Naive and Memory T Lymphocytes Migrate in Comparable Numbers Through Normal Rat Liver: Activated T Cells Accumulate in the Periportal Field

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Naive and Memory T Lymphocytes Migrate in Comparable Numbers Through Normal Rat Liver: Activated T Cells Accumulate in the Periportal Field

Birgit Luettig, Lars Pape, Ulrike Bode, Eric B. Bell, Sheila M. Sparshott, Siegfried Wagner, and Jürgen Westermann

Although the liver is known to contain a significant number of lymphocytes, migration of these through the compartments of the liver, parenchyma and periportal field, has not been studied. The periportal field, in particular, is affected in several immunological disorders of the liver. Populations of labeled naive, activated, and memory T cells were injected into congenic rats. The recipient livers and draining lymph nodes were removed at various time points, and cryostat sections were analyzed for the presence of donor cells using quantitative immunohistology. Donor cell proliferation and apoptosis were examined in vivo by BrdU (5 μM 5-bromo-2-deoxyuridine) incorporation and the TUNEL technique, respectively. Early after injection (0.5–1 h), naive, activated, and memory T cells were localized to the parenchyma and periportal field in comparable numbers. With time, all T cell subsets left the parenchyma but remained or, in the case of activated T cells, significantly accumulated in the periportal field. Furthermore, 12% of activated donor T cells proliferated in vivo within the periportal field, and 0.5% showed evidence of apoptosis. Taken together, not only activated and memory, but also naive T cells continuously migrate through the liver, showing a preference for the periportal field, and activated T cells mainly proliferate there. This may explain why many immunological liver diseases predominantly affect the periportal field. The Journal of Immunology, 1999, 163: 4300–4307.

Many immunologically mediated liver diseases, such as primary biliary cirrhosis and chronic hepatitis C, as well as acute rejection after liver transplantation, are characterized by lymphocytic cell infiltrates mainly in the periportal field, but not in the parenchyma of the liver (1–3). It is known that especially T cells play an important role in these situations. For example, there is an increase of activated T cells in the periportal field in primary biliary cirrhosis (4), and mainly memory T cells are found in the inflammatory lesions in the periportal field during chronic hepatitis C (2). Furthermore, the number of memory T cells increases in the periportal field during liver allograft rejection (5). Presently, it is poorly understood why T cells predominantly accumulate in the periportal field during these diseases.

From animal studies it is well known that T cells continuously migrate through lymphoid and nonlymphoid organs, including the liver (6–8). Interestingly, about as many lymphocytes migrate into the liver as into the lymph nodes (LNs) (6, 8–12). Furthermore, T cells leave the liver both via the hepatic veins into the blood (13), and via the afferent lymphatics into the draining celiac LN (12). Upon activation, the distribution of migrating T cells changes, and many of them are found in the liver (6, 14). It is very likely that the situation in humans is comparable with that in animal models. Even the normal human liver contains significant numbers of T cells (15, 16). In addition, following liver transplantation, many donor-derived lymphocytes are found in the blood, showing that human liver lymphocytes are able to leave the liver (13, 17).

To date, it is not known whether, even under nonpathological conditions, T cells migrate in comparable numbers and with similar kinetics through the two compartments of the liver, the parenchyma and the periportal field. In addition, it is unclear whether naive, activated, and memory T cells are different in this respect. This would be important to know since they differ in their activation requirements and the cytokines they secrete into the tissue. Understanding the migration of T cell subsets under physiological conditions through the compartments of the liver could help to elucidate why many immunological liver diseases initiate and perpetuate primarily in the periportal field. Therefore, in the present study, naive, activated, and memory T lymphocytes were separated, labeled, and injected i.v. into rats. Using quantitative immunohistology (18, 19), the migration of these T cell subsets through the parenchyma and periportal field of the liver and into the draining lymph node was investigated. Furthermore, the present study analyzes both the rate of proliferation (BrdU incorporation) and apoptosis (TUNEL technique) among activated T cells that have migrated either into the parenchyma or the periportal field of the liver.

Materials and Methods

T cell preparation and injection

T cells were prepared by two methods, depending on whether naive and memory T cells or activated T cells were studied. To obtain naive and memory T
cells, thoracic duct CD4+ T cells were separated into naive and memory phenotype according to the high and low molecular isoform of CD45R, respectively. Activated T cells were generated in vitro by stimulating lymphocytes from pLNs and mLNs via the TCR and CD28.

**Naive (CD45RC-) and memory (CD45RC+) CD4+ T cells**

Concgenic rats from the inbred PVG.7A (RT7a) and PVG.7B (RT7b) strains were bred and maintained under barrier conditions in the Animal Unit at the University of Manchester Medical School. Details of the purification procedure were described previously (7). Briefly, thoracic duct lymphocytes from PVG.7B donors (6–13 wk old) were depleted of B cells, CD8+ T cells, and CD90+ recent thymic emigrants using a mixture of specific mouse mAbs and anti-mouse Ig-conjugated immunomagnetic particles (20). The resulting population (>99% CD4+) was 80% CD45RC+. The CD45RC+ subset was prepared as above, but with the additional depletion of CD45RC- cells. Cell purity was routinely >97%. After i.v. injection of naive (CD45RC-) or memory (CD45RC+) T cells (20 × 10^6 cells), the livers of recipients were removed at various time intervals.

In addition, purified subsets of CD4+ T cells from the thoracic duct were labeled with 51Cr (sodium chromate CJS 1P; Amersham, Amersham, U.K.) using 10 μCi (0.37 MBq) per 10^6 subset cells. The livers of recipients were removed at various time points after injection, and the number of injected cells present in the whole liver was determined by gamma counting, as described (21). It is known that memory cells (CD45RC-) may revert back to the CD45RC+ phenotype and reexpress the high m.w. isoform of CD45R (22, 23). Nevertheless, linking CD45RC- and CD45RC+ with naive and memory is a useful division; it distinguishes "cells waiting to encounter Ag (naive) from those that have recently seen Ag (memory)" (22).

**In vitro activated T cells**

Cell suspensions were prepared from LEW.7B (RT7+) rat pLNs (pooled axillary, brachial, and cervical LNs) and mLNs (24). The cells were stimulated in vitro via the TCR-αβ (mAb R73) and CD28 (mAb J319), as described (25). During the activation period, the cells were cultured in the presence of 5 μM BrdU for 72 h (26). BrdU is incorporated into the DNA during the S phase of the cell cycle and can be detected in cytoplasmic and histological preparations (27). To localize naive and memory Ab (summarized in Ref. 27). Cytochrome preparations were made from pLN and mLN cells after stimulation (before injection), and the incorporated BrdU was detected in T cells (Ab R73) using the APAAP and peroxidase antiperoxidase techniques (27), showing that 79 ± 5% had incorporated BrdU and that 81 ± 3% of them are T cells (n = 10). There was no difference in the incorporation of BrdU or the proportion of T cells between pLN and mLN cells. In addition, our group recently demonstrated that expression of LEW.7B phenotype (H-2^d^) and CD44 is similar for activated pLN and mLN (26, 28). Among the T cells, 47.5 ± 5.7% were CD8- and 52.5 ± 5.7% were CD4+. Then a mean of 60 × 10^6 BrdU T cells originating from pLN or mLN was injected over 2 min i.v. into LEW.7A (RT7+) rats (5–6 wk old) (26). At various time points after injection, the liver, the celiac LN draining the liver, and the axillary LN draining the skin were removed.

**Preparation and injection of T cells activated in vitro to test for proliferation in vivo**

To study the local proliferation of activated T cells in the liver compartments, congenic donor cells from LEW.7B (RT7+) rats were activated as described, but in the absence of BrdU. Three days after injection of the activated cells into LEW.7A (RT7+) rats, the animals received 5 mg BrdU/100 g body weight i.v., and 1 h later the liver and the celiac LN draining the liver and the axillary LN draining the skin were removed. Thus, only those lymphocytes that were in the S phase of the cell cycle due to local stimulation within the respective microenvironment were labeled.

**Detection of donor cells in the recipient organs**

At various times after injection, the rats were anesthetised with ether and exanguinated. The liver and the celiac and axillary LNs draining the liver and skin, respectively, were removed, frozen in liquid nitrogen, and stored at −80°C. Crystallite sections were made (thickness = 5 μm), air dried, wrapped in aluminum foil, and stored at −20°C. Regarding the distribution of naive, activated, and memory T cells, no differences were found in the various lobes of the liver. Therefore, the same part of the right lobe was always removed for further analysis.

To detect naive and memory cells in the organ, the sections were incubated with a mAb directed against the injected cells (LEW.7B phenotype; HIS 41 (29)). After washing with Tris-buffered saline containing 0.05% Tween-20 (TBS/Tween), the bound Ab was revealed using a second Ab (rabbit anti-mouse; Dako, Hamburg, Germany) and a mouse Ab complex (APAAP; Dako) for 30 min. Each of the last two steps was repeated for 15 min. To visualize the Abs, a mixture of APAAP substrate (Dako) and fast blue (Sigma) in Tris buffer, pH 8.2, was used. The positive cells appeared blue. Next the slides were washed in TBS/Tween, incubated in 70% ethanol for 30 min, and then air dried for at least 30 min. To detect incorporated BrdU in activated lymphocytes, DNA was denatured with formamide (Sigma) and NaOH (18). Formamide (190 ml) was warmed to 70°C, and 1 N NaOH (10 ml) was added. The solutions were then mixed for 8 min. The slides were immersed in this solution for 30 s. After washing with TBS/Tween, the slides were immersed in formamide-containing 7.5 M urea and 4 M guanidine thiocyanate for 15 min. These steps were then done at 70°C. After this, the cells were washed in ice-cold TBS/Tween and fixed in 1% formaldehyde (30 min) and 0.2% glutaraldehyde (10 min). Slides were incubated overnight with the mAb anti-BrdU (Becton Dickinson, Mountain View, CA) dissolved in TBS/Tween. After washing with TBS/Tween, the bound Ab was revealed using rabbit anti-mouse (Dako) and the mouse Ab complex (APAAP; Dako) for 30 min. Each of the last two steps was repeated for 20 min. The sections were then incubated with a mixture of APAAP substrate (Dako) and fast red for 25 min, resulting in red staining of the BrdU+ cells (27). The slides were counterstained with hematoxylin and mounted in glycerogel (Dako). To test for proliferation of injected cells in the organs, first the injected cells were identified by using a mAb directed against the congeneric LEW.7B phenotype (HIS 41), and visualized in blue, as described above. BrdU incorporation was demonstrated by the TdT-biotin technique in blue. Proliferating donor cells appeared blue and red. Before the LN sections were stained for BrdU, they were stained with low concentrations of a mAb against B cells (HIS 14) concomitantly with HIS 41. Using the APAAP method with fast blue, the B cell areas appeared in light blue, and the injected cells in dark blue. This allowed a clear identification of the injected cells in the different organ compartments of the LN. Since both the applied Abs and the activity of the alkaline phosphatase were destroyed by the denaturation procedure, the incorporated BrdU could be revealed using the same system without leading to unwanted cross-reaction. At each time point, both the number and the phenotype of the injected cells were determined in the parenchyma and the periporal field (excluding the lumen of the vessels) of liver.

**Identification of apoptotic cells in the liver**

Cryostat sections were fixed with 4% paraformaldehyde (pH 7.4 in PBS) for 20 min. To localize donor cells in the liver, the sections were incubated with mAb directed against the congeneric LEW.7B phenotype (HIS 41) and visualized in blue by the APAAP technique, as described (18). To detect apoptotic cells by the TUNEL method (26, 35), the sections were incubated with 70% ethanol for 30 min at room temperature. After washing (0.05% Tween in Tris-buffered saline), the sections were incubated with digoxigenin-labeled UTP (1.7 nM) and TdT (12.5 U) in the TdT buffer (200 mM potassium cacodylate, 25 mM Tris-HCL, 1.25 mg/ml BSA, pH 6.6, 5 mM cobalt chloride solution) for 60 min at 37°C. After washing, the incorporated UTP was revealed by a peroxidase-conjugated anti-digoxigenin Fab fragment Ab (Boehringer Mannheim, Indianapolis, IN) and subsequent immunohistology. Diaminobenzidine was used as a substrate. Therefore, infected cells appeared blue and apoptotic cells brown.

**Statistics**

The data were analyzed using SPSS for Windows. The number of T cell subsets per area of the respective compartment was determined and expressed as means ± SD. Differences between time points, compartments, or organs were analyzed using either the Mann-Whitney U test or the Wilcoxon matched-pairs signed ranks test. p < 0.05 was considered significant.
Identification of organ compartments and injected cells in the liver

The different compartments of the liver, parenchyma and periporal field, could be clearly identified (Fig. 1A). When determined morphometrically (18), they made up 95.6 ± 1% and 5.6 ± 1% of a liver section, respectively (n = 10). In addition, within the different compartments both individual injected cells and their phenotype could be identified (Fig. 1A). Furthermore, it could be analyzed whether the injected cells were in the S phase of the cell cycle (Fig. 1B) or were dying by apoptosis (Fig. 1C).

Different kinetics of naive and memory CD4+ T cells in liver compartments

CD4+ T cells showed different kinetics in the parenchyma and the periporal field of the liver. Although naive T cells entered the parenchyma and periporal compartments in about similar numbers (0.5 h), after 2 h naive T cells left the parenchyma (Fig. 2). In contrast, the number of naive T cells in the periporal field remained constant. A comparable pattern was seen for memory T cells (Fig. 2). Using 51 Cr-labeled subsets, it was further confirmed that the numbers of naive and memory CD4+ T cells in the liver were comparable at each time point after injection (Table I). Since

Table 1. Localization of naive and memory CD4+ T cells in the liver

<table>
<thead>
<tr>
<th>CD4+ T Cell Subset</th>
<th>Time After Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
</tr>
<tr>
<td>Naive</td>
<td>2.6</td>
</tr>
<tr>
<td>Memory</td>
<td>3.2</td>
</tr>
</tbody>
</table>

*51 Cr-labeled CD4+ T cell subsets from thoracic duct lymph were injected i.v. and the recipients killed as indicated. Radioactivity was counted and expressed as percent injected dose per gram of liver. Values are from single recipients.
the liver of these rats weighed 10 g (9), our data show that the liver contained at least 20% of the injected cells at each time point.

**Accumulation of activated T cells in the periportal field of the liver**

Activated T cells were generated in vitro by stimulating the TCR and CD28. They were injected i.v. and the distribution in the liver was comparable to the number of injected cells at each time point. At 1 h, the number of activated T cells in the parenchyma and the periportal field was comparable (Fig. 3). Then the activated T cells left the parenchyma, a pattern that was also observed with naive and memory CD4 T cells. The means ± SD are given (n = 6–9). The asterisks refer to significant differences between the value at 1 h and the corresponding values at 9, 24, and 96 h (p < 0.05; Mann-Whitney U test).

When looking at the cells that had migrated into the liver, 81 ± 4% (n = 10) expressed the TCR-αβ. These cells were 53 ± 8% CD8+ (n = 10) and 47 ± 8% CD4+ T cells. However, the kinetics of migration within the periportal field was very different. After 9, 24, and 96 h, the number of T cells in the periportal field was significantly higher (up to 10-fold) compared with the number of cells at 1 h after cell injection.

When comparing the data for the parenchyma and the periportal field, there were differences in the expression of T cell activation markers between the liver compartments. At all time points, there were significantly more activated T cells expressing IL-2Rα in the periportal field (Table II). Similarly, the periportal field also contained significantly increased numbers of activated cells expressing MHC class II.

**Table II. IL2Rα and MHC class II expression on donor cells found in the compartments of the liver after injection of activated T cells**

<table>
<thead>
<tr>
<th>Time After Injection (h)</th>
<th>Parenchyma</th>
<th>Periportal field</th>
<th>Parenchyma</th>
<th>Periportal field</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 ± 10</td>
<td>52 ± 22*</td>
<td>46 ± 6</td>
<td>73 ± 8*</td>
</tr>
<tr>
<td>9</td>
<td>36 ± 10</td>
<td>63 ± 11*</td>
<td>49 ± 8</td>
<td>83 ± 10*</td>
</tr>
<tr>
<td>24</td>
<td>32 ± 6</td>
<td>46 ± 9*</td>
<td>44 ± 12</td>
<td>86 ± 3*</td>
</tr>
<tr>
<td>96</td>
<td>17 ± 7</td>
<td>33 ± 12*</td>
<td>23 ± 5</td>
<td>91 ± 15*</td>
</tr>
</tbody>
</table>

* Values are means ± SD of the percentage of IL2Rα or MHC class II-positive cells among injected cells found in the liver (n = 6–9). The data after injection of mLNs and pLN were comparable and were, therefore, pooled.

* All data for the periportal field were significantly different as compared to the parenchyma (p < 0.05; Wilcoxon matched-pairs signed ranks test).
Activated T cells also die in the liver and leave via lymphatics

To study the fate of T cells in the liver, we investigated whether the injected T cells die in situ. The rate of apoptosis among injected cells (Fig. 1C) was about 0.5% in the liver (among 2912 donor cells from mLNs, 16 cells were TUNEL positive; data pooled from eight animals).

The question arose as to whether most donor T cells die there or whether some activated T cells also leave the liver. Therefore, the number of donor lymphocytes in the celiac LN draining the liver was studied. As a control for the entrance via blood, the number of cells in the celiac LN was compared with that of the axillary LN draining the skin. Under normal circumstances, only very few lymphocytes reach the axillary LN via afferent lymph (14, 36). Many more injected cells were found in the celiac LN with time than in the axillary LN (Fig. 5A). This was true for both CD4⁺ and CD8⁺ T cells (data not shown). When the values were expressed as a ratio (Fig. 5B), about 2 times as many donor cells were found in the T cell region of the celiac LN draining the liver at 1 h. This increased to a factor of 10 at 9 h and 12 at 24 h. Comparable observations were made when comparing the B cell region and the medulla of both LNs (data not shown). The difference in the two LNs most likely suggests that the greater number of injected T cells in the celiac LN draining the liver is due to their exit from the liver via the lymphatics.

Discussion

It has been known for many years that immunologically mediated liver diseases such as primary biliary cirrhosis and chronic hepatitis C, as well as acute rejection of liver transplants, mainly manifest themselves in the perportal field of the liver and that T cells are involved (1–4, 37) (review Ref. 38). However, the mechanisms of disease initiation and manifestation are poorly understood. Studying the migration of naive, activated, and memory T cells under physiological conditions could help to elucidate these mechanisms.

T cell subsets migrate with different kinetics through the parenchyma and perportal field of the liver

Previous investigations reported that T cells recirculate through the liver (6, 11, 12). However, these studies did not consider the liver compartments that are differently affected in various immunological liver diseases. The present study demonstrates that T cells migrate through both the parenchyma and the perportal field. Although it is known that damaged cells are retrieved in the liver (10, 39, 40), the donor cells we found were clearly intact, as seen by immunohistochemistry. With the method that was applied it was not possible to differentiate where precisely the donor T cells are located in the parenchyma: in the sinusoids, between the endothelium and the hepatocytes, or between the hepatocytes. However, it is unlikely that they are in the sinus since both kinetics and proliferation rate of injected T cell subsets in the blood were different from those of donor T cells in the parenchyma (data not shown). T cells leave the parenchyma quickly, but accumulate in the perportal field of the liver. The mechanisms that are involved are unclear. It is known that the liver receives its blood from the hepatic artery (one-third) and the portal vein (two-thirds) (41), which preferentially supply the perportal field and the parenchyma, respectively (42). This might partly explain the different migration kinetics of the perportal field and parenchyma. In addition, adhesion molecules are constitutively expressed mainly in the perportal field of the liver (43). Whatever the mechanism, our study demonstrates that in vivo the perportal field of the liver receives relatively more T cells than the parenchyma, which could partially explain the initiation and manifestation of immunological liver diseases in this region compared with that of the parenchyma.

Naive and memory CD4⁺ T cells migrate with comparable kinetics through the liver compartments

Interestingly, we found that both naive and memory CD4⁺ T cells migrate through the liver. This is in agreement with reports that...
naive and memory T cells are also present in the human liver (2, 44, 45), and together this clearly demonstrates that naive T cells continuously migrate through nonlymphoid organs such as the liver. In our study, the injected naive T cell suspension contained some memory T cells (20%), because we opted to tolerate this contamination to avoid purification by positive selection, a procedure that would coat the CD45R+C subset with mAb. It is unlikely, however, that the cells found in the liver after naive cell injection all belong to the contaminating memory T cell population, because if this were true, the absolute number of cells we would have found would have been only one-fifth of the number of memory T cells. This was clearly not the case, since we found no difference in migration of naive or memory CD4 T cells through the liver (Fig. 2). Tietz and Hamman (14) reported that memory CD4 T cells have a preference for the liver similar to that of activated lymphocytes. However, they did not exclude activated T cells from their memory cell preparation. Thus, when the migration of naive and memory T cells was followed directly through the parenchyma and the perportal field, we found no evidence to indicate that there are different pathways. This underlines the importance of directly following the traffic of labeled lymphocyte subsets to define their migration routes in vivo (8, 19, 46, 47) and shows that the notion that naive T cells preferentially migrate through lymphoid organs and memory T cells preferentially through nonlymphoid organs is not as strict as previously thought (48, 49). In addition, since the liver contains dendritic cells (42, 50, 51) capable of presenting Ag to naive CD4 T cells, the liver may not merely be an effector site (immigration of memory CD4 T cells), but may also be a site of primary immune responses (immigration of naive CD4 T cells). Further studies must show whether similar kinetics apply to naive and memory CD8 T cells.

**Preferential proliferation of activated T cells in the perportal field**

After injection and migration, activated T cells of both phenotypes (CD4+ and CD8+) are found in the liver and are able to proliferate in situ. The proliferation rate is comparable or even higher than in the T cell area of LNs. The liver sections from rats injected with donor cells, however, are histologically indistinguishable from normal livers and do not contain readily detectable cell infiltrates. This shows that it is necessary to analyze BrdU incorporation at the single cell level within the respective compartments to demonstrate the proliferation of activated T cells in the liver. Thus, in addition to preferential migration into the perportal field, the differential proliferation of activated T cells in the liver compartments, which is more pronounced than that for naive and memory T cells, is also responsible for the accumulation of activated T cells in the perportal field compared with the parenchyma. This correlates well with the higher activation state of these cells in the former compartment (IL-2Ra and MHC class II).

T cells derived from mLNs show a proliferation rate in the perportal field that was 3 times as high as cells originating from pLNs and 3 times as high as endogenous proliferation. This augmented proliferation of mLN T cells is apparently governed by the perportal microenvironment, for when these same mLNs and pLNs T cells were examined before injection (26) and after injection in the celiac LN draining the liver (present study), there was no difference in the rate of proliferation between these two sources of T cells. In accordance with the preferential proliferation in the liver, it was recently shown that in the mLNs, activated donor T cells show enhanced proliferation if the cells originated from the same tissue (26). This also indicates that the presence of characteristic accessory molecules such as B7.1 and B7.2 (52, 53), various adhesion molecules (16), and different concentrations of various cytokines (54, 55) may drive the preferential proliferation of activated T cells in a microenvironment-specific fashion (28, 47). This is in line with recent observations in mice, in which it was shown that lymphocytes from pLN and Peyer’s patches significantly differ in the amount and ratio of IL-2, IL-4, and IFN-γ they produce (56, 57). In addition, extracellular matrix compounds, dendritic cell subsets (58), adhesion molecules (43), and even innervation by the central nervous system (59, 60) might play a role in the microenvironment-characteristic regulation of the proliferation of activated T cells.

Taken together, activated T cells have an even greater preference for the perportal field of the liver than naive or memory T cells, and they preferentially proliferate there. This could partly explain the preferential initiation and manifestation of some immunological liver diseases in the perportal field.

**Activated T cells survive in and are able to leave the liver**

Our study suggests that the preferential proliferation of activated T cells in the perportal field of the liver contributes to the accumulation of these cells in this compartment. However, other possibilities such as an increased entry or reduced cell death and exit should be considered. Entry into the liver does not seem to be important for the preferential accumulation in the perportal field, since regardless of whether they are naive, activated, or memory T cells, the number found initially (0.5–1 h after injection) in both compartments is comparable.

Injected cells die by apoptosis in the liver, showing that not only hepatocytes die by this pathway during viral hepatitis and primary biliary cirrhosis (61), but also activated T cells after migration into the normal liver. In a recent study, it was found that after antigenic stimulation many Ag-specific T cells accumulate in the liver, where they undergo apoptosis (39, 62). Due to the low number of apoptotic cells among the injected T cells in the liver, we can neither confirm nor exclude that preferential apoptosis in the parenchyma may be involved in the accumulation of activated T cells in the perportal field. In addition, the amount of cell death among activated T cells in the liver caused by apoptosis is unknown. However, the present study shows that a substantial number of activated T cells survives, and both CD4+ and CD8+ T cells are able to leave the liver. This is demonstrated by the fact that about 10 times more activated T cells are found in the celiac LN draining the liver than in the axillary LN draining the skin 9, 24, and 96 h after injection. It is known that under normal circumstances only very few lymphocytes reach the axillary LN via the afferent lymphatics (14). When assuming that the number of activated T cells entering the celiac and axillary LN via the high endothelial venules is roughly similar (19, 26), then a large difference in the number of activated T cells found in both LNs must be due to the arrival of activated T cells via the afferent lymphatics, a route that was previously described for dendritic cells (63).

**Conclusions**

Naive, activated, and memory T cells continuously migrate through the parenchyma and the perportal field of the liver. All migrating T cell subsets investigated in the present study have a preference for the perportal field of the normal liver, and activated T cells mainly proliferate there. Together, this may partly explain why many immunologically mediated diseases predominantly affect the perportal field and not the parenchyma. Future studies should aim at identifying the as yet unknown factors that are involved in regulating the compartment-characteristic migration and proliferation of T cells.
Traffic of T Cell Subsets in Liver Compartment

In addition, it is necessary to analyze how these subsets migrate through the compartments of the liver during various diseases. Modifying migration and proliferation of the different T cell subsets may represent a way to increase or decrease their numbers in the liver, and could have clinical relevance for the treatment of immunologically mediated diseases of the liver.

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