A Key Role for Poly(ADP-Ribose) Polymerase-1 Activity during Human Dendritic Cell Maturation

Alessandra Aldinucci, Gianni Gerlini, Silvia Fossati, Giulia Cipriani, Clara Ballerini, Tiziana Biagioli, Nicola Pimpinelli, Lorenzo Borgognoni, Luca Massacesi, Flavio Moroni and Alberto Chiarugi

*J Immunol* 2007; 179:305-312; doi: 10.4049/jimmunol.179.1.305
http://www.jimmunol.org/content/179/1/305

**References**

This article cites 66 articles, 25 of which you can access for free at: http://www.jimmunol.org/content/179/1/305.full#ref-list-1

**Subscription**

Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
A Key Role for Poly(ADP-Ribose) Polymerase-1 Activity during Human Dendritic Cell Maturation

Alessandra Aldinucci, † Gianni Gerlini, ‡§ Silvia Fossati,* Giulia Cipriani,* Clara Ballerini, † Tiziana Biagioli, † Nicola Pimpinelli, ‡ Lorenzo Borgognoni, ‡ Luca Massacesi, † Flavio Moroni,* and Alberto Chiarugi2*

Poly(ADP-ribose) (PAR) polymerase (PARP)-1 is a nuclear enzyme regulating protein that functions by targeting PAR chains. Besides its classic role in DNA repair, PARP-1 is emerging as a key transcriptional regulator in different cell types including the immune ones. In this study, we investigated the role of PARP-1 in human dendritic cell (DC) function. We report that both PARP-1 mRNA and protein levels significantly increased during in vitro DC differentiation from monocytes. Of note, inhibitors of PARP-1 such as phenanthridinone and thieno[2,3-c]isoquinolin-5-one reduced expression of CD86 and CD83 in a concentration-dependent manner, having no effects on expression of CD80 and HLA-DR in mature DCs. In the same cultures, PARP-1 inhibitors also reduced production of IL-12 and IL-10. Addition of exogenous IL-12 to the culture medium partially restored CD86 expression in DCs exposed to PARP-1 inhibitors. In line with the role of PAR formation in NF-κB-dependent transactivation, we also report that phenanthridinone and thieno[2,3-c]isoquinolin-5-one impaired NF-κB and AP-1 subunit DNA binding activity in cellular extract of activated DCs. Finally, we show that PARP-1 inhibitors reduced the T cell allostimulatory activity of mature DCs, and that this reduction was prevented when DCs matured in the presence of PARP-1 inhibitors plus IL-12. Of note, nonproliferating T cells exposed to PARP-1 inhibitor-challenged DCs could undergo efficient proliferation when exposed to a subsequent activation stimulus such as anti-CD3 plus anti-CD-28. Together, data provide evidence for a key role of PARP-1 and poly ADP-ribosylation in DC immunocompetence and underscore the relevance of PARP-1 inhibitors to treatment of immune disorders. *The Journal of Immunology, 2007, 179: 305–312.

Poly ADP-ribosylation is a posttranslational modification of proteins operated by poly(ADP-ribose) (PAR) polymerases (PARPs), a growing family of enzymes forming chains of PAR from NAD (1, 2). PARP-1 is the oldest and best characterized member of the PARP family, accounting for ∼80% of cellular PAR formation and targeting the negatively charged PAR polymers to numerous nuclear proteins, affecting their functioning through steric hindrance and electrostatic repulsion. PAR in turn is very rapidly degraded by PAR glycohydrolase, thereby allowing fast and dynamic signaling (3).

Although originally thought to be only involved in DNA repair, mounting evidence now points to PARP-1 as a key regulator of gene transcription. In particular, numerous reports demonstrate that PARP-1 and PAR formation positively regulate transcription in several types of immune cells (4–7), as well as macrophage activation (8, 9), granulocyte migration (10, 11), and T cell proliferation (6, 12, 13). Also, PARP-1 inhibition increases Ab class-switching (14), and alters Ab production during the autoimmune response in the CNS (15). Additional reports underscore the active role of poly ADP-ribosylation in immune activation of microglia and astrocytes (5, 7, 16). These findings taken together point to binding of PARP-1 and PAR synthesis at the levels of transcription regulating complexes as prerequisites for efficient expression of proinflammatory genes and immune cell activation (4, 17).

In the last several years, dendritic cells (DCs) emerged as professional APCs able to present antigenic peptides in the context of MHC molecules to lymphocytes (18, 19). Upon Ag interaction, a maturation program is triggered in DCs that migrate from peripheral tissues into afferent lymphatics to reach full activation into regional lymph nodes (18). This process is accompanied by immunophenotypic changes characterized by up-regulation of MHC class I-class II and costimulatory molecules (i.e., CD80 and CD86) as well as by neo-expression of CD83, a hallmark of DC maturation (19). Given the ability of DCs to induce immunity or tolerance, mostly depending on their activation profile, a great deal of efforts is now being directed to the pharmacologic manipulation of DCs to boost the immune response to external Ags or to promote T cell tolerance during autoimmune immune reactions (20). The ability of DCs to properly present tumor Ags, thereby favoring tumor vaccination and eradication, is also intensively investigated and exploited for antitumor therapy (21). Hence, in light of the key role of PARP-1 in transcriptional and functional activation of immune cells, as well as the availability of powerful chemical inhibitors of the enzyme, this study seeks to determine whether PARP-1 regulates DC immunocompetence.
Materials and Methods

**DC differentiation and stimulation**

PBMC were isolated from buffy coats by density gradient centrifugation using Lymphoprep (Axis-Shield). Monocytes were isolated from PBMC using MACS anti-CD14 microbeads and a Midi-MACS device (both from Miltenyi Biotec). Cells were cultured in medium supplemented with GM-CSF (1000 U/ml; Chemicon International) and IL-4 (1000 U/ml; R&D Systems), for 7 days. DC activation was induced by LPS (1 μg/ml/day; Sigma-Aldrich). After 7 days, monocyte-derived DCs have been monitored for DC maturation markers by flow cytometry. The PARP-1 inhibitors phanethridinridone (PHE), thieno[2,3-c]isooquinolin-5-one (TIQ-A), and the inactive analog UPF698 (22) have been directly added to the culture medium, while controls received vehicle.

**Surface markers**

DC surface markers have been evaluated by means of mouse anti-human-conjugated mAb (ImmunoTech). Cells have been analyzed on a four-color Epics XL cytometer (Expo32 software; Beckman Coulter). Abs used were against the following: CD80 (FITC), CD86 (PE), HLA-DR (ECD), CD83 (PCS), CD14 (FITC), CD11c (PE), CD3 (PE), and CD4 (FITC). Cell viability has been tested by means of propidium iodide (Molecular Probes). Briefly, staining has been done in PBS 1% FCS for 25 min at 4°C followed by two washes. Later, cells were analyzed by flow cytometry.

**Phagocytic activity**

DC macrophagocytosis capability was examined as described (23). Briefly, 2 × 10³ not activated and LPS-activated DCs were resuspended in 10% FCS RPMI 1640 medium and equilibrated at 37° or 0°C for 10 min. Cells were then pulsed with dextran-FITC (1 mg/ml, 40,000 m.w.; Molecular Probes) for 45 min at 37° or 0°C. After incubation, cells were washed four times with cold PBS buffer containing 0.01% sodium azide (Sigma-Aldrich) and 1% FCS and analyzed by flow cytometry using propidium iodide to exclude dead cells. Background is represented by cells incubated at 0°C.

**Western blotting**

For Western blot analysis, cells were scraped, collected in Eppendorf tubes, centrifuged (1500 × g for 5 min at 4°C) and resuspended in lysis buffer (50 mM Tris (pH 7.4), 1 mM EDTA, 1 mM PMSF, 4 μg/ml aprotinin and leupeptin, 1% SDS). Then 20–40 μg of protein/lane were loaded. After 4–20% SDS-PAGE and blotting, membranes (Hybond-ECL, Amersham Biosciences) were blocked with PBS containing 0.1% Tween 20 (PBS-T) and 5% dry milk and incubated with primary Abs (1/1000 in PBS-T/5% milk) followed by secondary Ab (1/2000). After washing in TPBS, ECL (Amersham Biosciences) was used to visualize the peroxidase-coated bands.

**Immunocytochemistry**

DCs were spun on poly-lysine-coated slide coverglass and then fixed with 4% paraformaldehyde. Cells were then permeabilized and blocked with 10% horse serum in PBS/0.3% Triton X-100. Later on slides were incubated for 3 h with 2% horse serum in PBS/0.3% Triton X-100 containing an anti-PARP-1 Ab (clone C2-10; Alexis). After washing, binding was revealed with a Cy2-conjugated anti-mouse Ab (1/200). Cells were counterstained with Hoechst 33342 (2 μg/ml/10 min) and then mounted and visualized using a Nikon TE-2000-U fluorescence microscope and a CCD camera equipped with Metafluor-Metamorph software.

**Evaluation of NF-kB DNA binding activity**

The DNA binding activity of NF-kB was investigated in cells scraped, pelleted, and resuspended in buffer A (containing 10 mM HEPES (pH 7.8), 10 mM KCl, 1 mM EDTA, 0.1 mM EGTA, 1 mM PMSF, and 4 μg/ml aprotinin and leupeptin). Cells were kept on ice for 15 min, vortexed every 3 min, and then centrifuged at 5000 × g for 5 min at 4°C. The nuclear pellet was resuspended in 50 μl of buffer B, analogous to buffer A plus 400 mM NaCl, and incubated for 10 min on ice. The mixture was centrifuged at 14,000 × g for 10 min at 4°C, and the supernatant aliquoted and stored at −80°C. The DNA binding activity was tested by incubating 10 μg of protein of the nuclear extract in 20 μl of a buffer containing 10 mM Tris (pH 7.4), 4% glycerol, 1 mM MgCl₂, 0.5 mM EDTA, 1 mM DTT, 50 mM NaCl, 0.05 mg/ml polyniinosine-polycytidylic acid, and 10,000 cpm of specific ³²P-labeled oligonucleotide for 20 min at room temperature. The mixture was electrophoresed in 6% nondenaturing polyacrylamide gels that, after drying, was exposed to x-ray film (Amersham Biosciences). The double-stranded oligonucleotide 5'-AGTTGAGGGGACTTTCCCAGGC-3' for NF-kB was used. For supershift experiments, 2 μl of the Abs raised against p65/RelA (Santa Cruz Biotechnology) were added to the binding mixture during incubation. DNA binding activity of p65, p50, c-Rel, and c-Fos was quantified by means of an ELISA kit (Active Motif), according to the manufacturer’s instruction.

**Semi quantitative RT-PCR**

Total RNA (1 μg) extracted with Trizol (Invitrogen Life Technologies) was reverse transcribed into DNA and subjected to PCR using the following software-designed oligonucleotide primers: PARP-1, 5'-CTGACATAGGAGACCTGG-3' (sense) and 5'-TCCCTGGAGAAGGAAAAGCTCT-3' (antisense); and β-actin, 5'-GTTGTCAAGAGATCTTCTAGT-3' (sense) and 5'-GG GTCTCAAACATGATCTGGG-3' (antisense). The number of PCR cycles (94°C at 30 s, 58°C at 30 s, 72°C at 1 min, and 5 min for the last extension) for the amplification of reverse transcribed products, selected after determining the linear working range for the reaction, was 24 (β-actin) and 31 (PARP-1). PCR amplification products were separated on a 1.8% agarose gel.

**MLR analysis**

MLR was performed in a 96-well U-bottom plate (Nunc). Three different dilutions (10⁴, 5 × 10⁴, 5 × 10³ cells/well) of LPS-activated DCs, exposed or not during maturation and activation to PARP-1 inhibitors (30 μM), were cultured 4 days with allogeneic CD4⁺ T cells (10⁵ cells/well). Experiments were conducted in quadruplicate. At day 5, the proliferative response was measured by [³H]thymidine incorporation test. [³H]Thymidine (1 μCi/well; Amersham Biosciences) was added and pulsed the last 8 h. Plates were then harvested (Tomtec MacII) on glass fiber filter...
(PerkinElmer), and thymidine uptake was measured by liquid scintillation in a MicroBeta 1450 Trimux counter (Wallac).

**CD4+ T cell separation**

T cells were isolated from buffy coats of healthy donors with CD4+ T cell isolation kit II (Miltenyi Biotec) and immediately used for MLR. Isolated cells were checked for purity by flow cytometry, and purity level was between 94 and 98%.

**T cell restimulation**

T cells were harvested at day 5 during MLR, washed three times in PBS buffer, plated with fresh medium at 37°C for 24 h. Later, cells were counted and seeded at the density of 1 x 10^6 well in a 96-well flat-bottom plate coated with anti-CD3 (clone BB11; Euroclone) and anti-CD28 (clone BT3; Euroclone) mAbs (both at 1 μg/ml). Cell proliferation was evaluated by [3H]thymidine incorporation after 48 h. T cell viability was assessed by trypan blue exclusion.

**Cytokine measurement**

Supernatants from LPS-activated (1 μg/ml/24 h) DCs were collected, and IL-12 and IL-10 were measured by means of an ELISA kit (BioSource International).

### Results

**PARP-1 expression during DC differentiation and maturation**

We first sought to determine PARP-1 expression in in vitro-differentiated DCs. We found that levels of PARP-1 were higher in monocyte-derived DCs exposed to GM-CSF/IL-4 for 7 days than in freshly seeded monocytes. A 24-h challenge with LPS reduced PARP-1 expression in DCs (Fig. 1A, a). Time course analysis of PARP-1 expression during in vitro DC differentiation showed that PARP-1 levels were barely detectable after 4 days in vitro, although increased at 6 days and remained stable for the following 2 days (Fig. 1A, b). Immunohistochemical analysis confirmed these findings by showing a strong increase of PARP-1 immunoreactivity in the nucleus of DCs with respect to that found in the nucleus of monocytes (Fig. 1B). The finding that PARP-1 expression levels changed during DC differentiation might be due to transcriptional and/or posttranscriptional events. We therefore determined whether PARP-1 transcript levels coherently changed with those of the protein. As shown in Fig. 1C, RT-PCR analysis showed that PARP-1 mRNA
increased in DCs compared with monocytes and decreased upon LPS stimulation.

Effects of PARP-1 inhibitors on DC differentiation

Given the augmented expression of PARP-1 in DCs, we next wondered whether the enzyme plays any role in monocyte differentiation into DCs. To address this issue, monocytes were exposed to potent inhibitors of PARP-1 activity such as PHE (24) or TIQ-A (22) at 30 μM, a concentration consistent with their IC₅₀ on PARP-1 (22). Exposure to the two drugs did not affect the number of CD11c⁺/HLA-DR⁺ DCs at the end of the differentiation period (Fig. 2A).

As shown in Fig. 2, A and B, these compounds neither increased the number of propidium iodide-positive cells (a prototypical marker of cell death), nor affected GM-CSF/IL-4-induced loss of CD14 in DCs, a classic event during differentiation from monocytes. Accordingly, DCs exposed to PARP-1 inhibitors for 7 days did not differ morphologically from vehicle-treated DCs (Fig. 2C and data not shown). On the contrary, PHE or TIQ-A selectively altered membrane Ag expression. Specifically, the drugs reduced the number of cells expressing CD86, although they did not affect that of HLA-DR and CD80 (Fig. 2D). As expected, CD83 was not highly expressed in immature DCs (Fig. 2D). Finally, chemical inhibition of PARP-1 did not affect phagocytic activity of immature DCs (Fig. 2E).

Effects of PARP-1 inhibitors on DC maturation

We then investigated the effect of pharmacologic inhibition of PARP-1 on the immunophenotype of LPS-matured DCs. Upon 24-h exposure to LPS (1 μg/ml), the number of CD86⁺ cells increased from 28 ± 3% to 78 ± 13%. Similarly, that of CD83⁺ cells increased from 1 ± 0.5% to 44 ± 12% and that of cells expressing CD80 raised from 66 ± 11% to 88 ± 9%. Notably, exposure (8 days) to PHE and TIQ-A reduced the number of cells expressing CD86 (to 41 ± 16% and 49 ± 17%, respectively, p < 0.05, determined using Student’s t test) and CD83 (to 28 ± 13% and 24 ± 12%, respectively, p < 0.05, Student’s t test) (Fig. 3).

PHE and TIQ-A did not significantly change the expression of HLA-DR and CD80 by mature DCs (Fig. 3). Phagocytic activity of mature DCs was reduced compared with that of nonmature DCs, but again it was not affected by exposure to PARP-1 inhibitors (Fig. 3E).

The effect of PARP inhibitors on CD86 and CD83 expression was dose-dependent (Fig. 4A), whereas UPF698 (3,4-dihydro-5-benzoyl-isochinolin-2(1H)-one), an inactive PARP-1 inhibitor structurally similar to PHE and TIQ-A (22), had no effect on the immunophenotype (Fig. 4B). Exposure of DCs to PARP-1 inhibitors only during the 24-h LPS challenge did not alter the LPS-induced expression of CD86, CD83, and CD80 (data not shown).

PARP-1 inhibitors reduce IL-12 and IL-10 cytokine production by activated DCs with implication on the immunophenotype

We also sought to determine whether PHE and TIQ-A had any effect on IL-12 and IL-10, key cytokines produced by DCs. As
shown in Fig. 5A, exposure to PARP-1 inhibitors during LPS stimulation significantly reduced IL-12 and IL-10 production by DCs.

It is well appreciated that autocrine and paracrine stimulation by proinflammatory cytokines including IL-12 concur to DC maturation (18, 20). We therefore investigated whether reduction of IL-12 production by PARP-1 inhibitors was responsible for decreased CD86 and CD83 expression. To this end, we exposed DC to LPS and PARP-1 inhibitors in the presence or absence of IL-12 directly added to the medium (5 ng/ml). Interestingly, addition of IL-12 counteracted the effect of PARP-1 inhibitors on CD86 expression, while having no effect on expression of CD83, CD80, and HLA-DR (Fig. 5B).

**Effect of PARP-1 inhibition on p38 kinase activation and DNA binding activity of NF-κB and AP-1 subunits in mature DCs**

Several reports point to p38 kinase as a key factor in DC differentiation and maturation. Notably, the immunophenotype of DCs exposed to a pharmacological inhibitor of p38 resembles that induced by PARP-1-inhibiting drugs (25–27). Under our experimental settings, however, p38 was constitutively active and neither LPS nor the PARP-1 inhibitors altered p38 activation levels (Fig. 6A). A large body of evidence indicates that suppression of PARP-1 activity reduces the inflammatory response because of impairment of transcription factor function involved in immune activation such as NF-κB and AP-1 (17, 28, 29). Accordingly, PARP-1 and PAR regulate NF-κB-dependent transactivation (30), whereas pharmacologic or genetic suppression of PARP-1 reduces NF-κB and AP-1 DNA binding activity and ensuing expression of proinflammatory mediators (5–7, 9, 16, 31). We therefore determined the effect of PARP-1 inhibition on the DNA binding activity of NF-κB and AP-1 subunits in DCs. Constitutive binding of NF-κB (p65 subunit) was present in extracts of immature DCs, and reduced by competitor cold oligoprobe (data not shown), indicating specificity of binding. Pharmacological inhibition of PARP-1 by PHE and TIQ-A did not alter the constitutive binding activity, although reduced that induced by LPS (1 h, 1 μg/ml) (Fig. 6B, a, and data not shown). The DNA binding activity of NF-κB and AP-1 subunits was also analyzed by means of ELISA. Data shown in Fig. 6B, b, confirm the findings on p65 obtained with the gel-shift assay. They also indicate that the binding activity of NF-κB subunit p50 but not that of c-Rel is reduced by chemicals inhibiting PARP-1, in line with previous findings with purified proteins (32). Likewise, the DNA binding activity of AP-1 subunit c-Fos was decreased in extracts of DCs challenged with PHE and TIQ-A (Fig. 6B, b, and data not shown).

**Inhibitors of PARP-1 reduce allostimulatory capacity of DCs**

Given the relevance of membrane Ags such as CD86 and CD83 to DC immunocompetence (33, 34), we then sought to determine whether inhibition of PARP-1 in DCs impaired their allostimulatory capacity. To this end, we measured proliferation of lymphocytes exposed to different concentrations of mature DCs previously exposed or not to PHE or TIQ-A (see Materials and Methods). In line with the immunophenotype studies, allogenic proliferation was reduced when lymphocytes were mixed with DCs previously challenged with PARP-1 inhibitors (Fig. 6C, a). Given that IL-12...
mean binding activity is reduced.

During differentiation and activation the LPS-induced stimulatory activity of activated DCs (5000 tions.

Fos under resting and LPS-activation condi-
tions. PARP-1 as a key regulator of the immune response (4, 17). Spe-

tivity of NF-kB subunit p65 in DC extracts. In cells exposed to PHE (30 μM, during differ-

tentiation and activation) the LPS-induced binding activity is reduced. Bb, ELISA eval-

uation of the effect of PHE on the DNA binding activity of p65, p50, c-Rel, and c-

Fos under resting and LPS-activation conditions. Ca, MLR reveals that the T cell allo-

stimulatory activity of activated DCs (5000 or 500 DCs/well) exposed to PHE or TIQ-A (both 30 μM, during differentiation and activ-

ation) is reduced compared with activity in vehicle-treated controls. The presence of IL-12 during maturation of DCs challenged with PARP-1 inhibitors fully restores their allostimulatory activity. Ch. T cells prolifer-

ating after exposure to DCs and T cells that did not proliferate because of exposure to PHE- or TIQ-A-challenged DCs have been exposed to the proliferative stimulus anti-

CD3 plus anti-CD28 (see Materials and Methods). Proliferation does not differ among these T cell populations. One experiment representative of two is shown in A and Ba. Data in Bb and C represent the mean ± SEM of at least three experiments conducted in triplicate.

Discussion

During the last several years a great deal of effort has been directed at promoting or repressing DC immunocompetence. A deeper insight in DC biology allowed designing of pharmacological strat-

egies able to target functional checkpoints of DCs, such as differ-

entiation, Ag uptake, and processing, migration, and maturation (20). In the present study, we show that PARP-1 expression is induced during DC differentiation in vitro, whereas chemicals inhibiting PARP-1 activity alter specific parameters of phenotypic and functional DC maturation. To our knowledge, this evidence is the first that formation of PAR is a prerequisite for efficient stim-

ulatory activity by DCs.

Our data are consistent with numerous reports indicating PARP-1 as a key regulator of the immune response (4, 17). Spe-

cifically, the enzyme positively regulates activation of macrophages (8, 9, 35), microglia (7, 36), granulocytes (37), lympho-

cytes (6, 13, 14), as well as TNF-α-challenged endothelial cells (38). Also, parp-1 null mice are resistant to endotoxic shock (39), and PARP-1 inhibitors provide protection in models of pleuritis (38). Also, arthritis (41), asthma (42), colitis (43, 44), allergic enceph-

alomylitis (6, 15, 45), and other autoimmune disorders (46). In this context, the present findings point to the negative regulation of DCs by PARP-1 inhibitors as a key mechanism through which this class of drugs suppresses the inflammatory response. As for the molecular mechanisms though which PARP-1 inhibition impairs DC immunocompetence, we show that its enzymatic inhibitors reduce NF-κB and AP-1 subunit DNA binding activity in mature DCs. Evidence that binding of c-Rel NF-κB to its recog-

nizing oligoprobe is not affected by chemicals inhibiting PARP-1 indicates that the enzyme specifically regulates transcription factor DNA binding. Data are consistent with the regulation of NF-κB- and AP-1-dependent transactivation by PAR in macrophages and lympho-

cytes (5–7, 36, 39, 47), as well as with the key role of these transcription factors in DC maturation and Ag presentation (48–51).

We originally report that PARP-1 mRNA and protein levels significantly increase during in vitro DC differentiation. Intrigu-

ingly, a 10-fold increase in PARP-1 transcript levels has been re-

ported during human lymphocyte activation (52), and a role for PARP-1 activity in cell differentiation has been proposed (53). To date, factors regulating parp-1 expression at the promoter level still wait to be clearly understood. Different binding sites for YY1
and Sp1 transcription factors, as well as multiple transcription initiation sites are present in the parp-1 promoter region (54, 55). Genetic variants within this region may therefore affect PARP-1 expression and inducibility. In this context, it is puzzling that specific haplotypes of parp-1 promoter confer higher risk of autoimmune disorders such as rheumatoid arthritis (56), celiac disease (57), and systemic lupus erythematosus (58). It is therefore tempting to speculate that induction of PARP-1 in DCs and/or other immune cells participates in development of autoimmunity. As for the decreased levels of PARP-1 in DCs exposed to LPS, we hypothesize that it could be due to as-yet unknown LPS-dependent specific signaling and/or PARP-1-dependent autorepression of its own promoter (55). Overall, these findings, along with evidence that PARP-1 activity is increased in IFN-γ-activated macrophages (8) and LPS-challenged microglia (7), suggest that induction of poly ADP-ribosylation plays a role in immune cell activation. This assumption is corroborated by our finding that chemical inhibition of PARP-1 affects the immunophenotype of DCs. In particular, we report that PARP-1 inhibitors reduce expression of CD86 and CD83, whereas they do not affect expression of HLA-DR and CD80 in activated DCs (Fig. 3). Data therefore suggest that poly ADP-ribosylation is not a general regulator of transcription in DCs but assists expression of specific genes. Remarkably, down-regulation of the immunomodulatory molecules CD86 and CD83 is in keeping with the reduced proliferation of lymphocytes cultured with DCs pre-exposed to PARP-1 inhibitors (Fig. 6).

The finding that PARP-1 inhibitors reduce production of IL-12 and IL-10 by DCs indicates that poly ADP-ribosylation modulates not only the phenotypic but also the functional maturation of DCs. Interestingly, we show that addition of IL-12 restores CD86 expression (Fig. 5B, b) as well as allostimulatory capacity (Fig. 6B, a) of DCs exposed to PARP-1 inhibitors. These results suggest that reduction of DC immunocompetence by drugs inhibiting PARP-1 is mainly due to reduced production of IL-12 and ensuing suppression of its autocrine/paracrine actions. Although c-Rel regulates IL-12 expression in DCs (59), results shown in Fig. 6B, b suggest that impairment of its DNA binding activity does not underlie down-regulation of the cytokine by PARP-1 inhibitors. Conversely, in keeping with the role of both p65 and p50 in IL-12 gene transactivation (60–62), our data point to the impairment of these two NF-κB subunits as a mechanisms contributing to reduction of IL-12. It is worth noting that classic anti-inflammatory drugs including corticosteroids reduce release of activating cytokines, whereas increase release of immunosuppressive cytokines from DCs (20). The ability of PHE and TIQ-A to suppress production of both IL-12 and IL-10, therefore, points to PARP-1 and its inhibitors as peculiar immunoregulators. On the contrary, PARP-1 inhibitors share with well-known immunosuppressive compounds such as cyclosporine, FK506, and rapamycin the capability of reducing expression of only specific markers of DC maturation (20).


16. Ullrich, O., A. Diestel, I. Y. Eyupoglu, and R. Nitsch. 2001. Regulation of microglial enzyme release in response to anti-CD28. Although it is known that PARP-1 inhibition represses lymphocyte proliferation (13), the fact that DCs were exposed to the chemicals only during maturation, and that IL-12 restored their stimulatory activity, rules out the possibility that suppression of T cell proliferation occurred because of DC-dependent “drug-carrier.” Hence, data lend support to the hypothesis that PARP-1 inhibitors compromise the T cell stimulatory capacity of DCs. The present findings, along with evidence that suppression of PAR formation also affects immunocompetence of T and B lymphocytes (6, 13, 14), help to explain why inhibitors of PARP-1 exert widespread immunosuppression (65). Finally, although the effects of PARP-1 inhibitors on DCs in vivo remains to be evaluated, we infer that these compounds, currently tested in different clinical trials in humans (66), can be harnessed to develop innovative therapies aimed at promoting graft survival or combating autoimmune disorders.

Disclosures

The authors have no financial conflict of interest.

References


