A Novel Migration Pathway for Rat Dendritic Cells from the Blood: Hepatic Sinusoids-Lymph Translocation

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Summary

The migration pathways for dendritic cells (DC) from the blood are not yet completely resolved. In our previous study, a selective recruitment of DC progenitors from the blood to the liver was suggested. To clarify the role of the hepatic sinusoids in the migration of blood DC, relatively immature DC and mature DC were isolated from hepatic and intestinal lymph, and intravenously transferred to allogeneic hosts. It was then possible to detect small numbers of DC within secondary lymphoid tissues either by immunostaining for donor type major histocompatibility complex class I antigen or, at much higher sensitivity, for bromodeoxyuridine incorporated by proliferating cells (mainly T lymphocytes), which responded to the alloantigen presented by the administered DC. The intravenously injected DC accumulated in the paracortex of regional lymph nodes of the liver via a lymph-borne pathway. Intravenously injected fluorochrome-labeled syngeneic DC behaved similarly. In contrast, very few DC were found in spleen sections and were hardly detectable in other lymph nodes or in other tissues. An in situ cell binding assay revealed a significant and selective binding of DC to Kupffer cells in liver cryosections. It is concluded that rat DC can undergo a blood-lymph translocation via the hepatic sinusoids, but not via the high endothelial venules of lymph nodes. Hence the hepatic sinusoids may act as a biological concentrator of blood DC into the regional hepatic nodes. Kupffer cells may play an important role in this mechanism.

The traffic as well as the significance of dendritic cells (DC) in the blood are not yet completely resolved (1). When particulates are injected intravenously, particle-laden DC appear initially in the blood marginating pool and then in the peripheral lymph draining the liver (2). These cells are proven to be relatively immature DC with a temporary phagocytic activity (2). These and other observations suggest that DC progenitors may be selectively recruited to the liver after intravenous injection of particulates (3), and that they subsequently translocate from the liver vasculature to the draining hepatic LNs. There have been few other reports concerning the involvement of the liver vasculature in the recruitment and translocation of cells of the DC lineage. After intravenous administration of radiolabeled mouse splenic DC (4) or of rat lymph DC (5) to syngeneic hosts, a high radioactivity was found not only in the spleen but also in the liver. Although the accumulation of DC in the liver was not explained in these reports, it may imply a specific affinity of DC for the liver vasculature. Fossum (5) noticed subsequent migration of DC to the celiac nodes, but only mature DC were administered and the significance of this pathway was unclear.

The aim of the present study is to understand the role of the hepatic sinusoids in the traffic of the rat DC lineage from the blood. Paramagnetic latex-laden DC in the hepatic lymph were isolated and used as relatively immature DC (2). Mature DC of either hepatic or intestinal lymph (6) were also examined. Since a trace number of DC was difficult to detect in host tissues, the isolated DC were intravenously transferred to allogeneic hosts. DC were then traced immunohistologically on tissue sections by immunostaining for either donor type MHC class I molecules or for 5-bromo-2′-deoxyuridine (BrdU) incorporated by proliferating cells that had responded to the alloantigen presented by the donor DC. An in situ cell binding assay (7, 8) was also performed to examine the capacity of DC to bind to the liver vasculature; the samples were further immunostained with some cell markers to reveal whether or not DC bind to a specific cell type.

Abbreviations used in this paper: BrdU, 5-bromo-2′-deoxyuridine; DC, dendritic cells.

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Materials and Methods

Animals. Inbred male DA rats (RT1a) were supplied by the Laboratory Animal Center for Experimental Research (Kumamoto University School of Medicine, Kumamoto, Japan). Lewis rats (RT11) were purchased from Sekisui Yosohito, Ltd. (Fukuoka, Japan). Both were reared under specific pathogen-free conditions.

Antibodies. Mouse mAbs specific for rat determinants, including antibodies against CD2 (OX34), TCR-αβ (R73), macrophage-related antigens (ED1, ED2, and ED3), and a polymorphonuclear MHC class I of DA rat (anti-RT1A\(^a\), M\(_{94,41}\)I), were obtained from Sera-Lab Ltd. (Crawley Down, Sussex, UK). mAbs to a pan-B cell marker (reference 2; HIS14; provided by Dr. F.G.M. Kroese, University of Groningen, Groningen, The Netherlands) and IgM (MARM-4; provided by Dr. H. Basin, Louvain University, Brussels, Belgium) were donated. An mAb against BrdU was purchased from Novocastra Laboratories Ltd. (Newcastle upon Tyne, UK).

Isolation of DC. Collection of DC from either the hepatic or the intestinal lymph has been described in detail (2,6). Briefly, the cells were collected from central thoracic duct lymph of either hepatic or mesenteric lymphonectomized rats. Immature DC that had ingested paramagnetic latex (0.8\(\mu\)m diameter, 0.5 ml/200 g body wt; L0898; Sigma Chemical Co., St. Louis, MO) in the blood were isolated from the hepatic lymph after intravenous administration of particulates (2). Lymph cells were treated with mitomycin C before isolation. The purity of latex-laden DC in the blood were isolated from the hepatic lymph after intravenous administration of particulates (2). Lymph cells were treated with mitomycin C before isolation. The purity of latex-laden DC in the blood were isolated from the hepatic lymph after intravenous administration of particulates (2).

Isolated DC from DA rats were intravenously transferred to hosts. Host rats were killed 3 d after cell transfer. Cryosections of the tissues were examined for presence of fluorescent cells under a fluorescence microscope at a wavelength exciting H33342 (365 nm).

Figure 1. RT1A\(^a\) immunostaining of cytosmeans of paramagnetic latex-laden DC from hepatic lymph of a DA rat. More than 95% of isolated latex-laden DC were RT1A\(^a\) (blue) with clearly recognizable particles in their cytoplasm. \(\times 1,300\).

Figure 2. Accumulation of latex-laden DC in the celiac LN 1 d after cell transfer. Double immunostaining of RT1A\(^a\) (blue) and CD2 and TCR-αβ (brown); (a) under low magnification, RT1A\(^a\) cells are localized in the paracortex (PC), which are populated with host T cells (brown), F; lymph follicle in the superficial cortex. (b) At higher magnification of the paracortex, four RT1A\(^a\) cells with dendritic cytoplasmic processes are clustering with host T cells (brown). Most of them contain recognizable particles in their cytoplasm (arrows). (a), \(\times 115\); (b), \(\times 730\).

Figure 3. Proliferative response in the celiac LN 3 d after cell transfer detected by BrdU labeling. Compared to normal state, (a) \(10^5\) unseparated cells did not induce a significant increase in the number of BrdU\(^+\) cells (red), but (b) \(10^5\) latex-laden DC induced a significant proliferation (see also Fig. 3) in the paracortex (PC). G; germinal center in the superficial cortex. \(\times 115\).

Figure 8. Phenotype of proliferating cells 3 d after transfer of 3 \(\times 10^5\) allogeneic DC. Paracortex near the medulla of the celiac LN's. Double immunostaining of CD2 and TCR-αβ (blue) and BrdU (red). Note proliferating cells were mainly T cell lineage (double positive cells). \(\times 520\).

Figure 10. In situ cell binding assay followed by double immunostaining with RT1A\(^a\) (blue) and CD2 (brown). (a) Under lower magnification, DC (RT1A\(^a\)) show a distribution pattern similar to that of Kupffer cells (ED2\(^+\)) in the liver lobules. (b) At higher magnification of the liver lobule examined under a differential interference microscope, associations of bound DC with Kupffer cells are frequently observed (arrows). Ratio of RT1A\(^a\) cells associated with ED2\(^+\) cells to those without the association was 2.3–4.0. (a), \(\times 64\); (b), \(\times 250\).
numbers of allogeneic cells, $10^4$ to $10^6$ for latex-laden DC, or $10^5$ to $10^6$ for unseparated cells, were intravenously transferred to hosts. The celiac LN s were examined 3 d after cell transfer. For the time kinetics study, $3 \times 10^5$ of allogeneic latex-laden DC were intravenously injected into hosts. Immediately (0 d), 1, 2, 3, 4, 5, and 7 d after cell transfer, the celiac LN s were examined. To examine cell phenotypes, mAb cocktails to CD2 and TCR-αβ for T cells; pan-B and IgM for B cells; or ED1, ED2, and ED3 for macrophages were used. The celiac LN s were removed 3 d after intravenous transfer of $10^6$ allogeneic latex-laden DC, and double immuno-staining of cell marker (blue) and BrdU (red) was performed as described (11). The number of double-positive cells was counted with respect to the three structural domains of the celiac LN s, namely, the superficial cortex, paracortex, and medulla, and the ratio of BrdU$^+$ T cells, BrdU$^+$ B cells, and BrdU$^+$ macrophages in each domain was estimated.

In situ Cell Binding Assay. Binding of DC to frozen sections was studied with a slight modification of previous reports (7, 8). Briefly, unfixed cryosections (6 μm) of liver and other tissues of either Lewis or DA rats were air dried for 1–4 h. Either latex-laden DC, mature DC, or unseparated cells from lymph of DA rats were resuspended in RPMI 1640 containing 5% FCS and 5 mM Hepes at a concentration of 10^6/ml. Cryosections were overlaid with 50 μl of cell suspension/section and incubated horizontally at 37°C in a humidified incubator for 30 min. Cell suspensions were then carefully aspirated, washed gently, and samples were fixed in formal calcium solution (2) for 3 min and fixed further with 1% glutaraldehyde in PBS for 1 min. Some sections were directly examined under a differential interference light microscopy. Other sections were double immunostained with RT1A$^+$ (blue-black) and ED2 (brown) to detect allogeneic DC and Kupffer cells (2), respectively, as described (6).

Results

Distribution of Transferred DC. One cytosmear $>95%$ of isolated paramagnetic latex-laden DC of DA rats were RT1A$^+$ with clearly recognizable latex particles in their cytoplasm (Fig. 1). 1 d after intravenous transfer of $10^6$ latex-laden DC to allogeneic hosts, RT1A$^+$ cells located mainly in the paracortex of both celiac (Fig. 2) and parathymic LN s, the number being 66.7 ± 30.4 and 17.4 ± 9.9 cells/section, respectively. Both celiac and parathymic LN s are regional LN s of the liver (2, 9). At an early stage after cell transfer (12 h), donor DC were already found in the marginal sinus and interfollicular area of the celiac LN s. The numbers of donor DC quickly declined by 2 d and almost disappeared by 3 d. Comparable numbers of mature allogeneic DC of either hepatic and intestinal lymph were also found in the regional hepatic LN s (not shown). In contrast, very few DC were found in the spleen (approximately one cell/section), and they were hardly detectable in other LN s or in other tissues. Syngeneic fluorochrome-labeled DC were easily detected by their blue fluorescent nuclei under a fluorescence microscope. They accumulated mainly in the hepatic LN s (35.5 ± 18.1 cells/section) of the celiac LN s in a similar manner as allogeneic DC.

Proliferative Response to Transferred Allogeneic DC. After transfer of $10^6$ allogeneic latex-laden DC, a significant increase in host cell proliferation as detected by BrdU-labeling was observed in the celiac LN s, parathymic LN s, and periaortal lymphoid sheath of the spleen, but not in other LN s or other tissues ($P < 0.05$ when compared with $10^6$ syngeneic latex-laden DC) (Figs. 3 and 4). An insignificant but relatively higher proliferative response was seen in the posterior mediastinal LN s, which may be due to the presence of lymphatic communications of these LN s with ascending lymphatics of the liver (2). BrdU$^+$ cells were mostly observed in the paracortex of the LN s (Fig. 5) and within the...
periarterial lymphoid sheath and the terminal arteriolar region in the red pulp of the spleen. The terminal arteriolar region is a protrusion of the periarterial lymphoid sheath and functionally equivalent to the latter (11). Allogeneic mature DC also induced proliferative response similar to allogeneic latex-laden DC (not shown). Allogeneic unseparated cells did not induce a significant proliferative response in most tissues; however, the spleen showed a proliferative response slightly weaker but comparable to that after transfer of allogeneic DC (Fig. 4). The dose–response study of the celiac LNs (Fig. 6) revealed that as few as \(10^5\) allogeneic DC induced significant proliferation, and that the maximum response at 3 d was achieved by transfer of \(3 \times 10^5\) and \(10^6\) allogeneic DC. On the other hand, allogeneic unseparated cells showed much weaker stimulation in that 100 times more unseparated cells were required to induce a similar level of proliferative response as for DC. The time kinetic study of the celiac LNs (Fig. 7) showed a slight increase of BrdU\(^+\) cells at 1 d and a significant proliferation 2–3 d after 3 \(\times 10^5\) allogeneic transfer. The ratio of BrdU\(^+\) T cells, BrdU\(^+\) B cells, and BrdU\(^+\) macrophages was 85:14:1 in the superficial cortex, 91:4:7 in the paracortex, and 82:11:7 in the medulla. This indicates that proliferating cells were mainly of the T cell lineage (Fig. 8).

**In Situ Cell Binding Assay.** Isolated lymph DC showed preferential binding to either allogeneic or syngeneic liver cryosections (Fig. 9) compared with other tissues such as spleen, lung, thymus, and LN. The number of bound DC/mm\(^2\) section in the liver was approximately two to three times more than those in the other tissues. The same concentration of unseparated cells showed less binding to the liver cryosections than DC (Fig. 9 b). The ratio of RT1A\(^+\) cells associated or not with ED2\(^+\) cells was 2.3–4.0, indicating a significant and selective binding of DC to Kupffer cells in the sections (Fig. 10).

**Discussion**

In this study, T cell proliferation was selectively induced in the thymus-dependent area of the regional hepatic LNs after intravenous transfer of allogeneic immature latex-laden DC, but not of allogeneic unseparated cells or syngeneic latex-laden DC. This probably represents a specific immune response against alloantigen presented by transferred DC. Together with the RT1A\(^\ast\) immunostaining study, the results demonstrate a preferential accumulation of allogeneic immature DC in the paracortex of the regional hepatic LNs. Since mature allogeneic DC and syngeneic DC also showed a similar accumulation pattern, this traffic is common among all types of transferred DC examined and not affected by MHC barriers, as for DC traffic to the mouse spleen (4). The absence of transferred DC in LNs other than the regional hepatic LNs and the initial appearance of DC in the marginal sinus of the regional hepatic LNs at an early stage after cell transfer suggest that the cells enter the LN via the afferent lymph after undergoing blood–lymph translocation in the liver. These and other (4) results, as well as the insignificant binding of DC to LN sections, also indicate that direct entry of DC via the high endothelial venules is unlikely. In this respect, DC are considered to lack some cell membrane constituent or receptor that would allow them to interact with the high endothelial venules (4).

This unique migratory pattern of DC in the blood also supports the speculation in the previous study (2) that the particle-laden DC in the hepatic lymph represent cells that have been recruited to the liver and undergone the blood–lymph translocation after phagocytosing the intravenously administered particles. The cells may be recruited as DC...
Hepatic Sinusoids–Lymph Translocation of Rat Dendritic Cells

progenitors, possibly in response to the intravenous particulates (3), and then develop into a phagocytic stage before the translocation event.

The initial appearance of latex-laden DC in the sinusoidal area, but not in the portal or hepatic vein area, after intravenous injection of latex particles (2) implies that the site for the blood-lymph translocation is the sinusoid. Localization of labeled mouse DC in the hepatic sinusoids after intravenous transfer has also been described (4). In mitogen-stimulated mouse liver, accumulation of lymphocytes in the space of Disse and then in the Glisson’s sheath is observed (12). Hence, it is suggested that DC attach to the vessel wall in the sinusoidal area, pass through the space of Disse to the connective tissue stroma of either the portal or hepatic vein area, and then enter the initial lymphatic ducts located there. Taken together, the present study demonstrates that both mature and immature rat DC in the blood preferentially undergo hepatic sinusoids-lymph translocation in an MHC-independent fashion and accumulate in the regional hepatic LNs, and further suggests that autologous blood DC may behave similarly to the transferred DC studied here.

The mechanisms and the factors that regulate this translocation are unknown. Selective translocation may imply that sinusoidal lining cells express certain adhesion molecules by which DC can attach the sinusoidal wall before initiating translocation. The in situ cell binding assay suggests that Kupffer cells in the hepatic sinusoids are capable of selectively trapping DC from the blood. Frequent observations of the close association of DC with Kupffer cells in the liver section in normal steady state and after latex injection (2) also support this idea. The cell transfer and cell binding assays have revealed that this translocation event can occur even in a donor-host combination of normal steady state, suggesting that de novo elaboration of migratory stimuli such as cytokines (13) may not be necessary. On the other hand, Kupffer cell activation might be essential for the recruitment of DC progenitors to the liver, since hepatic lymph under the steady state contain mostly mature DC (6) and the recruitment was induced by intravenous injection of particulate matters (2). In this respect, the accumulation of CFU-spleen in the mouse liver after estrogen treatment is considered to be due to trapping of CFU-spleen by Kupffer cells that have been activated by estrogen, a potent activator of the mouse macrophage lineage (14). Lymphocytes also translocate from the sinusoid to hepatic lymph (15), possibly by similar mechanisms, although an involvement of Kupffer cells is not certain.

The significant proliferative response was induced even when only a trace number of allogeneic DC was detectable.

Figure 9. In situ cell binding assay examined under differential interference light microscopy. DC showed preferential binding to either allogeneic (a) or syngeneic (not shown) liver cryosections compared to other tissues. In spleen (c), DC attached mainly to the marginal zone (Z) but not to the white pulp (W). In LN (d), bound DC were not obviously localized to specific areas. The same concentration of unseparated cells showed less binding to the liver cryosections than DC (b). P, portal area; M, medulla; C, cortex. ×160.
in lymphoid tissues, e.g., in the spleen, where approximately one cell/section was found. In addition, a minimum number (10^5) of intravenous allogeneic DC could induce a significant proliferative response in the regional hepatic LNs of the host. Usually 10^5 transferred cells are difficult to trace in vivo because they are diluted after entering the systemic circulation. Therefore, the result demonstrates that the proliferation assay is a very sensitive technique for detecting not only the presence of an immune response, but also the presence of a trace number of antigen presenting cells, and that blood DC are very effectively concentrated in the regional hepatic LNs. In other words, the hepatic sinusoids may act as a biological concentrator of DC into regional LNs. In contrast, the proliferative response in the spleen to allogeneic unseparated cells, which was comparable to that of DC, may be due to preferential accumulation of recirculating lymphocytes in the white pulp (4, 5). Together, the result indicates more efficient accumulation of DC in the hepatic LNs than in the spleen, especially when small number were transferred.

The reason for the presence of only few DC in spleen sections in this study compared with the considerable accumulation of radiolabeled DC in other reports (4, 5) is unclear. Since the spleen is much bigger than the hepatic LNs, DC may be more dispersed in the splenic tissues than in the hepatic LN, and therefore, it might be more difficult to find DC in the spleen sections even if total number of DC within the spleen is high. In a preliminary study of rat liver allotransplantation, a significant number of donor DC were observed in the parietal lymphoid sheath of the host spleen within a few days after transplantation (Matsuura, K., S. Kudo, N. Miyani, T. Ezaki, and M. Ogawa, unpublished data). This suggests that resident DC in the liver also migrate to the spleen via the blood if the liver is transplanted to allogeneic hosts as in transplantation of other organs (1), although we cannot examine the hepatic LNs since lymphatic connections are interrupted by surgery. Alternatively, some difference in trafficking of DC to either the spleen or the hepatic LNs may exist among different types of DC. Accumulating evidence concerning heterogeneities of DC (13) may support this possibility. Migration pattern of different types of DC is currently under study.

Fossum (5) found that the labeled DC proceeded from the liver to the celiac LNs via afferent lymphatics, but the significance of this DC translocation was not clear. The present study demonstrates this traffic as a novel and definite migration pathway for rat DC from the blood. The regional hepatic LNs might become an important site for immunoproliferation in response to blood-borne antigen, including gut-derived antigen carried by DC via the portal vein, or to allograft-derived DC as the extrinsic sensitization outside of graft (1). The transfer study would also be useful as a simulation model for studying blood-borne migration kinetics of donor DC from transplants.

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