Increasing evidence suggests that reactive oxygen species (ROS) have important signaling properties in many cells, including dendritic cells (DC). ROS affect the maturation state, the production and secretion of cytokines, and the antigen-presenting capacity of DC, and the cells become functionally more efficient when exposed to ROS (1, 11, 13, 16). Like monocytes and neutrophils, for example, DC are equipped with a membrane-localized electron transport system, NADPH-oxidase, that reduces molecular oxygen to superoxide anions at the expense of NADPH (6, 11, 17). Activation of DC may thus initiate/include ROS-mediated autocrine/paracrine regulatory functions. NADPH-oxidase is activated by a large number of receptor-binding agonists, including particles that trigger phagocytosis, chemokine receptors, and other “danger molecules.” The precise receptor repertoire thus determines the responsiveness of the cells to a particular agonist.

DC represent a heterogeneous population of leukocytes derived from several different progenitors, which also have different functional properties, depending on their maturation status (3, 8, 9). Due to the scarcity of DC in human blood, most functional studies of DC are made with in vitro-generated monocyte-derived DC (moDC). However, expression of pathogen recognition receptors on DC may vary according to their hematopoietic origins and degrees of maturation. We therefore set out to characterize the abilities of human DC of different origins to respond to three agonists and degrees of maturation. We therefor set out to characterize the abilities of human DC of different origins to respond to three formyl peptide receptor (FPR) family agonists: fMLF (N-formyl-Met-Leu-Phe), WKYMVm (Trp-Lys-Tyr-Met-Val-D-Met-NH₂), and WKYMVM (Trp-Lys-Tyr-Met-Val-L-Met-NH₂) (7). The human FPR family consists of three receptors, FPR, FPR-like receptor 1 (FPRL1), and FPRL2, which are all expressed on moDC (18–20). FPR is a high-affinity receptor for formyl peptides but can also be activated by WKYMVm. FPRL1 is a promiscuous receptor that can be activated by numerous and chemically unrelated ligands, including WKYMVm and WKYMVM, and this receptor can also bind fMLF with low affinity. FPRL2 has a low affinity for several FPRL1 ligands, e.g., WKYMVm and WKYMVM, but it does not bind fMLF (4, 7).

Human plasmacytoid DC (pDC), myeloid DC (mDC), and monocytes were isolated from peripheral blood mononuclear cells, using a BDCA-4 cell isolation kit, a CD1c (BDCA-1) dendritic cell isolation kit, and CD14 microbeads (all from Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), respectively. ROS responses in monocytes and DC to the FPR family agonists fMLF (Sigma Chemical Co., St. Louis, MO), WKYMVm, and WKYMVM (both synthesized and HPLC-purified; Alta Bioscience, University of Birmingham, UK) were measured by luminol-enhanced chemiluminescence (CL) as previously described (5), using 4 × 10⁴ cells for each experiment.

All three FPR family agonists induced responses in both mDC and pDC, but the overall strength of the responses was severalfold higher in mDC than in pDC (Table 1). The responses differed very little between the cell types, however, with respect to the relative responsiveness among the three agonists (Fig. 1A and B and Table 1) and the kinetics of the agonist-specific responses (Fig. 2). Thus, WKYMVm, which binds all three human FPR family receptors (FPR, FPRL1, and FPRL2), was the strongest agonist for both mDC and pDC, with 50% inhibitory concentration (IC₅₀) values of 13 and 3.7 nM, respectively. The FPR agonist fMLF had a three-times-lower IC₅₀ value than the FPRL1/FPRL2 agonist WKYMVM in both cell types.

Compared to that in monocytes, however, ROS responses in mDC and pDC differed with respect to strength and sensitivity as well as to the relative responsiveness to the three agonists, implying that DC and monocytes differ in their FPR densities and/or distributions (Table 1 and Fig. 1C). Monocytes displayed an overall-higher level of responsiveness than DC (Table 1). Like DC, monocytes had the strongest response to WKYMVm, of which the 50% effective concentration was 1...
nM. Furthermore, fMLF and WKYMVM were equally efficient at inducing ROS responses in monocytes (while fMLF was a stronger agonist in DC) and induced peak responses at 5- to 10-times-lower doses than in DC.

The majority of studies of DC-generated ROS production have been performed using in vitro-generated human moDC (1, 10, 12, 14). Since moDC differ from mDC in several aspects, we wanted to investigate whether moDC are representative of in vivo DC with respect to FPR family agonist-induced ROS responses. Isolated monocytes were therefore cultured for 7 days with recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (800 U/ml; Leucomax; Sher- ring Plough) and recombinant human interleukin-4 (500 U/ml; R&D) before being analyzed. We found that moDC have an impaired response to fMLF and perhaps also to WKYMVM compared to those for mDC (Table 1 and Fig. 2), even though both mDC and moDC responded strongly to WKYMVM. One explanation for this could be that mDC express higher levels or a different composition of FPR family receptors than moDC. Furthermore, it was evident from comparing moDC to monocytes that the 7-day culture in GM-CSF-containing medium led to severely impaired responses to both WKYMVM and fMLF and a partially impaired response to WKYMVM, implying that the in vitro culture profoundly down-modulates the expression of at least some of the FPR family receptors. Other studies have shown that many other pathogen recognition molecules, e.g., the Toll-like receptors 1, 2, 4, and 5, which are present in monocytes and mDC, are virtually absent in moDC (15). An alternative (or complementing) explanation could be that the 7-day culture leads to a decrease in the amount and/or function of NADPH-oxidase.

Our data show that directly isolated human mDC and pDC exhibit similar levels of ROS responsiveness to the three FPR family receptor agonists tested. However, in vitro-generated moDC differed considerably from in vivo DC in their responses to these agonists and are thus not universal substitutes for mDC in this field of research. This is to our knowledge the first study to show not only that directly isolated immature human mDC and pDC can respond to FPR agonists but also that mDC have stronger responses to the agonists tested, even though the relative pattern of responsiveness was almost identical. This is interesting, as mDC and pDC have very different expression levels of other pathogen recognition receptors, such as the Toll-like receptors (9), and also since they belong to two distinct hematopoietic lineages, the myeloid and the lymphoid, respectively (2). Thus, even though mDC are more closely related to monocytes than to pDC, they differ from monocytes but not from pDC in FPR expression.

In our study of FPR family receptor expression and the induced ROS responses of the FPR family, moDC were not an

### Table 1. Maximal peak values of chemoattractant-induced NADPH-oxidase responses in monocytes and dendritic cells

| Cell type (no. of expt) | fMLF | WKYMVM | WKYMVM |
|------------------------|------|--------|--------|
| Monocytes (n = 3)      | 23.9 ± 10.3 (0.5 µM) | 26.0 ± 12.1 (0.01 µM) | 26.1 ± 14.3 (0.5 µM) |
| mDC (n = 3)            | 8.4 ± 1.7 (1 µM)     | 8.3 ± 2.2 (1 µM)      | 8.0 ± 1.8 (1 µM)      |
| pDC (n = 2)            | 1.2 ± 0.7 (1 µM)     | 1.2 ± 0.7 (1 µM)      | 1.3 ± 0.5 (1 µM)      |
| moDC, cultured (n = 4) | 2.1 ± 1.2 (1 µM)     | 5.6 ± 2.7 (1 µM)      | 2.6 ± 1.4 (1 µM)      |

* Given in Mcpm (10^6 counts per minute). The lowest concentration at which the maximal CL response was achieved is given in parentheses.

FIG. 1. Dose responses of NADPH-oxidase activity induced by FPR family agonists in purified human DC and monocytes. Human mDC (A), pDC (B), and monocytes (C) purified from peripheral blood were stimulated with different concentrations of fMLF, WKYMVM, or WKYMVM, and superoxide release was measured by luminol-enhanced CL. The figures show the mean peak CL responses at different concentrations of each agonist, given as percentages of the maximal CL response (achieved at different concentrations for the different agonists). The figure also shows the IC_{50} value for each agonist, calculated as described by Christophe et al. (4).
agonists but that the amounts of ROS produced differed considerably between both monocytes and mDC in that ROS responses were poor in moDC and that these cells had a particularly low ability to respond to FMLF.

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FIG. 2. NADPH-oxidase responses in mDC, pDC, and moDC. Purified mDC (A), pDC (B), and cultured, monocyte-derived moDC (C) were stimulated with 1 μM FMLF, WKYMVm, or WKYMVM, and superoxide (O₂⁻) release was measured continuously by CL. The insets show mean peak values (n = 3) of the CL responses as percentages of the responses to WKYMVM.

identical substitute for mDC, and they also differed from monocytes. It has previously been shown that propagation of moDC is associated with a specific down-regulation of FPR1 but not of FPR and FPRL2 (18–20). The NADPH-oxidase components p47phox and gp91 phox are also down-regulated during monocyte differentiation to DC (16), which further adds to their diminished responsiveness vis-à-vis monocytes. That pDC can secrete ROS has previously been shown, using purified and interleukin-3-activated pDC (16).

In summary, we found that human monocytes, mDC, and pDC had similar response patterns to FPR family receptor agonists but that the amounts of ROS produced differed considerably, with monocytes producing more than mDC, which produced more than pDC. moDC differed considerably from both monocytes and mDC in that ROS responses were poor in moDC and that these cells had a particularly low ability to respond to FMLF.