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Polymorphonuclear Neutrophil-Derived Ectosomes Interfere with the Maturation of Monocyte-Derived Dendritic Cells

Ceylan Eken, Olivier Gasser, Gabriela Zenhaeusern, Ineke Oehri, Christoph Hess, and Jürg A. Schifferli

Polymorphonuclear neutrophils (PMNs) are a key component of the innate immune system. Their activation leads to the release of potent antimicrobial agents through degranulation. Simultaneously, PMNs release cell surface-derived microvesicles, so-called ectosomes (PMN-Ect). PMN-Ect are rightside-out vesicles with a diameter of 50–200 nm. They expose phosphatidylycerine in the outer leaflet of their membrane and down-modulate monocyte/macrophage-activation in vitro. In this study, we analyzed the effects of PMN-Ect on maturation of human monocyte-derived dendritic cells (MoDCs). Intriguingly, exposing immature MoDCs to PMN-Ect modified their morphology, reduced their phagocytic activity, and increased the release of TGF-β1. When immature MoDCs were incubated with PMN-Ect and stimulated with the TLR4 ligand LPS, the maturation process was partially inhibited as evidenced by reduced expression of cell surface markers (CD40, CD80, CD83, CD86, and HLA-DR). Inhibition of cytokine-release (IL-8, IL-10, IL-12, and TNF-α), and a reduced capacity to induce T cell proliferation. Together these data provide evidence that PMN-Ect have the ability to modify MoDC maturation and function. PMN-Ect may thus represent an as yet unidentified host-factor influencing MoDC maturation at the site of injury, thereby possibly impacting on downstream MoDC-dependent immunity.

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PMN-Ect share important biological properties with apoptotic cells, including the expression of PS (9–11, 16). Apoptotic cells have been identified as major regulators of DC function both in vitro and in vivo (12–15, 17–20). PS, both on apoptotic cells as well as when incorporated into artificial liposomes, has been identified as a major factor influencing monocyte-derived dendritic cell (MoDC) maturation and function (21–24). Furthermore, vesicles expressing PS released by tumor cells have recently been shown to down-regulate the activation of DCs, thus impairing the possible immune response against tumor Ags (25).

Although it is plausible that during the early phase of an immune response PMN-Ect interact with DCs, no data characterizing such interactions exist. In this study, we investigated the impact of other Ect, PMN-Ect have recently been shown to inhibit the inflammatory properties of human monocyte-derived macrophages in vitro. Induction of TGF-β1 secretion by macrophages and the exposure of PS on the surface of PMN-Ect were shown to contribute independently to this effect (11).

Dendritic cells (DCs) function as sentinels of the immune system, bridging innate and acquired immunity. In their tissue of residence, immature DCs (iDCs) internalize and proteolytically process self- and non-self Ags. When Ag uptake and processing occurs under inflammatory conditions, for example, conditions characterized by concomitant pattern recognition signals delivered to iDCs via pathogen-derived products, iDCs change their morphology, shut down phagocytosis, and increase expression of co-stimulatory molecules and secretion of cytokines. Simultaneously, DCs migrate into secondary lymphoid organs (i.e., spleen or lymph nodes). DCs activated and induced to mature under inflammatory conditions are then capable of priming and fully activating naïve CD4+ and CD8+ T cells. By contrast, partially and/or “inappropriately” activated iDCs are thought to induce immunological tolerance to Ags presented on their surface (12–15). The precise factors determining immunogenic vs tolerogenic DC-mediated priming remain to be defined.

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PMN-Ect on MoDCs. Specifically, we examined the maturation of MoDCs in the presence/absence of PMN-Ect and the functional activity of MoDCs that were matured in the presence of PMN-Ect.

Materials and Methods

Collection of PMN-Ect

To isolate PMNs, a fresh buffy coat was diluted 1/1 (v/v) with PBS-EDTA (2 mM), mixed with 0.25 vol of 4% dextran T500 (GE Healthcare Biomedicals), and left for 30 min for erythrocyte sedimentation. Leukocyte-rich supernatant was aspirated and centrifuged for 10 min at 200 × g. The pellet was resuspended in 9 ml of ultrapure water to lyse erythrocytes. Isotonicity was restored by addition of 3 ml of KCl (0.6 M) and 40 ml of NaCl (0.15 M). Cells were then centrifuged for 10 min at 350 × g and resuspended in 20 ml of PBS-EDTA. This suspension was layered over 20 ml of Histopaque-1077 (Sigma-Aldrich) and centrifuged for 30 min at 350 × g. The PMN-rich pellet was recovered and washed twice in PBS-EDTA. All manipulations were performed at 4°C, thus minimizing PMN activation (10, 11).

For stimulation, pooled PMNs (1 × 10⁷ cells/ml) from healthy blood donors were diluted 1/1 (v/v) in prewarmed (37°C) RPMI 1640 (Invitrogen Life Technologies) with 1 μM IMLP and incubated for 20 min at 37°C. PMNs were removed by centrifugation (4000 × g for 15 min at 4°C), and PMN-Ect contained in the supernatant were concentrated with Centriprep centrifugal filter devices (molecular mass 10,000 kDa cutoff; Millipore) and stored in aliquots at −80°C until use (10, 11).

Isolation, culture, and maturation of MoDCs

MoDCs were derived from monocytes isolated from fresh buffy coats. A buffy coat was diluted 1/1 (v/v) with HBSS (Invitrogen Life Technologies), layered over Histopaque-1077, and centrifuged for 30 min at 350 × g. PBMCs were washed and cultured in complete medium (RPMI 1640, 1% NaCl (0.15 M). Cells were then centrifuged for 1 h at 37°C in 6-well plates or in 75-cm² flasks. After incubation, nonadherent cells were removed by washing twice with prewarmed RPMI 1640. The remaining adherent cells were then cultured in complete medium supplemented with 50 ng/ml GM-CSF and 50 ng/ml IL-4 (ImmuNoTools). On days 2 and 5, the media including the supplements were replaced. On day 6, nonadherent immature MoDCs (iMoDCs) were harvested, counted, and plated in 6- or 24-well plates (1 × 10⁶ cells/ml) in fresh medium containing GM-CSF and IL-4 (50 ng/ml each) (26, 27). After incubation, nonadherent cells were removed by washing twice with prewarmed RPMI 1640. The remaining adherent cells were then cultured in complete medium supplemented with 50 ng/ml GM-CSF and 50 ng/ml IL-4 (ImmuNoTools). On days 2 and 5, the media including the supplements were replaced. On day 6, nonadherent immature MoDCs (iMoDCs) were harvested, counted, and plated in 6- or 24-well plates (1 × 10⁶ cells/ml) in fresh medium containing GM-CSF and IL-4 (50 ng/ml each). After 24 h of LPS maturation (10, 11).

Flow cytometric analysis

Flow cytometric analyses of cell surface markers were performed using the following mouse mAbs conjugated with FITC: CD14, CD40, CD80, CD86, and HLA-DR (BD Biosciences/BD Pharmingen). In each experiment, parallel stainings with isotype-matched controls IgG1-FITC, IgG2a-FITC, and IgG2a-PE (BD Biosciences/BD Pharmingen) were performed. After each incubation, cells were spun down, resuspended in PBS/1% BSA, and labeled for 30–45 min at 4°C with appropriate Abs. After labeling, cells were washed twice in PBS/1% BSA and data were acquired with a FACS Calibur flow cytometer (BD Biosciences) and analyzed using Summit software (DakoCytomation). A minimal of 10,000 events were collected per dataset.

Detection of apoptosis

Detection of apoptotic and/or necrotic cells was performed using FITC-conjugated annexin V (AnV; BD Biosciences/BD Pharmingen) and Via-Probe (BD Biosciences/BD Pharmingen), a nucleic acid dye (7-aminoactinomycin D) used for the exclusion of nonviable cells. MoDCs were washed twice with cold PBS and resuspended in AnV-binding buffer (BD Biosciences/BD Pharmingen) at a concentration of 1 × 10⁶ cells/ml. Aliquots of 100 μl were stained with 5 μl of AnV-FITC and 2 μl of Via-Probe and incubated for 15 min at room temperature in the dark. Samples were then diluted in 400 μl of binding buffer and analyzed by flow cytometry.

Endocytic activity

Endocytic activity of MoDCs was measured by assessing uptake of FITC-conjugated dextran (molecular mass, 40,000 kDa; Molecular Probes) (27). To that end, cells were incubated with 0.5 mg/ml FITC-conjugated dextran in complete medium for 15, 30, or 45 min at 37 and 4°C to measure specific uptake vs nonspecific binding, respectively. MoDCs were then washed three times and analyzed by flow cytometry.

Quantitation of cytokines by ELISA

Relevant supernatants were collected and spun for 10 min at 500 × g at 4°C to remove cellular debris. The concentrations of IL-8, IL-10, IL-12p70, TNF-α, and TGF-β1 were measured using OptEIA ELISA kits (BD Biosciences) according to the manufacturer’s instructions. All samples were measured in duplicates.

T cell proliferation assay

MoDCs incubated for 24 h with 1) medium alone, 2) medium and PMN-Ect, 3) medium and LPS, and 4) medium and LPS and PMN-Ect were collected and washed twice to remove excess PMN-Ect and LPS. CD3⁺ T cells, obtained by positive magnetic selection with CD3⁺ microbeads (Miltenyi Biotec), were labeled with 0.25 mM CFSE at room temperature for 10 min in the dark. The reaction was stopped by adding cold complete medium. Cells were then washed twice with cold medium and seeded at a 1:1 ratio (if not stated otherwise) with MoDCs. After 5 days of culture, proliferation of CFSE-labeled T cells was assessed by flow cytometry.

PS blocking assay

On day 6, before coincubation with MoDCs, PMN-Ect were preincubated for 30 min at 4°C with recombinant AnV (50 μg/ml final concentration; BD Biosciences/BD Pharmingen) and then washed. MoDCs and supernatants were collected after 24 h.

Statistical analysis

Datasets were tested for normality. For normally distributed data, parametric analysis (two-tailed paired Student’s t test) and for non-normally distributed data nonparametric analysis (Wilcoxon-matched pairs test) were performed using GraphPad Prism software. Data are expressed as mean ± SEM. A p < 0.05 was considered statistically significant.

Results

PMN-Ect modified the morphology of MoDCs

We first assessed the effects of PMN-Ect on MoDC morphol- ogy. Before LPS exposure, iMoDCs were round, whereas after 24 h of LPS maturation the name-giving dendritic morphology became evident (Fig. 1A). The major finding was that the formation of dendrites was inhibited by PMN-Ect when MoDCs were matured with LPS. In line with the literature, there were no significant scatter modifications between iMoDCs and LPS-matured MoDCs (mMoDCs) (Fig. 1B): Ø vs LPS (21, 28). Intriguingly, we found that PMN-Ect down-regulated the endocytic activity of MoDCs. At 4°C no incorporation of phagocytosed dextran particles (Fig. 2). PMN-Ect significantly reduced the endocytic capacity of mMoDCs (Fig. 2A) as well as mMoDCs (Fig. 2B). Strikingly, PMN-Ect reduced iMoDC phagocytosis to the level of PMN-Ect on MoDC viability, tested both via AnV and Via-Probe binding (data not shown).

PMN-Ect down-regulated the endocytic activity of MoDCs

We next examined whether PMN-Ect have an impact on the endocytic activity of MoDCs. MoDCs were incubated with FITC-conjugated dextran at 37°C to measure specific uptake and at 4°C to quantify nonspecific binding. As expected, mMoDCs lost partially their capacity to phagocytose dextran particles (Fig. 2). PMN-Ect significantly reduced the endocytic capacity of iMoDCs (Fig. 2A) as well as mMoDCs (Fig. 2B). PMN-Ect down-regulated the phenotypic maturation of MoDCs

Having shown that PMN-Ect influence MoDC morphology and endocytic capacity, we next asked the question whether PMN-Ect impact on expression of surface markers of nonactivated vs nonactivated MoDCs as well. The data of six independent experiments were...
PMN-Ect were significantly modified when exposed to PMN-Ect. A. When observed by light microscopy (original magnification, ×20), MoDCs were round, whereas after LPS activation dendrites became apparent in a large fraction of the cells (indicated by arrows). The appearance of dendrites by mMoDCs was largely abolished by PMN-Ect. B. Forward scatter (FSC)/side scatter (SSC) characteristics were used as a quantitative readout of changes in MoDC morphology. To define the modifications of FSC/SSC, the percentage of gated cells in an arbitrary circle are indicated. The FSC/SSC characteristics of MoDCs were significantly modified when exposed to PMN-Ect (illustrated in Fig. 3), although these differences did not reach statistical significance.

As expected, coincubation of iMoDCs with LPS induced significant up-regulation of surface markers indicative of MoDC maturation (mMoDCs): CD40 (mean fluorescence intensity (MFI), 72.05 ± 10.02 vs 175.9 ± 36.74; p = 0.026), CD83 (6.308 ± 0.681 vs 17.48 ± 2.991; p = 0.007), CD86 (104.5 ± 18.11 vs 121 ± 20.01; p = 0.021), HLA-DP DQ DR (1049 ± 62.92 vs 1402 ± 74.28; p < 0.001). Up-regulation of CD80 was evident as well, but did not reach statistical significance (7.795 ± 1.019 vs 9.487 ± 1.282; p = 0.14; Fig. 3).

MoDCs coincubated with PMN-Ect, in contrast, expressed significantly less CD40 (MFI, 175.9 ± 36.74 vs 127.1 ± 40.73; p = 0.027), CD80 (9.487 ± 1.282 vs 7.665 ± 0.9681; p = 0.019), CD83 (17.48 ± 2.991 vs 11.74 ± 1.932; p = 0.042), CD86 (121 ± 20.01 vs 78.93 ± 19.23; p = 0.002), and HLA-DP DQ DR (1402 ± 74.28 vs 1014 ± 124.8; p = 0.029) than mMoDCs alone (Fig. 3).

Together these data are evidence that PMN-Ect have the potential to modify MoDC maturation as judged by the expression pattern of various cell surface markers.

**PMN-Ect inhibited the cytokine release of MoDCs**

We next assessed whether the effect of PMN-Ect on MoDC phenotype was accompanied by changes in their release of cytokines (IL-8, IL-10, IL-12p70, and TNF-α). Levels of IL-8, IL-10, IL-12p70, and TNF-α remained unchanged when iMoDCs were incubated with PMN-Ect as compared with iMoDCs alone (Fig. 4). Compared with iMoDCs alone, secretion of each of these cytokines was up-regulated when iMoDCs were matured with LPS (IL-8: 274.8 ± 44.39 vs 24 346 ± 9410 pg/ml; p = 0.002; IL-10: 30.27 ± 12.98 vs 241.5 ± 51.72 pg/ml; p = 0.002; IL-12p70: 3.71 ± 0.52 vs 23.8 ± 3.14 pg/ml; p = 0.002; TNF-α: 107 ± 24.83 vs 3168 ± 912.9 pg/ml; p = 0.002).

Importantly, and in line with the effect of PMN-Ect on cell surface maturation markers, coincubation of mMoDCs with PMN-Ect strongly down-modulated the release of IL-10 (241.5 ± 51.72 vs 144.4 ± 56.56 pg/ml; p = 0.002), IL-12p70 (23.80 ± 3.140 vs 9.053 ± 1.629 pg/ml; p = 0.014), and TNF-α (3168 ± 912.9 vs 983.8 ± 361.9 pg/ml; p = 0.002) and slightly but significantly...
The indicated numbers represent the mean MFIs of six independent experiments.

reduced the release of IL-8 (24 346 ± 9410 vs 17 237 ± 7071 pg/ml; p = 0.01).

Of note, variability in the absolute concentrations of cytokines was important and somewhat unpredictable. This variability might originate largely from the fact that for each experiment cells and PMN-Ect from different donors were used. For example, as shown in Fig. 4, cells from one donor reacted very strongly to LPS and, compared with other donors, released huge amounts of IL-8, IL-10, and TNF-α. Importantly, however, also in this donor a relative reduction was observed when iMoDCs were matured in the presence of PMN-Ect.

Because our results might have been modified by the fact that the PMN-Ect were from different donors than the MoDCs, we repeated the same studies using an autologous system. However, similar differences in the expression of surface markers and cytokine release were observed using PMN-Ect and MoDCs from the same donor (four independent experiments, data not shown).

FIGURE 3. PMN-Ect inhibit up-regulation of key surface markers of human MoDCs. iMoDCs were incubated for 24 h with 1) medium alone (Ø), 2) medium + PMN-Ect, 3) medium + LPS (10 ng/ml), and 4) medium + LPS + PMN-Ect. Cells were then collected, washed, and analyzed by flow cytometry for cell surface expression of CD14, CD40, CD80, CD83, CD86, and HLA-DP DQ DR (filled histograms). Open gray lines represent staining with matched control Abs. The results shown are from one representative experiment. The indicated numbers represent the mean MFIs of six independent experiments.

FIGURE 4. PMN-Ect inhibit the release of inflammatory cytokines by LPS-matured human MoDCs. iMoDCs were incubated for 24 h with 1) medium alone (Ø), 2) medium + PMN-Ect, 3) medium + LPS (10 ng/ml), and 4) medium + LPS + PMN-Ect. Concentrations of IL-8, IL-10, IL-12, and TNF-α were analyzed in supernatants. The results of five experiments done in duplicates are shown.
MoDCs exposed to PMN-Ect released TGF-β1 and expressed less CCR7

Since TGF-β1 is known to be a central down-regulator of DCs, we measured its release when these cells were exposed to PMN-Ect. Strikingly, PMN-Ect increased the release of TGF-β1 from MoDCs in all experiments performed, whether the cells were immature (43.42 ± 19.64 vs 217 ± 46.61 pg/ml; p = 0.002) or LPS matured (90.63 ± 24.43 vs 260.3 ± 59 pg/ml; p = 0.002). Identical results were found whether the MoDCs and the PMN-Ect were from the same donor or not (Fig. 5A).

Since the expression of the chemokine receptor CCR7 in DCs is inhibited by TGF-β1 (29), we measured surface expression of CCR7 in different cells and found that it was reduced when MoDCs were LPS matured in the presence of PMN-Ect (21.13 ± 4.25 vs 12.7 ± 4.67% positive cells, p = 0.003; Fig. 5B).

MoDCs exposed to PMN-Ect stimulated T cell proliferation poorly

Given that PMN-Ect were found to impact on phenotypic maturation and the amount of inflammatory cytokines released by MoDCs, we next examined the immunostimulatory capacity of MoDCs exposed to PMN-Ect. When MoDCs were incubated with PMN-Ect without LPS, no significant effect on T cell proliferation was observed. By contrast, MoDCs coincubated with PMN-Ect at the time of LPS exposure induced significantly less T cell proliferation than their non-PMN-Ect-exposed counterparts (mean decrease in percent proliferating cells: 20.4%; p < 0.001; Fig. 6). The effect was seen using MoDCs suppressed by autologous and allogeneic PMN-Ect. The results observed in a MoDC:T cell ratio of 1 was also observed at a ratio of 1:10 (data not shown).

The activities of PMN-Ect were reversed by AnV binding

PS exposure on PMN-Ect has previously been shown using AnV binding (10). Since PS might be involved in the functional property of PMN-Ect to down-regulate the maturation of MoDCs, we coated first the PMN-Ect with recombinant AnV before adding them to the MoDCs. For mMoDCs, this coating reversed the inhibitory effects of PMN-Ect on expression of surface markers (for CD40, CD83, CD86, and HLA-DP DQ DR) (Fig. 7, cf LPS plus PMN-Ect and LPS plus PMN-Ect/AnV). We could not analyze the release of cytokines, because AnV per se induced an activation of MoDCs.

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**FIGURE 5.** PMN-Ect increase the release of anti-inflammatory cytokine TGF-β1 and decrease the chemokine receptor CCR7 by human MoDCs. iMoDCs were incubated for 24 h with 1) medium alone (Ø), 2) medium + PMN-Ect, 3) medium + LPS (10 ng/ml), and 4) medium + LPS + PMN-Ect. A, Concentrations of TGF-β1 were analyzed in supernatants (n = 10). •, Allogeneic experiments (n = 5) and ○ and dotted lines autologous experiments (n = 5). B, Surface expression of CCR7 was analyzed by flow cytometry and results are indicated in percent positive cells. CCR7 was significantly reduced when MoDCs were LPS-matured in the presence of PMN-Ect (n = 6).

**FIGURE 6.** Human MoDCs matured in the presence of PMN-Ect are less efficient inducers of T cell proliferation. iMoDCs were incubated for 24 h with 1) medium alone (Ø), 2) medium + PMN-Ect, 3) medium + LPS (10 ng/ml), and 4) medium + LPS + PMN-Ect. Cells were washed and incubated with CFSE-stained CD3+ T cells. A, Proliferation of T cells was assessed by flow cytometry after 5 days of coculture (representative of n = 12). B, T cells proliferated significantly less (p < 0.001) when exposed to MoDCs coincubated with PMN-Ect at the time of LPS activation. •, Allogeneic experiments (n = 8) and ○, autologous experiments (n = 4).
leased TGF-
activate T cells. In addition, iMoDCs exposed to PMN-Ect re-
maturation of iMoDCs by LPS and induced new morphological
(12, 15, 33). In this study, we showed that PMN-Ect inhibited the
expression of costimulatory molecules such
(12, etc.) and increased expression of costimulatory molecules such
induce maturation with release of specific cytokines (TNF-
sponsive inflammation and prevent autoimmunity. For instance, apop-
totic PMNs may have a protective role in allowing the termination of
acute inflammation due to their overexpression of CCR5, which
may adsorb CCL3 and CCL5 away from their targets, and thus act as “terminators” of chemokine signaling during the resolution of
inflammation (36, 37). Ect are released at the early phase of PMN
activation, when much phagocytic and inflammatory activity is
still needed at the site of injury, whether this injury is related to cell
necrosis and/or infection. But such local inflammation requires
control as well and does not need systematically DCs to provoke
T cell stimulation and an acquired immune response. Our results
indicate that such early down-regulation is a property of PMN-Ect,
which in the local context may participate in the control of auto-
immune responses, similarly to what has been suggested for apo-
potic cells (15, 19, 20, 22). However and by contrast to apoptotic
cells, PMN-Ect have the particularity to be involved very early in
inflammation, a time point, which might be crucial for determining
later aspects of the cascade responsible for acquired immunity, in
that sense not terminator of inflammation, but responsible for con-
trolling the immune response.

Two aspects merit attention. First the effects of PMN-Ect on
resting iMoDCs and then those on the maturation process of
MoDCs induced by LPS. The morphological and phenotypic changes of iMoDCs cocultured with PMN-Ect were subtle. There
was a minimal change in the forward and side scatters seen by
FACS analysis and a nonsignificant but repeatedly observed very
slight reduction of the expression of costimulatory molecules
(CD40, CD86) and HLA class II molecules. However, iMoDCs
induced by LPS. The morphological and phenotypic changes of iMoDCs, as observed by others (15, 34), and of
surface expression of CCR7 (29). During DC maturation, the up-
regulated CCR7 is responsible for directing the migration of DCs
to the lymph nodes. It has been shown that CCR7 controls the
cytoarchitecture, the rate of endocytosis, the survival, the migratory
speed, and the maturation of the DCs (35), so that a reduced
expression of CCR7 might interfere with normal immune re-
response. PMN-Ect have already been shown to induce the release of
TGF-β1 from macrophages, a release, which was responsible in
part for the inhibition of macrophage activation by LPS and zy-
omosan (11). Hence, it appears that PMN-Ect have down-regulating
properties at different levels in the inflammatory process, which
lead to T cell activation. Interestingly, similar properties have
been ascribed to cells undergoing apoptosis. Good evidence indicates
that exposure of iDCs to apoptotic cells induces a tolerogenic – as
opposed to an immunogenic-DC phenotype (12, 13, 15, 18, 20).

Together these observations highlight the complexity of the in-
flammatory process, which on one hand has to be amplified so as
to trigger a specific immune response, but also has to limit exces-
sive inflammation and prevent autoimmunity. For instance, apop-

![Image](http://www.jimmunol.org/)

**FIGURE 7.** The effects of PMN-Ect were reversed by AnV binding. iMoDCs were incubated for 24 h with
1) medium alone (Ø), 2) medium + PMN-Ect, 3) medium + PMN-Ect preincubated with AnV (PMN-Ect/
AnV), 4) medium + LPS (10 ng/ml), 5) medium + LPS + PMN-Ect, and 6) medium + LPS + PMN-Ect/AnV.
Cells were then collected, washed, and analyzed by flow
cytometry for cell surface expression of CD40, CD83,
CD86, and HLA-DP Q DQ DR (filled histograms). Open
gray lines represent staining with matched control Abs.
The results shown are from one representative experi-
ment (n = 4). The indicated numbers represent the MFI
for CD40, CD86, and HLA-DP Q DQ DR and the percen-
tage of positive cells for CD83.

**Discussion**
In the present study, we identified a new pathway by which ac-
vated human polymorphonuclear leukocytes, through the release
of Ect, skew DC differentiation. It is likely that PMN-Ect released in
vivo interact with tissue-resident iDCs at the site of injury or
infection, i.e., when iDCs are exposed to maturation-inducing sub-
stances released from bacteria (14, 30). LPS and zymosan trigger,
respectively, TLR4 and TLR2 receptors on iDCs (13, 31, 32) and
infection, i.e., when iDCs are exposed to maturation-inducing sub-
stances released from bacteria (14, 30). LPS and zymosan trigger,
in addition, iMoDCs exposed to PMN-Ect re-
leased TGF-β1 and it might well be that to some or a larger extent
TGF-β1 was responsible for the down-regulation of TLR4-medi-
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to trigger a specific immune response, but also has to limit exces-
sive inflammation and prevent autoimmunity. For instance, apop-
The mechanisms underlying the biological effects of PMN-Ect on DCs remain speculative. In many respects, they may be similar to those proposed for the down-regulation of DCs by apoptotic cells, including the high expression of PS, which in multiple experiments has been shown to allow specific binding of apoptotic cells to macrophages or DCs (16, 22, 43–46). AnV is known to interfere with the binding of PS-expressing cells/particles to macrophages (11). In the experiments performed here, we could observe the down-regulation of surface markers of MoDCs by PMN-Ect by incubating first the PMN-Ect with AnV, suggesting that the expression of PS on PMN-Ect was responsible for their property to modify MoDCs. However, PMN-Ect, which had bound AnV, induced by themselves the release of TNF-α by iDCs, indicating that AnV might have induced/blocking other interactions not directly related to PS as well.

Apoptotic cells and Ect released by the same type of cells express different sets of proteins. For instance, PMN-Ect express high levels of complement receptor 1 and CD66b (9, 10), whereas these molecules are down-regulated on apoptotic PMNs (47). Apoptotic cells express on their surface nuclear components (48), which will not be present on Ect released by live, activated cells. Thus, it is likely that diverse sets of proteins on dying cells vs Ect will allow different functional activities, although many of the basic properties might be very similar.

For instance, the specific release of TGF-β1 induced by the binding of PMN-Ect to MoDCs is most likely one of the essential mediators reprogramming the DCs so that it has a lower reactivity to LPS. Indeed TGF-β is a major player in modulating the activity of iDCs and their maturation (15, 25, 49, 50). It is produced by iDCs exposed to apoptotic cells as well and under such conditions reprograms the DCs to become tolerogenic (15, 20). Whether iDCs exposed to PMN-Ect have similar properties, i.e., become tolerogenic, remains to be tested.

In conclusion, we suggest that, in addition to regulating macrophage activation (11), PMN-Ect may have the potential to influence the outcome of Ag-specific immunity by playing an active role in shaping DC-dependent immunity. In vivo models of inflammation/infecction will now have to test the relevance of the here-proposed activities of PMN-Ect.

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