Human monocyte-derived and CD34$^+$ cell-derived dendritic cells express functional receptors for platelet activating factor

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Abstract Dendritic cells (DC) are a heterogeneous population of specialized antigen presenting cells that exhibit complex trafficking properties. DC differentiated in vitro from both peripheral monocytes and CD34$^+$ cells expressed mRNA for platelet activating factor (PAF) receptor. Expression of PAF receptor was increased by TNFα, a prototypic inflammatory cytokine that induces differentiation and in vivo mobilization of DC. PAF induced in vitro directional migration of DC obtained from both precursor cells through its specific receptor. Since DC are highly motile cells, protein chemoattractants as well as bioactive phospholipids are likely to contribute to tissue localization of DC, in vivo.

Key words: Dendritic cell; Platelet activating factor receptor

1. Introduction

Dendritic cells (DC) are bone marrow-derived leukocytes specialized in antigen uptake, processing, and presentation to T lymphocytes. DC are believed to be indispensable for the initiation of a primary immune response [1,2]. Recently, techniques that allow the production of large quantities of DC, in vitro, were developed. DC cells can be derived from either CD34$^+$ precursor cells (CD34-DC) or from circulating monocytes (mono-DC) by culturing them in the presence of a mixture of inflammatory cytokines and growth factors [3,4]. Although these cells share the key functions of specialized antigen presenting cells, an extensive comparison of DC derived from different precursors has yet to be performed [4,5].

It has been known for a long time that DC are highly motile cells. DC progenitors enter the blood and seed non-lymphoid tissues, where they develop into immature DC, with high ability in antigen uptake and processing, and yet low ability in T cell stimulation. Locally produced inflammatory cytokines (e.g. TNF and IL-1) and the encounter with an antigen promote the maturation and migration of DC to regional lymph nodes via afferent lymphatics, where they present the antigens to unprimed T lymphocytes. Alternatively, DC can migrate to the spleen through the blood [1,6]. A number of peptide chemotactic factors induce directional migration of DC, in vitro. These include 'classical' chemotactic factors, such as formylated peptides and C5a and a selected pattern of CC (RANTES, MIP-1α, MIP-1β, MIP-3α, MIP-5/HCC2, MCP-3, and MDC) and CXC (SDF-1) chemokines [7–11]. In a limited series of experiments, the chemotactic response of mono-DC and CD34-DC was comparable [8]. However, only CD34-DC, but not mono-DC, expressed the chemokine receptor CCR6 and migrated in response of MIP-3α, its ligand [11,12].

In addition to proteins, some lipids/phospholipids are known to be potent chemotactic factors, in vitro and in vivo [13–16]. No data are so far available on lipid chemotactic factors for DC. The goal of the present study was to investigate whether platelet activating factor (PAF), a bioactive phospholipid that plays a key role in leukocyte extravasation [14–16], could induce directional migration of mono-DC and CD34-DC, in vitro.

2. Materials and methods

2.1. Reagents

Human recombinant RANTES was from PeproTech Inc. (Rocky Hill, NJ, USA). Human recombinant GM-CSF and TNFα were a generous gift from Sandoz (Basel, Switzerland), and BASF (Knoll, Germany), respectively. Cytokines were endotoxin free as assessed by the Limulus amebocyte assay. Platelet activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphochokine; cat. no. P9525) was from Sigma (St. Louis, MO, USA). WEB 2187 and 1-*O*-octadecyl-2-acetyl-(*S*)-glycero-3-phosphocholine (S-PAF) were from Boehringer Ingelheim KG (Ingelheim am Rhein, Germany).

2.2. DC culture

Highly enriched blood monocytes (>95% CD14$^+$) were obtained and purified from buffy coats as previously described and cultured for 7 days in RPMI (Biochrom, Berlin, Germany), 10% FCS (Hyclone, Logan, UT, USA) supplemented with 50 ng/ml GM-CSF and 10 ng/ml IL-13. These cells (mono-DC) were >80% CD1a$^+$, >90% MHC class II$^+$, <10% CD14$^+$, <2% CD3$^+$ and <4% CD20$^+$ [3,7–9]. CD-34$^+$ cells were purified from cord blood using Minimacs columns (Milteny Biotec, Germany). CD-34$^+$ cells were then cultured in RPMI 1640, 10% FCS in the presence of 50 ng/ml GM-CSF, 10 ng/ml TNFα, and 50 ng/ml stem cell factor (Amgen Biological, Thousand Oaks, CA, USA). After 14 days of culture, cells (CD34-DC) were 90% CD1a$^+$, 95% MHC class II$^+$, 81% PAM-1$^+$, and 85% CD80$^+$ [4].

2.3. Northern blot analysis

Total RNA was extracted by the guanidinium thiocyanate method, blotted and hybridized as described [8,9]. PAF receptor cDNA [17] was labeled with the Megaprime DNA labeling system (Amersham, Buckinghamshire, UK) with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham) as previously described [8,9].

2.4. Chemotaxis assay

Cell migration was evaluated using a chemotaxis microchamber

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technique as previously described [7–9]. Twenty-seven microliters of chemoattractant solution or control medium (RPMI 1640 with 1% FCS) were added to the lower wells of a chemotaxis chamber (Neuroprobe, Pleasanton, CA, USA). A polycarbonate filter (5 μm pore size; Neuroprobe) was layered onto the wells, covered with a silicon gasket and with the top plate. Fifty microliters of cell suspension (0.7–1×10^6/ml) were seeded in the upper chamber. The chamber was incubated at 37°C in humidified atmosphere in the presence of 5% CO_2 for 90 min. At the end of the incubation, filters were removed, stained with Diff-Quik (Baxter s.p.a., Rome, Italy) and five high power oil-immersion fields (100×) were counted. Results are expressed as the mean number of migrated cells in 10 high power fields. Each experiment was performed in triplicate.

3. Results and discussion

PAF is a biologically active phospholipid that plays an important role in leukocyte recruitment [13–16]. To investigate whether PAF could also activate DC, cells were generated from monocyte-enriched circulating mononuclear cells and from $CD34^+$ cells. Fig. 1 shows that both mono-DC and CD34-DC express specific mRNA for PAF receptor of the expected size (~4.5 kbp), evaluated by Northern blot analysis. The level of expression was comparable to that observed in human monocytes and polymorphonuclear cells, two leukocyte populations known to be activated by PAF [14,15]. It is interesting to note that 24 h stimulation with 20 ng/ml TNFα increased the expression of PAF receptor in mono-DC. This is in agreement with previous results obtained with human monocytes [18].

Fig. 2 shows that DC express functional PAF receptors. PAF induced a concentration-dependent migration of mono-DC, in vitro (Fig. 2A). The effect was statistically significant at 10 nM ($P<0.01$) and reached maximal levels at 100 nM PAF with a migration that was $73\pm3\%$ ($n=3$) of that observed in response to an optimal concentration of RANTES (12 nM), a reference chemotactic factor [7,8,10]. PAF showed the bell-shaped dose-response curve typical for chemotactic agonists and was active in a concentration range similar to other phagocytic cells (not shown). The effect of PAF was completely inhibited by WEB 2187, a PAF receptor antagonist. Furthermore, the inactive PAF enantiomer, S-PAF [14,15], did not induce DC migration (Fig. 2B). These results

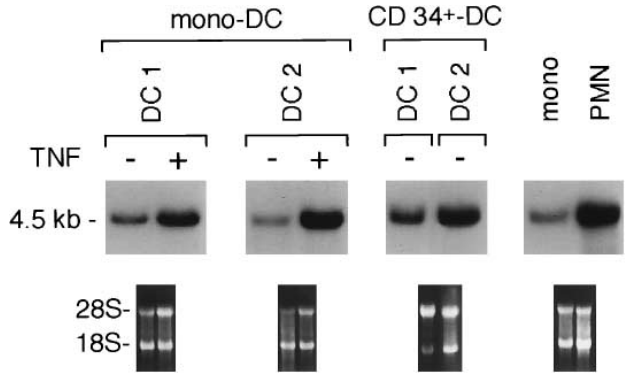

Fig. 1. Expression of PAF receptor in mono-DC and CD34-DC. Ten micrograms of total RNA was purified from DC obtained from blood circulating monocytes (mono-DC), from cord blood $CD34^+$ cells ($CD34^+$-DC) or from human monocytes (mono) and human granulocytes (PMN) and used in Northern blot analysis. Where indicated, mono-DC were incubated with 20 ng/ml TNFα for 24 h. Results of two independent cultures of DC are shown. Ethidium bromide staining is reported below.

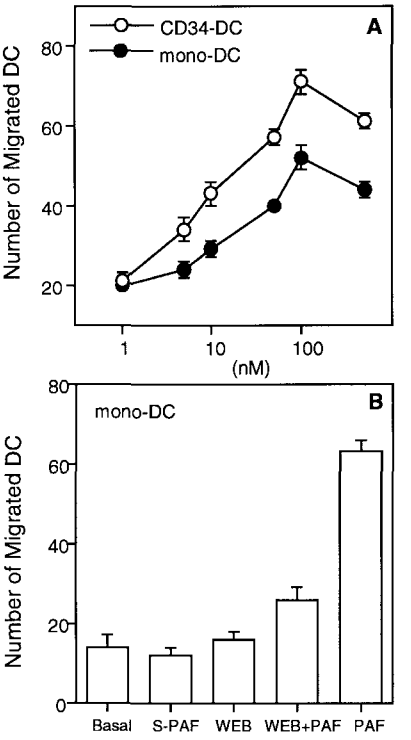

Fig. 2. Chemotactic activity of PAF for mono-DC and CD34-DC. A: DC (1×10^6/ml) were tested for their ability to migrate across a polycarbonate filter in response to different concentrations of PAF. B: DC were tested for their migration to 100 nM PAF or 100 nM S-PAF. WEB 2187 (5 μM) was added directly to the cells and left for the entire duration of the assay. At the end of the incubation (90 min), the number of cells in 10 high power microscope immersion fields was evaluated. Results of one experiment representative of three are shown.

indicated that the effect of PAF on mono-DC was due to the activation of its specific receptor and not to a membrane effect. As expected on the basis of previous studies performed in other cell types, PAF also induced a rapid and transient increase in intracellular calcium concentration in fura-2-loaded mono-DC (data not shown). To obtain indications as to the relative role of chemotaxis versus chemokinesis in induction of DC migration across filters, checkerboard analysis was performed. In these experiments, PAF was added only in the upper or in the lower compartment of the well, or at equal concentrations on both sides of the filter. As shown in Fig. 3A, significant cell migration was observed only in the presence of a positive concentration gradient (higher concentration in the lower well) of the chemoattractant, an observation consistent with a chemotactic effect.

Fig. 2A shows that also CD34-DC migrated in response to PAF. The concentration curve and the magnitude of response of CD34-DC and mono-DC were comparable with respective chemotactic indices of 3.2 ± 0.7 and 2.8 ± 0.2 ($n=5$). Similar results were obtained with $CD34^+$ cells obtained from cord blood and bone marrow (data not shown). $CD34^+$ progenitor cells can differentiate along multiple differentiation lineages [1,2,4]. To confirm that the cells migrating to PAF were indeed DC and not possibly contaminating leukocytes, the expression of CD1a, a marker for immature DC [1], was used to obtain a purified DC population by fluorescent activated cell sorting. Fig. 3B shows that $CD1a^+$-sorted cells efficiently migrated to PAF and to an optimal concentration of RANTES.

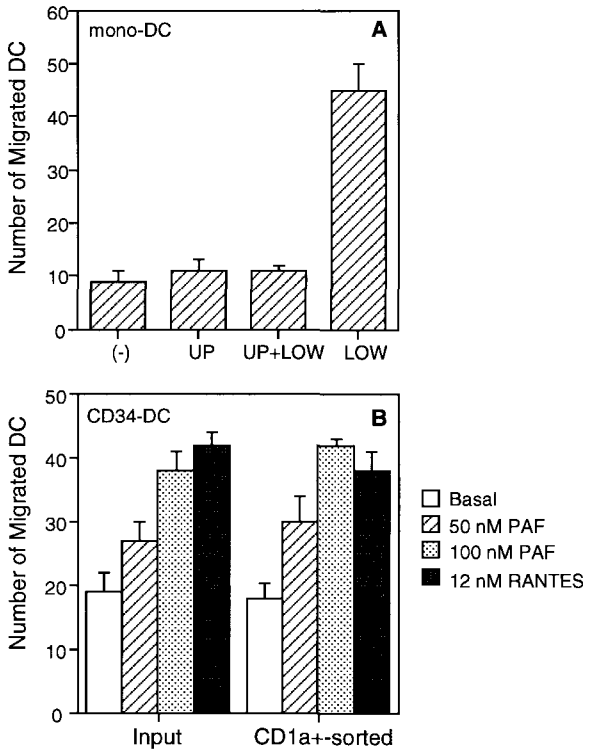

Fig. 3. Analysis of chemotactic response of mono-DC and CD34-DC to PAF. A: For checkerboard analysis PAF (100 nM) was placed in the lower (positive gradient), in the upper (negative gradient) or in both compartments (no gradient) of the chemotactic chamber. B: CD1a$^+$ CD34-DC were purified by FACS (CD1a$^+$-sorted) and tested for their migration to PAF and to RANTES. Input cells (before purification) were tested in parallel. The average numbers ± S.D. of triplicate determinations of one experiment, representative of three, are shown.

Fig. 3B also shows that the level of response of CD1a$^+$-sorted cells was comparable to that obtained with the starting input cell population.

This study reports that mono-DC and CD34-DC express specific receptors and migrate to PAF. It is interesting to note that PAF receptor expression is upregulated by TNF. It was shown that TNF induces DC mobilization, in vivo [6], and that it has a profound effect on the stage of maturation of DC [3]. PAF is known to be involved in proliferation and differentiation of various cells, including neurons and B lymphocytes [15,19]. The possible effect of PAF on DC cells differentiation has yet to be investigated.

DC localize in peripheral tissues and subsequently migrate to lymphoid organs [1,6]. Rapid DC recruitment is a hallmark of inflammation caused by bacteria and their products at mucosal surfaces [20]. Pro-inflammatory signals (e.g. LPS, IL-1 and TNF) induce a rapid mobilization of DC, in vivo [6]. The same inflammatory signals also induce the production of PAF and CC chemokines by different cell types [10,14,15]. DC produce chemokines [8–10,21] and in preliminary experiments we found that they also release PAF in response to a calcium ionophore (0.3 and 0.75 pmol/10^6 cells after 60 min incubation with medium or 1 μM A23187, respectively). PAF and chemokines were shown to act in a synergistic manner in the induction of leukocyte migration [22,23]. It is likely that chemotactic signals of both protein and lipid nature contribute to the rapid accumulation of these cells observed at sites of infection and tumor growth [1,2,6,20].

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