Hematopoiesis

The influence of lysophosphatidic acid on the immunophenotypic differentiation of human monocytes into dendritic cells

Lysophosphatidic acid (LPA), a naturally occurring phospholipid, has been suggested to have an immunoregulatory role. We investigated the effect of LPA on differentiation of human monocytes into dendritic cells (DC). We found that LPA affects DC differentiation from monocytes by blocking the expression of CD1a molecules on their surface in a dose-dependent manner.

Haematologica 2006; 91:1273-1274
(*http://www.haematologica.org/journal/2006/09/1273.html*)

Lysophosphatidic acid (LPA) is a naturally occurring water-soluble phospholipid identified as a key molecule in the *de novo* synthesis of lipids.[1] LPA is stored within the cells at concentrations of up of to 50 μM and may accumulate in extracellular fluids as an inflammatory exudate where it has been suggested to have an immunoregulatory role. Recently, Panther *et al*. demonstrated that LPA has biological activity on human differentiated dendritic cells (DC) stimulating an increase of Ca^{2+}, actin polymerization and chemotaxis without effects on DC phenotype.[2] DC reside in peripheral tissues in an immature state where they capture antigens and undergo a maturation process migrating to lymphoid tissues.[3] They express high levels of CD83, MHC class I and II and co-stimulatory molecules to activate naive T lymphocytes and providing belated protection against pathogens or tumors. At the site of inflammation, chemokines promote the recruitment of DC precursors such as monocytes, which differentiate into DC or macrophages depending on the environmental factors.[4,5] Since LPA is accumulated in sites of inflammation in several pathophysiological states,[6] we investigated the effect of LPA on differentiation of human monocytes into DC. Peripheral circulating monocytes were purified from buffy-

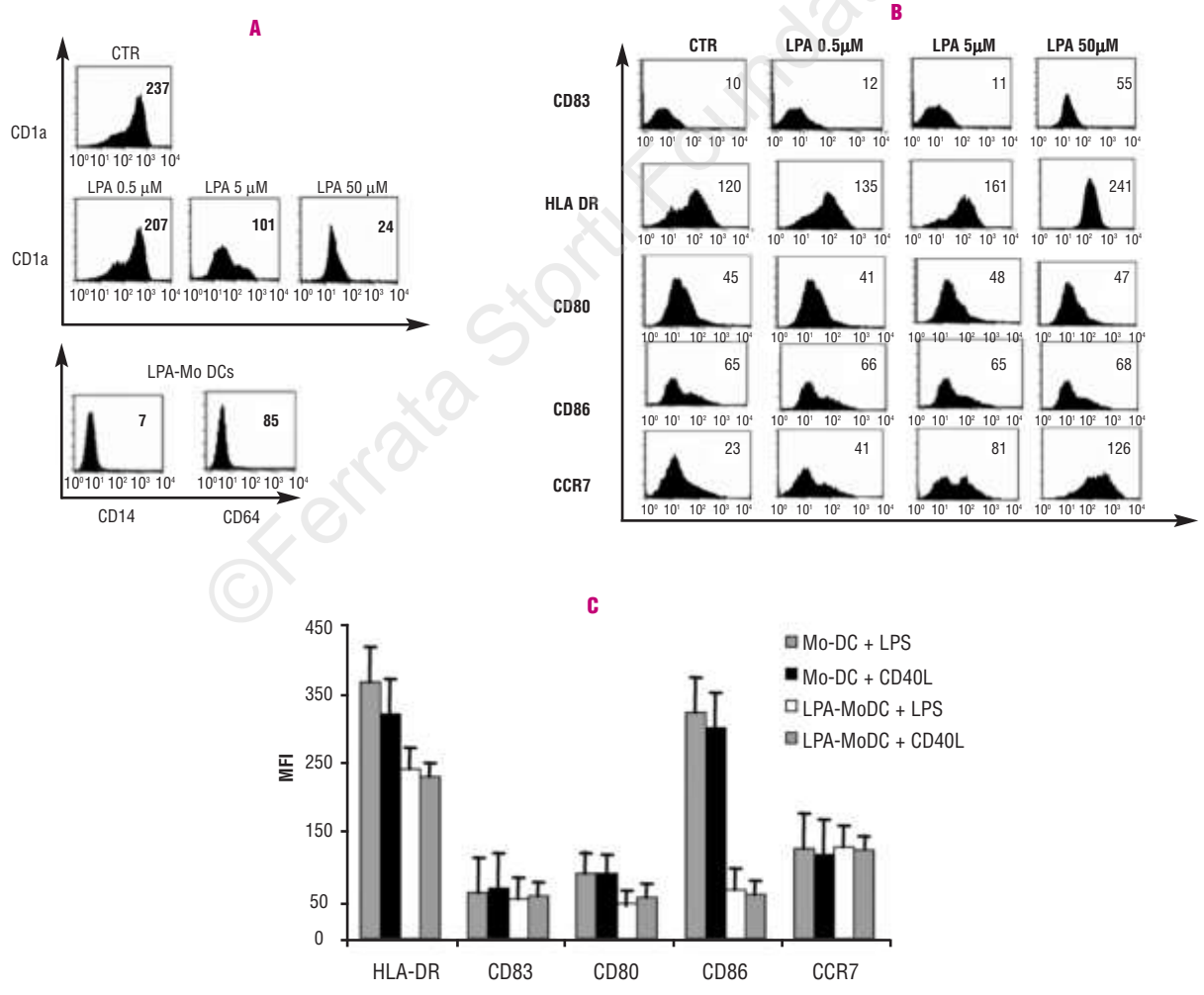

Figure 1. A. *Flow cytometric analysis of CD1 isoform expression.* Staining for CD1a was performed on DC derived from untreated or LPA-treated monocytes on the fifth day of culture with GM-CSF (200 U/mL) and IL-4 (10 ng/mL). Numbers indicate the mean fluorescence intensity (MFI) for histograms plots. **B.** *Flow cytometric analysis of DC maturation markers.* Staining for CD80, CD83, CD86, MHC class II and CCR7 was performed on DC derived from untreated or LPA-treated monocytes on the fifth day of culture. Numbers indicate the MFI for histogram plots. **C.** *DC maturation marker expression.* The MFI of phenotypic markers of LPA-treated or untreated monocyte DC after 48 hours of stimulation with lipopolysaccharide (LPS) (200 ng/mL) or CD40L (1 μg/mL) is shown as the mean of four independent experiments ± SD.

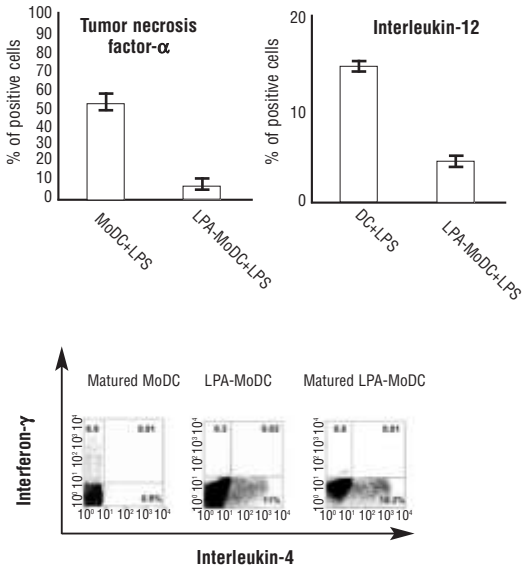

Figure 2. A. ***Cytokine production by LPA-treated monocyte DC (LPA-MoDC) and untreated monocyte DC (MoDC).*** **DC producing TNF-α and IL-12 were analyzed by flow cytometry after 12h of LPS stimulation. Results are expressed as means of the percentages of cytokine-positive cells in four independent experiments. B.** ***Naive CD4 T cell polarizing capacity.*** **DC derived from untreated or LPA-treated monocytes were matured with LPS/CD40L and cultured with purified naïve CD4 T-cells for 8 days. Intracellular staining for IFN-γ and IL-4-producing T cells was performed and analyzed by cytometry. Numbers indicate the percentage of cells. A representative result out of four independent experiments is shown.**

coats of healthy donors by using magnetic beads and stimulated with three doses of LPA (0.5, 5, 50 μM), covering the physiological to pathological range, for 5 days in complete medium containing granulocyte-monocyte colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4), widely used for the generation of immature DC *in vitro*.

We found that LPA affects DC differentiation from monocytes by blocking the expression of CD1a molecules on their surface in a dose-dependent manner as assessed by cytometry (Figure 1A). However, this DC population derived from LPA-treated monocytes was $CD14^-$, $CD64^-$, suggesting that the cells were not blocked at the monocyte/macrophage stage. Furthermore, no apoptotic effect was observed during the treatment (*data not shown*).

In the absence of maturation stimuli, LPA-treated monocytic DC expressed higher levels of MHC class II, CD83, and CCR7 than did immature DC derived from untreated monocytes, while no significant differences were observed in the expression of CD80 and CD86 (Figure 1B). When stimulated with maturation stimuli such as lipopolysaccharide or CD40L, no change was observed in the expression of maturation markers in the LPA-treated monocyte DC (Figure 1C). This phenotype correlates with impaired immunological function of the DC. Indeed, by intracellular staining, LPA-treated monocyte DC showed significantly lower frequencies of tumor necrosis factor-α^+/interleukin (IL)-12^+ cells than did untreated monocyte-derived DC after lipopolysaccharide stimulation (Figure 2A) or CD40L stimulation (*data not shown*). According with this, we speculated that LPA-treated monocyte DC have different capabilities to drive naïve T-cell polarization. LPA-treated and untreated monocyte DC matured or not with lipopolysaccharide/CD40L were cultured with purified $CD4^+CD45RA^+$ T cells for 8 days. Then, naïve T cells were stimulated with anti-CD3 over night in the presence of brefeldin A and the IL-4/interferon (IFN)γ^+ cells were stained by an intracellular stain. Matured DC induced a high frequency of IFNγ-producing T cells and a low frequency of IL-4-producing T cells (Figure 2B). In contrast, LPA-treated monocyte DC induced a high percentage of IL-4^+ T cells independently of the maturation stimuli. Thus LPA is able to regulate an immune program in circulating monocytes, which cannot be reverted during DC differentiation and maturation. It is known that DC and macrophages play different roles in immunity. While DC initiate specific immune responses, macrophages exhibit potent bactericidal and antitumor activity.[7] Increasing evidence indicates that the microenvironment influences the commitment of monocytes towards DC.[8] In particular, monocytes permeate inflamed tissues and receive stimuli to differentiate into competent DC. Thus, it is evident that in pathological conditions, in which phospholipids play an important role, the influence on the generation of immune cells is crucial. Furthermore, LPA has been identified as a potent natural ligand for the transcription factor peroxisome-proliferator-activated receptor-γ that promotes lipid accumulation in phagocytes *in vitro* and atherogenesis *in vivo*.[9] In this context, we observed that the stimulation of monocytes with LPA affects DC phenotype during their differentiation generating $CD1a^-$ DC. In contrast to $CD1a^+$ DC, this peculiar population has been previously described to be involved in Th2 immunity or have a suppressive role on immune response.[5,10] In summary, LPA is able to modify the immune functions of differentiated DC or their precursors generating a DC population that can alter the immune system homeostasis with important consequences in pathological states.

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Key words: CD1a, dendritic cells, LPA, differentiation

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