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Liver sinusoidal endothelial cells (LSEC) have been reported to express MHC class II, CD80, CD86, and CD11c and effectively stimulate naive T cells. Because dendritic cells (DC) are known to possess these characteristics, we sought to directly compare the phenotype and function of murine LSEC and DC. Nonparenchymal cells from C57BL/6 mice were obtained by collagenase digestion of the liver followed by density gradient centrifugation. From the enriched nonparenchymal cell fraction, LSEC (CD45−) were then isolated to 99% purity using immunomagnetic beads. Flow cytometric analysis of LSEC demonstrated high expression of CD31, von Willebrand factor, and FcγRs. However, unlike DC, LSEC had low or absent expression of MHC class II, CD86, and CD11c. LSEC demonstrated a high capacity for Ag uptake in vitro and in vivo. Although acetylated low-density lipoprotein uptake has been purported to be a specific function of LSEC, we found DC captured acetylated low-density lipoprotein to a similar extent in vivo. Consistent with their phenotype, LSEC were poor stimulators of allogeneic T cells. Furthermore, in the absence of exogenous costimulation, LSEC induced negligible proliferation of CD4+ or CD8+ TCR-transgenic T cells. Thus, contrary to previous reports, our data indicate that LSEC alone are insufficient to activate naive T cells. The Journal of Immunology, 2004, 173: 230–235.

The liver possesses several unique immunologic functions, including the induction and maintenance of peripheral tolerance (1, 2). For instance, allogeneic liver transplants may be accepted across MHC barriers without the need for immunosuppression and the delivery of Ag via the portal vein may induce tolerance (3). The microscopic architecture of the liver is likely to contribute to its immunologic properties. Specifically, sluggish and intermittent blood flow through the narrow lumens of the hepatic sinusoids facilitates interaction between blood-borne leukocytes and resident liver cells (4). The immune functions of cells within the liver sinusoids are therefore of particular interest, since they may be responsible for certain aspects of liver immunity.

Liver sinusoidal endothelial cells (LSEC) constitute the predominant cell type within the liver sinusoids. LSEC form a fenestrated barrier between the blood and hepatocytes and are therefore strategically situated to interact with leukocytes as they traverse the liver. In addition to anatomic and rheologic factors favoring interaction of LSEC with lymphocytes, LSEC have high Ag uptake and processing capacity, a property associated with professional APC (5). LSEC are also believed to express the costimulatory moieties CD80 and CD86 and stimulate T cells through peptide presentation in the context of MHC class I and II molecules (6, 7). LSEC have been shown to stimulate CD4+ T cell cytokine production and to stimulate CD8+ T cells via cross-presentation of soluble Ag (7, 8). However, LSEC have also been found to express CD4, CD11b, and CD11c, markers generally associated with T cells, myeloid cells, and dendritic cells (DC) (7, 9, 10).

Because many of the phenotypic and functional features attributed to LSEC are also properties of DC, we speculated that previous studies of LSEC may have been confounded by contamination with DC. Previous isolation techniques, such as counterflow elutriation, may not have adequately discriminated DC from LSEC. In addition, given that DC take up a wide variety of macromolecules, we hypothesized that the methods used to confirm LSEC purity, such as uptake of acetylated low-density lipoprotein (AcLDL), may not be specific (7, 11–17). To test our hypotheses, we designed a more specific LSEC isolation protocol based on surface marker expression and then re-evaluated the phenotype and function of pure LSEC. We isolated LSEC from nonparenchymal cells (NPC) using immunomagnetic beads to exclude CD45+ cells. We showed that this isolation process resulted in <1% contamination by CD45+ NPC and that nearly all CD45+ NPC were von Willebrand factor positive (vWF+) and CD31+. In contrast to previous reports, we found LSEC to express negligible levels of MHC class II, CD80, CD86, and CD11c. Consistent with this phenotype, LSEC induced minimal allogeneic and Ag-specific T cell stimulation when compared with DC. Therefore, although LSEC capture and process Ag, they are not sufficient for activation of naive T cells.

Materials and Methods

Mice

Adult 6- to 10-wk-old male C57BL/6 (B6, H-2Kb) and BALB/c (H-2Kb) mice were purchased from Taconic Farms (Germantown, NY), OT-I (B6.Cg-RAG2tm1Fwa-TgN) and OT-II (B6.Cg-RAG2tm1Alt-TgN) TCR-transgenic Rag-2−/− mice on a B6 background were also obtained from Taconic Farms. All animals were maintained in a pathogen-free animal housing facility at Memorial Sloan-Kettering Cancer Center. All procedures were approved by the Institutional Animal Care and Use Committee.

LSEC and DC preparation

Liver NPC were isolated as previously described, with modifications (18). Briefly, animals were euthanized and the portal vein was isolated, cannulated with a 24-gauge catheter (BD Biosciences, Franklin Lakes, NJ), and

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2 Abbreviations used in this paper: LSEC, liver sinusoidal endothelial cell; DC, dendritic cell; AcLDL, acetylated low-density lipoprotein; NPC, nonparenchymal cell; vWF, von Willebrand factor; TEM, transmission electron microscopy.
injected with 2 ml of 1% (w/v) collagenase D (Sigma-Aldrich, St. Louis, MO) in HBSS. We then mechanically disrupted the liver before a 20-min incubation in 20 ml of 1% collagenase at 37°C. The resulting cell suspension was then passed through a sterile 100-μm nylon mesh filter (Falcon; BD Biosciences) and spun three times at 30 × g for 5 min to remove hepatocytes. The supernatant was centrifuged (300 × g for 10 min) and the pellet containing NPC was resuspended in 1.5 ml of complete RPMI 1640 medium (10% FCS, 2 mM l-glutamine, 50 μM 2-ME, 100 U/ml penicillin, and 100 U/ml streptomycin) and added to 2.8 ml of 30% (w/v) metrizamide (Sigma-Aldrich; product recently discontinued) or 3.0 ml of 40% (w/v) Optiprep (Sigma-Aldrich) to remove debris and enrich the NPC. With metrizamide this suspension was layered under 5 ml of HBSS in a 15-ml centrifuge tube (Falcon) and spun at 1500 × g for 20 min. When Optiprep was used, the cell suspension was layered under 4 ml of HBSS and spun at 400 × g for 15 min. The layer of low-density cells at the interface was harvested. The cells were incubated with 1 μg of anti-FcyRII/III (FcγR) mAb 2.4G2 (Fc block; mAb Core Facility, Sloan-Kettering Institute, New York, NY) per 1 × 107 cells and then fractionated based on CD45 expression using immunomagnetic beads (Miltenyi Biotec, Auburn, CA) as follows. Enriched NPC were incubated with anti-CD45 immunomagnetic beads and then passed through a single-positive selection column (Miltenyi Biotec). The effluent (CD45− NPC) was then further purified by passing it through a high-affinity depletion column (Miltenyi Biotec) which has a slower expansion rate and removes residual CD45+ cells with greater efficiency. Liver DC were isolated by incubation of NPC with anti-CD11c immunomagnetic beads and passage through two consecutive positive selection columns. Splenic DC were isolated similarly with anti-CD11c beads following mechanical disruption of the organ and erythrocyte lysis (18).

Flow cytometry

Four-color flow cytometry was performed on a FACSCalibur flow cytometer (BD Biosciences). Voltages were based on unstained cells and compensation was set using single-stained positive controls for each color. All samples were incubated with Fc block before staining. We labeled 1 × 10⁶ cells with 0.1 μg FITC-, PE-, allophycocyanin-, or biotin-conjugated Ab (BD Pharmingen, San Diego, CA, except as indicated). Biotinylated Abs were secondarily stained with streptavidin PerCP. Cells were stained for endothelial markers (CD31 (WM-59), VWF (Serotec, Raleigh, NC)); CD4 (RM4-4); FcγRII (2.4G2), Mac-3 (M3/84); CD11c (HL-3); MHC class II (AF6-120.1, I-A b ); MHC class I (AF6-88.5, H-2K b ); CD54 (3-E2); and costimulatory molecules (CD40 (3/23), CD80 (16-10A1), and CD86 (GL-1)).

Electron microscopy

For transmission electron microscopy (TEM), LSEC were pelleted and fixed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.075 M cacodylate buffer for 1 h followed by postfixation in 2% OsO₄ for 1 h. The samples were rinsed in distilled water and serially dehydrated in ethanol (50, 75, 95, and then 100%), followed by propylene oxide. The cells were placed in propylene oxide/PolyBed 812 (1/1) overnight (Polysciences, Warrington, PA). The samples were embedded in PolyBed 812 in a 60°C oven. Ultrathin sections were obtained with a Reichert Ultracut S-microtome (Leica, Vienna, Austria). The thin sections were photographed using a JEOL 1200-EX electron microscope (Peabody, MA). For scanning electron microscopy, the cells were placed on poly-L-lysine-coated Thermaxon (Nunc, Rochester, NY) plastic coverslips. The cells were fixed in 2.5% glutaraldehyde/2% paraformaldehyde in 0.075 M cacodylate buffer for 1 h, rinsed in buffer, and dehydrated in a graded series of ethanol as above. The samples were then freeze dried in a Pierce-Edwards dryer (BOC Edwards, Wilmington, MA). The samples were subsequently coated with gold/palladium in a Technics Hummer VI sputtering system. The samples were photographed using a JEOL JSM 35 scanning electron microscope.

Ag uptake

To measure in vitro Ag uptake, we incubated purified LSEC or CD45⁺ NPC with 100 μg of FITC-conjugated dextran, OVA, DQ-OVA, or BODIPY AcLDL in duplicate wells at 37°C (Molecular Probes, Eugene, OR). At various time points, the reactions were stopped and the cells washed with cold PBS and then stained with anti-CD31 (LSEC) or anti-CD11c (CD45⁺ NPC) before measurement of mean fluorescence of the captured FITC-labeled Ags.

To measure in vivo Ag uptake, we injected 100 μg of FITC-dextran, FITC-OVA, or FITC-DQ-OVA or 50 μg BODIPY-AcLDL into the lateral tail vein of mice and the livers were harvested 5 or 30 min later. CD45⁺ and CD45⁻ NPC were purified as above. Ag uptake was then assessed by measuring mean fluorescence on a flow cytometer and LSEC were identified as CD45− CD31− cells while DC were CD45⁺ CD11c⁺.

**FIGURE 1.** CD45⁺ and CD45⁻ NPC have similar physical properties. A. When bulk, enriched NPC were stained for CD45 (solid line) or with isotype control Ab (dotted line), 77% of the cells were CD45⁺. B. Enriched NPC were stained with fluorescent anti-CD45 and gated into positive and negative fractions to analyze their light scatter properties.

**T cell assays**

MLR was performed as previously described (19). Briefly, LSEC and DC from C57BL/6 mice were added in various numbers to 1 × 10⁵ BALB/c T lymphocytes purified using Thy1.2 (CD90.2) immunomagnetic beads according to the manufacturer’s protocol (Miltenyi Biotec) in 96-well U-bottom plates (Falcon). Assays were performed in complete RPMI 1640 and CpG oligonucleotide (oligodeoxynucleotide 1826, 10 μg/ml; Oligos Etc., Wilsonville, OR) or LPS (1 μg/ml; Sigma-Aldrich) as needed. Wells were pulsed with [³H]thymidine (1 μCi/well) on day 3 and radioactive uptake was measured 20 h later. In vitro Ag-specific CD8⁺ T cell activation was assayed with OT-I CD8⁺-transgenic T cells specific for OVA257-264 (20). In vitro Ag-specific CD4⁺ T cell activation was assayed with OT-II CD4⁺ transgenic T cells specific for OVA237-248 (20). OT-I and OT-II T cells were isolated with Thy 1.2 immunomagnetic beads (Miltenyi Biotec) from splenocytes. The Trp-2, H-2g, I-A, and I-Etc., Wilsonville, OR) or LPS (1 μg/ml; Sigma-Aldrich) were added as needed. LPS was then added to the wells at a concentration of 20 μg/ml to some wells, in which case LSEC or DC were preincubated with OVA237-248 for 1 h and then washed before culture with T cells. T cell proliferation was determined as for the MLR experiments and cell culture supernatant was tested by cytokine bead array for IL-2 and IFN-γ according to the manufacturer’s protocols (BD PharMingen).

**Results**

**LSEC have a phenotypic profile distinct from DC**

Much of the published data on LSEC derives from cells isolated with elutriation. We sought to develop a more specific method of LSEC purification based on cell surface marker expression. We started by examining bulk liver NPC from mice. To remove cellular debris that results from collagenase digestion of the liver, we used density gradient centrifugation. Because LSEC are not of bone marrow origin (8), we used the common leukocyte marker CD45 to distinguish LSEC from other NPC, including Kupffer cells.

**FIGURE 2.** Phenotypic characterization of CD45⁺ NPC. NPC from B6 mice were enriched using density gradient centrifugation and analyzed by flow cytometry. CD45⁺ NPC were compared with CD45⁻ NPC. CD45⁺ NPC (shaded histogram) expressed notably higher levels of vWF, CD31, Mac-3, and FcγRII/III than CD45⁻ NPC (solid line). CD31 and CD45 were found only on CD45⁺ NPC. Isotype controls are shown as dotted lines.
cells, B cells, T cells, NK cells, and DC. We found that 77% of enriched NPC lacked expression of CD45 (Fig. 1A). Furthermore, there was considerable overlap in the forward and side scatter profiles of CD45<sup>+</sup> and CD45<sup>-</sup> NPC (Fig. 1B). To determine what proportion of CD45<sup>-</sup>-enriched NPC were actually LSEC, we tested markers of endothelial cell origin. Nearly all CD45<sup>-</sup> cells expressed CD31 and vWF (Fig. 2), which have been reported to be expressed by LSEC (21–23). As expected, CD45<sup>-</sup> cells, which we have previously shown to contain liver DC (Fig. 2) (18), had only slight expression of vWF and CD31. Furthermore, LSEC demonstrated high levels of FcγR expression (Fig. 2), which distinguishes LSEC from other NPC types including endothelial cells from central veins and portal vessels of the liver (24). In contrast to findings from previous reports (7, 25), we found that LSEC did not express the murine DC marker CD11c or the T cell marker CD4, but did express the macrophage marker Mac-3 (Fig. 2). Taken together, these data demonstrated that enriched CD45<sup>-</sup> NPC were predominantly comprised of LSEC. Furthermore, previous LSEC preparations may have been contaminated by DC and other leukocytes because LSEC and CD45<sup>-</sup> NPC are similar in size as approximated by forward light scatter and are recovered from the same layer in a density gradient.

To assess the functional potential of LSEC, we next determined their expression of MHC and costimulatory molecules (Fig. 3). LSEC expressed MHC class I molecules, as did CD45<sup>-</sup>-enriched NPC controls. However, in contrast to previous reports (7, 10, 13, 25), we found that LSEC had negligible expression of MHC class II, CD80, CD86, and CD40. Consistent with previous observations, LSEC demonstrated high levels of ICAM-1 and Fas (data not shown) (4).

**LSEC can be purified based on lack of CD45 expression**

Based on our finding that enriched CD45<sup>-</sup> NPC were predominantly LSEC, we used anti-CD45 immunomagnetic beads to isolate LSEC to study their function. To eliminate contaminating CD45<sup>-</sup> NPC and DC, in particular, we developed a two-step procedure for LSEC isolation. First, we labeled enriched NPC with anti-CD45 immunomagnetic beads and passed them through a positive selection column. The effluent was then run through a high-affinity depletion column and was found to be 99% pure by lack of CD45 staining. The mean yield of LSEC from a mouse liver was 10<sup>6</sup> cells, B cells, T cells, NK cells, and DC. We found that 77% of enriched NPC lacked expression of CD45 (Fig. 1A). Furthermore, there was considerable overlap in the forward and side scatter profiles of CD45<sup>-</sup> and CD45<sup>+</sup> NPC (Fig. 1B). To determine what proportion of CD45<sup>-</sup>-enriched NPC were actually LSEC, we tested markers of endothelial cell origin. Nearly all CD45<sup>-</sup> cells expressed CD31 and vWF (Fig. 2), which have been reported to be expressed by LSEC (21–23). As expected, CD45<sup>-</sup> cells, which we have previously shown to contain liver DC (Fig. 2) (18), had only slight expression of vWF and CD31. Furthermore, LSEC demonstrated high levels of FcγR expression (Fig. 2), which distinguishes LSEC from other NPC types including endothelial cells from central veins and portal vessels of the liver (24). In contrast to findings from previous reports (7, 25), we found that LSEC did not express the murine DC marker CD11c or the T cell marker CD4, but did express the macrophage marker Mac-3 (Fig. 2). Taken together, these data demonstrated that enriched CD45<sup>-</sup> NPC were predominantly comprised of LSEC. Furthermore, previous LSEC preparations may have been contaminated by DC and other leukocytes because LSEC and CD45<sup>-</sup> NPC are similar in size as approximated by forward light scatter and are recovered from the same layer in a density gradient.

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**LSEC avidly capture Ag but AcLDL uptake is not specific for LSEC**

Once we developed a reliable method of LSEC purification, we then tested their Ag uptake capacity, an integral component of in vivo T cell stimulation by APC. To provide a context in which to interpret the results, we compared LSEC directly to DC. We found that both LSEC and DC took up comparable levels of dextran and OVA in vitro, both of which are captured by macropinocytosis (Fig. 5A). In fact, LSEC took up more OVA in vivo than did DC (Fig. 5B), a finding which may be due to their relatively greater contact exposure to blood in the sinusoids. In addition to Ag uptake, we measured a component of Ag processing with DQ-OVA, which becomes fluorescent only after lysosomal acidification and proteolytic cleavage. By this measure, LSEC and DC functioned similarly in vitro and the results were comparable in vivo when normalized to the amount of captured OVA. Finally, we tested
AcLDL uptake because it is purported to be a specific function of LSEC. As we expected, we found that DC were also able to capture Ag efficiently and their expression of MHC class I, we repeated the OT-1 proliferation assay following addition of a stimulating anti-CD28 Ab. Surprisingly, the presence of the anti-CD28 Ab markedly enhanced the ability of LSEC to stimulate proliferation of naive CD8\(^+\) T cells (Fig. 7D). Finally, given that IFN-\(\gamma\) has been shown to activate other endothelial cell types (28-30), we exposed LSEC to IFN-\(\gamma\) (20 ng/ml; R&D Systems, Minneapolis, MN) for 16, 36, or 60 h. IFN-\(\gamma\) did not induce MHC class II or costimulatory molecule expression on LSEC, while these moieties were up-regulated on splenic macrophages under the same experimental conditions. Moreover, pretreatment with IFN-\(\gamma\) for 60 h did not enable LSEC to stimulate allogeneic T cells (data not shown).

**Discussion**

LSEC have been reported to possess several properties of DC, including the expression of MHC class II, costimulatory molecules, and CD11c, in addition to the ability to activate naive T cells (6–10, 13, 25, 31). Therefore, we sought to compare LSEC and DC directly. We found that LSEC expressed surface markers reflective of their endothelial nature. Indeed, nearly all of the enriched NPC that were CD45\(^+\) expressed the endothelial markers CD31 and vWF (Fig. 2), consistent with previous reports of LSEC (21, 23, 24). However, LSEC lacked expression of CD11c, the most widely used marker of murine DC (Fig. 2). Furthermore, in contrast to others (6, 7, 10), we found that LSEC had undetectable levels of CD40, CD80, and CD86 and only minimal MHC class II (Fig. 2). We confirmed these findings with multiple Ab concentrations and several different fluorochromes (data not shown). Consistent with their lack of costimulatory molecule expression, we

**FIGURE 5.** LSEC take up high levels of Ag in vitro and in vivo and AcLDL uptake is nonspecific. A. In vitro Ag uptake was performed by incubating 1 \(\times\) 10\(^6\) freshly isolated LSEC or CD45\(^+\) NPC with fluorescent dex- 

trin, OVA, DQ-OVA, or AcLDL at 37°C for various times. Reactions were quenched with cold PBS. Cells were subsequently washed and mean fluorescence was measured by flow cytometry. LSEC were isolated by immunomagnetic exclusion of CD45\(^-\) NPC and were analyzed by gating on CD31\(^+\) cells. Liver DC were isolated by immunomagnetic isolation of CD45\(^+\) NPC and analyzed by gating on CD11c\(^+\) cells. For each sample, 1 \(\times\) 10\(^5\) cells were collected for determination of mean fluorescence. B. In vivo Ag uptake was performed by tail vein injection of fluorescent dextran (100 \(\mu\)g), OVA (100 \(\mu\)g), DQ-OVA (100 \(\mu\)g), or AcLDL (50 \(\mu\)g). Animals were sacrificed at 5 or 30 min. Liver NPC were then harvested as above and stained with anti-CD31, -CD45, and -CD11c for analysis of LSEC and DC. For each sample, 1 \(\times\) 10\(^5\) cells were collected for determination of mean fluorescence.

**FIGURE 6.** LSEC do not stimulate allogeneic T cells. LSEC and splenic DC from B6 mice were isolated with immunomagnetic beads. Varying numbers of LSEC or DC were incubated with 1 \(\times\) 10\(^5\) BALB/c T cells for 72 h, at which time \([\text{^3}\text{H}]\)thymidine was added. After an additional 24 h, thymidine uptake was measured. Proliferation of stimulators and responders cultured alone was minimal (0.15 \(\times\) 1000 cpm). Averages and SDs of triplicate wells are shown.
FIGURE 7. LSEC are poor stimulators of Ag-specific CD4+ and CD8+ T cells. LSEC and splenic DC were isolated and incubated at various concentrations with 2 × 10^4 OT-I T cells in the presence of OVA257-264 (A) or 1 × 10^4 OT-II T cells in the presence of OVA323-339 for 72 h (B), at which time [3H]thymidine was added. Thymidine uptake was counted 20 h after addition. Proliferation of CD8+ T cells, LSEC, and DC alone was negligible (<0.14 × 10^4 cpm). The mean proliferation for CD4+ T cells alone was 1.6 × 10^4 cpm. C, Supernatant from the CD8+ stimulation cocultures was removed at 48 h and assayed for IL-2 and IFN-γ. D, LSEC and DC were incubated with 2 × 10^4 OT-I T cells in the presence of OVA257-264 and a stimulating anti-CD28 Ab (20 μg/ml). Proliferation of stimulators cultured alone and responders cultured alone were each negligible (<0.16 × 10^4 cpm). Means and SDs of triplicate wells are shown.

There are a number of potential explanations for why our data do not adequately exclude other populations of NPC, including liver DC. It is especially important to remove DC from LSEC preparations since we and others have previously shown liver DC to be potent inducers of immunity (18, 19, 32). Instead of using cell density or size, we purified LSEC by their lack of CD45 expression. Using negative selection columns to deplete CD45+ cells from enriched NPC were LSEC (Fig. 2). We further validated our isolation method with transmission and scanning electron microscopy (Fig. 4) by identifying the characteristic membrane fenestrae of LSEC (26).

In addition to our method of LSEC isolation, there are other factors that may account for our discrepant data. LSEC are highly autofluorescent, which can confound the interpretation of flow cytometry analysis. We tailored our analyses of surface marker expression by choosing appropriate voltages according to the level of autofluorescence. Furthermore, we routinely blocked FcγR during bead sorting and flow cytometric phenotype analysis. It is unclear whether anti-FcγR was used previously. Because LSEC are known to express high levels of FcγR, considerable nonspecific binding of Abs would be expected if these receptors were not blocked. Lastly, we used freshly isolated cells exclusively, while others often assessed LSEC phenotype and function after 3 days of culture (13, 25). However, we did not detect any noteworthy phenotypic changes by flow cytometry when LSEC were analyzed after culture for 3 days, even when treated with IFN-γ (data not shown).
 Taken together, our findings that LSEC do not express MHC class II, costimulatory molecules, or CD11c, and do not activate T cells raises the possibility that LSEC preparations used in previous studies were contaminated with DC. In fact, we found that even low levels (i.e., <10%) of contamination by CD45+ cells resulted in T cell stimulation (data not shown). Furthermore, not only are the physical properties of LSEC inadequate to separate them from other NPC populations, we have shown that the uptake of AcLDL, an indicator of LSEC purity used widely by others, is not a function unique to LSEC (Fig. 5). Again, we believe that distinguishing LSEC from other NPC populations is more reliably accomplished by surface marker expression.

Although our results show that LSEC are unlikely to stimulate T cells directly in vivo through presentation of peptides in the context of MHC molecules, LSEC may still contribute to the induction of immunity or tolerance within the liver. Given the factors favoring cellular contact within the hepatic sinusoids, a neighboring cell may provide costimulation or a necessary cytokine during CD8+ TCR binding to Ag in the context of LSEC MHC class I molecules. Furthermore, the absence of MHC class II expression does not prohibit presentation of exogenous protein to T cells because LSEC may be able to cross-present soluble Ag (8). Moreover, LSEC may affect T presentation of exogenous protein to T cells because LSEC may be capable of binding to Ag in the context of LSEC MHC class I molecules. Fur-}

ular contact within the hepatic sinusoids, a neighboring cell may pro-

mote the development of T cells bearing receptors and lowered surface expression of accessory molecules. This suggests that LSEC may still contribute to the induction of immunologic function of the liver.

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