ELSEVIER

An Endothelial Cell-Derived Chemotactic Factor Promotes Transendothelial Migration of Human Dendritic Cells

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DENDRITIC CELLS (DC) are specialized antigen presenting leukocytes that take up antigens within peripheral tissues and migrate via the lymph or blood, to draining lymph nodes or the spleen respectively, where they present processed antigens to T cells and deliver unique activating signals.[1] It is known that DC migration is a regulated process[2] and that inflammatory mediators appear to promote mobilization of DC.[3] Entry of DC into lymph or blood by transendothelial migration (TEM) is a key point at which DC function may be regulated but, in contrast to other leukocytes, little is known about the molecular signals that regulate migration of DC. To define these signals we developed in vitro assays of DC TEM and DC-endothelial cell (EC) adhesion and we report that an endothelial cell supernatant stimulates both adhesion and TEM of human cytokine-derived DC.

MATERIALS AND METHODS

Human DC were cultured from buffy coats under the influence of GMCSF and IL-4.[4] Day 5 to 6 cultured cells (typically ~70% DC by MHC class II and CD1a expression) were used. Adhesion was assayed by retention of ^{51}Cr-labelled cytokine-derived cells on monolayers of a human microvascular endothelial cell line (HMEC-1)[5] after a 4 hour incubation at 37°C and washing (×5) to remove nonadherent cells. The phenotype (HLA-DR and CD1a expression) of the adherent cells was determined by flow cytometry after mobilization of the EC-monolayers/adherent cells using EDTA. A variety of cell supernatants or recombinant chemokines were tested in the adhesion assay by coincubation with either the EC monolayer or the cytokine-derived DC, for 16 to 18 hours before performing the assay. For TEM assays, HMEC-1 monolayers were cultured on porous collagen-coated polycarbonate membranes in transwell inserts (Costar, 5 μm pore size). Typically 2×10^5 DC were added to transwell inserts and after 4 hours at 37°C the number and phenotype of cells that had migrated through the EC monolayer under the influence of a variety of supernatants or recombinant chemokines was determined by direct counting, immunocytochemistry, and flow cytometry. Recombinant chemokines (kindly provided by Dr T Schall, DNAX, Palo Alto, Calif) were used at 50 ng/mL. Biological activity at this concentration was confirmed in adhesion or chemotaxis assays using blood leukocytes (PBMC or PMN).

RESULTS

Supernatant from IL-1β-activated EC (aECS) but not IL-1β alone promoted both adhesion and migration of DC. Adhesion was dependent on preincubation of DC but not the EC-monolayer with aECS. DC TEM was also detectable in response to unstimulated EC supernatant but this was quantitatively less potent than aECS. A "checkerboard" analysis, in which aECS was placed above, below or both above and below the EC-monolayer, suggested that the DC response was both chemotactic and chemokinetic. Recombinant chemokines (MCP-1, MIP-1α and β, RANTES, C-10, Eotaxin, IL-8, IP-10, PF-4, MGSA, Lymphotactin) did not promote DC adhesion or migration.

DISCUSSION AND CONCLUSIONS

Endothelial cells are known to produce a variety of chemokines in response to activating stimuli[6] but we have not yet attempted to define which factor(s) in the endothelial supernatant are active on DC. It has been reported that DC respond to a variety of chemokines in chemotaxis experiments[7] but in the absence of endothelium the relevance of this is uncertain. The studies reported here have made use of a transformed endothelial cell line and cytokine-derived DC and require confirmation using primary endothelial cell cultures and a variety of DC populations, isolated directly from tissue or blood.

In conclusion, we developed in vitro models to define key molecular signals regulating human DC migration and demonstrated DC TEM and DC-EC adhesion in response to an EC supernatant. Individual recombinant chemokines did not share this activity, suggesting either that DC require signals from multiple chemokines, or that a novel chemotactic factor may be present in aECS. Ultimately it may be possible to define a unique set of signals which selectively promote (or inhibit) migration of DC and this may suggest new ways in which the initiation and development of primary T and T-dependent immune responses may be controlled or modified (to potentiate vaccination strategies or inhibit rejection following organ transplantation for example).

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0041-1345/97/$17.00
PII S0041-1345(96)00460-5

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