Peritoneal dialysis solutions inhibit the differentiation and maturation of human monocyte-derived dendritic cells: effect of lactate and glucose-degradation products

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Abstract: Peritoneal dialysis (PD) is a well-established therapy for end-stage renal failure, but its efficiency is limited by recurrent peritonitis. As PD solutions impair local inflammatory responses within the peritoneal cavity, we have analyzed their influence on the in vitro maturation of human monocyte-derived dendritic cells (MDDC). Evaluation of MDDC maturation parameters [expression of adhesion and costimulatory molecules, receptor-mediated endocytosis, allogeneic T cell activation, production of tumor necrosis factor α, interleukin (IL)-6 and IL-12 p70, and nuclear factor (NF)-κB activation] revealed that currently used PD solutions differentially inhibit the lipopolysaccharide (LPS)-induced maturation of MDDC, an inhibition that correlated with their ability to impair the LPS-stimulated NF-κB activation. Evaluation of PD components revealed that sodium lactate and glucose-degradation products impaired the acquisition of maturation parameters and NF-κB activation in a dose-dependent manner. Moreover, PD solutions impaired monocyte-MDDC differentiation, inhibiting the acquisition of DC markers such as CD1a and DC-specific intercellular adhesion molecule-3 grabbing nonintegrin (CD209). These findings have important implications for the initiation of immune responses under high lactate conditions, such as those occurring within tumor tissues or after macrophage activation. *J. Leukoc. Biol.* 73: 482–492; 2003.

Key Words: *NF-κB · IL-12 · IL-6 · TNF-α · immune response*

INTRODUCTION

Dendritic cells (DC) are professional antigen-presenting cells that play an essential role in innate immunity and in the initiation of primary immune responses [1–3]. Immature DC are found in most tissues and organs and exhibit an efficient antigen-capturing ability but a weak T lymphocyte-stimulatory activity [4]. In vivo, monocytes/macrophages appear to differentiate into immature DC [5], a transition that can be recapitulated in vitro in the presence of granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin (IL)-4 [6]. In vivo and in vitro, signals delivered by inflammatory cytokines [e.g., tumor necrosis factor α (TNF-α), IL-1] or pathogen-associated molecules [e.g., lipopolysaccharides (LPS), CpG DNA] set off a maturation process, whereby DC lose their antigen-capturing ability; migrate into secondary lymphoid organs; up-regulate adhesion (CD54), major histocompatibility complex (MHC), and costimulatory molecules (CD80, CD83, CD86, CD40); and display the unique capacity to activate "naive" T lymphocytes [1–6, 7]. Depending on the maturation stimulus, mature DC can also synthesize and release IL-12, which critically contributes to T helper cell type 1 (Th1) lymphocyte polarization [3]. The acquisition of all these maturation parameters is absolutely dependent on the activity of the nuclear factor (NF)-κB family of transcription factors [8].

DC have been identified within the peritoneal cavity and characterized morphologically and phenotypically [5, 9–11]. DC account for 1–2% of the total peritoneal cells [9, 10], and peritoneal monocytes/macrophages acquire DC phenotypic properties during transport of exogenous agents into adjacent lymph nodes [5]. In the steady state, mesothelial cells could affect DC differentiation and maturation in the peritoneum through their capacity to secrete GM-CSF [12], IL-1 [13], and TNF-α [14].

Continuous ambulatory peritoneal dialysis (CAPD) has become an effective replacement therapy for treating patients with end-stage renal failure. However, CAPD duration is limited, as some patients exhibit a gradual loss of peritoneal membrane functionality and the periodic occurrence of perito-

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Received September 11, 2002; revised December 24, 2002; accepted January 13, 2003; doi: 10.1189/jlb.0902451.

nitis [15, 16], which is the leading cause of discontinuation of PD [17]. Adequate, immunologic responses within the peritoneal cavity are vital to eradicate infection. The instillation of PD solutions interferes with the local immune and inflammatory response in the peritoneal cavity [18, 19] and negatively affects the involvement of mesothelial cells and macrophages in the peritoneal immune response against invading pathogens [20]. In fact, PD solutions alter the viability, phenotype, and function of peritoneal cells (e.g., mesothelial cells and macrophages) in a phenomenon commonly referred to as bioincompatibility [21]. Furthermore, PD solutions are acidic and hypertonic and contain high concentrations of glucose and glucose degradation products (GDPs), and these four parameters are major determinants for the peritoneal membrane damage that results in altered peritoneal permeability and loss of ultrafiltration capacity [15, 21–23].

PD solutions inhibit cytokine production by mesothelial cells and monocytes/macrophages [14] and consequently, might impair normal DC development. In this regard, it has been shown that PD solutions inhibit TNF-α mRNA expression and NF-κB DNA-binding activity in LPS-treated macrophages [14]. The present study was undertaken to analyze the influence of currently used PD solutions on monocyte-derived DC (MDDC) maturation and mature DC-effector functions. Analysis of PD solutions and their components revealed that sodium lactate and GDPs have a profound, negative impact on several phenotypic and functional maturation parameters. These findings have important implications for the initiation of primary immune responses within the peritoneal cavity and imply that DC maturation might be impaired in tissues exhibiting high concentrations of lactate (e.g., tumors) or GDPs (uremic or hyperglycemic conditions).

MATERIALS AND METHODS

PD solutions

PD fluids used in the present study included bicarbonate (25 mM)-/lactate (15 mM)-buffered solutions (pH 7.4) with 1.3%, 2.6%, or 3.8% glucose (hereafter termed P1, P2, and P3, respectively), and heat (D)- or filtered (Df)-sterilized lactate (40 mM)-containing solutions (pH 5.5) with equivalent glucose concentrations (D1, D2, D3 and D1f, D2f, D3f). The composition and main characteristics of the different solutions are indicated in **Table 1**. All solutions were kindly provided by Baxter Healthcare (Brussels, Belgium). Sodium lactate and other chemicals and reagents were obtained from Sigma (Barcelona, Spain). GDPs [methyl-glyoxal, 3-deoxyglucosone (3-DG), acetaldehyde, formaldehyde] were obtained from Sigma or Toronto Research Chemicals (Ontario, Canada).

Generation of MDDC

MDDC were obtained as described previously [4, 6, 24]. Briefly, peripheral blood monocytes were isolated from buffy coats from healthy donors by density centrifugation on Lymphoprep (Nycomed Pharma, Oslo, Norway) and were allowed to adhere to six-well tissue-culture plates. Nonadherent cells were removed by washing, and adherent cells were maintained in 3 ml RPMI medium with 10% fetal bovine serum, 2 mM glutamine, and 50 μg/ml gentamicin (complete medium) at 37°C in a humidified atmosphere with 5% CO_2. Differentiation into immature MDDC was accomplished by the addition of GM-CSF (Leucomax, Schering-Plough, Kenilworth, NJ) and IL-4 (PreProtech, Rocky Hill, NJ), both at 1000 U/ml. Medium was replaced, and new cytokines were added every 2 days. After 5–7 days, cells were in suspension and exhibited the phenotypic and functional characteristics of immature DC, as described [4, 6, 24]. For MDDC maturation, cells were treated with TNF-α (at 100 U/ml) or *Escherichia coli* 055:B5 LPS (10 ng/ml) and were allowed to mature for 48 h.

To assess the effect of PD fluids on DC maturation, immature MDDC were subjected to the standard maturation protocol [6, 24] and were cultured in a 1:1 dilution of each PD solution in complete medium. To that end, 3 ml of the corresponding PD solution was added onto 3 ml cultures of immature MDDC ($5x10^5$ cells/ml), and after a 1-h incubation, TNF-α (100 U/ml) or LPS (10 ng/ml) was added to the culture medium. In all cases, the pH of the resulting media was within the 7.2–7.6 range. After 24–48 h, cells were harvested for phenotypic and functional analysis, and cell supernatants were collected for determination of cytokine levels. As a control, immature MDDC were diluted 1:1 with RPMI. Evaluation of the influence of sodium lactate and GDPs on MDDC maturation was performed by addition of the different reagents onto the wells, where immature MDDC had been differentiated. Similarly, the effect of PD fluids on DC differentiation was done by culturing monocytes with GM-CSF and IL-4 (both at 1000 U/ml) in a 1:1 dilution of each PD solution in complete medium, and phenotypic measurements were performed after 48 h.

Flow cytometry and antibodies

Twenty four or 48 h after TNF-α or LPS stimulation, cells were collected, washed in ice-cold phosphate-buffered saline (PBS), and processed for indirect immunofluorescence. Cells were resuspended in 100 μl complete medium containing 50 μg/ml human immunoglobulin G (IgG) and were incubated for 15 min at 4°C to prevent binding through the Fc portion of the antibodies. Then, 100 μl of a solution containing 10 μg/ml of the indicated monoclonal antibodies (mAb) was added and incubated for 30 min on ice. After three washing steps in PBS, cells were resuspended in 100 μl complete medium containing fluorescein isothiocyanate (FITC)-labeled $F(ab')_2$ rabbit anti-mouse IgG, kept on ice for 30 min, washed, and resuspended in 200 μl PBS for flow cytometry. mAb included anti-CD1a (BL6), anti-CD83 (HB1/5; Immunotech, Marseille, France), anti-CD86 (B-T7; Diaclone Research, Besancon, France), anti-CD49d (ALC1/6.3), anti-CD40 (MAB89), anti-MHC class II (TS1/2), anti-CD11a (TS1/11), anti-CD151 (kindly provided by Dr. Francisco Sánchez-Madrid, Hospital de la Princesa, Madrid), and anti-CD209 (MR-1). Cells were also incubated with isotype-matched control antibodies to determine the basal level of fluorescence. Flow cytometry analysis was performed with an EPICS-CS (Coulter Científica, Madrid, Spain) using log amplifiers.

Measurement of MDDC phagocytic activity

Mannose receptor-mediated endocytosis was measured after 24 or 48 h of maturation. Mature MDDC ($5x10^5$ cells/ml) were incubated in 200 μl solution

TABLE 1. Characteristics of PD Solutions

		pH	mOsm/l	[Glucose] (g/l)	[Sodium lactate] (g/l)	Sterilization	GDPs
Physioneal glucose 1.36%	P1	7.4	344	13.6	1.68 (15 mM)	Heating	Yes
Physioneal glucose 3.86%	P3	7.4	483	38.6	1.68 (15 mM)	Heating	Yes
Dianeal glucose 1.36%	D1	5.5	344	13.6	4.5 (40 mM)	Heating	Yes
Dianeal glucose 3.86%	D3	5.5	483	38.6	4.5 (40 mM)	Heating	Yes
Dianeal glucose 1.36% F	D1f	5.5	344	13.6	4.5 (40 mM)	Filtration	No
Dianeal glucose 3.86% F	D3f	5.5	483	38.6	4.5 (40 mM)	Filtration	No

buffered with 25 mM HEPES and containing 1 mg/ml FITC-dextran (Sigma). After a 1-h incubation at 37°C, cells were washed four times in ice-cold PBS and analyzed in a EPICS flow cytometer. As control, cells from each culture condition were maintained in the same solution for 1 h at 4°C.

Determination of cytokine levels

The level of IL-12 (p70) released in each culture condition was determined using the IL-12 Eli-pair system from Diaclone Research (capture mAb B-T21; detection biotynilated antibody B-P24) or the OptiEIA human IL-12 p70 set (PharMingen, San Diego, CA), following the manufacturer's recommendations. Based on preliminary experiments, supernatants from MDDC were assayed, undiluted or diluted 1:3 in complete medium. Culture supernatants were assayed in triplicate for TNF-α and IL-6 by enzyme-linked immunosorbent assay with Ready-SET-Go sets (eBioscience, San Diego, CA).

MDDC-induced allogeneic T lymphocyte proliferation

Allogeneic lymphocytes were obtained from the peripheral blood of healthy adults after density centrifugation on Lymphoprep (Nycomed Pharma) and monocyte removal by an adherence step. Lymphocytes ($2x10^5$) were stimulated in a flat-bottom 96-well plate with 200, 400, 10^3, 2 x 10^3, or 5 x 10^3 allogeneic, irradiated (150 rads/min for 10 min) MDDC, matured under the distinct culture conditions. Each stimulator/responder ratio was assayed in triplicate. After a 6-day incubation period, [^{3}H]thymidine was added (1 μCi/well) during the last 16 h of coculture, and thymidine incorporation was determined to evaluate the level of T cell proliferation.

Electrophoretic mobility shift assay (EMSA)

EMSA was performed essentially as described [25], using 5 μg nuclear extracts prepared according to Schreiber et al. [26]. For competition experiments, unlabeled oligonucleotide (100-fold molar excess) was preincubated with nuclear extracts at 4°C for 30 min before the addition of the probe. EMSA probe was NF-κB-CONS (5′-AGTTGAGGGGACTTTCCCAGGC-3′), which contains a consensus-binding site for NF-κB.

Western blot

Total cell lysates were obtained in 50 mM HEPES, pH 7.5, 250 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 0.5 mM dithiothreitol, 10 mM NaF, 1 mM Na_3VO_4, 20 mM Pefabloc, and 2 μg/ml aprotinin, antipain, leupeptin, and pepstatin. Each sample (10 μg) was resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions and was transferred onto an Immobilon polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking the unoccupied sites with 5% bovine serum albumin in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1% Tween-20, IκBα detection was accomplished with an anti-IκBα polyclonal antiserum from Santa Cruz Biotechnology (Santa Cruz, CA), a horseradish peroxidase-labeled goat anti-rabbit antiserum (Dako A/S, Denmark), and the Supersignal West Pico chemiluminescent system (Pierce, Rockford, IL).

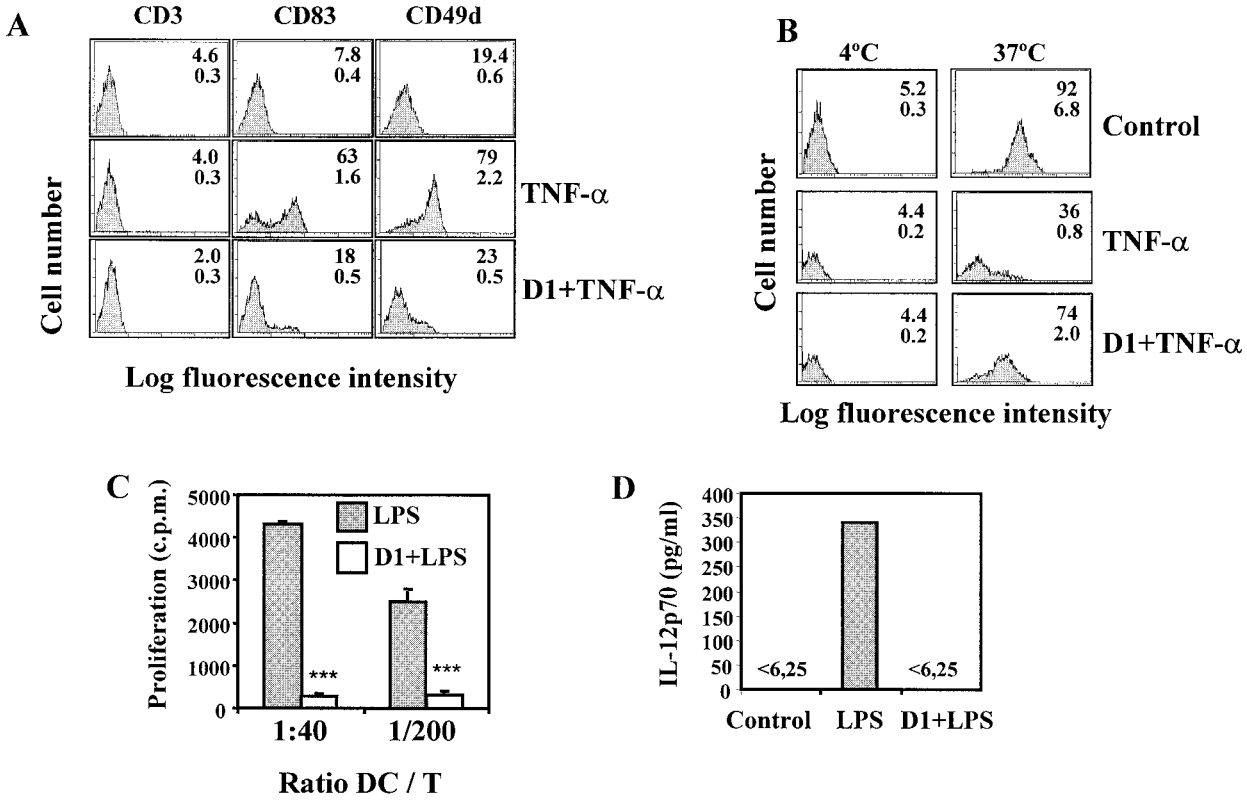

Fig. 1. Phenotypic and functional changes during MDDC maturation: effect of the GDP-rich, high lactate-containing D1 solution. Immature MDDC were treated with TNF-α (A, B) or LPS (C, D) in complete medium or in medium diluted 1:1 with D1 PD solution. (A) Expression of maturation markers. After 48 h, cell-surface expression of the maturation markers CD83 and CD49d was determined by indirect immunofluorescence, as well as the fluorescence of a negative control antibody (CD3). The percentage of marker-positive cells (upper number) and the mean fluorescence intensity (lower number) is indicated in each case. (B) Mannose receptor-mediated endocytosis. Forty-eight hours after TNF-α addition, endocytosis was determined by flow cytometry using FITC-dextran. In all cases, endocytic activity was also measured at 4°C to control for nonspecific fluorescence. The percentage of FITC-dextran-positive cells and the mean fluorescence intensity is indicated in each case. (C) T cell-stimulatory capacity. 48 h after LPS treatment cells were irradiated and used to stimulate 2×10^5 allogeneic T lymphocytes at the indicated stimulator/responder ratios. After a 6-day coculture, ^{3}H-thymidine was added to the culture for 16 h, and T cell proliferation was determined by measuring incorporated thymidine. Data represent mean ± SD of triplicate determinations (***, $P<0.001$). (D) IL-12 p70 production 24 h after LPS addition. Cell supernatants were removed, and IL-12 p70 concentration was determined using the IL-12 Eli-pair system. All experiments were performed at least twice on independent donors, and a representative experiment is shown.

Statistical analysis

Data from individual representative experiments were presented as mean values ± SD, and data from multiple experiments were represented as mean group values ± SD. Statistical significance of the observed differences between groups was determined by ANOVA and paired Student's *t*-test, and $P < 0.05$ values were considered significant. Statistical analysis of the data was performed with MPM III 1.34 (BioRad, Hercules, CA) and GraphPad Prism (GraphPad Software, San Diego, CA) software.

RESULTS

PD solutions differentially impair the phenotypic and functional maturation of MDDC

As PD solutions interfere with inflammatory responses within the peritoneal cavity, we reasoned that they could affect the development and/or effector functions of DC. To test this hypothesis, MDDC maturation was performed in the presence of currently used PD solutions. Initially, immature MDDC were diluted 1:1 with the D1 PD solution, and maturation was induced with TNF-α (100 U/ml) or LPS (10 ng/ml). Addition of the D1 solution prevented the expression of maturation markers CD83 and CD49 (**Fig. 1A**) and impaired the loss of mannose receptor-mediated endocytosis, an effect that could be observed 24 and 48 h after LPS addition (Fig. 1B; and data not shown). More importantly, D1 significantly inhibited the DC-mediated, allogenic T cell proliferation at two distinct effector:target ratios (Fig. 1C) and blocked the maturation-dependent production of IL-12 p70 (Fig. 1D). Therefore, maturation of MDDC in response to TNF-α or LPS is inhibited by the D1 PD solution.

Initial experiments revealed that PD fluids did not alter the maturation state of immature DC, as demonstrated by the lack of induction of CD83, the failure to up-regulate CD86 and CD54, and the absence of IL-12 p70 release (data not shown). Subsequently, DC maturation was induced with TNF-α or LPS and in the presence of PD solutions differing in their glucose, lactate, and GDP content (Table 1). Except for the D3 solution, MDDC viability was not affected by exposure to PD solutions, as propidium iodide and annexin V staining revealed that more than 90% of the cells remained viable for up to 48 h (data not shown). In the case of the D3 solution, 70% of the cells remained viable cells after 48 h, and flow cytometry analysis was performed after gating out dead cells. As shown in **Table 2**, all PD solutions inhibited the LPS-triggered production of IL-12 p70 and differentially affected the expression of maturation markers and costimulatory molecules and the loss of FITC-dextran phagocytosis. More importantly, allogenic T cell proliferation induced by mature DC was also differentially inhibited by the tested PD solutions. Comparison of the relative composition of the distinct PD fluids and their inhibitory effects revealed that heat-sterilized, high lactate-containing solutions (D1, D3) produced higher inhibitory effects than heat-sterilized, low lactate solutions (P1, P3), and filtered, GDP-free solutions (D1f, D3f) always exerted a lower inhibitory effect than heat-sterilized, GDP-rich counterparts (D1, D3; Table 2). Altogether, these experiments indicated that PD solutions differentially inhibit the acquisition of MDDC maturation parameters and suggested that lactate and GDPs are major contributors to the inhibitory effect of PD solutions on MDDC maturation.

The inhibitory effect of PD solutions on MDDC maturation correlates with inhibition of NF-κB activity

Besides IL-12 p70, LPS-matured MDDC synthesize cytokines such as TNF-α and IL-6 [27], and PD solutions were analyzed for their influence on the maturation-dependent release of both cytokines. MDDC released less TNF-α when maturation took place in the presence of high lactate-containing solutions (D1, D3, D1f, D3f), whereas solutions with lower levels of lactate (15 mM; P1, P3) did not affect cytokine production (**Fig. 2A**). For IL-6, a strong, inhibitory effect was also observed for the D1 and D3 solutions, and P1 and D1f solutions exerted a weak inhibition (Fig. 2A). Therefore, PD solutions differentially inhibit the production of TNF-α and IL-6 by LPS-matured

TABLE 2. Effect of PD Solutions on Phenotypic and Functional Changes during MDDC Maturation[1]

	Cell surface expression					Allogenic T cell proliferation		
DC treatment	CD83	CD86	CD40	MHC II	Endocytosis	1:40	1/200	IL-12 p70 (pg/ml)
Untreated	4.22 (0.3)	(1.0)	—	(4.4)	95.7 (5.5)	N.D.	N.D.	<7.8
LPS	47.89 (1)	(9.4)	(20.7)	(4.4)	26.7 (0.7)	4326 ± 50.5	2473 ± 250.5	256.5
P1 + LPS	29.16 (0.7)	(7.7)	(24.3)	(4.1)	46.45 (1.1)	3903 ± 49.6 (**)	705 ± 161.6 (**)	<7.8
P3 + LPS	22.28 (0.6)	(8.2)	(14.5)	(3.9)	42.93 (1)	1301 ± 167.4 (***)	370 ± 54.5 (***)	<7.8
D1 + LPS	11.76 (0.5)	(6.1)	(16.6)	(3.8)	75.58 (1.8)	294 ± 35.4 (***)	315 ± 79.4 (***)	<7.8
D3 + LPS	21.55 (0.6)	(6.4)	(11.5)	(3.4)	51.85 (1.1)	224 ± 8.4 (***)	309 ± 176.9 (**)	<7.8
D1f + LPS	20.68 (0.5)	(5.4)	(16.9)	(4.7)	62.85 (1.5)	3576 ± 476.3 (NS)	1556 ± 179 (**)	<7.8
D3f + LPS	28.19 (0.7)	(6.6)	(12)	(4.8)	50.35 (1.1)	2829 ± 530.2 (*)	893 ± 237.8 (**)	<7.8

[1] Immature MDDC were treated with LPS (10 ng/ml) in the presence of the distinct PD solutions as indicated in Materials and Methods. Cell-surface expression of CD83, CD86, CD40, and MHC class II was determined by indirect immunofluorescence and flow cytometry analysis. Mannose receptor-mediated endocytosis was evaluated with FITC-dextran and flow cytometry. In both cases, the percentage of marker-positive cells is indicated, with mean fluorescence intensity values shown in parentheses. Allogenic T cell proliferation was performed using the indicated DC/T ratios. Thymidine incorporation was determined after a 6-day culture period, and the mean ± SD is shown for each culture condition. Comparison with LPS-treated cells yielded the indicated *P* values (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant; N.D., not determined). IL-12 p70 was determined in culture supernatants after 48 h as described in Materials and Methods. Each individual determination was performed at least three times, and representative experiments are shown.

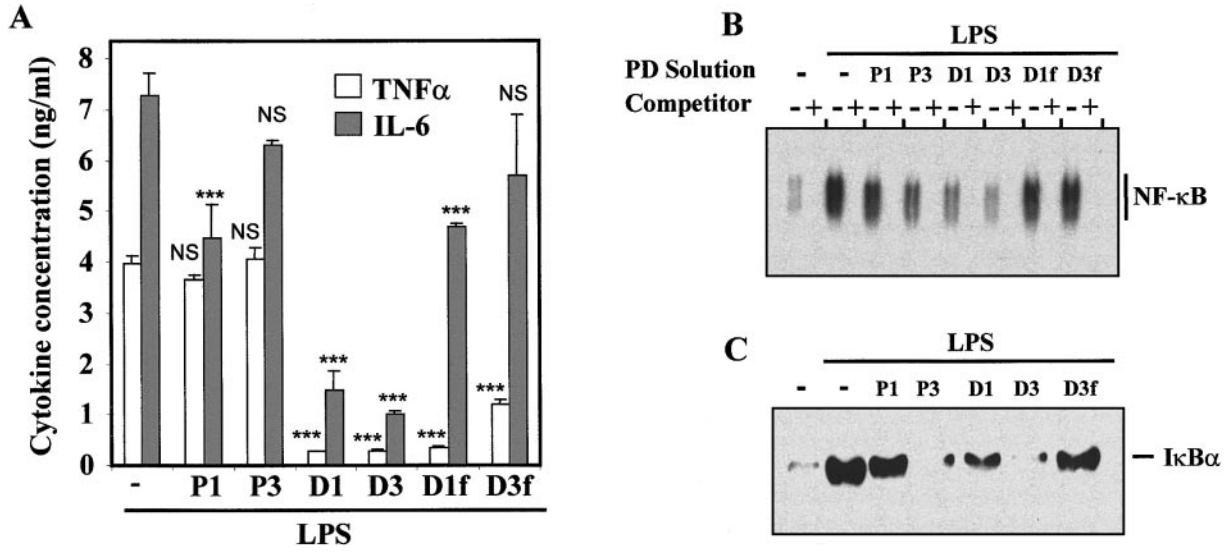

Fig. 2. Influence of PD solutions on cytokine production, NF-κB activation, and IκBα levels during LPS-induced MDDC maturation. Immature MDDC were left untreated or cultured with LPS for 48 h in complete medium (–) or in medium diluted 1:1 with the indicated PD solutions. (A) TNF-α and IL-6 levels. Cell supernatants were collected and evaluated for their content of TNF-α and IL-6 using Ready-SET-Go sets. Data represent mean ± SD of triplicate determinations (***, $P<0.001$; NS, not significant). (B) NF-κB activity. NF-κB DNA-binding activity was determined in total cell extracts. EMSA was performed on the NF-κB consensus oligonucleotide and in the absence (− lanes) or presence (+ lanes) of unlabeled competitor oligonucleotides at a 100-fold molar excess. (C) IκBα levels. Cytoplasmic extract (10 μg) from each culture condition was subjected to Western blot using a rabbit polyclonal antiserum specific for IκBα. Experiments were performed on two distinct donors with identical results, and representative experiments are shown.

MDDC, and high lactate-containing solutions exhibited a higher inhibitory effect.

DC maturation is dependent on NF-κB transcription factors, whose activity increases during the process [3, 8, 27–29]. As PD solutions impair MDDC functional maturation, their influence on NF-κB activity during LPS-induced MDDC maturation was determined. As expected, immature MDDC exhibited a moderate level of NF-κB DNA binding, and a great increase in NF-κB-dependent DNA binding was found in mature MDDC (Fig. 2B) [28]. However, MDDC maturation in the presence of certain PD solutions resulted in reduced levels of NF-κB activity (Fig. 2B). The maturation-associated increase in NF-κB activity was strongly reduced by D1 and moderately inhibited by P1, and GDP-free solutions showed a minor inhibitory effect (D1 vs. D1f and D3 vs. D3f; Fig. 2B), thus implying that high lactate and GDPs have a detrimental effect on the increase in NF-κB DNA-binding activity during MDDC maturation. Previous studies have also shown increased expression of IκBα in mature MDDC [28]. The level of IκBα was also greatly reduced in MDDC matured in the presence of D solutions, and GDP-containing solutions (P3 and D3) had a stronger inhibitory effect than the corresponding GDP-free solution (D3f; Fig. 2C). These results indicate that the differential, inhibitory effect of PD solutions on MDDC maturation reflects their distinct ability to prevent the LPS-induced increase in NF-κB activity.

Inhibitory effect of lactate and GDPs on the phenotypic and functional maturation of MDDC

As lactate and GDPs were potentially responsible for the observed, inhibitory effect of PD solutions, their individual effects on MDDC maturation were evaluated. In the case of lactate, experiments were performed using 15 or 40 mM sodium lactate, and the pH of the lactate-containing media remained within the 7.4–7.6 range. Lactate dose-dependently inhibited the LPS-induced up-regulation of CD83 and weakly affected the CD86 up-regulation but had no influence on the CD151 expression (**Fig. 3A**). Lactate also inhibited IL-12 p70 production in a dose-dependent manner: IL-12 p70 production was reduced 5–10 times in the presence of 15 mM lactate, and 40 mM lactate reduced IL-12 p70 production to undetectable levels (Fig. 3B). Sodium lactate significantly inhibited the LPS-induced production of TNF-α, had no effect on the maturation-dependent production of IL-6 (Fig. 3C), and reduced NF-κB activation in immature and LPS-treated MDDC (Fig. 3D). As a whole, these results demonstrate that sodium lactate impairs the LPS-induced phenotypic and functional MDDC maturation by inhibiting NF-κB activation in a dose-dependent manner.

A similar approach was undertaken to evaluate the effects of GDPs on MDDC maturation. Previous studies detected high concentrations of acetaldehyde and 3-DG in PD fluids and the effluent of PD patients [30, 31]. Preliminary analysis revealed that acetaldehyde, formaldehyde, and glyoxal had no influence on MDDC maturation (data not shown), and 3-DG exhibited moderate effects when used at the concentrations usually found in PD fluids. Subsequent experiments were performed using 486 μM acetaldehyde or 3-DG at 50, 150, and 450 μM, all of which are similar or lower than their corresponding concentrations in glucose-containing PD solutions [31]. 3-DG had a weak inhibitory effect on the up-regulation of CD83 and CD86, and acetaldehyde and formaldehyde were without effect (**Fig. 4A**; and not shown). The inhibitory effect of 15 mM lactate was further increased in the presence of 3-DG (Fig. 4A), suggesting

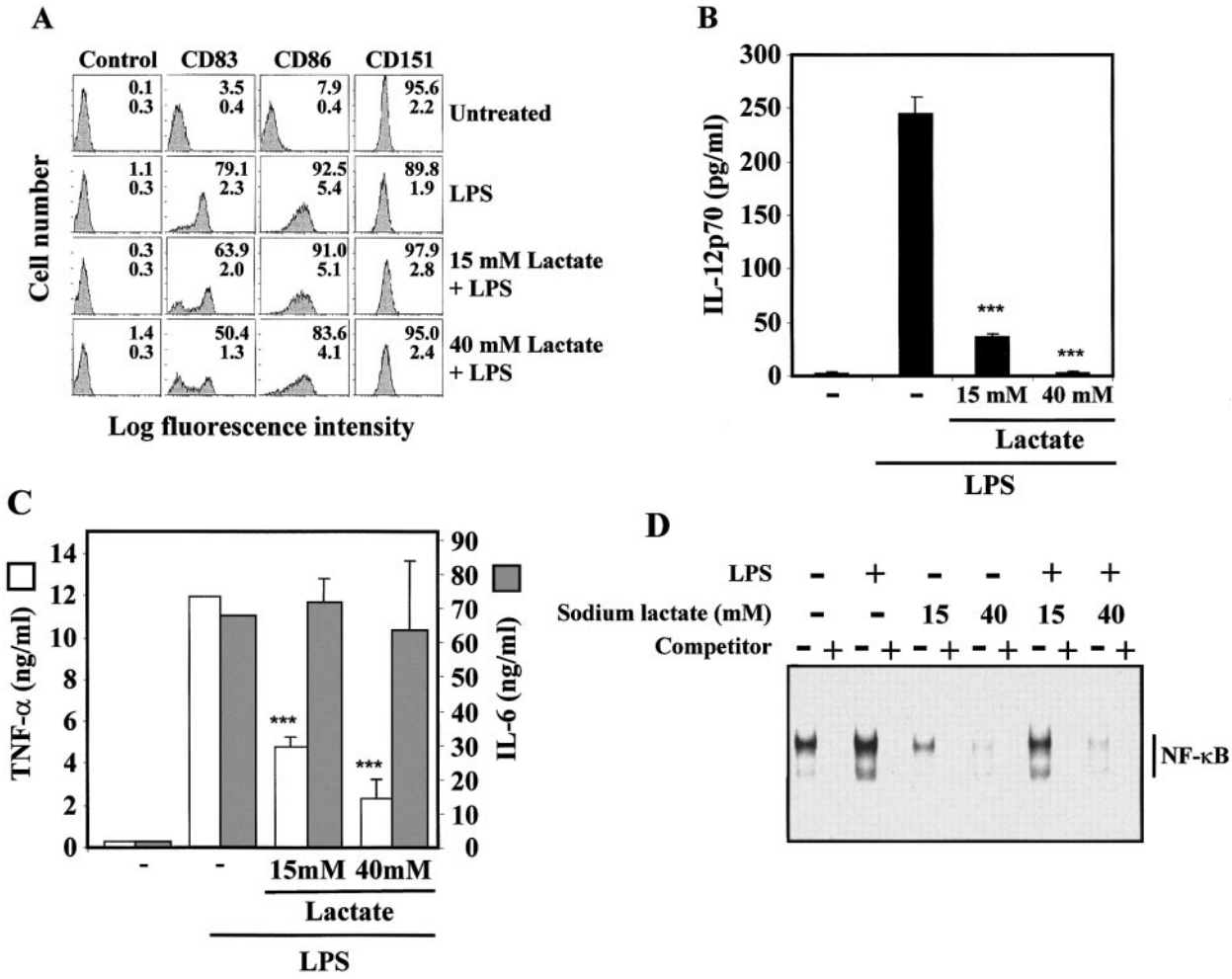

Fig. 3. Phenotypic and functional changes during LPS-induced MDDC maturation: effect of lactate. Immature MDDC were treated with LPS in complete medium or in complete medium containing 15 mM or 40 mM lactate. (A) Expression of maturation markers. After 48 h, indirect immunofluorescence determined cell-surface expression of the maturation markers CD83 and CD86, as well as the fluorescence of an isotype-matched antibody (Control) and that of CD151. The percentage of marker-positive cells (upper number) and the mean fluorescence intensity (lower number) is indicated in each case. (B) IL-12 p70 production. Twenty-four hours after LPS addition, cell supernatants were removed, and IL-12 p70 concentration was determined using the IL-12 Eli-pair system. Data represent mean ± SD of triplicate determinations (***, $P<0.001$). (C) TNF-α and IL-6 levels. Forty-eight hours after LPS addition, cell supernatants were collected and evaluated for their content of TNF-α and IL-6 using Ready-SET-Go sets. Data represent mean ± SD of triplicate determinations (***, $P<0.001$). (D) NF-κB activity. NF-κB DNA-binding activity was determined in total cell extracts from each culture condition. EMSA was performed on the NF-κB consensus oligonucleotide and in the absence (− lanes) or presence (+ lanes) of unlabeled competitor oligonucleotides at a 100-fold molar excess. All experiments were performed at least twice on independent donors, and representative experiments are shown. L15, 15 mM lactate; L40, 40 mM lactate.

that both agents have an additive, negative influence on MDDC maturation. Regarding IL-12 p70 production, acetaldehyde (486 μM) and 3-DG (450 μM) inhibited the production of IL-12 p70 in response to LPS (Fig. 4B). Compared with 15 mM lactate, 3-DG was a stronger inhibitor, and the simultaneous addition of 15 mM lactate and any GDP resulted in higher levels of inhibition (Fig. 4B). The molecular basis for the inhibitory effect of GDPs was analyzed by determining its influence on NF-κB activity during MDDC maturation. 3-DG impaired the increase in NF-κB during MDDC maturation, with a higher inhibitory effect than lactate, and acetaldehyde had no effect (Fig. 4C). Finally, experiments were set up to evaluate the influence of lactate and 3-DG on the acquisition of T cell-stimulatory activity. Lactate moderately inhibited the T cell stimulatory activity, and 40 mM lactate caused a 25% reduction (**Fig. 5**). The parallel analysis of 3-DG revealed that it reduced allogenic T cell proliferation to a higher extent than 40 mM lactate, as it brought allogenic proliferation down to 50% at a 1:40 MDDC:T ratio, and it caused an almost complete blockade at a 1:200 ratio (Fig. 5). Altogether, these results demonstrate that lactate and 3-DG negatively affect LPS-induced MDDC maturation and might contribute to the inhibitory action of PD solutions.

PD solutions inhibit monocyte differentiation into MDDC

As peritoneal monocytes/macrophages acquire DC phenotypic properties during transport of exogenous agents into adjacent lymph nodes [5], we also analyzed the influence of PD fluids on the GM-CSF + IL-4-driven differentiation of monocytes toward DC. Monocytes were subjected to the MDDC in vitro differentiation protocol in the presence of PD fluids, and differentiation

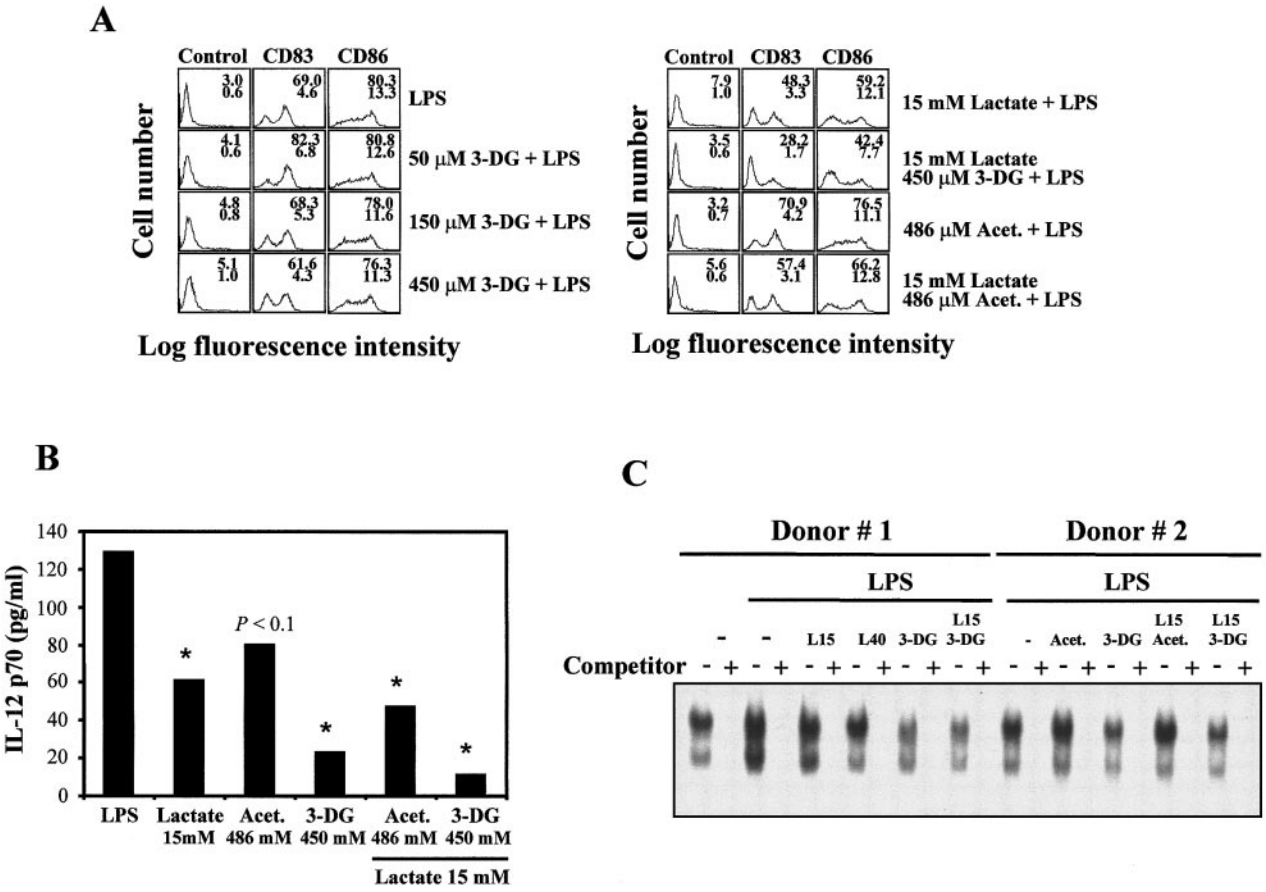

Fig. 4. Phenotypic and functional changes during LPS-induced MDDC maturation: effect of glucose degradation products. Immature MDDC were treated with LPS in complete medium or in complete medium containing 15 mM lactate and/or acetaldehyde (Acet.) or 3-DG. (A) Expression of maturation markers. After 48 h, cell-surface expression of the maturation markers CD83 and CD86 was determined by indirect immunofluorescence, as well as the fluorescence of an isotype-matched antibody (Control). The percentage of marker-positive cells (upper number) and the mean fluorescence intensity (lower number) is indicated in each case. (B) IL-12 p70 production. Twenty-four hours after LPS addition, cell supernatants were removed, and IL-12 p70 concentration was determined using the IL-12 Eli-pair system. Data represent mean ± SD of triplicate determinations (*, $P<0.05$). (C) NF-κB activity. NF-κB DNA-binding activity was determined in total cell extracts from each culture condition. EMSA was performed on the NF-κB consensus oligonucleotide and in the absence (− lanes) or presence (+ lanes) of unlabeled, competitor oligonucleotides at a 100-fold molar excess. All experiments were performed at least twice on independent donors, and a representative experiment is shown.

was evaluated through the expression of the DC markers CD1a and DC-specific intercellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin (DC-SIGN), which are induced at the early stages of the monocyte–DC transition [32, 33]. As described [32, 33], CD1a and DC-SIGN were not expressed by monocytes but were induced after 48 h in the presence of GM-CSF and IL-4 (**Fig. 6A**). CD1a induction was very sensitive to the presence of PD solutions, and its induction was almost similarly prevented by heat-sterilized or filtered PD solutions (D3 and D3f in Fig. 6A). By contrast, PD fluids variably impaired DC-SIGN induction, and heat-sterilized, high lactate-containing solutions (D1, D3) produced the highest inhibitory effects, whereas GDP-free solutions (D3f) were without effect (Fig. 6A). Therefore, PD solutions differentially prevent monocyte differentiation into MDDC, and heat-sterilized, high lactate-containing solutions exhibited the stronger inhibitory effect. Microscopical examination confirmed this finding, as heat-sterilized solutions prevented the polarization and acquisition of cytoplasmic projections that characterize differentiating MDDC (Fig. 6B). The above results suggested that DC-SIGN induction might be GDP-sensitive (cf., D3 and D3f in Fig. 6A), and factors other than GDP interfere with CD1a induction. In accordance, sodium lactate inhibited the differentiation-dependent induction of CD1a in a dose-dependent manner but did not significantly affect DC-SIGN upregulation or the constitutive expression of CD14 (Fig. 6C). Thus, acquisition of CD1a expression is specifically impaired in the presence of sodium lactate.

DISCUSSION

As a first step toward understanding the relationship of biocompatibility to peritoneal host defense and susceptibility to infection, we have analyzed the effect of currently used PD fluids on the differentiation and functional maturation of MDDC. All the tested PD solutions completely prevented the production of IL-12 p70 by LPS-treated MDDC and differentially affected the differentiation and maturation-dependent acquisition of costimulatory molecules and the increase in

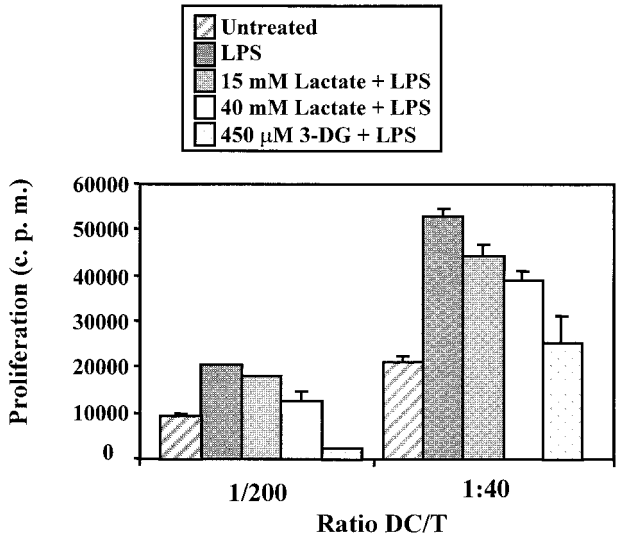

Fig. 5. Influence of lactate and 3-DG on the T cell-stimulatory capacity of mature MDDC, which was induced with LPS in the absence or in the presence of 15 mM lactate, 40 mM lactate, or 450 μM (3-DG). After 48 h, cells were irradiated and used to stimulate 2×10^5 allogeneic T lymphocytes at the indicated stimulator/responder ratios in 96-well plates. After a 6-day coculture, ^{3}H-thymidine was added to the culture for 16 h, and T cell proliferation was determined by measuring the incorporated thymidine. The experiment was performed twice on separate donors, and a representative experiment is shown.

allogenic T cell stimulation. Evaluation of PD solution components revealed that sodium lactate and the GDP 3-DG have a direct, negative effect on MDDC maturation and significantly contribute to the inhibitory action of PD solutions by preventing NF-κB activation induced by maturation agents. Although the experimental conditions used in the current studies may not mimic the changing kinetics of different solutes in clinical conditions, they provide a clear demonstration that different PD solutions and certain PD solution constituents (sodium lactate, GDPs) can inhibit differentiation programs at the genetic and functional levels. In addition, the protocols implemented in our studies may serve to illustrate the compounded effect of these solutions upon cyclical exposure. To the best of our knowledge, the present manuscript provides the first experimental evidence that currently used PD solutions exert a potent and differential inhibitory effect on DC differentiation and maturation.

Recent studies have investigated the presence and biological relevance of DC within the peritoneal cavity. DC account for 1–2% of the total peritoneal cell pool [5, 10, 11] and participate in the initiation of immune responses against bacteria [34]. However, the number of functionally effective peritoneal DC could be considerably higher in an infectious situation, as monocytes and macrophages can differentiate in vivo

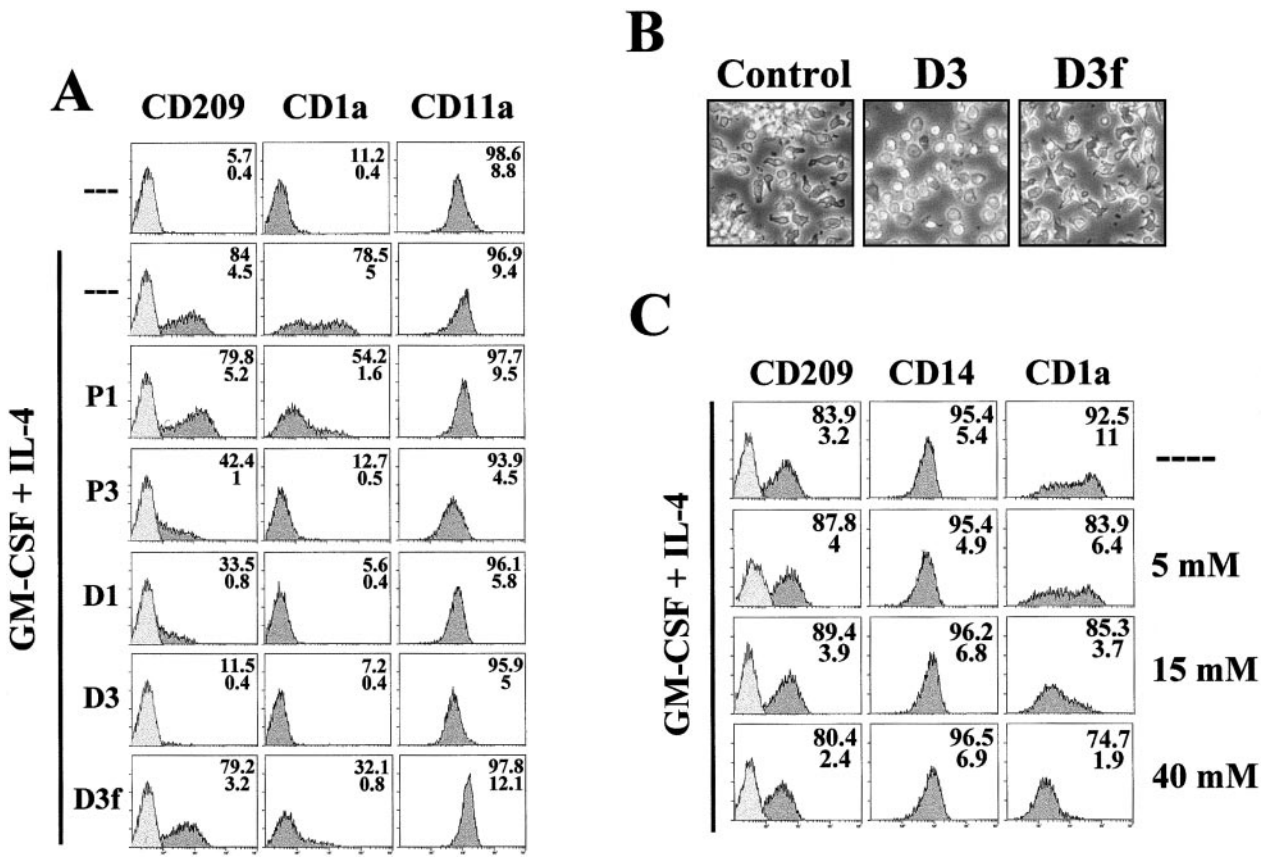

Fig. 6. Influence of PD solutions on MDDC differentiation from monocytes, which were left untreated or cultured with GM-CSF + IL-4 in the absence or in the presence of various PD solutions (A, B) or the indicated sodium lactate concentrations (C). After 48 h, cell-surface expression of CD14 (C) and the DC differentiation markers CD1a and DC-SIGN (A) was determined by indirect immunofluorescence. Lightly shaded profiles illustrate the fluorescence of an isotype-matched, negative control antibody. The percentage of marker-positive cells (upper number) and the mean fluorescence intensity (lower number) is indicated in each case. (B) Cells were photographed after 48 h of culture in the indicated conditions and at a 40X original magnification. Experiments were performed on three independent donors, and a representative experiment is shown.

into fully mature DC [5, 11]. Previous studies have also illustrated the capacity of murine peritoneal cells to differentiate in vivo along the dendritic pathway in the presence of GM-CSF and/or TNF-α, leading to the proposal that peritoneal cells constitute a reservoir of late DC precursors [35], and the number of functionally mature DC is dramatically increased in the peritoneal cavity after Flt3L treatment [36]. Our results indicate that PD solutions impair monocyte differentiation toward DC, affecting cellular morphology and the acquisition of the DC markers CD1a and DC-SIGN, which not only mediates transient DC–T interactions and DC rolling on endothelial cells by interacting with ICAM-3 and ICAM-2 [32, 37] but is also involved in HIV and Ebola virus capture [38, 39] and pathogen recognition [40] and can be considered as a pathogen-recognition receptor. Conversely, the CD1 family of antigen-presenting molecules binds and presents a variety of mammalian and microbial glycolipids for specific recognition by T cells [41]. Therefore, PD solutions, which inhibit the differentiation-associated induction of CD1a and DC-SIGN, might disrupt peritoneal immunocompetence by impairing the acquisition of the antigen-presenting and the pathogen-recognition capabilities normally exhibited by DC. In consequence, pharmacological treatment with differentiation- or maturation-promoting cytokines might improve peritoneal immunocompetence during exposure to PD fluids.

The inhibitory effect of PD solutions on the maturation-dependent release of IL-12 p70 and IL-6 is another relevant finding. IL-12 p70 is critically involved in the process of polarization of naive T lymphocytes into the Th1 lymphocyte differentiation pathway and the cellular branch of the immune response [27, 42]. Consequently, if the inhibitory action of lactate and 3-DG takes place in vivo, peritoneal DC from CAPD patients would be deficient in their Th1-polarizing capability and would preferentially trigger pro-tolerogenic or Th2-mediated humoral-immune responses [1, 3, 42], thus impairing the initiation of cellular immune responses within the peritoneal cavity. In this regard, it is worth mentioning that Th cells from CAPD patients have been found to show a dysregulated, differentiation profile characterized by a major increase in the percentage of Th2 cells [43]. Conversely, IL-6 production is more strongly inhibited in the presence of PD solutions containing high levels of GDP (Fig. 2A), which suggests a role for GDPs in IL-6 inhibition. However, direct evaluation of the individual effects of 3-DG and acetaldehyde on the maturation-dependent IL-6 production revealed no effect (data not shown). Several GDPs have been shown previously to weakly inhibit IL-6 production by human peritoneal mesothelial cells but only when six distinct GDPs were present [44]. Therefore, although GDPs might influence IL-6 production, their inhibitory action might require the combination of various GDPs or might even require the concomitant presence of other components of the PD solutions (including glucose and lactate). Along the same line, the inhibitory effect of lactate on NF-κB activity and its differential effect on the production of TNF-α and IL-6 (Fig. 3C) suggest that their expression is differentially affected by NF-κB inhibition. In fact, although TNF-α expression is extremely sensitive to NF-κB inhibition, the IL-6 promoter in DC is positively regulated by additional transcription factors such as activated protein-1, CCAAT/enhancer binding protein (C/EBP), or cyclic adenosine monophosphate response element-binding protein [45]. These factors, whose sensitivity to lactate is currently under investigation, might compensate for the diminished NF-κB activity and explain the lack of effect of lactate on the maturation-dependent IL-6 production. Alternatively, lactate might interfere with signaling pathways differentially affecting the expression of IL-6 and TNF-α and whose involvement in the stimulus-specific MDDC maturation has been recently demonstrated [46].

As a metabolite from activated macrophages, physiologically relevant concentrations of lactate (10–30 mM) are similar to those found to inhibit CD1a expression and MDDC maturation (15–45 mM) and have been previously found to modulate T cell receptor signaling [47]. However, the negative effect of lactate on MDDC maturation has additional implications in other pathological situations. High lactate concentrations are normally found within solid tumors, and lactate concentration correlates with the incidence of metastasis [48]. As tumor-associated DC usually have low allostimulatory capacity [49] and are phenotypically immature [50], it is tempting to speculate that lactate might contribute to the defective, functional maturation of DC in cancer patients. By contrast, GDP concentrations in PD fluids (500 μM for 3-DG in 3.86% glucose-containing solutions) are higher than those found in plasma under physiological conditions (1 μM) or even under diabetic conditions (7–10 μM) [51]. Although we have demonstrated its inhibitory effect on NF-κB activation, it remains to be established whether 3-DG also impairs MDDC maturation through its glycation capabilities, as 3-DG accelerates advanced glycation end-product formation [52] and induces growth-factor gene expression at 500 μM [53]. Regardless of the mechanism, 3-DG and sodium lactate appear to contribute to the inhibitory activity of PD fluids and consequently, might directly impair the onset of adequate immune responses within the peritoneal cavity and its surrounding tissues, a hypothesis that deserves further investigation.

ACKNOWLEDGMENTS

This work was supported by grant SAF2002-04615-C02-01 from Ministerio de Ciencia y Tecnología (to A. L. C.), grants 08.3/0026/2000.1 and 08.3/0026/2000.2 from Comunidad Autónoma de Madrid (to A. L. C. and P. S-M.), grant 01/0063-01 from Fondo de Investigaciones Sanitarias (to A. L. C.), and grants from the Canadian Institutes of Health Research, the Kidney Foundation of Canada, and the Ontario Research and Development Challenge Fund (ORDCF; to J. M.). G. C. is an ORDCF fellow, and J. M. holds a Canada Research Chair in Transplantation and Immunobiology. A. P-K. and O. M-P. contributed equally to this work, and the order of authorship is arbitrary. The authors gratefully acknowledge Dr. L. Skoufos and P. G. Blake for critical reading of the manuscript and helpful suggestions and Dr. Paola Elisabettini and Dr. Anne Leyssens for generous reagent supply.

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