ANIT toxicity toward mouse hepatocytes in vivo is mediated primarily by neutrophils via CD18

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Kodali, Pratima, Ping Wu, Parshawn A. Lahiji, Eric J. Brown, and Jacquelyn J. Maher. ANIT toxicity toward mouse hepatocytes in vivo is mediated primarily by neutrophils via CD18. Am J Physiol Gastrointest Liver Physiol 291: G355–G363, 2006. First published April 13, 2006; doi:10.1152/ajpgi.00458.2005.—α-Naphthylisothiocyanate (ANIT) is a hepatotoxicant that causes acute cholestatic hepatitis with infiltration of neutrophils around bile ducts and necrotic hepatocytes. The objective of this study was to determine whether the β2-integrin CD18, which plays an important role in leukocyte invasion and cytotoxicity, contributes to ANIT-induced hepatic inflammation and liver injury. Mice with varying levels of leukocyte CD18 expression were treated with ANIT and monitored for hepatic neutrophil influx and liver injury over 48 h. Mice that were partially deficient in CD18 (30% of normal levels) developed periporal inflammation and widespread hepatic necrosis after ANIT treatment in a pattern identical to that in wild-type (WT) mice. In contrast, mice that completely lack CD18 (CD18 null) were resistant to ANIT toxicity. Forty-eight hours after ANIT, CD18-null mice displayed 60% lower serum alanine aminotransferase (ALT) levels and 75% less hepatic necrosis, as shown by morphometry, than WT mice. This was true despite evidence that ANIT still provoked hepatic neutrophil influx in CD18-null mice. WT mice could also be protected from ANIT-induced hepatocellular necrosis, by depleting the animals of neutrophils. Notably, neither CD18-null mice nor neutrophil-depleted WT mice exhibited any attenuation of bile duct injury or cholestasis due to ANIT. We conclude from these experiments that neutrophils invade ANIT-treated livers in a CD18-independent fashion but utilize CD18 to induce hepatocellular cytotoxicity. The results emphasize that neutrophil-mediated amplification of ANIT-induced liver injury is directed toward hepatocytes rather than cholangiocytes. In fact, the data indicate that the majority of ANIT toxicity toward hepatocytes in vivo is neutrophil driven.

bile duct; cholangiocyte; hepatocyte; hepatotoxicity; inflammation; liver

NEUTROPHILIC INFLAMMATION is a common feature of toxic liver injury. It occurs in response to a variety of xenobiotic agents, including carbon tetrachloride (14), acetaminophen (39), galatosamine/endotoxin (29), and monocrotaline/endotoxin (57). Drug-related hepatic inflammation can result from drug-induced oxidant stress or the local upregulation of inflammatory mediators (19); whatever the stimulus, neutrophil invasion often worsens the liver damage caused by the xenobiotic itself (13, 29, 57). One compound known for its ability to cause neutrophilic liver injury is α-naphthylisothiocyanate (ANIT). This drug provokes an acute cholestatic hepatitis, due in part to its recycling through repeated rounds of glutathione conjugation and biliary excretion (12). Although ANIT can kill liver cells directly (27, 32, 47, 56), it also induces intense neutrophilic inflammation around injured hepatocytes and bile ducts (10, 11, 56). The marked infiltration of neutrophils into ANIT-treated treated livers is believed to play an important role in the toxicity of the drug in vivo (10).

Transmigration of neutrophils from the circulation into the liver is a process facilitated by β2-integrins (23). Expressed on the cell surface, β2-integrins enable neutrophils to firmly adhere to the sinusoidal endothelium by binding endothelial ICAM-1 (15, 22). Adherent neutrophils invade the liver in response to chemotactic signals from the parenchyma (18, 38); once infiltrated, they again utilize β2-integrins and ICAM-1 to adhere to hepatocytes, stellate cells, or biliary cells (24, 36, 50). Engagement of β2-integrins stimulates not only adhesion and migration of neutrophils, but also cellular activation including degranulation and the respiratory burst (46). In addition, β2-integrin ligation is a prerequisite for neutrophils to respond fully to cytokines such as GM-CSF and IL-8 (33). Like all other members of the integrin superfamily, β2-integrins are heterodimers comprising one α-chain and one β-chain (23). Four separate heterodimers have been described, in which one of four possible α-subunits (CD11a, CD11b, CD11c, or CD11d) partners with a common β-chain (CD18) (hence the alternative designation CD11/CD18 integrins). A rare syndrome exists in which human beings have severely reduced or absent expression of CD18 due to mutations in the β2-chain (CD18) (10, 11, 56). The marked infiltration of neutrophils into ANIT-treated treated livers is believed to play an important role in the toxicity of the drug in vivo (10).

Although β2-integrins are critical for normal neutrophil function, not all neutrophil responses are CD18 dependent (4, 25, 37, 44, 54). The objective of this study was to determine whether the severe neutrophilic inflammation that occurs in the liver in response to ANIT treatment is mediated by CD18. We also wanted to determine the extent to which neutrophils and CD18 enhance ANIT toxicity in vivo. The results indicate that CD18 is not required for neutrophils to invade ANIT-treated treated livers, but it is a key mediator of ANIT toxicity, much of which is neutrophil dependent.

MATERIALS AND METHODS

Induction of acute ANIT toxicity in mice. Male mice that express low levels of CD18 (B6.129S7-Hbb2<sub>deficit</sub>/J) (hereafter called “partially deficient” or “CD18 deficient”) and wild-type (WT) C57BL/6J con-

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trols were purchased from the Jackson Laboratory (Bar Harbor, ME). CD18-null mice were bred from a colony originally provided by Arthur Beaudet (Baylor College of Medicine, Houston, TX; Ref. 51). All animals were 8 wk old at the beginning of study. Mice were fasted overnight and then treated with a single dose of ANIT by gavage (10 mg/ml in olive oil, 50 mg/kg). Controls received olive oil alone (5 ml/kg). In some experiments, mice were pretreated with anti-neutrophil antibody (125 μg ip; no. 553122, Pharmingen) or nonspecific IgG (125 μg ip; no. 556968, Pharmingen) immediately prior to ANIT. Mice were killed at various intervals from 6 to 48 h after ANIT administration. All procedures involving live animals were approved by the Institutional Animal Care and Use Committee at the University of California, San Francisco.

Analysis of whole blood and serum. Complete blood counts were performed on EDTA-treated whole blood (IDEXX Laboratories, West Sacramento, CA). Absolute neutrophil counts were calculated from automated total leukocyte counts and manual differentials. Serum was collected separately for measurement of liver enzymes; alanine aminotransferase (ALT), alkaline phosphatase, and bilirubin levels were quantitated using a BAX autoanalyzer (Bayer, Tokyo, Japan) in the clinical chemistry laboratory at San Francisco General Hospital.

Histology and immunohistochemistry. Formalin-fixed liver sections were stained with hematoxylin and eosin. Immuno-histochemical staining was performed on frozen tissue; neutrophils were identified with rat anti-mouse Ly-6G antibody (BD Pharmingen, San Diego, CA) (1:100), and CD18 was identified with rat anti-mouse CD18 (BD Pharmingen). The proteins of interest were detected with biotinylated secondary antibodies followed by incubation with avidin-biotin-peroxidase (Vectastain Elite; Vector Laboratories, Burlingame, CA). 3,3’-Diaminobenzidine was used as a colorimetric substrate. All histological sections were viewed and photographed with a Nikon Microphot-FXA microscope (Nikon, Tokyo, Japan).

Quantitation of neutrophils in liver sections. Neutrophils were quantitated by direct cell counting in liver sections stained with

Fig. 1. Liver histology of wild-type (WT) and CD18-deficient mice after α-naphthylisothiocyanate (ANIT) treatment. Photomicrographs illustrate livers from WT mice (a, c, e, and g) and CD18-deficient mice (b, d, f, and h) at 24–48 h after ANIT treatment. a and b: hematoxylin and eosin staining at 24 h reveals prominent periductal inflammation in both groups (arrowheads). c and d: low-power view of hematoxylin and eosin-stained liver at 48 h shows large foci of hepatic necrosis of comparable severity in WT and CD18-deficient mice. e and f: neutrophils are present, as shown by immunohistochemistry, at 24 h in the portal tracts and necrotic areas (N) of WT as well as CD18-deficient mice. g and h: immunostaining for CD18 at 24 h shows numerous positive cells in portal tracts and necrotic areas, even in CD18-deficient mice. Original magnification ×10 (a, b, and e–h) and ×5 (c and d).
anti-Ly-6G. In some experiments, region-specific neutrophil counts were performed to document the number of cells within portal tracts, parenchyma, and necrotic zones. Group means were calculated from the average cell counts for each mouse. Data are reported as the mean neutrophil count per region or per ×20 microscopic field per mouse.

Quantitation of hepatic necrosis in liver sections. The amount of necrosis in ANIT-treated livers was measured morphometrically as a fraction of total liver area (Simple PCI software; Compix, Cranberry Township, PA). Nine microscopic fields (×4) were evaluated per mouse and averaged before calculating a mean for each treatment group. Data are reported as the mean percent of liver area occupied by necrosis per mouse.

Quantitation of CD18 expression. CD18 expression was assessed by fluorescence-activated cell sorting (FACS) in Ly-6G-positive cells obtained from mouse bone marrow. Briefly, bone marrow was collected from the femur and tibia of mice into sterile HBSS, treated with hypotonic saline to remove red blood cells, and spun through 62% Percoll (Amersham Biosciences, Piscataway, NJ) at 1,000 g for 30 min. The leukocyte pellet was washed twice and resuspended in HBSS-1% BSA; aliquots of one million cells were then incubated with phycoerythrin (PE)-conjugated Ly-6G antibody (no. 553128, Pharmingen) and FITC-conjugated CD18 antibody (no. 553292, Pharmingen) for 1 h. Antibody-treated cells were analyzed for fluorescence on an EPICS XL flow cytometer (Coulter, Miami, FL).

Statistical analysis. Experiments included three to five animals per study group. Mean data from each study group were compared by analysis of variance. P values <0.05 were considered statistically significant.

RESULTS

ANIT toxicity is identical in WT mice and mice partially deficient in CD18. Acute ANIT toxicity in mice is marked by destruction of intrahepatic bile ducts, necrosis of adjacent hepatocytes, and infiltration of inflammatory cells into areas of tissue damage (56). Within 48 h of ANIT administration, WT mice displayed each of these abnormalities. Cholangitis was the most prominent histological finding in WT livers at 24 h (Fig. 1a); by 48 h, large foci of hepatocyte necrosis were also present (Fig. 1c). Mice partially deficient in CD18 also developed significant liver abnormalities after ANIT treatment. Bile duct destruction and hepatocellular necrosis were indistinguishable from the lesions observed in WT mice; in addition, periductal inflammation was grossly evident (Fig. 1, b and d). Hepatic neutrophils, when highlighted by immunohistochemistry, were as abundant in CD18-deficient mice as in WT mice (Fig. 1, e and f). A time-course study showed that liver enzymes began to rise 18 h after ANIT treatment in both groups of mice (Fig. 2, top). Portal tract neutrophils did not accumulate further between 18 and 48 h, but the number of neutrophils in necrotic zones increased more than twofold as the necrotic zones themselves enlarged.

CD18 expression is only moderately reduced in CD18-deficient mice. Because CD18-deficient mice displayed absolutely no attenuation of hepatic injury or inflammation in
CD18-null mice was
WT mice (filled curve) and CD18-null mice (open curve). CD18 expression in
deficient mice (open curve). CD18 expression in CD18-deficient mice was
CD18 expression in granulocytes from WT mice (filled curve) and CD18-
less cell-surface CD18 than WT granulocytes (Fig. 3
granulocytes from CD18-deficient mice displayed significantly
response to ANIT, we measured CD18 expression in these
mouse granulocytes identified by Ly-6G (see MATERIALS AND METHODS ).
expression was quantitated by fluorescence-activated cell sorting (FACS) in
Fig. 3. Measurement of CD18 expression in mouse granulocytes. CD18
eexpression was quantitated by fluorescence intensity, 162 ± 27 vs. 551 ± 60, P <
0.0005). After ANIT treatment, CD18-positive cells were readily detected in the livers of CD18-deficient mice by immuno-
These cells localized to portal tracts and necrotic foci, exactly as they did in WT mice (Fig. 1, e and f).
CD18-null mice are protected from ANIT-induced hepato-
cellular necrosis. Our initial experiment made it clear that a
70% reduction of leukocyte CD18 expression was inadequate for assessing the impact of CD18-mediated inflammation on
ANIT toxicity. Consequently, we performed a second study in CD18-null mice, whose granulocytes have no cell-surface
CD18 expression (Fig. 3B). When CD18-null mice were treated with ANIT, their serum ALT rose to only 40% of the
level observed in WT mice (1.032 vs. 2,705 IU/l at 24 h; 2,189 vs. 6,138 IU/l at 48 h, P < 0.0002, Fig. 4). Liver histology
confirmed that hepatocellular necrosis was markedly attenuated in CD18-null mice (1.1 vs. 4.4% necrosis at 24 h, P <
0.02; 2.7 vs. 11.6% necrosis at 48 h, P < 0.01) (Fig. 5, a and b). By contrast, serum markers of cholestasis were equivalent in both groups (Fig. 4, top), as was histological bile duct
damage (Fig. 5, c–f). Notably, neutrophils were readily identified within portal tracts and necrotic zones of CD18-null mice
after ANIT treatment (Fig. 5h). None of the inflammatory cells within the livers of CD18-null mice showed any CD18 immu-
noreactivity (data not shown).
Because hepatic neutrophil invasion was unexpected in CD18-null mice after ANIT treatment, we analyzed the distri-

Fig. 3. Measurement of CD18 expression in mouse granulocytes. CD18
expression was quantitated by fluorescence-activated cell sorting (FACS) in
mouse granulocytes identified by Ly-6G (see MATERIALS AND METHODS ). A: CD18 expression in granulocytes from WT mice (filled curve) and CD18-deficient mice (open curve). CD18 expression in CD18-deficient mice was
measured at 30% of that in WT mice. B: CD18 expression in granulocytes from WT mice (filled curve) and CD18-null mice (open curve). CD18 expression in CD18-null mice was <1% of that in WT mice.

Neutrophil depletion alleviates ANIT-induced hepato-
cellular necrosis. The fact that CD18-null mice were afforded
substantial protection against ANIT-induced hepatic necrosis indicates that CD18-mediated leukocyte activation signifi-
cantly amplifies hepatic injury in response to this toxicant. To determine whether neutrophils are the cells responsible for this effect, we selectively depleted WT mice of neutrophils and monitored their outcome after ANIT treatment. Using an anti-
neutrophil antibody, we reduced the number of circulating neutrophils in ANIT-treated mice by 90% without altering monocyte or lymphocyte counts (Table 1). Neutrophil-depleted mice, like CD18-null mice, exhibited much lower serum ALT
levels than controls at both 24 and 48 h after ANIT treatment but no improvement in alkaline phosphatase or bilirubin (Table 1). Neutrophil depletion considerably reduced the extent of hepatocellular necrosis in liver sections (0.9% vs. 5.2% at 24 h, P < 0.02; 1.4% vs. 9.4% at 48 h, P < 0.004) (Table 1; Fig. 6, a and b). Despite the near-complete absence of neutrophils from the liver, however, cholangiocyte destruction persisted (Table 1; Fig. 6, c–h).

DISCUSSION
The above experiments demonstrate that CD18, the common β-subunit of leukocyte β2-integrins, is central to the pathogen-
esis of ANIT-induced liver injury in mice. The CD18-expressing cells of greatest importance to ANIT toxicity are neutro-
phils, based on experimental evidence that neutrophil depletion affords the same degree of protection against ANIT-induced liver injury as genetic deletion of CD18. CD18 is not required for neutrophils to infiltrate the liver after ANIT treatment; however, CD18 is clearly important for neutrophils to amplify ANIT-mediated destruction of hepatocytes. Indeed, CD18-
dependent activation of neutrophils triggers more than 50% of the hepatocellular necrosis that occurs in mice after ANIT
treatment in vivo. β2-Integrins on neutrophils are known to play an important role in many types of liver disease. They have been implicated in the development of hepatic injury in response to ischemia-reperfusion (30), galactosamine-endotoxin challenge (29), alcohol consumption (3), and bile duct
Although $\beta_2$-integrins are not required for neutrophils to accumulate in sinusoids after a hepatic insult (31), they are essential for neutrophil transmigration into the hepatic parenchyma and the subsequent destruction of hepatocytes (15, 16, 31, 42). $\beta_2$-Integrin engagement on neutrophils can provoke hepatocellular necrosis by stimulating the release of proteinases (cathepsins and elastase) and reactive oxygen species (46, 48, 52). Proteinases are the predominant mediators of neutrophil-mediated hepatocyte toxicity in cell culture (20, 26, 43), whereas neutrophil-derived oxidants contribute significantly in vivo (9, 40, 55). In the setting of ANIT treatment, hepatocytes may be particularly sensitive to neutrophil-derived oxidants, because ANIT profoundly depletes hepatocellular glutathione (12).

The current study extends previous observations regarding the participation of neutrophils in ANIT toxicity. In an earlier study involving rats, Dahm et al. (9) reported that neutrophils were critical to ANIT toxicity, because neutrophil depletion reversed ANIT-induced transaminase release at 24 h. Our data confirm that neutrophils enhance transaminase release in ANIT-treated mice and demonstrate that the effect is $CD18$ dependent. In rats, neutrophils also contributed to ANIT-induced cholestasis (9); the same is not true of mice, as our experiments uncovered no impact of neutrophils or $CD18$ on ANIT-induced bile duct destruction or elevation of alkaline phosphatase or bilirubin. Why $CD18$-positive neutrophils would kill hepatocytes but not biliary cells after ANIT treatment is uncertain. Hepatocytes and biliary cells upregulate ICAM-1 expression in response to injury (6, 8, 28, 50), and thus both are potential targets of $CD18$-mediated cytotoxicity (2). It is possible that ICAM-1 is upregulated more strongly on hepatocytes than biliary cells after ANIT treatment. This has been reported after bile duct ligation (35) and may explain why neutrophils from bile duct-ligated mice show a similar tendency to kill hepatocytes more readily than cholangiocytes (22). Alternatively, ICAM-1 may be upregulated significantly.

Fig. 4. Liver enzymes and hepatic neutrophil counts in WT and CD18-null mice after ANIT treatment. Top: histograms depict serum ALT, alkaline phosphatase, and bilirubin at 24 and 48 h after ANIT treatment in WT mice (black bars) and CD18-null mice (gray bars). ALT, alkaline phosphatase, and bilirubin were each significantly higher than normal in the serum of WT mice at both time points. ALT levels in CD18-null mice were significantly lower than those in WT mice at 24 and 48 h after ANIT. Alkaline phosphatase was lower in CD18-null mice at 24 h but reached WT levels by 48 h. Bilirubin was comparable between the two groups at both time points. Values are means $\pm$ SE for $n = 5$. *$P < 0.05$. Bottom: histograms depict region-specific hepatic neutrophil counts in WT mice (black bars) and CD18-null mice (gray bars) after ANIT treatment. Portal tract neutrophils were equally abundant in WT and CD18-null mice. Parenchymal (sinusoidal) neutrophils were initially more prominent in CD18-null mice, but by 48 h the numbers were equivalent in both groups. Necrotic zone neutrophils were more numerous in WT mice than CD18-null mice, but necrotic foci themselves were significantly larger in WT mice (see text and Fig. 5). When necrotic zone neutrophil counts were normalized to necrosis area, the numbers were identical. Values are means $\pm$ SE for $n = 5$. *$P < 0.05$ for CD18-null mice vs. WT mice.
Fig. 5. Liver histology in WT and CD18-null mice after ANIT treatment. Photomicrographs illustrate routine histology and neutrophil immunohistochemistry in the livers of WT mice (a, c, e, and g) and CD18-null mice (b, d, f, and h) 24–48 h after ANIT treatment. a: a low-power view of WT liver stained with hematoxylin and eosin at 48 h demonstrates numerous large foci of necrosis. b: a low-power view of CD18-null liver shows fewer and smaller foci of necrosis at 48 h. c and e: higher-power views of WT liver at 24 h show extensive bile duct destruction and marked ductal inflammation. d and f: similar views of CD18-null liver at 24 h demonstrate severe bile duct injury but possibly fewer inflammatory cells. g and h: neutrophil staining reveals similar degrees of hepatic neutrophil infiltration in both groups. CD18 immunohistochemistry showed numerous positive cells in WT livers but virtually no stained cells in the livers of CD18-null mice (not shown). Original magnification ×5 (a and b), ×20 (c, d, g, and h), and ×40 (e and f).

Table 1. Effect of neutrophil depletion on α-naphthylisothiocyanate toxicity

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<th>ANIT, 24 h</th>
<th>ANIT, 48 h</th>
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<tr>
<td></td>
<td>Control Antibody</td>
<td>Anti-Neutrophil Antibody</td>
</tr>
<tr>
<td>Neutrophils, cells/mm³</td>
<td>1,653±301</td>
<td>1,88±52*</td>
</tr>
<tr>
<td>Monocytes, cells/mm³</td>
<td>138±56</td>
<td>133±39</td>
</tr>
<tr>
<td>Lymphocytes, cells/mm³</td>
<td>2,359±212</td>
<td>2,085±117</td>
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<tr>
<td>Hepatic neutrophils, cells per × 20 field</td>
<td>45±11</td>
<td>11±1.2*</td>
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<tr>
<td>ALT, IU/l</td>
<td>1,291±168*</td>
<td>681±15</td>
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<tr>
<td>Alkaline phosphatase, IU/l</td>
<td>187±24</td>
<td>130±8</td>
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<tr>
<td>Bilirubin, mg/dl</td>
<td>3.9±0.9</td>
<td>1.1±0.4</td>
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<tr>
<td>Necrosis, %area</td>
<td>5.2±1.1</td>
<td>0.9±0.1*</td>
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All mice were gavaged with 50 mg/kg α-naphthylisothiocyanate (ANIT) and injected with 125 μg anti-neutrophil or control antibody at time 0. For the 48-h study, mice received a booster injection of anti-neutrophil or control antibody at 24 h. Values are means ± SE for n = 3 (24 h) and n = 8 (48 h). *P < 0.05 vs. control antibody.
on cholangiocytes in ANIT-treated mice but with little impact, because the drug has such pronounced direct toxicity toward cholangiocytes that it leaves little room for amplification by CD18-positive neutrophils. Regardless of the explanation, the mouse model of ANIT toxicity in vivo reveals that cholangiocyte injury is direct, whereas hepatocyte injury is both direct and indirect. The pronounced indirect cytotoxicity of ANIT toward hepatocytes is attributable to neutrophil activation via CD18.

Noteworthy in our study is that neutrophils penetrated the livers of ANIT-treated mice in a CD18-independent fashion. CD18-independent neutrophil invasion has been reported to occur in the liver following disruption of the sinusoidal endothelium (34); ANIT, however, is not known to damage sinusoidal endothelial cells (58). CD18-independent mechanisms are at times utilized by neutrophils to invade other tissues such as lung (41, 49) and heart (5). Some evidence suggests that CXC chemokines are particularly effective at activating this behavior (41, 45, 49). This is of interest because dying liver cells produce CXC chemokines (18), and thus these cells have the potential to recruit neutrophils to sites of liver injury in a CD18-independent fashion. There has been some speculation that CD18-null mice, which exhibit significant neutrophilia (44, 51), do not exhibit true directed migration of neutrophils into damaged tissues but instead display the appearance of inflammation due to passive extravasation of circulating neutrophils into the lung or peritoneum (17). Although we cannot refute that in our experiments, some neutrophils may have migrated passively into the livers of CD18-null mice after ANIT treatment, the specific distribution of neutrophils in areas of hepatic injury in these mice suggests that their migration was at least in part directed. Thus our findings are most

Fig. 6. Liver histology in WT and neutrophil-depleted mice after ANIT treatment. Photomicrographs illustrate routine histology and neutrophil immunohistochemistry in the livers of WT mice (a, c, e, and g) and neutrophil-depleted mice (b, d, f, and h) 24–48 h after ANIT treatment. Views are comparable to those in Fig. 5. WT mice again demonstrate large foci of hepatic necrosis at 48 h together with bile duct destruction and periductal inflammation at 24 h. (b, d, and f) Neutrophil-depleted mice exhibit much less hepatic necrosis and periductal inflammation than WT mice after ANIT treatment; bile duct injury, however, remains severe. g and h: neutrophil immunohistochemistry documents that neutrophils are abundant in WT livers (g) but nearly absent from neutrophil-depleted livers (h). Original magnification ×5 (a and b), ×20 (c, d, g, and h), and ×40 (e and f).
consistent with evidence that CD18-null mice are capable of exhibiting CD18-independent migration into tissues following appropriate stimuli (44).

The fact that we found no attenuation of ANIT toxicity in CD18-deficient mice but substantial protection in CD18-null mice underscores the difference between these two genetically engineered strains. CD18-deficient mice were originally reported to exhibit only 2–16% of normal CD18 expression on granulocytes (53); animals that are now sold commercially display much more of the B2-integrin subunit (30% of normal, Fig. 3). In our hands, this residual amount of CD18 present on the neutrophils of CD18-deficient mice was sufficient to render the cells functionally immunocompetent with regard to ANIT toxicity. Although it was recently reported that partial deficiency of CD18 is sufficient to suppress neutrophil-mediated liver injury after bile duct ligation (21), our experience indicates that total elimination of CD18 is required to alleviate ANIT toxicity. Taken together, these results emphasize that CD18 contributes differentially to neutrophilic liver injury depending on the insult and that the molecule may need to be completely absent for its role to become clear.

In summary, our results demonstrate that the toxicant ANIT induces hepatic neutrophil infiltration in a CD18-independent fashion. Although ANIT-induced hepatic inflammation is CD18 independent, a substantial portion of ANIT-related liver injury is CD18 dependent. CD18-positive neutrophils augment murine ANIT toxicity in a specific manner, worsening hepatocellular necrosis but having little impact on ANIT-related biliary cell damage or cholestasis. Importantly, only a fraction of normal neutrophil CD18 expression is required to trigger significant amplification of ANIT-induced liver injury in vivo.

GRANTS

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