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ISOLATION, PHENOTYPE, AND ALLOSTIMULATORY ACTIVITY OF MOUSE LIVER DENDRITIC CELLS[1]

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Donor liver-derived dendritic cells (DC) have recently been identified within various lymphoid and nonlymphoid tissues of organ allograft recipients, including nonimmunosuppressed mice transplanted with and permanently accepting major histocompatibility complex (MHC)–disparate hepatic allografts. These findings have raised questions about the basis of the tolerogenicity of the liver—and, in particular, about the properties of liver-derived DC. To study further the structure, immunophenotype and allostimulatory activity of leukocytes resident in normal mouse (B10.BR; H-2^k, I-E^k) liver, a procedure was developed to maximize the yield of viable, nonparenchymal cells (NPC) obtained following collagenase digestion of perfused liver fragments and density centrifugation (Percoll). These cells comprised populations expressing lymphoid and myeloid cell surface antigens. As compared with spleen cells, they proved good allostimulators of naive (B10; H-2^b, I-E$^-$) splenic T cells when tested in primary mixed leukocyte reactions (MLR). After overnight (18-hr) incubation of the NPC, enrichment for transiently adherent, low-density (LD) cells on metrizamide gradients permitted the recovery of low numbers of cells (approx. 2–5 × 10^6 per liver), many of which displayed distinct DC morphology. Flow cytometric analysis revealed that these cells were CD3$^-$, CD4$^-$, CD8$^-$, and B220$^-$, but strongly expressed CD45 (leukocyte-common antigen), and mild-to-moderate levels of CD11b, heat-stable antigen, and CD44. The cells also expressed moderate intensity of NLDC 145 but not 33D1, DC restricted markers which have been shown to be differentially expressed on mouse DC isolated from various organs. This DC-enriched population was more strongly MHC class II(I-E^k)$^+$ than NPC, as determined by immunocytochemistry and flow cytometry and exhibited much more potent allostimulatory activity for naive T cells. These findings demonstrate that freshly isolated murine liver NPC, and perhaps their counterparts in situ, exhibit allostimulatory activity that is enhanced in the nonadherent, low-density (DC-enriched) fraction after overnight culture. They further suggest that the maturation of liver DC may play a key role in determining the immunogenicity and or tolerogenicity of hepatic allografts.

Dendritic cells (DC)* are a minor population of large, bone marrow–derived leukocytes that are distributed ubiquitously throughout the body (*1, 2*). DC resident in the interstitial connective tissue of nonlymphoid organs, such as the kidney, heart, or liver, are believed to be important "passenger" (donor-derived) leukocytes (*3*), that migrate to T dependent areas of host lymphoid tissue following organ transplantation (*4*). They constitute potential members of a hemopoietic lineage of potent antigen-presenting cells (APC) that, on maturation, express abundant cell surface major histocompatibility complex (MHC) products. Their isolation depends largely on physical characteristics and on the expression of lineage-specific markers. Freshly isolated DC from lymphoid organs are potent stimulators of primary MLR (*5*), whereas freshly isolated epidermal DC of skin (Langerhans cells) are unable to initiate primary T cell responses, but develop this capacity following in vitro culture with granulocyte-macrophage colony-stimulating factor (GM-CSF) (*6*). It is thus thought that the DC resident within nonlymphoid tissue may be functionally immature (*7*) and/or more heterogeneous than the potent stimulators of T cell activation that can be isolated from lymphoid organs. The phenotype and properties of liver DC, which are located within the portal triads, have been described both in the rat (*8–10*) and in man (*11*). Little is known, however, about the properties of DC present within mouse liver, although presumptive DC have been described in portal triads (*12*).

Recently, it has been suggested that the chimeric cells observed in various organs of recipients of liver or other allografts include, predominantly, cells of DC lineage that may play an important role in modulation of the immunological interaction between host and donor (*13–15*). Since the liver is the most tolerogenic of all transplanted organs (*16–18*) and can be spontaneously accepted across MHC barriers in mice without the need for immunosuppressive therapy (*19*), studies on mouse liver DC may provide important clues to mechanisms underlying tolerance induction. We have isolated DC-enriched cell populations from normal mouse liver and describe herein their immunophenotype and in vitro allostimulatory activity compared with freshly isolated liver nonparenchymal cells (NPC).

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* Abbreviations: DC, dendritic cell; FBS, fetal bovine serum; HD, high-density; LD, low-density; mAb, monoclonal antibody; MHC, major histocompatibility complex; NPC, nonparenchymal cell.

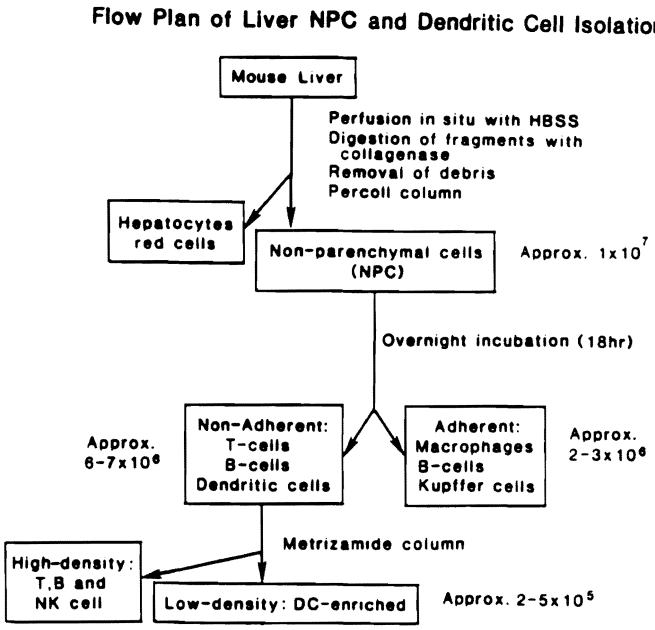

FIGURE 1. Flow plan for the isolation of nonparenchymal cells from normal mouse liver and for the preparation of DC-enriched suspensions.

TABLE 1. Monoclonal antibody panel

Antigen (CD)	Isotype	Supplier/clone name (ATCC No.)
Leukocyte-common Ag		
CD45	Rat IgG2a	M1/9.3.4 (TIB 122)
CD45RA;B220	Rat IgM	RA3–3A 1/6.1 (TIB 146)
Lymphoid (primarily)		
Thy 1.2	Rat IgG2a	Pharmingen; 53–2.1
CD3-ε	Hamster IgG	Pharmingen; 145–2C11
CD4	Rat IgG2a	Pharmingen; RM–4–5
CD8a	Rat IgG2a	Pharmingen; 53–6.7
Heat stable antigen	Rat IgM	J11D (TIB 183)
MHC: class II; $I\text{-}E^{k,d,p,r}$	C3H IgG2a	Pharmingen; 14–4–4S (HB32)
DC-restricted		
Lymphoid DC	Rat IgG2b	33D1 (TIB 227)
Interdigitating cell	Rat IgG2a	NLDC-145; Dr. R.M. Steinman
Myeloid (primarily): macrophage	Rat IgG2b	F 4/80 (HB 198); Dr. R.M. Steinman
Receptors/adhesins		
CD32, FcγRII	Rat IgG2b	Pharmingen; 2.4G2 (HB 197)
CD11b, Mac-1α unit; C3biR	Rat IgG2b	M1/70. (TIB 128)
CD11c, p150/90	Hamster IgG	N418; Dr. R.M. Steinman
CD44, Pgp-1	Rat IgG2a	Pharmingen; 2D2C (TIB 235)

MATERIALS AND METHODS

Animals. Adult 8–12-week-old male B10.BR ($H\text{-}2^k$, $I\text{-}E^+$) and C57BL/10SnJ (B10, $H\text{-}2^b$, $I\text{-}E^-$) mice were purchased from the Jackson Laboratory, Bar Harbor, ME. They were maintained in the specific pathogen–free facility of the University of Pittsburgh Medical Center.

FIGURE 2. Cryostat section of normal B10.BR mouse ($H\text{-}2K^kI\text{-}E^+$) liver, showing strongly MHC class II–positive cells in a portal area. All positive cells appear to be associated with blood vessel components. There is no staining of bile duct epithelium, hepatocytes, or endothelial cells (avidin-biotin peroxidase, counterstained with hematoxylin; ×400).

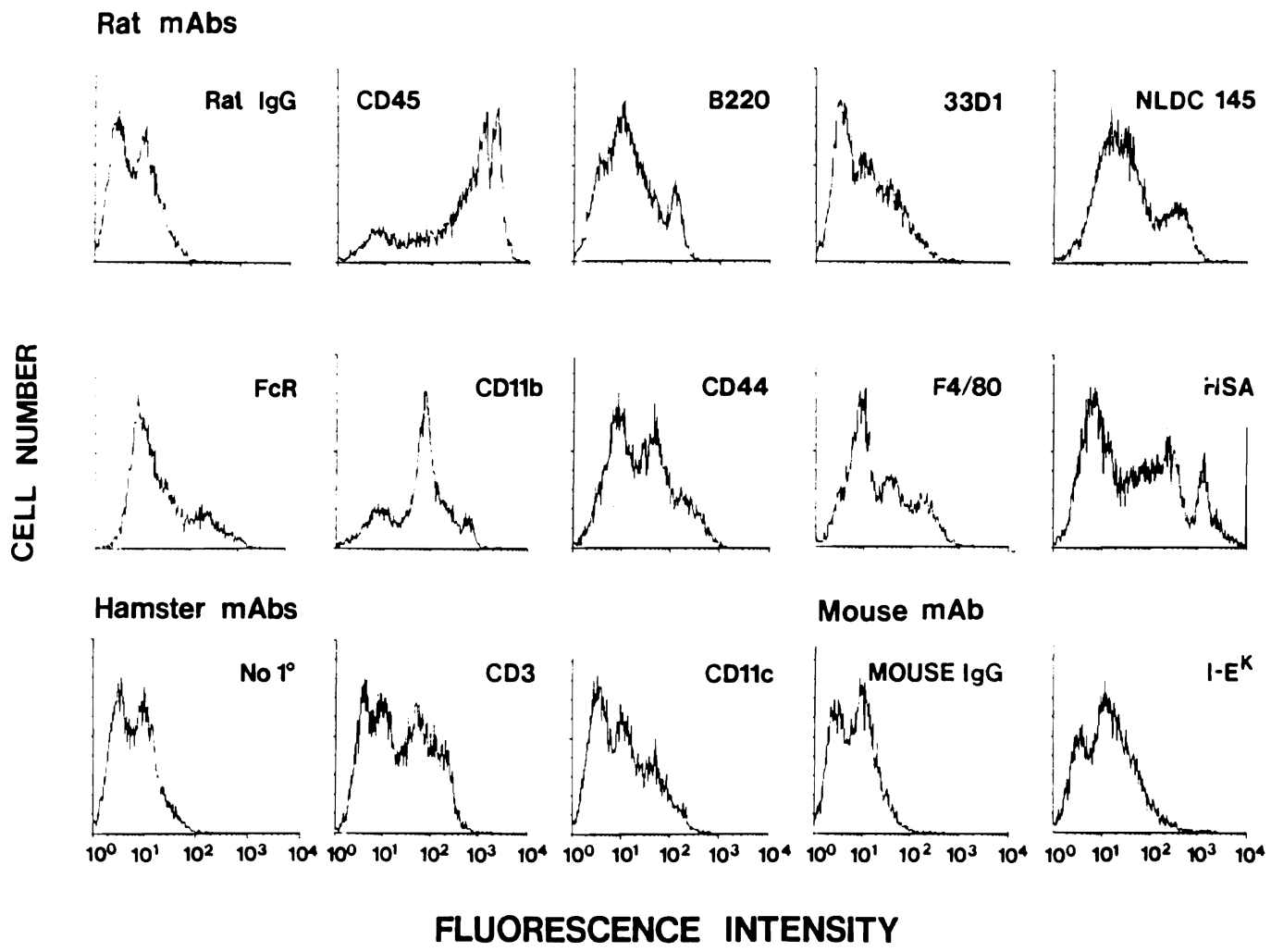

FIGURE 3. Representative flow cytometric analysis of cell surface markers expressed on the NPC population freshly isolated from normal (B10.BR) mouse liver. The cells were stained by direct or indirect immunofluorescence and five thousand gated events were acquired for each mAb.

Isolation of NPC from liver (Fig. 1). Mice were anesthetized with metofen and swabbed with 70% ethanol, and an abdominal mid-line incision was performed. The liver was perfused for 3 min in situ via the inferior vena cava, using 30 ml Hanks' balanced salt solution (HBSS; Gibco, Grand Island, NY) and a 22 G intravenous catheter (Critikon, Tampa, FL). Collagenase solution (2 ml [Sigma, St. Louis, MO] type IV, 1 mg/ml in HBSS) was then injected; the liver was excised immediately, diced into small pieces, and digested in collagenase solution (20 ml/liver) for 30 min at 37°C, with constant stirring. To increase the yield of cells, 2–4 livers were usually pooled. The digested tissue was filtered through sterile nylon mesh (0.1 mm) to remove debris and connective tissue.

The cell suspension was then centrifuged and washed twice in RPMI-1640 (Gibco), supplemented with glutamine and antibiotics at 400 $\times g$ for 5 min. The cells were resuspended in 7 ml sterile self-generating Percoll solution (Sigma; 1.079 relative density) and centrifuged at 4°C for 10 min at 39,000 $\times g$ in an ultracentrifuge (Beckman Instruments, Palo Alto, CA). The top layer of cells, containing intact hepatocytes and hepatocyte fragments was removed and discarded. The cell suspension between the upper and lower (erythrocyte) layer, constituting the freshly isolated nonparenchymal cell (NPC) population, was then removed and washed $\times 2$ (400 $\times g$; 5 min) in RPMI-1640.

Enrichment for dendritic cells (DC). To enrich for DC, NPC were placed in 25-cm^2 tissue culture flasks and incubated overnight (18 hr) at 37°C in 5% CO_2 in air. Nonadherent cells were recovered and resuspended in 8 ml RPMI-1640, supplemented with 10% heat-inactivated fetal bovine serum (FBS) (complete medium) then layered onto 2 ml columns of hypertonic (14.5% w/v) metrizamide (Sigma; grade 1, approx. 99% pure) dissolved in complete medium in 15 ml conical tubes. The cells were sedimented at 600 $\times g$ for 15 min at room temperature. The low-density (LD), DC-enriched interface population was removed carefully using a disposable pasteur pipette. These cells, subsequently referred to as "DC-enriched cells," were washed twice and finally resuspended in complete medium. The cell pellet (high-density; HD) was also recovered for subsequent functional studies.

Immunocytochemistry and histochemistry. Cytocentrifuge preparations of isolated cells or 10-μm cryostat sections of the liver were stained using the avidin-biotin-peroxidase complex (ABC) staining procedure. Specimens were air-dried at room temperature (RT) before fixation in acetone for 5 min. The slides were washed in phosphate-buffered saline (PBS), treated using a blocking kit (Vector Labs., Burlingame, CA), then incubated for 1 hr at RT with predetermined optimal dilutions of the appropriate biotinylated primary monoclonal antibody (mAb). After further washes in PBS (3×, 5 min each), the slides were incubated with streptavidin-biotin-peroxidase complex (ABC-P) (Boehringer Mannheim, Indianapolis, IN) diluted in PBS for 30 min, and the color reaction developed for 6 min using a peroxidase chromogen kit (AEC; Biomedia, Foster City, CA). Cells

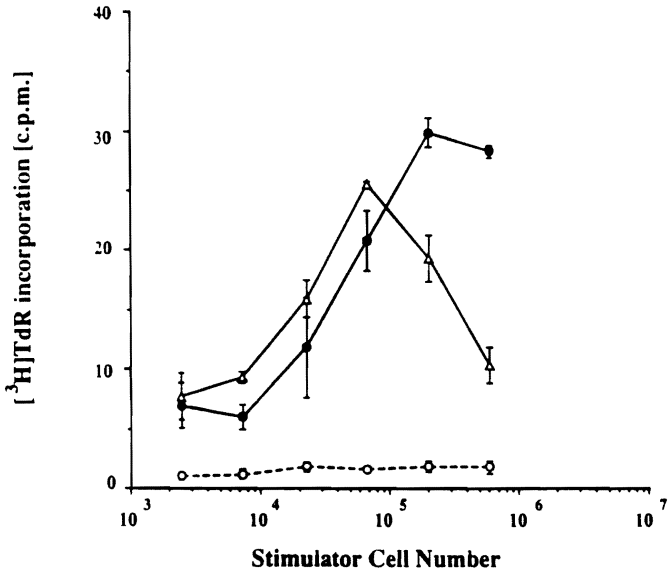

FIGURE 4. Allostimulatory activity for 2.10^5 naive B.10 (H-2^b) mouse splenic T cells of variable numbers of γ-irradiated, freshly isolated NPC prepared from normal B10.BR (H-2^k) mouse liver. Cells were cultured for 72 hr; [^{3}H]TdR was added to the cultures 18 hr before harvesting. Results are mean cpm ± 1 SD and representative of 3 separate experiments. (△) freshly isolated NPC; (●) freshly isolated B10.BR spleen cells; (○) unstimulated B10 splenic T cells. Syngeneic (B10) liver NPC stimulators gave results almost identifical to those obtained with unstimulated T cells.

were counterstained lightly with hematoxylin. Controls included the omission of primary antibody and the use of isotype-matched irrelevant mAb.

Flow cytometric analyses. Liver NPC or DC-enriched cells (5×10^5/tube) in HBSS with 1% w/v bovine serum albumin (BSA; Sigma) and 0.01% sodium azide (Sigma) were stained either by direct or indirect immunofluorescence. T lymphocytes were identified using FITC-conjugated rat antimouse Thy 1.2, PE-conjugated rat antimouse CD4 or CD8α, or hamster antimouse CD3-ε mAbs (PharMingen, San Diego, CA). To detect B cells, rat antimouse B220 (CD45RA; TIB146, ATCC) was used and was identified by FITC-conjugated affinity-purified goat antirat IgM (Jackson Immunoresearch, West Grove, PA). Anti–leukocyte-common antigen (CD45; TIB122, ATCC), antimacrophage antibody (F4/80, HB198; ATCC), anti–heat-stable antigen (HSA, J11D, TIB 183; ATCC), and antibodies directed against DC-restricted markers (33D1, TIB227; ATCC, NLDC-145; and CD11c, N418; kindly provided by Dr. R.M. Steinman, Rockefeller University, New York, NY) were employed to further characterize the lineages of the isolated cells. The expression of certain receptors/adhesins was analyzed using mAb against FcγRII (CD32), CD11b (MAC-1α unit, M1/70; TIB 128, ATCC), and Pgp-1 glycoprotein (CD44, TIB235; ATCC). FITC-conjugated antirat, antihamster, or antimouse antibodies, as appropriate, were used as secondary antibodies. Normal hamster IgG or the appropriate rat Ig isotypes were used as negative controls. Biotin-conjugated mouse antimouse I-$E^{k,d,p,r}$ (PharMingen) was used with FITC-conjugated streptavidin (Jackson) as the secondary reagent. For the latter mAb, biotin-conjugated mouse IgG2a, together with FITC-conjugated streptavidin was used as a negative control. After staining, cells were fixed in 1% paraformaldehyde in saline before flow cytometric analysis was performed in a FACSTAR flow cytometer (Becton Dickinson, San Jose, CA). Five thousand events were acquired for each sample.

Mixed leukocyte cultures. One-way mixed leukocyte cultures in 96-well, round-bottomed microculture plates were performed with variable numbers of γ-irradiated (20 Gy) allogeneic (B10.BR) or syngeneic (B10) liver NPC or splenocytes or DC-enriched populations as stimulators (S). Naive T cell enriched B10 spleen responders (R)

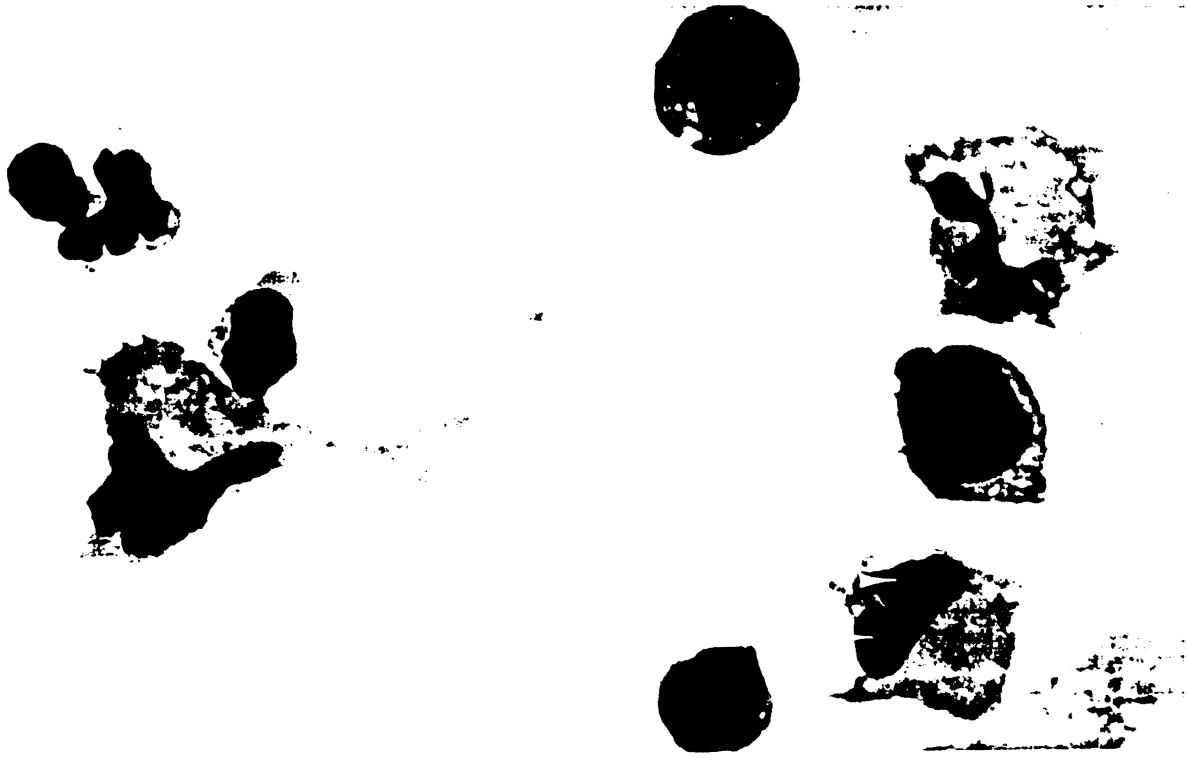

FIGURE 5. Giemsa-stained cytocentrifuge preparations of nonadherent, low-density, liver-derived cells recovered from metrizamide gradients after overnight culture of NPC from normal B10.BR mouse livers. The cells are agranular, with variable degrees of cytoplasmic vacuolation and irregularly shaped, eccentric nuclei. Some cells display distinct cytoplasmic projections. One of the cells (right panel) exhibits prominent nucleoli (arrows) ×1000.

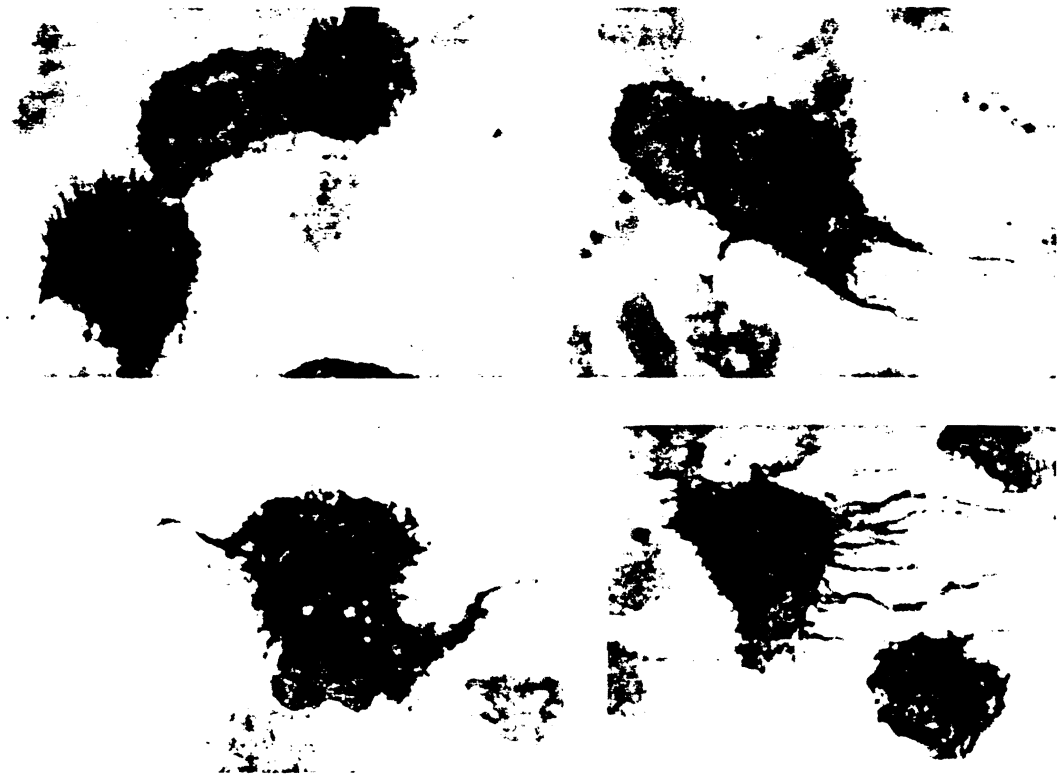

FIGURE 6. Strongly MHC class II$^+$ cells with distinct dendritic morphology present in the nonadherent, LD fraction recovered from overnight-cultured B10.BR mouse liver NPC. Cells were stained using a mAb to I-E^k and the avidin-biotin-peroxidase procedure (counterstained with hematoxylin, ×1000).

(2.10^5 in 100 μl/well) were from normal, naive adult animals. Cultures were maintained in RPMI-1640 with 10% heat-inactivated FBS and 2.10^{-5} M 2-mercaptoethanol for 72 hr in 5% CO_2 in air; for the final 18 hr, 10 μl [^{3}H]TdR (1 μCi) was added to each well. Cells were harvested onto glass fiber disks using a multiple cell harvester, and the degree of thymidine incorporation was determined in a liquid scintillation counter. Results were expressed as mean counts per minute (cpm) ± 1 SD.

RESULTS

Localization of liver DC in situ. Since it has been shown previously that presumptive interstitial DC of rat (*8, 10*), or human (*11*), or mouse liver (*12*) are localized predominantly around portal triads, and since mature DC strongly express MHC class II in situ, we examined cryostat sections of normal B10.BR liver stained for MHC class II (I-E^k) to confirm the presence and localization of cells bearing this marker. As shown in Figure 2, and in keeping with a recent report (*12*), strongly I-E^k-positive cells with dendritic morphology were identified in portal areas. Morphometric analysis revealed that at least 90% of the class II$^+$ cells in liver sections were located in this region.

Isolation and immunophenotypic analysis of hepatic NPC. The number of NPC isolated routinely per normal mouse liver was approximately 1×10^7, with less than 5% hepatocyte contamination on microscopic examination of Giemsa-stained cytocentrifuge preparations. The cell surface phenotype of these cells was characterized by flow cytometric analysis. Figure 3 shows the FACSCAN profile obtained after gating on the entire live cell population. The cells were strongly positive for the leukocyte-common antigen (CD45^{bright}) but MHC class II (I-E^k) was low. They stained both for lymphoid markers (CD3$^-$, B220^{low}, and HSA^{bright}) and the macrophage cell surface antigen, F4/80; they also expressed the following receptors/adhesins: CD11b (MAC-1α unit)$^+$, CD11c^{low}, CD44 (Pgp-1)$^+$, and CD32 (FcγRII). Expression of the DC-restricted marker NLDC145 was also detected but that of 33D1 was low.

Allostimulatory activity of hepatic NPC. The allostimulatory activity of freshly isolated B10.BR (I-E$^+$) liver NPC was examined by culturing variable numbers of these γ-irradiated cells with a fixed number (2×10^5) of naive B10 (I-E$^-$) splenic T cells in 3-day mixed leukocyte cultures. As shown in Figure 4, the liver NPC induced proliferation of allogeneic T cells, and were similar in potency to freshly isolated allogeneic spleen cells. When an equivalent number or excess of NPC over T cells was used, however, the extent of the MLR was diminished.

Enrichment for DC. To enrich for DC, freshly isolated NPC were cultured overnight (18 hr), and the nonadherent, LD fraction was recovered (see Fig. 1 and *Materials and Methods*). This population consisted of approximately 45–50% mononuclear cells with distinct DC morphology. Giemsa-stained cytocentrifuge preparations exhibited cells with irregularly shaped eccentric nuclei, abundant cytoplasm, few cytoplasmic granules, and prominent cytoplasmic projections or "veils" (Fig. 5). Prominent nucleoli could be discerned in many of the dendritic-shaped cells. The degree of cytoplasmic vacuolation was variable; in most cells, there were no prominent vacuoles, while in others variable numbers could be seen.

Immunophenotypic analysis of DC-enriched populations. Immunocytochemical staining of cytospins for MHC class II (I-E^k) revealed that the LD cells with distinct DC morphology were strongly class II–positive (Fig. 6). More extensive immunophenotypic analysis of the DC-enriched cell population was undertaken by flow cytometry (Fig. 7). The cells were CD45^{bright}, and had markedly upregulated their MHC class II expression. They were Thy1.2$^-$, CD3$^-$, CD4$^-$, CD8α$^-$, B220$^-$, HSA$^+$, CD32$^-$, CD11c$^+$, F4/80$^-$, CD44^{dim}, and showed moderate expression of CD11b and of the DC-restricted marker NLDC 145 but were 33D1^{dim}.

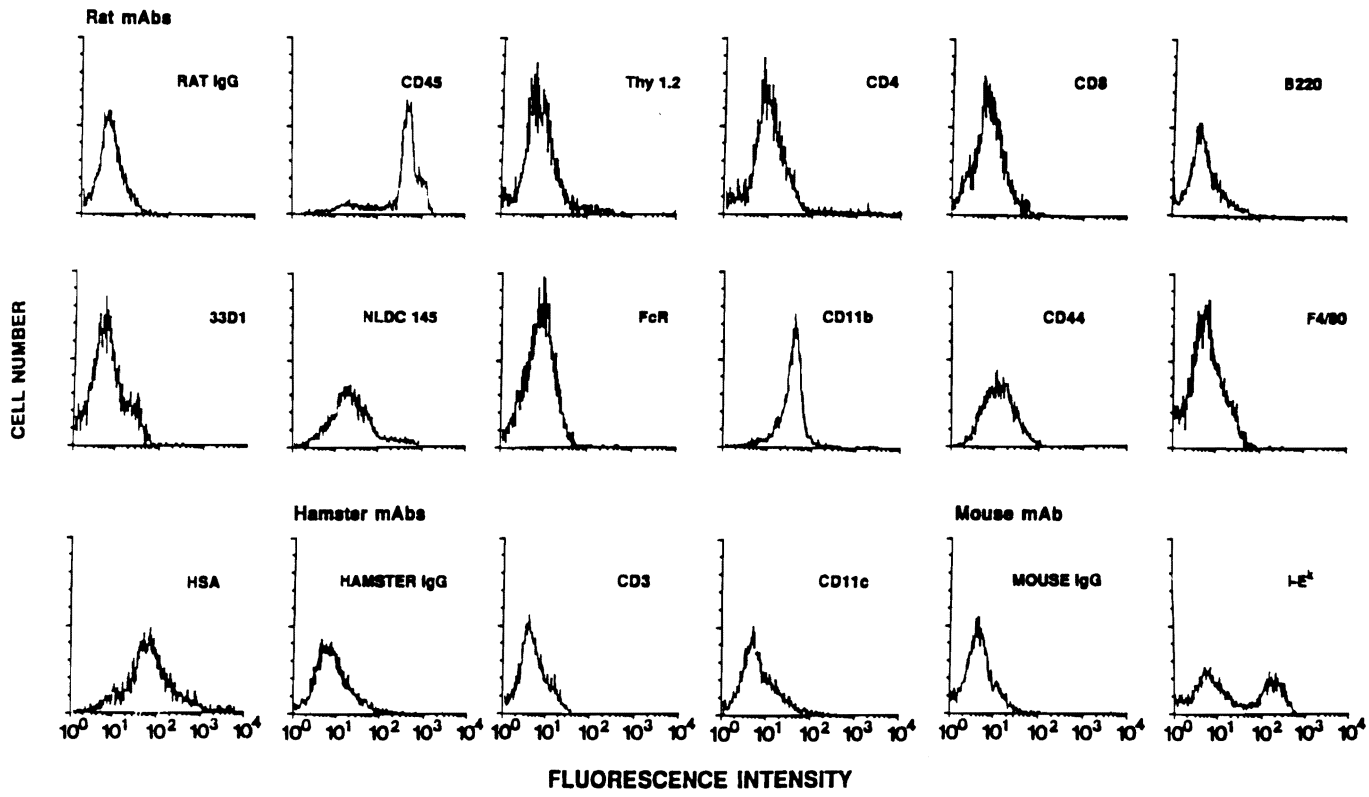

FIGURE 7. Cell surface markers on normal mouse liver DC-enriched cell populations stained by direct or indirect immunofluorescence and analyzed by flow cytometry. Nonadherent LD cells were harvested from metrizamide gradients after overnight culture of NPC from normal B10.BR mouse livers. Five thousand gated events were acquired for each mAb (detailed in Table 1). The results are representative of 3 separate experiments.

Comparative allostimulatory activity of overnight-cultured liver NPC- and DC-enriched populations. To test and compare the allostimulatory activity of the B10.BR (I-E^k) overnight-cultured nonadherent liver NPC and DC-enriched populations, variable numbers of γ-irradiated cells were used as stimulators of a fixed number (2×10^5) of naive B10 (I-E$^-$) splenic T cells in 3 day, one-way mixed leukocyte cultures. For comparative analysis, freshly isolated γ-irradiated B10.BR spleen cells were used as stimulators. As shown in Figure 8, comparatively low numbers of the LD, DC-enriched population were highly effective in inducing proliferation of alloreactive T cells. In contrast, the overnight-cultured HD liver NPCs or bulk NPC exhibited at least 9–27-fold less allostimulatory activity. As with freshly isolated NPC, a progressive reduction in the MLR was observed when an excess of overnight-cultured NPC (bulk cells or HD cells) over responder cells was used. The effect was especially marked with the bulk NPC population.

DISCUSSION

DC residing in the interstitial connective tissue of nonlymphoid organs are important donor-derived leukocytes which, after transplantation, migrate from the graft into the host and initiate primary T cell responses (*20*). The phenotype and functional properties of liver-derived DC have been extensively investigated in both the rat and human (*8–11*). There are, however, no published data on hepatic DC isolated from the mouse.

In these studies, the isolation of NPC from normal mouse liver following collagenase digestion yielded a population that was MHC class II^{dim}, and expressed FcγRII, F4/80, heat-stable antigen (J11d) and CD11b (C3biR), which are classically associated with immature mouse DC isolated from nonlymphoid tissue (*6, 7, 21*). Furthermore, these cells consistently expressed the DC-restricted marker NLDC145, but were 33D1^{low} and CD11c (N418)^{low}. When tested in a primary MLR, the freshly isolated liver NPC were as good as fresh spleen cells in initiating proliferation of naive T cells. These observations are similar to earlier reports that freshly isolated rat liver DC have the capacity to stimulate naive T cells, but only following removal of inhibitory macrophages (*9*). The latter may have accounted for the reduced MLR seen in this study when an excess of liver NPC stimulator cells over splenic responder cells was tested. Similar findings have been made for DC isolated from rat pulmonary tissues (*22*).

Isolation of nonadherent, LD cells after overnight (18-hr) culture of NPC permitted the recovery of cells, many of which had the morphological features of DC (*1, 23*). These included abundant cytoplasm with prominent projections, irregularly shaped nuclei, and few cytoplasmic granules. Approximately 50% of cells in these enriched populations were DC, with some contamination of lymphocytes and a small proportion of macrophages visible in cytocentrifuge preparations. This degree of enrichment is similar to that reported by Lautenschlager et al. (*24*), who analyzed rat liver DC after collagenase digestion of the tissue and enrichment by density gradient centrifugation. In keeping with findings on murine DC isolated from other sites, the liver DC-enriched population was strongly positive for CD45 and showed apparent upregulation of MHC class II expression with concomitant downregulation of FcγRII, F4/80 and CD11c. These phenotypic changes are consistent with earlier observations re-

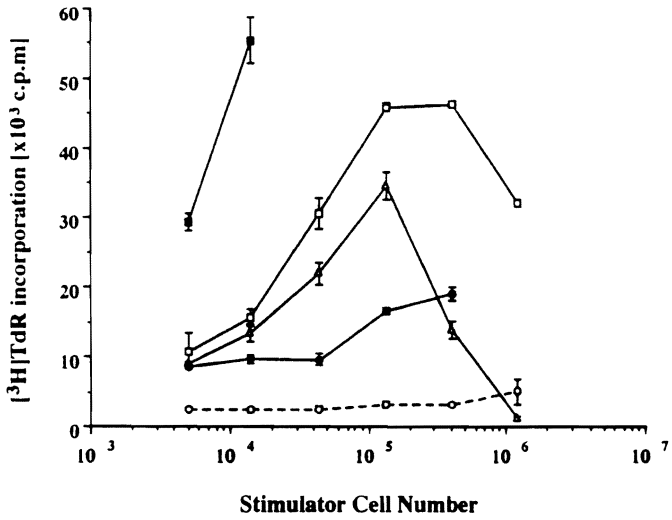

FIGURE 8. Allostimulatory activity for 2.10^5 naive B.10 (H-2^b) mouse splenic T cells, of variable numbers of γ-irradiated, overnight-cultured NPC or DC-enriched stimulator cell suspensions prepared from normal B10.BR (H-2^k) mouse liver. Cells were cultured for 72 hr; [^{3}H]TdR was added to the cultures 18 hr before harvesting. Results are mean cpm ± 1 SD and representative of 3 separate experiments. (■) DC-enriched cell population (nonadherent LD NPC population after overnight culture); (□) HD NPC population after overnight culture; (△) bulk NPC; (●) fresh B10.BR spleen cells; (○) unstimulated splenic T cells. Syngeneic (B10) liver NPC stimulators gave results almost identifical to those obtained with unstimulated T cells.

garding the in vitro maturation of murine Langerhans cells (*6*). Furthermore, the change in MHC class II—but not FcγRII, F4/80, and CD11c—expression after overnight culture has also been reported for DC isolated from other mouse nonlymphoid organs (*7*). Similar to freshly isolated NPC, the liver DC expressed NLDC 145, although not 33D1. The DC were positive for CD11b, CD44 (Pgp), and heat stable antigen (J11D), in common with the phenotype described recently for DC cultured from mouse blood (*25*).

Previously, differential antigen expression by DC isolated from mouse tissues—including spleen, thymus, and skin—has been described (*26*). Thus, most splenic DC, which are considered representative of mature cells, express 33D1 but not NLDC 145, whereas freshly isolated Langerhans cells (considered examples of immature DC) are 33D1$^-$, NLDC 145$^-$. The mouse liver NPC and DC, therefore, share similar characteristics with freshly isolated and cultured Langerhans cells, respectively.

In addition to strong MHC class II expression, the liver-derived DC-enriched cell population was highly active in stimulating allogeneic T cells. They were at least 9-fold more potent compared with freshly isolated NPC, nonadherent bulk or HD cells, or freshly isolated spleen cells. Freshly isolated liver NPC, and perhaps their counterparts in vivo, may be less mature and acquire more potent allostimulatory activity in culture. These findings are similar to evidence that freshly isolated DC populations from other nonlymphoid tissues—i.e., the skin, heart, kidney, and lung—acquire stimulatory function after a 24-hr period of culture (*6, 7, 21*).

These observations are not inconsistent with the presence within the liver of immature DC—which, as speculated recently by Steinman et al. (*28*), may be deficient in initiating immunity or may even be tolerogenic. We are examining this possibility and have recently propagated GM-CSF-stimulated DC progenitors (MHC class II$^{-/dim}$) from normal B10.BR mouse liver that fail to stimulate alloreactivity in vitro (*29*). Unlike skin, this inherent inability to propagate mature DC from liver in response to GM-CSF might reflect, in the mouse, the heterogeneity of the DC population residing in different nonlymphoid organs. It is conceivable that the ease with which the liver can be transplanted across MHC barriers in the mouse might reflect this heterogenicity or the relative abundance of DC progenitors with postulated tolerogenic potential.

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REVERSAL OF CHIMERA DONOR-TO-HOST TOLERANCE IN A TOLEROGEN-FREE ENVIRONMENT

EVIDENCE OF A NONDELETIONAL MECHANISM[1]

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Clonal deletion of self-antigen–reactive T cells is known to be a dominant mechanism for tolerance induction in animals with a normal immune system. This phenomenon is mediated intrathymically by macrophages and dendritic cells. Some recent data have shown that tolerance to antigens expressed on radioresistant thymic stromal cells results in clonal anergy. This report considers tolerance to host antigens in murine H-2-incompatible chimeras (H-2d→H-2k) where thymic stromal cells remained of the host origin while virtually all lymphoid cells were replaced by donor H-2d cells. To assess the mechanism responsible for donor-to-host tolerance induction and the possible role of tolerogens in this process, we transferred (H-2d→H-2k) chimeric lymphoid cells into lethally irradiated H-2d mice (a murine environment free of host H-2k antigens). Engrafted chimeric cells restored immunocompetence of secondary recipients without inducing a graft-versus-host reaction. H-2k skin test-grafts performed four weeks later were acutely rejected (median survival time = 9 days versus 11 days in controls). These results indicate that (A) donor-type lymphocytes reactive to host antigens in (H-2d→H-2k) chimeras are not deleted during tolerance induction; (B) the continuous presence of the H-2k tolerogens appears to be necessary for the maintenance of nonreactivity to these tolerogens; (C) the anamnestic-like response to the H-2k skin grafts suggests that, during tolerance induction, anti-host (anti-H-2k) memory cells developed, an interpretation consistent with the concept that tolerance can result from a powerful immune response.

Induction of tolerance can be achieved in adults using various protocols combining immunosuppression and administration of donor antigens. Improved understanding of the mechanisms of induction and maintenance of tolerance resulted from the availability of monoclonal antibodies to T cell

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