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DENDRITIC CELL PROGENITORS, mouse, liver, MIGRATION, CHIMERISM

PROPAGATION OF DENDRITIC CELL PROGENITORS FROM MOUSE LIVER AND THEIR IN VIVO MIGRATION TO T-DEPENDENT AREAS OF ALLOGENEIC LYMPHOID TISSUE

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We have recently succeeded in propagating dendritic cell (DC) progenitors from non-parenchymal cells harvested from normal mouse liver.[1] The cytokine granulocyte/macrophage colony stimulating factor (GM-CSF) was used to induce cell proliferation, using a modification of methods described by Inaba etal[2] for propagation of DC from mouse blood. The liver-derived cells were found to express DC-restricted cell surface phenotypic markers, but were avidly phagocytic, - a characteristic of immature

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DC, and were resistant to the induction of strong MHC class II antigen expression and allostimulatory activity.[1]

A well-recognized property of DC resident in non-lymphoid tissue is their capacity to migrate (or "home") to T-dependent areas of secondary lymphoid tissue[3,4] and therein to present antigen to T cells. We have compared directly the capacity of mouse liver-derived DC progenitors to home to the spleen of allogeneic recipients with that of similarly propagated mouse spleen-derived DC. The results suggest a possible link between hepatic tolerogenicity and the presence, within the liver, of DC progenitors (MHC class $II^{dim/-}$) that can proliferate rapidly, migrate to T-dependent areas of secondary lymphoid tissue of unmodified allogeneic recipients, and therein express MHC class II. These findings provide insight into the establishment of donor cell chimerism following liver transplantation.

MATERIALS AND METHODS

Animals

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 and were maintained in the specific pathogen-free facility of the University of Pittsburgh Medical Center.

Isolation of non-parenchymal cells (NPC) from normal mouse liver

B10.BR mouse livers were perfused in situ with Hank's balanced salt solution (HBSS), then 2 ml collagenase solution (Sigma, St. Louis, MO; type IV, 1mg/ml in HBSS) was injected via the portal vein. The liver was excised immediately, diced into small pieces and digested in collagenase solution (20ml/liver) for 30 min at 37°C, with constant

stirring. The digested tissue was filtered through sterile nylon mesh (0.1mm). Cells from 2-4 livers were pooled. The washed cell suspension was resuspended in 7 ml sterile, self-generating Percoll solution (Sigma; 1.130 relative density) and centrifuged at 4°C for 10 min at 39,000g. The top layer of cells, containing intact hepatocytes and hepatocyte fragments was removed and discarded. The cells between the upper and lower (erythrocyte) layer constituting the non-parenchymal cell population were harvested and washed twice. Control spleen cell populations were prepared using the same protocol.

Culture of Cells with GM-CSF

Liver NPC or spleen cells (2.5 x 10^5/well) were cultured in 24-well plates in 2 ml of RPMI-1640 complete medium, supplemented with 10% FCS and 0.4 ng/ml mouse GM-CSF (R&D Systems, Minneapolis, MN). Non-adherent, low buoyant density DC-lineage cells released from developing clusters were propagated and harvested as described[1], using a modification of the method of Inaba etal.[2] Cells were harvested after 7-10 days. In some experiments, GM-CSF-stimulated liver-derived cells were depleted of Ia^+ cells by complement-dependent lysis, using mouse anti-I-E^k monoclonal antibody (mAb) and low toxicity rabbit complement.

Immunophenotypic analyses

An extensive panel of mAbs, including antibodies directed against mouse DC-restricted markers (33D1, TIB227; ATCC, NLDC-145; and CD11c, N418) and MHC class II (I-$E^{k,d,p,r}$) was used to characterize the cells by both flow cytometric analysis of cell suspensions and immunocytochemical staining of cytocentrifuge preparations.

Dendritic Cell Homing

Non-depleted or Ia^+-depleted cultured B10.BR liver- or spleen-derived cells were washed in RPMI-1640 and injected s.c. (1 or 2.5 x 10^5 cells in 50μl) into one hind footpad of i.v. (1 x 10^6 in 200 μl) via the lateral tail vein of normal B10 mice. One to 5 d later, the draining popliteal lymph node (where appropriate) and spleen were removed, embedded in Tissue-Tek® (O.C.T. Compound, Miles Inc., Elkhart, IN) and frozen at -70°C. Cryostat sections were fixed in acetone before immunoperoxidase staining, using an avidin-biotin-peroxidase procedure.

RESULTS

Growth and Immunophenotype of Liver DC Progenitors

Non-adherent cells with typical dendritic shape harvested after 7 days of culture in GM-CSF expressed surface antigens associated with mouse DC, including CD45, heat stable antigen, ICAM-1, CD11b (MAC-1) and CD44. Staining of weak to moderate intensity was observed for the DC-restricted markers NLDC-145, 33D1 and N418 and for F4/80 and FcγRII. Liver-derived cells however, expressed only a low level of MHC class II ($I\text{-}E^k$) surface antigen molecule. GM-CSF-stimulated spleen cells propagated under the same conditions expressed a similar range and intensity of markers, except that they strongly expressed $I\text{-}E^k$ (Table 1).

Allostimulatory Activity by GM-CSF-Propagated Liver-Derived Cells

Compared with GM-CSF-stimulated spleen cells (MHC class II^{bright}) propagated and harvested under the same conditions, the cultured, liver-derived cells (MHC class $II^{dim/-}$) did not induce proliferation in naive, allogeneic splenic T cell (B.10; $I\text{-}E^-$) populations.

Similar observations were made whether Ia^{dim} or Ia-depleted liver-derived cells were examined.

Homing of Liver-Derived Developing DC

Liver-derived cells propagated in GM-CSF migrated after injection almost exclusively to the T-cell areas of recipients' spleens, where they were located in close proximity to arterioles (Fig. 1). Similar observations were made in the draining (popliteal) lymph nodes of footpad-injected mice (data not shown). Strong $I\text{-}E^k$ expression was detected on the liver-derived cells, many of which exhibited distinct dendritic morphology. Five days after injection, the liver-derived DC in the recipient's spleen were more abundant than spleen-derived DC observed in spleens of separate animals injected only with the latter cells. Similar findings were made when the injected liver-derived cells had been depleted of all Ia^+ cells, although the incidence of positive cells in recipients' spleens was reduced.

DISCUSSION

Survival and apparent maturation of cultured liver DC progenitors homing to T-cell dependent areas of allogeneic lymphoid tissue is consistent with the view[5,6] that the destination of cells, migrating after whole organ transplantation, is lineage-specific, following the same routes taken by syngeneic cells of the same lineages. After transplantation of the liver or other organs, immature cells of DC lineage may undergo maturation under the influence of endogenous GM-CSF and other cytokines, in a manner similar to that described for cultured cells.[2,7,8] The kinetics of this maturation process are likely to be influenced by the level of host immunosuppressive therapy, but

the general pathways have been shown to be much the same in rats treated with FK 506.[5] The possible tolerogenic implications of the rapid egress from transplanted whole organs (the liver above all) of postulated precursor or progenitor DC, exhibiting poor cell surface MHC class II expression and low T-cell stimulating activity, are considerable. Recently, the existence of subpopulations of murine DC with a veto function has been proposed.[9] Moreover, HLA-DR^{dim} allogeneic donor bone marrow cells, shown to exhibit veto cell activity (inactivation of T-helper cells or cytotoxic T-cell precursors) have been postulated to be immature DC.[10] The precise basis of DC-T-cell interactions leading to tolerance induction is uncertain, but would clearly depend on the relative affinity or avidity (compared with effective APC) of the donor DC-TCR interactions and on the expression, on the former cells, of adhesins and costimulatory molecules, such a B7/BB1. These aspects of developing liver and also bone marrow-derived DC are under further investigation in our laboratory.

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FIGURE LEGENDS

Fig 1. Development and isolation of mouse liver-derived dendritic cell (DC) progenitors in liquid cultures supplemented with GM-CSF. (a), an early aggregate of proliferating putative DC progenitors (day 4) attached to strongly adherent macrophages and showing typical, loosely-adherent cells (arrows) that were released from the aggregates. x 100. (b), Giemsa-stained cytocentrifuge preparation, showing released cells (day 6) which exhibit irregular-shaped, eccentric nuclei, variable degrees of vacuolation, absence of prominent cytoplasmic granules and distinct cytoplasmic processes. x 600. Reproduced with permission of the Rockefeller University Press.

Fig 2. Migration of GM-CSF-stimulated B10.BR liver-derived DC progenitors ($I\text{-}E^{dim/-}$) to T-dependent areas of allogeneic spleen. The cells were injected into one hind footpad of B10 ($I\text{-}E^{-}$) recipients and detected by immunohistochemistry, 1-5 days later. Strongly MHC class II ($I\text{-}E^{k}$)-positive cells are readily identified in close proximity to arterioles. x 400. Inset: dendritic morphology is evident in a high power view of a donor liver-derived cell in the recipient's spleen. x 1000. Reproduced with permission of the Rockefeller University Press.

Table 1. Immunophenotypic characteristics of non-adherent, GM-CSF stimulated mouse liver-derived DC progenitors harvested from liquid cultures: comparison with GM-CSF stimulated spleen-derived DC

Antigen (mAb)	Liver DC progenitors	Spleen-derived DC
Leukocyte Common Ag		
CD45	++	++
CD45RA;B220	-	-
MHC		
Class II; I-$E^{k.d.p.r}$	-/dim	+++
DC-restricted		
Lymphoid DC (33D1)	+	+
Interdigitating Cell (NLDC 145)	+	+
Myeloid (primarily)		
Macrophage (F4/80)	+	+
Lymphoid (primarily)		
$CD3_{\epsilon}$	-	-
Heat stable antigen (J11D)	++	++
NK cells		
NK1.1	-	-
Receptors/adhesins		
CD32, FcγRII	+	+
CD11b, MAC-1α unit; C3biR	++	+
CD11c, p150/90 (N418)	+	ND
CD44, Pgp-1	++	++
CD54, ICAM-1	++	++
CD25, p55; IL-2R	-	-

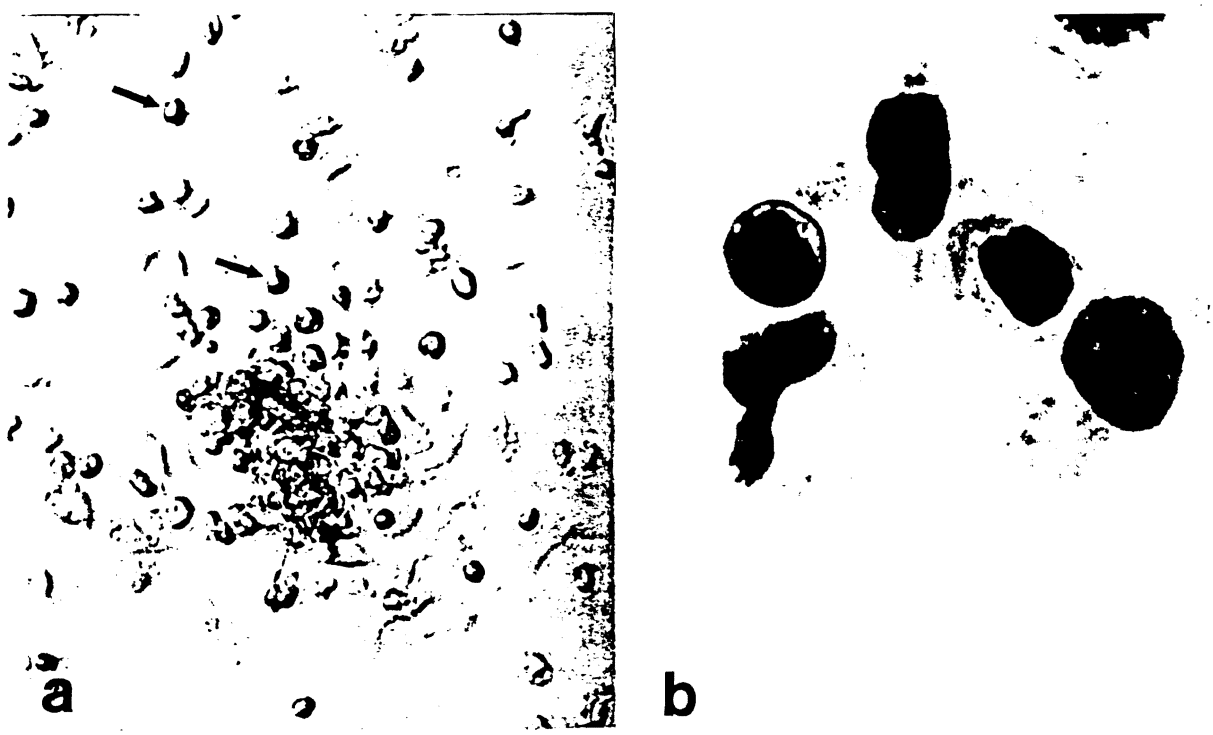

Figure 1

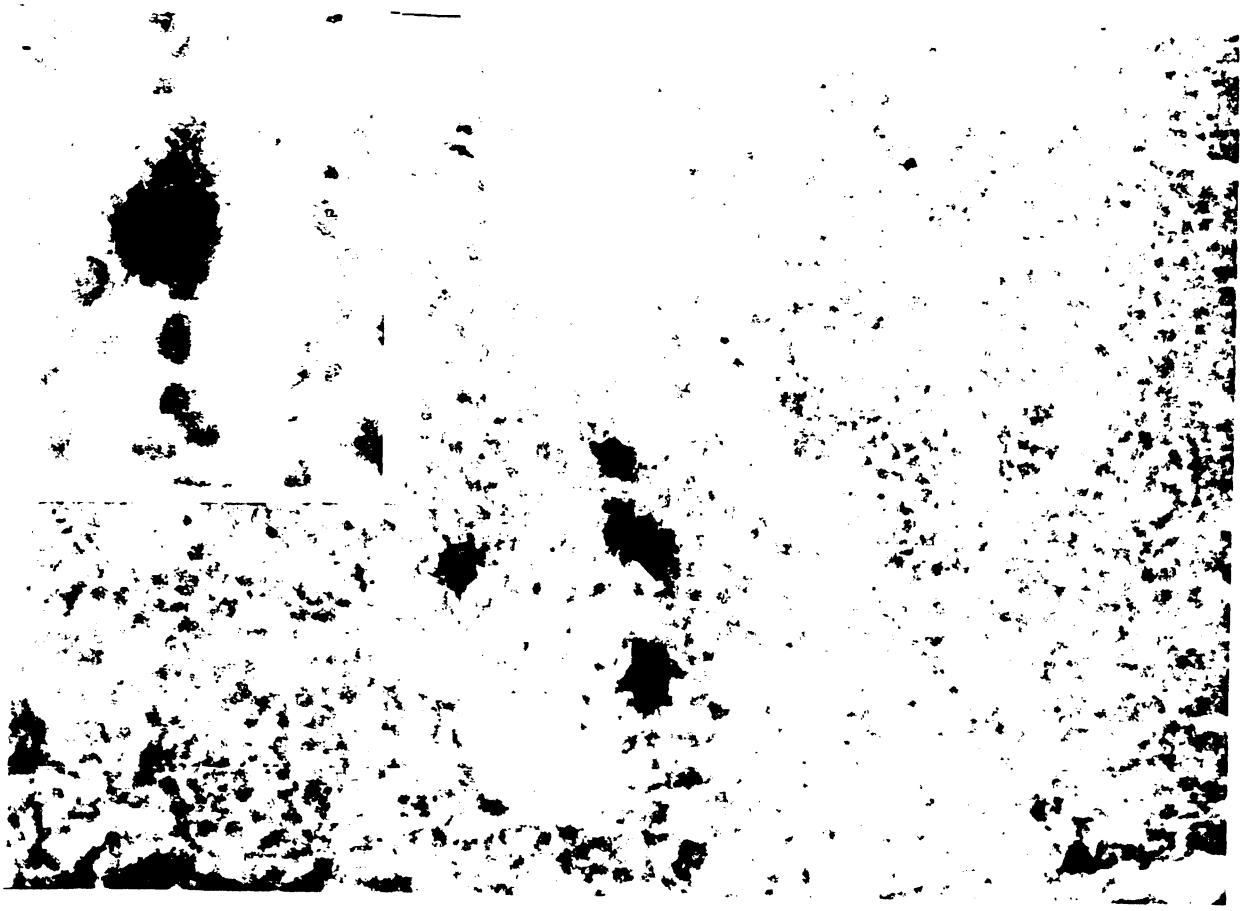

Figure 2