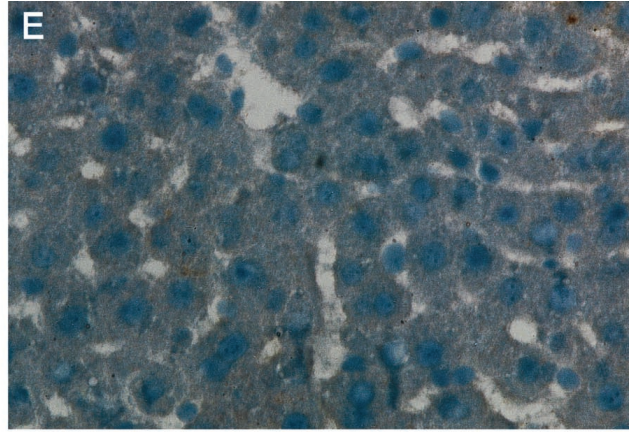
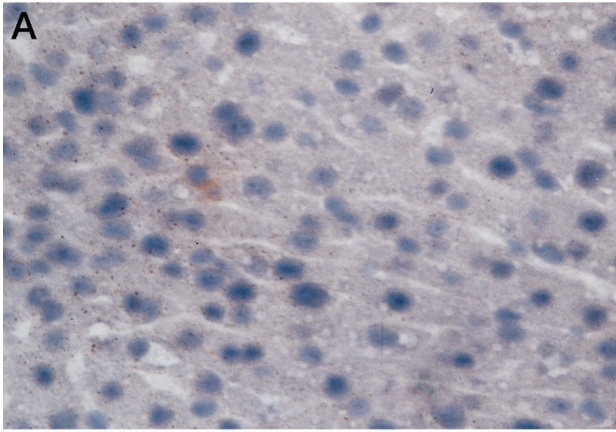
Figure 4. CD40 expression in a liver specimen of a patient with chronic HBV (MAb 5C3). Hepatocytes, Kupffer cells, SECs, and bile duct epithelia stain strongly positive for CD40 in the patient with chronic HBV. Magnification, $\times 400$.

cells and no SECs express the co-stimulatory molecules CD80 and CD86 in normal individuals and even in patients with acute or chronic active inflammatory liver disease. Antigens reaching the liver via the portal blood usually do not elicit an immune response.^{17,18} This observation may be explained by the specific way antigen is presented in the liver by Kupffer cells and SECs, which can take up, process, and present antigens¹⁹ but are only poor activators of T lymphocytes,²⁰ probably due to the lack of co-stimulation by SECs.

In FHF, this situation is changed dramatically. Our results demonstrate a marked increase in the numbers of CD80- and CD86-positive cells in FHF not only including macrophages but also SECs. Thus, antigens reaching the liver in FHF are presented not only by a higher number of macrophages but also by SECs. An expression of abundant co-stimulatory molecules may indicate that now both cell types have become competent in efficient co-stimulation. Thus, portal antigens may no longer be tolerated but instead trigger potent immunoactivation that contributes to further hepatocyte damage in FHF. In line with these observations, we found a significant increase in lymphocytes that express the CD80/CD86 counter-receptor CD28. Furthermore, there was a close correlation between the total number of CD80/CD86-positive cells and CD8-positive lymphocytes. Interestingly, within islets of surviving hepatocytes, a normal pattern of CD80 and CD86 expression exclusively on Kupffer cells was linked with a complete absence of CD28-positive and CD8-positive lymphocytes. It is tempting to speculate that these hepatocytes survived because control of immune activation had been maintained locally within the islets.

A collapse of liver tissue in FHF is unlikely to account for the higher numbers of CD80/CD86-positive cells, because their numbers were still increased when corrected for the number of portal tracts per visual field (data not shown).

Figure 5. CD86 (A to D, MAb GL1) and CD40 (E to H, MAb 3/23) expression in mice without (A and E) and 4 (B and F), 8 (C and G) and 12 (D and H) hours after treatment with GalN/TNF. Sinusoidal CD40 and CD86 expression is induced 8 hours after treatment with GalN/TNF. Similar expression could be observed in the GalN/LPS model (not shown).



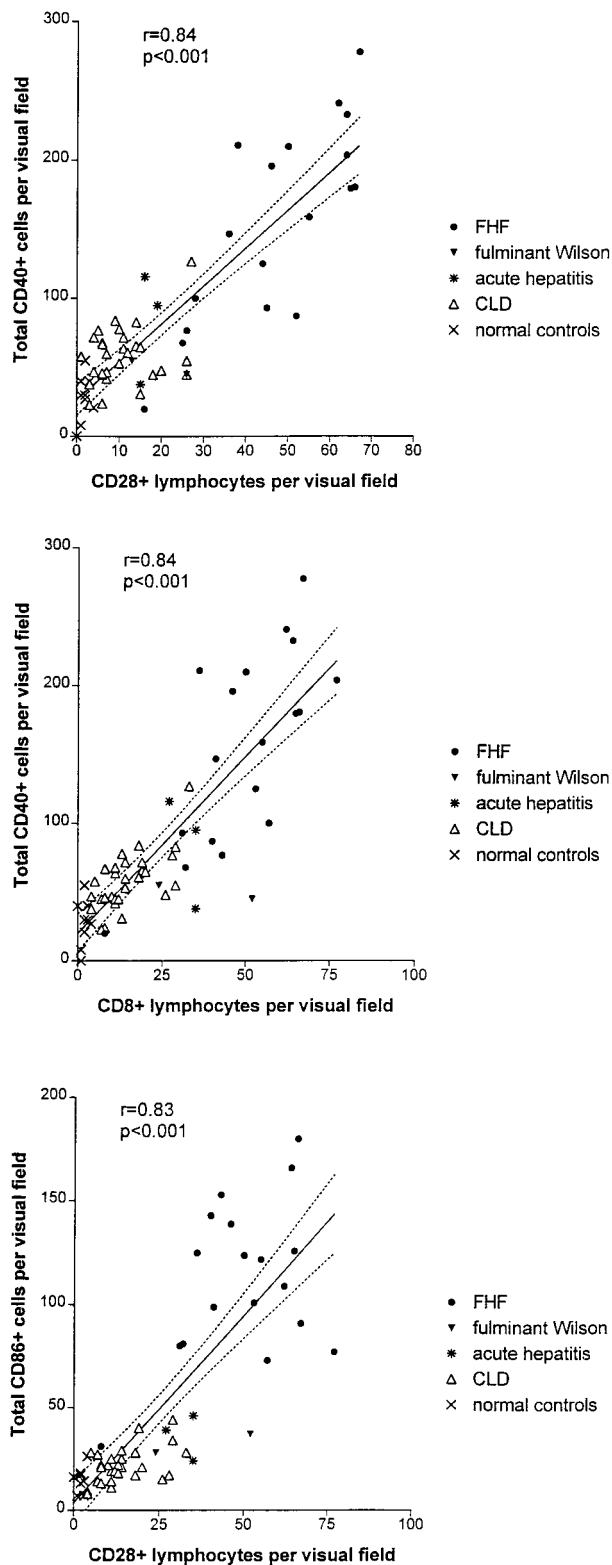


Figure 6. Correlation between the number of CD40-positive cells to CD8- and CD28- as well as CD86- to CD28-positive cells.

Nevertheless, our data in human livers cannot exclude the possibility that expression of co-stimulatory molecules may follow extensive hepatocellular necrosis, as serial specimens are not available in FHF in man. Thus,

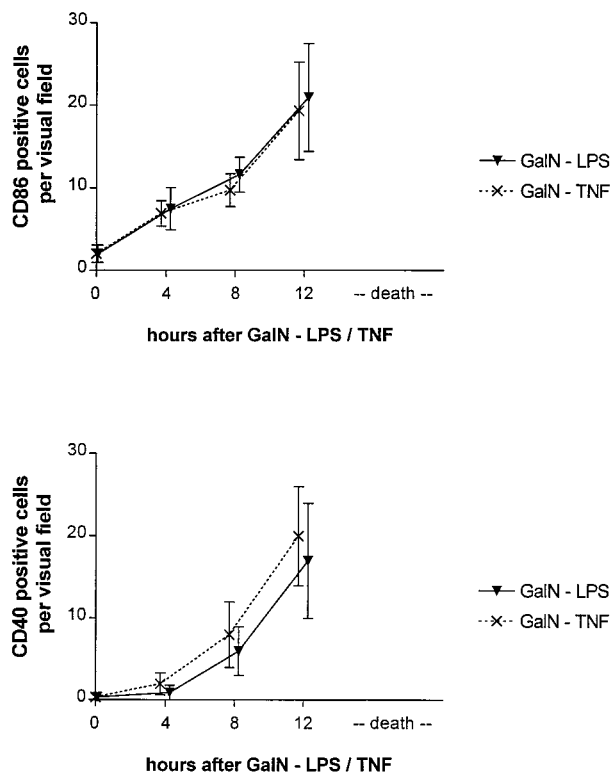


Figure 7. Number of CD86- and CD40-expressing cells after treatment with GalN/TNF or GalN/LPS.

we examined the temporal relationship of CD80 and CD86 expression in the GalN/LPS and in the GalN/TNF mouse models of FHF. In both of these well characterized mouse models we could demonstrate up-regulation of CD80 and CD86 expression on Kupffer cells and SECs. This up-regulation occurred before the onset of massive hepatocyte necrosis and hepatic infiltration by inflammatory cells. The results in the animal models therefore indicate that CD80 and CD86 up-regulation is involved as an early step in the pathogenesis of FHF.

The interaction of CD40 with its ligand CD154 is believed to be a key step in an up-regulation of CD80 and CD86 expression in APCs and has a strong impact on B-cell activation, the initiation of antigen-specific T-cell responses, and macrophage development.^{8,21,22} In liver tissue from patients with FHF we found markedly enhanced expression of CD40 on macrophages, Kupffer cells, SECs, hepatocytes, and bile ducts as well as an increased frequency of CD154-positive lymphocytes. This observation of enhanced CD40 expression in humans is confirmed in the murine models, where we could demonstrate an early and strong expression of CD40 on macrophages and SECs that accompanies the induction of CD80 and CD86. Up-regulation of CD40 occurred before tissue damage but apparently did not precede CD80/CD86 induction. However, hepatocytes and bile ducts were CD40 negative in mice in contrast to humans where these also stained positive in cases of chronic active liver disease or FHF.

There is circumstantial evidence that expression of CD80/CD86 or CD40 conveys potent immunostimulating

properties to the cells that express these molecules (overview in Ref. 7). Blocking these pathways with a CTLA-4Ig fusion protein or CD154 antibodies can prevent autoimmune disease, autoantibody production, antigen presentation, T cell activation, T cell-B cell interaction and clonal expansion of effector T-cell populations^{19,23-29} as well as rejection in most transplant models.³⁰⁻³⁴ Therefore, it is quite likely that also in human FHF, an up-regulation of CD80/CD86 and CD40 will result in a potent activation of immunocompetent cytotoxic effector cells. This concept is further supported by recent experiments by Adachi and co-workers.³⁵ Blocking the CD80/CD86-CD28 or the CD40-CD154 pathways with neutralizing CD80 and CD86 or CD154 antibodies prevented liver cell damage in their concanavalin A mouse model of fulminant hepatitis.

In summary, our observations provide evidence for abnormally up-regulated antigen presentation by non-parenchymal liver cells as an early step in the immune-mediated pathogenesis of FHF. In particular, expression of co-stimulatory molecules is markedly enhanced and may trigger and maintain the inflammatory processes leading to massive hepatocyte damage. Novel therapeutic strategies targeted at blocking these co-stimulatory molecules might therefore be a promising novel approach to the treatment and prevention of FHF in man.

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