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Loss of Type I IFN Receptors and Impaired IFN Responsiveness During Terminal Maturation of Monocyte-Derived Human Dendritic Cells

Maria Cristina Gauzzi,* Irene Canini,* Pierre Eid, † Filippo Belardelli,* and Sandra Gessani2*  

Type I IFNs are modulators of myeloid dendritic cell (DC) development, survival, and functional activities. Here we monitored the signal transduction pathway underlying type I IFN biological activities during in vitro maturation of human monocyte-derived DCs. IFN-inducible tyrosine phosphorylation of STAT family members was severely impaired upon LPS-induced DC maturation. This correlated with a marked reduction of both type I IFN receptor chains occurring as early as 4 h after LPS treatment. The reduced receptor expression was a post-transcriptional event only partially mediated by ligand-induced internalization/degradation. In fact, although an early and transient production of type I IFNs was observed after LPS treatment, its neutralization was not sufficient to completely rescue IFN receptor expression. Notably, neutralization of LPS-induced, endogenous type I IFNs did not interfere with the acquisition of a fully mature surface phenotype, nor did it have a significant effect on the allostimulatory properties of LPS-stimulated DCs. Overall, these data indicate that DCs strictly modulate their responsiveness to type I IFNs as part of their maturation program, underlining the importance of the IFN system in the regulation of DC physiology. The Journal of Immunology, 2002, 169: 3038–3045.

Dendritic cells (DCs) play a pivotal role in linking innate and adaptive immunity by their ability to induce appropriate immune responses upon recognition of invading pathogens. To exert their role, DCs pass through different functional states of activation. Resting DCs reside in peripheral tissues in an immature state, where they are adapted to capture and accumulate Ags. Upon receipt of a variety of activation signals, these cells are induced to mature and migrate to secondary lymphoid organs. This process is accompanied by a concomitant decrease in their capacity for Ag uptake and a marked increase in their ability to present Ags and activate naïve T cells. Activated DCs are also capable of directing the type of response made by T cells, a process that can be strongly influenced by the cytokine milieu where DC maturation takes place (1, 2).

Type I IFNs are cytokines spontaneously expressed at low levels under physiologic conditions (3) whose expression is highly enhanced soon after cell exposure to viruses or other stimuli (4). Although first characterized as potent antiviral molecules, type I IFNs are also endowed with immunoregulatory activities, and their important role in linking innate and adaptive immune responses has been unraveled (5, 6). A number of reports have recently demonstrated a profound effect of these cytokines on DC maturation, survival, and function. In this regard it has been shown that type I IFNs (in combination with GM-CSF) rapidly induced the differentiation of monocytes into partially mature DCs (mDCs) endowed with potent functional activities both in vitro and in vivo (7, 8). Likewise, IFN-α has been described to act as a potent maturation factor for CD11c+ DCs (myeloid DCs), directly purified from the peripheral blood (9) and to enhance the terminal differentiation of CD34+–derived DCs (10). Moreover, type I IFNs have been shown to act as adjuvants in the promotion of humoral immune responses through stimulation of DCs (11). On the other hand, a negative role of type I IFNs in the functional maturation of DCs induced by pro-inflammatory cytokines has also been described (12, 13).

Type I IFNs exert their biological activity through a broadly expressed heterodimeric receptor composed of the IFNAR1 and IFNAR2 subunits, whose mutual interaction activates a series of intracellular signaling molecules (14). The major signal transduction pathway used by type I IFNs is triggered upon receptor binding by the receptor-associated tyrosine kinases Janus kinase type 1 (JAK1) and tyrosine kinase type 2 (Tyk2). These kinases are activated by tyrosine auto/cross-phosphorylation and phosphorylate a number of substrates. Among them, a pivotal role is played by the transcriptional factors of the STAT family. Although STAT-1 and -2 are considered the major factors mediating type I IFN signaling, it has been reported that STAT-3 and, in humans, STAT-4 can be activated in response to these cytokines (15, 16).

Despite the growing evidence showing the importance of type I IFNs in the development, survival, and functional activities of DCs, no studies are currently available on the expression and signaling activity of type I IFN receptors during the differentiation/maturation of these cells. Moreover, although many of the stimuli promoting DC terminal maturation are known to induce the production of type I IFNs, the role of these endogenously produced IFNs in the modulation of DC functions is still poorly studied. In this study we report that upon LPS-induced maturation, DCs show a greatly impaired responsiveness to IFN-β, which correlated with a marked reduction of the expression of both IFN receptor subunits. Down-modulation of receptor chains was only partially mediated by
ligand-induced receptor internalization consequent to LPS-stimulated type I IFN secretion.

Overall, these data indicate that DCs strictly modulate their responsiveness to type I IFNs as part of their maturation program, strongly supporting the importance of the IFN system for the regulation of DC physiology.

Materials and Methods

Cell separation and culture

Monocytes were isolated from the peripheral blood of healthy donors by counterflow centrifugal elutriation and were further purified by depleting the nonmonocytic population by immunomagnetic beads selection (MACS monocyte isolation kit from Miltenyi Biotec, Auburn, CA) using the manufacturer’s procedure. To obtain immature DCs (iDCs), monocytes were cultured at 1 x 10^7 cells/ml in RPMI 1640 (Life Technologies, Gaithersburg, MD) medium containing 10% FBS supplemented with 50 ng/ml recombinant human GM-CSF and 500 U/ml recombinant human IL-4. Both cytokines were provided by Schering-Plough (Dandilly, France). GM-CSF and IL-4 were added to the cultures every 2–3 days. On days 4–6, iDCs were induced to final maturation by adding LPS (200 ng/ml; Sigma-Aldrich, St. Louis, MO) and were harvested 24 h later. In some experiments DCs were generated by culturing monocytes in the presence of GM-CSF (50 ng/ml) and IFN-α2b (PeproTech, Rocky Hill, NJ). To neutralize type I IFNs during the course of LPS-induced maturation, the following Abs were used: anti-IFNAR1 mAb 64G12 (20 μg/ml) (17, 18), or a mixture of sheep anti-human IFN-α and sheep anti-IFN-β (PBL; Biomedical Laboratories, New Brunswick, NJ) Abs, each at the concentration of 200 neutralizing units/ml. One neutralizing unit is defined as the amount of Ab that reduces 10 IU/ml IFN to 1 IU/ml.

Type I IFN determination

Type I IFNs secreted in culture supernatants were measured using a cytotoxicity reduction assay with HeLa cells (1 x 10^6 cells/well in 96-well microplates) and vesicular stomatitis virus at a multiplicity of infection of 0.1 PFU/cell as challenge virus. Human IFN-α reference standard (G23-902-530; National Institutes of Health, Bethesda, MD) was used at a dilution of 500 IU/ml. The sensitivity of the assay ranged from 5 to 15 IU/ml.

FACS analysis of DC surface phenotype

Cell staining was performed using FITC-conjugated mAbs (BD Pharmingen, San Diego, CA). The following mAbs were used: CD14 (M5E2), CD11a (HI149), CD80 (L307.4), CD86 (2331), CD83 (HB15e), HLA-DR (G46-6), and CD40 (5C5). Briefly, 1-2 x 10^6 cells were preincubated for 30 min on ice with PBS/10% human serum to block nonspecific Ig binding and then incubated for an additional 30 min with the appropriate mAb. Cells were washed in PBS/10% human serum, fixed in 1% formaldehyde, and analyzed by FACS flow cytometer (BD Biosciences, Mountain View, CA).

FACS analysis of type I IFN receptor expression

Surface receptor expression was monitored by staining cells with anti-IFNAR1 64G12 mAb (17, 18) or anti-IFNAR2 mAb (Calbiochem, La Jolla, CA), followed by biotinylated polyclonal anti-mouse IgG Ab (BD Pharmingen) and FITC-streptavidin (BD Pharmingen). Before staining, cells were blocked with PBS, 50% human serum, and 10% FBS. At the end of each incubation, cells were washed with PBS/10% human serum. After FITC-streptavidin incubation, cells were fixed and analyzed as described above.

RT-PCR

The total RNA used for the RT reaction was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer’s instructions. RT-PCR analysis of IFNAR1, IFNAR2, and GAPDH was performed as previously described (19). IFNAR1 cDNA was amplified using primers 2 and 3 as described by Abramovich et al. (20). For IFNAR2 cDNA amplification, primers DN11 and DN12 were used as previously described (21). The sequence of GAPDH primers has been previously described (22).

Immunoblotting

For the immunoblotting analysis of STAT tyrosine phosphorylation, cells were left untreated or were treated for 45 min with different doses of IFN-β (provided by Serono, Ardea, Italy), washed three times with ice-cold PBS, and lysed in RIPA buffer (150 mM NaCl, 50 mM Tris-Cl (pH 7.5), 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing a cocktail of protease and phosphatase inhibitors (10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 μg/ml pepstatin, 2 mM PMSE, 2 mM NaF, and 0.4 mM Na3VO4). The protein concentration was determined using the Bio-Rad protein assay (Hercules, CA) according to the manufacturer’s instructions. Forty micrograms of lysate was boiled for 4 min in Laemmli sample buffer, fractionated on an 8% SDS-PAGE, and electroblotted to nitrocellulose filter (Protran BA 85, Schleicher & Schuell, Keene, NH). The following Abs were used for the immunoblots: anti-phospho-STAT-1 (Y701) and rabbit anti-phospho-STAT-2 (Y699) (Upstate Biotechnology, Lake Placid, NY), rabbit anti-phospho-STAT-3 (Y705; Cell Signaling Technology, Beverly, MA), and anti-STAT-1, -2, and -3 Abs (Transduction Laboratories, Lexington, KY). An ECL Western blot detection system (Amer sham, Piscataway, NJ) was used according to the manufacturer’s instructions.

Immunoprecipitation

For IFNAR1 and IFNAR2 immunoprecipitation, cells were lysed with Nonidet P-40 lysis buffer (150 mM NaCl, 50 mM Tris-Cl (pH 8), 0.5% Nonidet P-40, 10% glycerol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 μg/ml pepstatin, and 2 mM PMSE). IFNAR1 and IFNAR2 were immunoprecipitated from 1 mg whole cell lysate with 10 μg anti-IFNAR1 64G12 mAb or 5 μg anti-IFNAR2 mAb (Calbiochem), respectively, followed by a mix of protein A and protein G (1/1)Sepharose. The immunoprecipitates were resolved on SDS-PAGE, electroblotted, and subjected to Western blot analysis as described above. The following mAbs were used for detection: 64G12 and anti-IFNAR2 (Calbiochem).

MLR assay

MLR was performed in RPMI culture medium supplemented with 5% heat-inactivated normal human AB serum. Allogeneic T cells (1–2 x 10^7/well) were cultured for 5 days in 96-well culture microplates as responder cells with 1–2 x 10^5 stimulatory cells (DCs). [3H]Thymidine incorporation (sp. act., 3 Ci/mmol; Amersham) was measured after a 16-h pulse with 0.5 μCi/well. Results are shown as mean counts per minute of triplicate determinations. [3H]Thymidine incorporation in negative control wells, with responder T cells, or with stimulators DCs alone was always <800 cpm.

Results

Type I IFN signaling is severely impaired in LPS-matured DCs

Human peripheral blood monocytes were cultured with GM-CSF/IL-4 to generate iDCs. Incubation of these cells with LPS for an additional 24 h led to a significant up-modulation of cell surface markers, such as CD80, CD86, HLA-DR, CD83, and CD40, indicating the acquisition of the well-defined phenotype of mDCs (Table I).

In a first series of experiments the signaling activity of type I IFN receptor during the course of DC maturation was evaluated by monitoring the IFN-β-induced tyrosine phosphorylation of STAT family members, which are among the most proximal targets of the

| Table I. Phenotypic characterization of immature and mature DC populationsa |
| --- | --- | --- | --- |
| Cell Surface Marker | % Positive cells | Fluorescence intensity | % Positive cells | Fluorescence intensity |
| iDCs | LPS-mDCs | iDCs | LPS-mDCs |
| CD14 (n = 5) | 6 ± 2 | 15 ± 0.5 | 3 ± 1 | 17 ± 2 |
| CD80 (n = 7) | 15 ± 3 | 16 ± 1 | 92 ± 2 | 46 ± 3 |
| CD86 (n = 7) | 9 ± 2 | 20 ± 2 | 93 ± 2 | 130 ± 24 |
| CD83 (n = 7) | 10 ± 4 | 15 ± 0.9 | 86 ± 3 | 36 ± 6 |
| CD40 (n = 8) | 73 ± 13 | 22 ± 0.6 | 91 ± 0.9 | 55 ± 3 |
| HLA-DR (n = 4) | 90 ± 4 | 67 ± 8 | 93 ± 3 | 148 ± 47 |

a Peripheral blood monocytes were cultured with GM-CSF (50 ng/ml) and IL-4 (500 U/ml) for 4–6 days. Cells were then stimulated with LPS (200 ng/ml) for an additional 24 h. At the end of incubation, cells were directly stained with specific Abs and analyzed by flow cytometry. The percentage of cells positive for the expression of each surface Ag is calculated by differences between the level of staining with a specific Ab and the baseline of the control isotype. The values represent the mean ± SE of n separate experiments.
receptor-associated tyrosine kinases, rapidly activated upon IFN binding.

Fig. 1 (A and B) shows the results of a representative experiment in which we analyzed the in vivo tyrosine phosphorylation of STAT-1, -2, and -3 in response to different doses of IFN-β in iDCs and LPS-mDCs 24 h after stimulation. In iDCs, a marked tyrosine phosphorylation of all STATs analyzed was observed at low IFN-β doses (ranging from 10 to 50 IU/ml), which only slightly increased at higher doses of this cytokine. On the contrary, LPS-mDCs displayed a constitutive level of tyrosine phosphorylation of both STAT-1, -2, and -3, which was not further induced even upon stimulation with high IFN-β concentrations (up to >1000 IU/ml). Notably, a higher expression of STAT-1 was consistently detected in LPS-mDCs with respect to iDCs.

Expression of type I IFN receptor chains is rapidly down-modulated during LPS-induced DC maturation

To gain insight into the mechanism(s) involved in the impaired STAT activation by LPS-mDCs in response to IFN-β, time-course experiments were conducted to investigate the surface expression of type I IFN receptor chains, IFNAR1 and IFNAR2, during LPS-induced DC maturation. As shown in Fig. 2, a remarkable percentage of iDCs (CD86low and CD83-) expressed both receptor components. However, as soon as 4 h after LPS stimulation, a marked reduction in the surface expression of both receptor chains was observed, concomitantly to a significant increase in CD86 and CD83 expression. At later time points (8 and 24 h), the surface expression of IFNAR1 and IFNAR2 remained barely detectable, whereas a progressive increase in the expression of costimulatory and maturation markers was observed.

Down-modulation of type I IFN receptor chains is mediated by post-transcriptional mechanisms

To characterize the mechanism(s) responsible for the reduced expression of type I IFN receptor chains at the plasma membrane, the steady-state level of expression of the receptor subunits was evaluated by immunoprecipitation assays. Both IFNAR1 and IFNAR2 are glycosylated proteins that migrate with an apparent molecular mass of 110–150 kDa (IFNAR1) and 90–100 kDa (IFNAR2), depending on the cell type. As shown in Fig. 3A, the total content of both IFN receptor subunits was severely reduced upon LPS-induced maturation. To establish whether the marked reduction in the total content of the IFN receptor chains was associated with a decreased expression of the corresponding mRNA, we performed semiquantitative RT-PCR analysis using RNA isolated from iDCs and LPS-mDCs. As shown in Fig. 3B, comparable levels of IFNAR1 and IFNAR2 mRNA accumulation were observed independently of the DC maturation stage.

LPS induces an early and transient production of type I IFNs, whose neutralization only partially restores type I IFN receptor expression

LPS is a well-known type I IFN inducer in a variety of murine and human cell models, including monocytes/macrophages and DCs (22, 23). Since it has been described that type I IFN binding to
their receptor induces IFNAR1 internalization and degradation (24), we asked whether the LPS-induced type I IFNs could be responsible for the down-modulation of the IFN receptor chains observed during DC maturation. To this aim, we initially analyzed the kinetics of type I IFN production by DCs stimulated with LPS. In our experimental cell model iDCs did not secrete any detectable level of biologically active type I IFNs (data not shown). On the contrary, biologically active type I IFNs were detected in the culture supernatant 4 h after LPS stimulation, reached a plateau at 8 h, and remained stable until 48 h poststimulation (Fig. 4). To discriminate between de novo production and accumulation of these cytokines in the culture medium, after supernatant collection 1, 2, 4, 8, and 24 h poststimulation, cells were reseeded in the presence of LPS and cultured until 24 or 48 h from the first stimulation, when supernatants were collected to measure the residual type I IFN production. As shown in Fig. 4, type I IFN production was barely detectable between 8 and 24 h poststimulation and was below detection limits between 24 and 48 h. These results suggest that type I IFN production is an early and transient phenomenon, starting between 2 and 4 h after LPS stimulation, peaking between 4 and 8 h, and rapidly extinguishing between 8 and 24 h.

We then conducted experiments aimed at evaluating the role of LPS-induced type I IFNs in the observed IFN receptor down-modulation. To this purpose, iDCs were stimulated to undergo maturation by LPS in the presence or the absence of a mixture of IFN-α- and IFN-β-neutralizing Abs. As shown in Fig. 5, the marked reduction in the percentage of cells expressing IFNAR1 or IFNAR2 observed in LPS-mDCs was only partially reverted when LPS-induced type I IFNs were neutralized by specific Abs. Notably, IFNAR2 expression appeared to be almost completely independent of the presence of type I IFNs. No changes were observed for IFNAR1 and IFNAR2 expression in cells cultured with a control Ab (Fig. 5).

Endogenous type I IFNs, secreted during LPS-induced maturation of iDCs, are not required for acquisition of the fully mature DC phenotype and allostimulatory capacity

We finally evaluated whether the LPS-induced endogenous type I IFNs played a role in phenotypic and functional DC maturation. Immature DCs were stimulated to undergo terminal maturation by LPS in the presence of Abs directed to the IFNAR1 subunit or in the presence of a control Ab. As shown in Fig. 6A, neutralization of the biological activity of endogenous type I IFNs did not result

FIGURE 3. Expression of type I IFN receptor mRNAs and proteins in iDCs and LPS-mDCs. A, Whole cell extracts (1 mg) were immunoprecipitated with anti-IFNAR1 or anti-IFNAR2 mAbs. Immunoprecipitates were fractionated on 8% SDS-PAGE, transferred to nitrocellulose filter, and immunoblotted with the indicated Abs. B, Total RNA was extracted from iDCs or LPS-mDCs and analyzed for the expression of IFNAR1 and IFNAR2 mRNA by semiquantitative RT-PCR. The level of expression of the housekeeping gene GAPDH is shown as internal control.

FIGURE 4. Kinetics of type I IFN production during the course of LPS-induced DC maturation. Immature DCs, generated as described in Fig. 1, were treated with LPS (200 ng/ml), and culture supernatants were collected for IFN titration at 1, 2, 4, 8, 24, and 48 h poststimulation. Cells were then reseeded in the presence of LPS and cultured 24 or 48 h. At the end of the incubation, culture supernatants were collected again and assessed for the presence of biologically active type I IFNs as described in Materials and Methods. A representative experiment of three performed is shown.

FIGURE 5. Effect of type I IFN neutralization on the down-modulation of IFNAR1 and IFNAR2 observed in the course of LPS-induced DC maturation. Immature DCs or LPS-mDCs generated in the presence or the absence of Abs directed to IFN-α and IFN-β were assessed 24 h after induction of maturation for the surface expression of IFNAR1 and IFNAR2 as described in Fig. 2. Filled histograms represent background staining of isotype-matched control Ab.
in any significant phenotypic change as the same pattern of surface marker expression was found independently of the presence of the anti-IFNAR1 Ab. Similar results were obtained when LPS maturation occurred in the presence of a mixture of Abs directed to IFN-α/H9251 and IFN-β/H9252 (data not shown). To exclude that the lack of effect on the LPS-induced DC phenotypic maturation could result from incomplete blockage of IFN activity, DCs were derived from monocytes cultured with GM-CSF and IFN-α/H9251 in the presence or the absence of 20 μg/ml of the anti-IFNAR1 neutralizing mAb or control IgG1. As previously described, this experimental procedure leads to the generation of partially mature DCs expressing CD83 and exhibiting marked allostimulatory properties (7). As shown in Fig. 6B, the addition of anti-IFNAR1 Ab to GM-CSF/IFN-α monocyte cultures completely reversed the IFN-induced up-modulation of CD86 and CD83 as well as the down-modulation of CD14. The addition of a control Ab had no effect on the phenotypic maturation induced by IFN-α.

We then performed functional experiments aimed at comparing the ability of DCs undergoing LPS-induced maturation under experimental conditions in which the biological activity of type I IFNs was abrogated to stimulate proliferation of allogeneic T cells. As shown in Fig. 7, at all stimulator/responder ratios, LPS-mDCs exhibited a higher capacity to stimulate the proliferation of allogeneic lymphocytes than iDCs. LPS-mDCs generated in the presence of Ab to IFNAR1 showed a similar allostimulatory capacity.
with respect to LPS-mDCs derived in the absence of the Ab. No differences were observed when LPS-mDCs were generated in the presence of a control Ab with respect to control cultures (data not shown).

Discussion
In this study we report that LPS-induced maturation of monocyte-derived DCs results in impaired activation of STAT-1, -2, and -3 in response to exogenous type I IFN, which correlated with an impressive and rapid down-modulation of both IFN receptor subunits, IFNAR1 and IFNAR2. The analysis of the signal transduction pathway typically triggered through type I IFN receptor revealed that STAT-1, -2, and -3 were tyrosine phosphorylated in a dose-dependent manner upon exposure to IFN-β in iDCs, whereas no inducible phosphorylation of any of these STATs was detected in LPS-mDCs. Moreover, STAT-1, -2, and -3 were constitutively activated in LPS-mDCs. These observations do not necessarily imply that DC maturation results in the complete loss of type I IFN sensitivity, but clearly indicate that the capacity of mDCs to respond to this cytokine by activating the relevant STATs is markedly reduced. This could be due to the fact that upon LPS stimulation a number of cytokines, including type I IFNs, are induced, many of which signal through the JAK/STAT pathway. This massive activation of STATs could transiently exhaust the capacity of mDCs to respond to cytokines through these transduction factors. Cytokine-mediated JAK/STAT signaling pathways have been little studied in monocyte-derived human DCs. Interestingly, it has been reported that LPS-mDCs fail to activate STAT-1 and -3 in response to IL-10 (25) and become unresponsive to IFN-γ by down-regulating membrane expression of IFN-γR1 (26), suggesting that modulation of receptor expression and/or STAT activation may represent common mechanisms to regulate cytokine responsiveness during the course of DC maturation. Of note, IFN-γ, IL-10, and type I IFN receptors all belong to the class II cytokine receptor family and share many similarities in their structure (14). Moreover, both IL-10 and type I IFN receptors use the same JAK, Tyk2, and JAK1 for signal transduction.

The absence of IFN-inducible STAT activation correlated with a clear-cut down-modulation of the membrane expression of IFNAR1 and IFNAR2, which occurred as early as 4 h after LPS treatment, and with a severe reduction of the total intracellular content of both receptor chains. The steady-state level of the corresponding transcripts was not affected, suggesting that down-modulation is mediated by post-transcriptional mechanisms. Although further studies are needed to precisely define the step(s) at which receptor down-modulation takes place, our data are consistent with the existence of multiple regulatory mechanisms operating during the course of DC maturation. In this regard, a common mechanism involved in the modulation of cytokine and chemokine responsiveness is represented by regulation of their receptor expression through autocrine ligand-mediated loops. With respect to the IFN system, it has been described that IFNAR1 is internalized and degraded following ligand binding in Daudi cells (24). Moreover, we have previously reported that IFNAR1 expression is regulated in human monocytes/macrophages by differentiation-dependent post-transcriptional mechanism(s) at least in part involving intracellular sequestration of receptor components (19), and more recently, an increase in type I IFN receptor binding sites has been observed upon in vitro differentiation of CD34+ cells (27). Finally, a role for the receptor-associated Tyk2 tyrosine kinase in sustaining IFNAR1 expression has been demonstrated (28, 29). The finding that LPS-stimulated DCs rapidly and transiently produce type I IFNs and that their maturation in the presence of a mixture of neutralizing Abs directed to IFN-α and IFN-β only partially restored IFNAR1 expression suggests that ligand-mediated receptor internalization/degredation is one of the mechanisms involved in the regulation of receptor expression during DC maturation. Although IFNAR1 down-modulation cannot be explained by a reduced Tyk2 expression in LPS-mDCs, as Tyk2 levels did not change upon LPS stimulation (data not shown), a reduced intracellular availability of Tyk2 for IFNAR1, due to competition with other cytokine receptor chains interacting with Tyk2, cannot be excluded (29). Notably, the expression of IFNAR1 and IFNAR2 appears to be independently regulated in this cellular model. In fact, neutralization of the endogenous LPS-induced type I IFNs did not rescue the surface expression of IFNAR2. To the best of our knowledge, this is the first report describing the differential regulation of type I IFN receptor chains and an IFN-independent regulation of IFNAR2 expression. The observation that a fine regulation of receptor expression occurs in DCs during the course of their maturation provides a strong additional evidence on the important role that type I IFNs may play in DC biology. In this regard, it is tempting to speculate that this reduced type I IFN sensitivity may contribute to protect mDCs from IFN-induced apoptosis. The pro-apoptotic activity of type I IFNs on DCs has been independently described by different groups (7, 8, 13) that observed a reduced cell recovery after DC culture in the presence of type I IFNs. In addition, it has been recently reported that type I IFNs in combination with LPS induce apoptosis of monocyte-derived DCs (30). Consistent with these reports we reproducibly observed a reduced number of viable cells in DC cultures after 24 h of LPS stimulation with respect to control iDCs. Moreover, when LPS-driven DC maturation took place in the presence of IFN-α- and IFN-β-neutralizing Abs, comparable numbers of viable cells were recovered in the two maturation stages (data not shown). Protection from type I IFN-induced apoptosis could be particularly important in the inflamed lymph node when, upon arrival of plasmacytoid DCs secreting large amounts of type I IFNs, the local concentrations of these cytokines become very high (31, 32).

In the last few years the role of type I IFNs in the regulation of DC development and functional maturation has been the object of an intense investigation that yielded apparently contrasting results (7–10, 12, 13, 23). Although many of the stimuli used for triggering DC maturation are well-known type I IFN inducers, very few data are available in the literature about the contribution of the endogenous type I IFNs to the maturation process of monocyte-derived DCs. In this regard, type I IFNs were characterized as...
soluble mediators released following measles virus infection of monocyte-derived iDCs, which could potentially contribute to DC maturation. However, their neutralization only partially reversed the induction of CD86 observed in DC stimulated with supernatants from infected cultures, and no effect was observed on DC allostimulatory activity (33). We report here that LPS-induced endogenous type I IFNs are dispensable for accomplishment of the phenotypic and functional changes associated with DC maturation. In fact, DCs induced to terminal maturation in the presence of phenotypic and functional changes associated with DC maturation. dogenous type I IFNs are dispensable for accomplishment of the tantants from infected cultures, and no effect was observed on DC monocyte-derived iDCs, which could potentially contribute to DC soluble mediators released following measles virus infection of 3044 DC MATURATION AND IFN RESPONSIVENESS within the in fl are not thought to be present at sites of pathogen entry (36). Later, cause the main type I IFN-producing cells, the plasmacytoid DCs, early production of type I IFNs appears particularly important be-fore be released when DCs have reached the lymph nodes (34). The strict temporal regulation of type I IFN production and re-interventions.

**References**


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