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Monocyte/Macrophage-Derived Microparticles Up-Regulate Inflammatory Mediator Synthesis by Human Airway Epithelial Cells[1]

Chiara Cerri,* Daniele Chimenti,† Ilaria Conti,* Tommaso Neri,* Pierluigi Paggiaro,* and Alessandro Celi[2]*

Cell-derived microparticles (MP) are membrane fragments shed by virtually all eukaryotic cells upon activation or during apoptosis that play a significant role in physiologically relevant processes, including coagulation and inflammation. We investigated whether MP derived from monocytes/macrophages have the potential to modulate human airway epithelial cell activation. Monocytes/macrophages were isolated from the buffy coats of blood donors by Ficoll gradient centrifugation, followed by overnight culture of the mononuclear cell fraction. Adherent cells were washed and incubated with the calcium ionophore, A23187, or with histamine. The MP-containing supernatant was incubated with cells of the human bronchial epithelial line BEAS-2B and of the human alveolar line A549. IL-8, MCP-1, and ICAM-1 production was assessed by ELISA and by RT-PCR. In some experiments, monocytes/macrophages were stained with the fluorescent lipid intercalating dye PKH67, and the supernatant was analyzed by FACS. Stimulation of monocytes/macrophages with A23187 caused the release of particles that retain their fluorescent lipid intercalating label, indicating that they are derived from cell membranes. Incubation with A549 and BEAS-2B cells up-regulate IL-8 synthesis. Ultrafiltration and ultracentrifugation of the material abolished the effect, indicating that particulate matter, rather than soluble molecules, is responsible for it. Up-regulation of MCP-1 and ICAM-1 was also demonstrated in A549 cells. Similar results were obtained with histamine. Our data show that human monocytes/macrophages release MP that have the potential to sustain the innate immunity of the airway epithelium, as well as to contribute to the pathogenesis of inflammatory diseases of the lungs through up-regulation of proinflammatory mediators. ***The Journal of Immunology,*** **2006, 177: 1975–1980.**

Microparticles (MP),[3] also referred to as ectosomes or microvesicles, are cell membrane fragments shed by eukaryotic cells upon activation and/or during apoptosis (1). MP were first discovered in platelets (2), but the existence of MP derived from other cell types has been demonstrated subsequently (3, 4). Originally considered in vitro artifacts or, at most, cell debris generated in vivo but devoid of physiological significance, MP have been since shown to play significant roles in different processes, including blood coagulation and inflammation (5, 6). Besides the relatively well-characterized role of platelet MP as a source of the negatively charged phospholipids essential for the assembly of the multimolecular complexes involved in blood coagulation (7), experiments in vitro have shown that also human monocytes shed MP capable of disseminating procoagulant activity (3). These observations were extended subsequently in in vivo models to show that circulating MP participate in thrombus formation (8). Studies aimed at investigating the role of cell-cell interaction in inflammation have demonstrated that endothelial cell activation by polymorphonuclear cells (PMN) in coculture is in part mediated by PMN-derived MP (9).

Once perceived as a passive barrier, the airway epithelium is in fact a complex, integrated structure that participates in a number of physiological phenomena. As the first point of contact for inhaled substances, it is not surprising that the epithelium lining the airways contributes to the innate immune system (10). Among a variety of molecules expressed as a first line of defense, an array of adhesion molecules and soluble mediators involved in the recruitment and activation of inflammatory leukocytes play a critical role to this end (11, 12).

The aim of this study was to investigate whether cell-derived MP carry the potential to contribute to the innate immune system of the airways. We demonstrate that human monocytes/macrophages shed MP that can up-regulate the synthesis of proinflammatory mediators by human airway epithelial cells.

Materials and Methods

Reagents and kits

RPMI 1640 medium, penicillin, streptomycin, L-glutamine, trypsin, trypsin inhibitor, trypan blue, formaldehyde, BSA, *o*-phenylendiamine, PBS, Ficoll-Hypaque, dextran, calcium ionophore A23187, FBS, histamine, ethidium bromide, and the dye PKH67 were obtained from Sigma-Aldrich. The BEGM Bullet kit was obtained from Cambrex. The IL-8 Flexa kit was purchased from BioSource International, and the human MCP-1 module set was obtained from Bender MedSystems. The substrate 3,3′,5,5′-tetramethylbenzidine was obtained from Calbiochem. All other chemicals were obtained from the hospital pharmacy and were of the best grade available.

Abs, cytokines, and chemokines

The mouse anti-human-ICAM-1 mAb (clone 15.2) was obtained from Ancell. Peroxidase-conjugate anti-mouse IgG (γ-chain specific), and IFN-γ were obtained from Sigma-Aldrich (Milan, Italy). Human rTNF-α was

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[3] Abbreviation used in this paper: MP, microparticle; PMN, polymorphonuclear cell; TF, tissue factor.

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obtained from Alexis Italy. Human MCP-1 used as the reference standard for the ELISA was obtained from Inalco.

Cell culture

Human A549 cells (ATCC CCL-195) were provided by Dr. R. Danesi (University of Pisa, Pisa, Italy). A549 cells were maintained in RPMI 1640 supplemented with 10% (v/v) FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified 95% air-5% CO_2 atmosphere at 37°C. The immortalized bronchial epithelial cells, BEAS-2B (ATCC CRL-9609) were provided by Dr. S. Carnevali (University of Modena, Modena, Italy). BEAS-2B cells were maintained in 50% RPMI 1640 and 50% bronchial epithelial cell growth medium in a humidified 95% air-5% CO_2 atmosphere at 37°C.

Monocyte isolation and MP generation

Monocytes were isolated either from fresh buffy coats obtained from the local blood bank or from the peripheral blood of normal volunteers as described previously (13). Briefly, a fresh buffy coat was diluted 1/1 with PBS-EDTA (2 mM), mixed gently with 0.25 volume of 4% Dextran T500, and left for 30 min for erythrocyte sedimentation. The leukocyte-rich supernatant was recovered and submitted to centrifugation for 10 min at $200 \times g$. The pellet was resuspended in 30 ml of PBS-EDTA, layered over 15 ml of Ficoll-Hypaque, and submitted to centrifugation for 30 min at $350 \times g$ at room temperature. The mononuclear cell-rich ring was recovered and washed twice in PBS-EDTA. Mononuclear cells were then resuspended in RPMI 1640/10% FBS and allowed to adhere for 18 h at 37°C on 24-well plates (10^6 cells/well). Adherent cells were washed three times with prewarmed serum-free RPMI 1640. For MP generation, A23187 (12 μM unless otherwise specified in serum-free RPMI 1640) was added; after 10 min at 37°C, the supernatant was recovered, cleared by centrifugation at $14,000 \times g$ for 5 min at room temperature to remove dead cells and big cell fragments that might have detached during the stimulation, and immediately used for additional experiments. In parallel experiments, cells treated as described were detached by trypsin/EDTA, put onto a slide using a cytocentrifuge, and stained with Diff-Quick. Typically, ~75–80% cells were monocytes/macrophages, with the contaminants being mostly lymphocytes.

In some experiments, histamine (30 μM) was used instead of A23187 for monocytes/macrophages stimulation and MP induction.

Cell membrane labeling

To label cell membranes for FACS analysis of MP the amphiphilic cell linker dye, PKH67, was used following the labeling procedure provided by the manufacturer. Briefly, adherent leukocytes prepared as described above, were detached with 0.25% trypsin, washed once with RPMI 1640/10% FBS, and resuspended at the concentration of 2×10^7/ml in diluent C. The suspension was mixed with 1 ml of diluent C/dye solution (dye diluted 1/200) for 2–5 min at room temperature, with gentle shaking. The staining reaction was stopped by adding an equal volume (2 ml) of RPMI 1640 with BSA 1% for 1 min at 22°C. The sample was diluted with an equal volume of RPMI 1640 and pelleted by centrifugation for 10 min at $400 \times g$ to remove the staining solution. The cells were further washed for three times and diluted in 1 ml of RPMI 1640. Cells were then incubated with A23187 as described above.

Flow cytometry analysis

The MP-containing supernatant of A23187-stimulated monocytes/macrophages was analyzed using a FACScan flow cytometer (BD Biosciences). The sheath fluid was Isoton II-balanced electrolyte solution (Beckman-Coulter). The light scatter was set at logarithmic gain. The forward light scatter setting was E-01, with a threshold of 16. A total of 10,000 events was analyzed. Based on the results of experiments in which MP were filtered through a 0.22-μm filter, a region (R1) was defined on forward vs side angle light scatter intensity dot plot representation that we deem contains biologically active MP (see *Results* and Fig. 1).

Epithelial cell activation by MP

Supernatants from stimulated and untreated monocytes were incubated with A549 and BEAS-2B cells grown to confluence in 200 μl/well (final volume) in 96-well plates for 24 h at 37°C. Following an 18-h incubation, the conditioned medium was harvested, cleared by centrifugation for 5 min at 12,000 rpm, and analyzed for IL-8 and MCP-1 content. Cells were fixed for analysis of surface ICAM-1 expression. In some experiments, mononuclear cell supernatants were filtered (0.22 μm), ultrafiltered (Amicon ultra-4 centrifugal filter devices; 100 kDa m.w. cut-off; Millipore), or submitted to ultracentrifugation ($160,000 \times g$, 40 min) before incubation with A549 and BEAS-2B cells.

ELISA for chemokine detection

IL-8 and MCP-1 in supernatants from A549 and BEAS-2B epithelial cells were measured by sandwich ELISA kits, according to the manufacturers' instructions.

Measurement of cell surface ICAM-1 expression

ICAM-1 expression on A549 was measured by direct cell ELISA as described with minor modifications (13). Briefly, cells were seeded at 7.5×10^4 cells/well in 0.1 ml of medium in 96-well plates. When the cells reached subconfluence, they were washed three times with HBSS and fixed with 3.7% formaldehyde (w/v). Following additional extensive washing, nonspecific Ab binding sites were blocked by incubating the cells with HBSS/2% BSA (w/v) for 2 h. Cells were then incubated with mouse anti-human ICAM-1 Ab (5 μg/ml) in HBSS 0.5% BSA (w/v) for 2 h at 37°C, then washed three times with wash buffer. Peroxidase-conjugated anti-mouse IgG Ab was then added at the concentration recommended by the manufacturer and incubated for 1 h at 37°C. After extensive washing, bound enzyme was determined by adding *o*-phenylendiamine, 1 mg/ml in 50 mM sodium citrate (pH 4), containing 0.015% H_2O_2. After blocking the reaction with 2 M H_2SO_4, the plate was read in a spectrophotometer (Titertek Multiskan MCC ELISA reader; Flow Laboratories) at 492 nm.

RT-PCR

RNA was extracted from the cells using the Qiagen RNeasy kit (Qiagen), according to the manufacturer's instructions. RNA concentration and purity were determined by spectrophotometric readings at 260/280 nm. RNA was then either reverse transcribed to cDNA or stored at −80°C for additional uses. The RNA to cDNA reverse transcription was performed using the Invitrogen Sensiscript Reverse Transcriptase First-Strand DNA Synthesis kit (Qiagen), according to the manufacturer's instructions. The obtained cDNA was amplified by PCR using specific primers for IL-8, MCP-1, ICAM-1, and GAPDH or stored at −20°C for further use. The sense and antisense primers for human IL-8 and MCP-1 and ICAM-1 designed in our laboratory, and for GAPDH, were obtained from Invitrogen Life Technologies and had the following sequences: IL-8—sense, ATGACTTCCAAGCTGGCCGT, and antisense, CCTCTTCAAAAACTTCTCCACACC; MCP-1—sense, GCCTCCAGCATGAAAGTCTC, and antisense, CAGATCTCCTTGGCCACAAT; ICAM-1—sense, GGCTGGAGCTGTTTGAGAAC, and antisense, ACTGTGGGGTTCAACCTCTG; and GAPDH—sense, CGATGCTGGCGCTGAGTA, and antisense CGTTCAGCTCAGGGATGACC.

PCR were set up using RedTaq DNA polymerase kit (Sigma-Aldrich). Amplification products were run on 1.5% agarose gel with 0.001% ethidium bromide, photographed, and analyzed with QuantityOne Software (Bio-Rad). PCR conditions: IL-8 and MCP-1: 94°C for 60 s, 55°C for 60 s, 72°C for 60 s for 35 times, followed by 72°C for 7 min and storage at 4°C; ICAM-1: 94°C for 30 s, 60°C for 30 s, 72°C for 30 s for 35 times, followed by 72°C for 7 min and storage at 4°C; and GAPDH: 95°C for 5 min, then 94°C for 45 s, 58°C for 45 s, 72°C for 45 s for 35 times, followed by 72°C for 5 min and storage at 4°C.

Data presentation and statistical analysis

All data are shown as mean ± SEM. Comparisons among groups were made by the Friedman test for repeated measures using Prism Software (GraphPad). Values of $p < 0.05$ were considered statistically significant.

Results

Flow cytometric analysis of MP formation induced by the calcium ionophore, A23187

Flow cytometric analysis of the supernatant of unstimulated monocytes/macrophages shows virtually no events (Fig. 1*A*). Incubation of adherent cells with A23187 (12 μM, 10 min) causes the appearance of events in two distinct regions. In both regions, most events are fluorescent (shown in gray), suggesting their origin from cell membranes (Fig. 1*B*). Nonfluorescent events (shown in black) may either represent membrane fragments that are too small to contain enough fluorescent dye or may have a different origin. Upon filtration of the supernatant through a 0.22-μm filter, one of the regions virtually disappears while the other remains mostly unchanged (R1) (Fig. 1*C*). The effect of A23187 on the generation of events in R1 is dose dependent (Fig. 2).

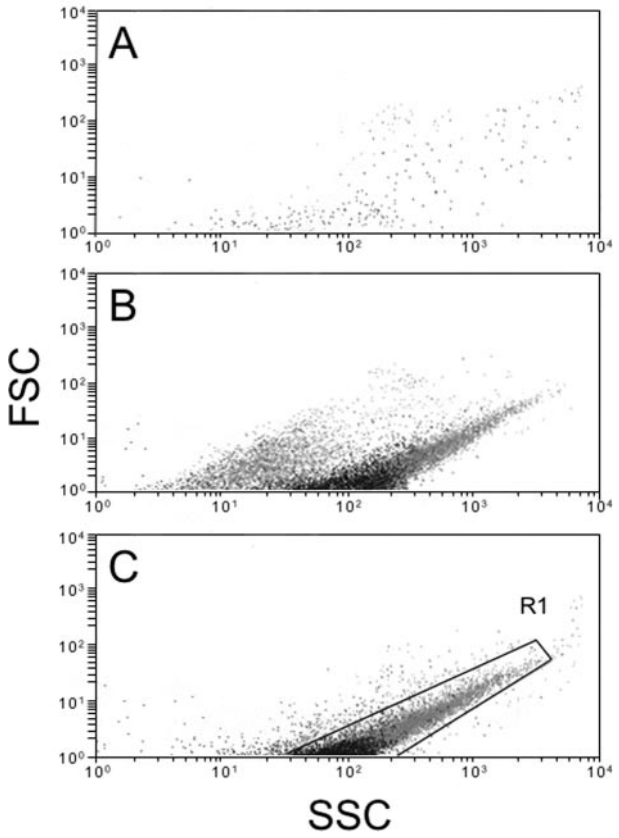

FIGURE 1. Flow cytometric analysis of the supernatant of monocytes/macrophages. *A*, Unstimulated cells; *B*, A23187-stimulated cells; and *C*, A23187-stimulated cells after filtration through a 0.22-μm filter. The gray dots represent fluorescent events (green in the original figure), whereas the black dots are fluorescence negative. R1 denotes a region arbitrarily chosen to denote events that are not influenced by 0.22-μm filtration (see text). Data from one experiment representative of three. SSC, side scatter; FSC, forward scatter.

MP originated by monocytes/macrophages up-regulate IL-8 secretion by airway epithelial cells

A549 cells constitutively express an average of 800 pg/ml IL-8 under our experimental conditions. Overnight incubation with the supernatant of unstimulated monocytes/macrophages causes an increase in IL-8 expression. An additional 4-fold increase in IL-8 expression is obtained when A549 cells are incubated with the supernatant of monocytes/macrophages stimulated with A23187 (Fig. 3*A*). To investigate whether such up-regulation could be solely explained by the release of soluble mediators by monocytes/macrophages upon stimulation with calcium ionophore, the MP-containing supernatant was filtered through a 0.22-μm filter and through an ultrafilter with a nominal m.w. cut-off of 100 kDa. Filtration through a 0.22-μm filter caused a small, not reproducible reduction in the stimulatory effect of the supernatant that did not reach statistical significance after seven consecutive experiments (Fig. 3*B*). However, ultrafiltration almost completely abolished this effect (Fig. 3*B*). As a control, ultrafiltration of a paradigmatic soluble molecule involved in A549 stimulation, TNF-α, did not impair its stimulatory effect (Fig. 4). To confirm that the effect of the supernatant required the presence of particulate material rather than of soluble molecules large enough to be retained by a 100-kDa filter, the material was submitted to ultracentrifugation. Ultracentrifugation abolished the stimulatory effect of the supernatant; furthermore, we were able to reconstitute such effect by

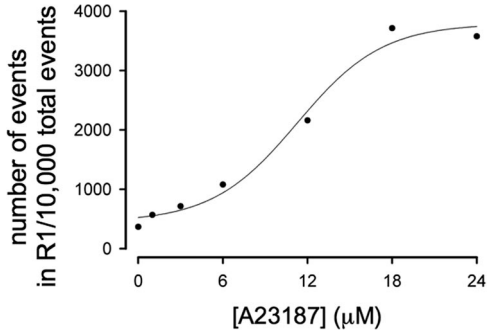

FIGURE 2. Dose-response curve of MP generation by human monocytes/macrophages upon stimulation with A23187. The supernatant of A23187-stimulated monocytes/macrophages was analyzed by flow cytometry as described in Fig. 1. The number of events in R1 per 10,000 total events was calculated. Data points represent mean values from two independent tubes within the same experiment representative of two performed under identical conditions.

resuspending the pellet in an equal amount of buffer (Fig. 3*C*), thus confirming that cell-derived MP, rather than large soluble molecules, are involved. The variations in IL-8 secretion were paralleled by variations in IL-8 mRNA (Fig. 5*A*). Control experiments using A23187 to directly stimulate A549 cells were performed to rule out the possibility that the stimulatory effect was due to A23187 that remains in the MP fraction (pellet and filter retentate) due to its lipid solubility, rather than to MP. The concentration of IL-8 in the conditioned medium of A549 cells went from 4,105 pg/ml in baseline conditions to 6,393 pg/ml upon direct stimulation with 12 μM A23187 to 52,835 pg/ml upon incubation with MP derived from monocytes/macrophages stimulated with 12 μM A23187 (data from one experiment representative of three).

Fig. 6 shows the effect of monocyte/macrophage-derived MP on IL-8 secretion by the bronchial epithelial cells, BEAS-2B. MP significantly increase IL-8 secretion by BEAS-2B cells, although to a much lesser extent than A549 cells. Again, ultrafiltration inhibits the stimulatory effect.

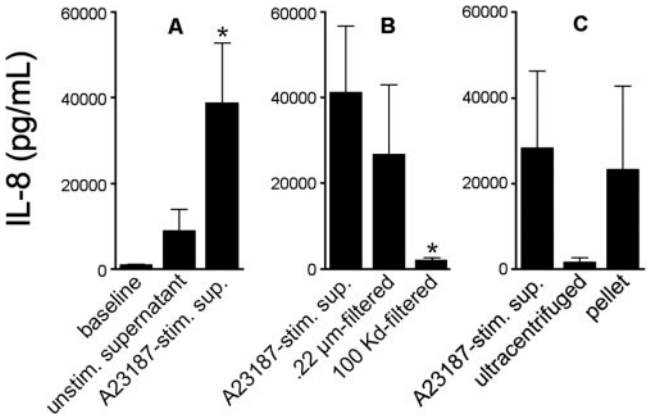

FIGURE 3. *A*, IL-8 secretion by A549 cells in baseline conditions and upon overnight incubation with the supernatant of unstimulated monocytes/macrophages or of A23187-stimulated monocytes/macrophages. *, $p < 0.05$ compared with baseline; $n = 7$. *B*, IL-8 secretion by A549 cells upon overnight incubation with the supernatant of A23187-stimulated monocytes/macrophages unfiltered and filtered through a 0.22-μm filter or through an ultrafilter with a cut-off of 100 kDa. *, $p < 0.05$ compared with unfiltered supernatant; $n = 7$. *C*, IL-8 secretion by A549 cells upon overnight incubation with the supernatant of A23187-stimulated monocytes/macrophages before and after ultracentrifugation and with the pellet resuspended in an equal volume of medium; $n = 3$.

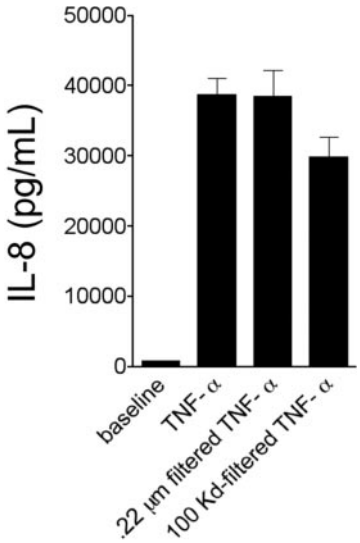

FIGURE 4. IL-8 secretion by A549 cells in baseline conditions and upon overnight incubation with TNF-α. Labels as in Fig. 3; $n = 3$.

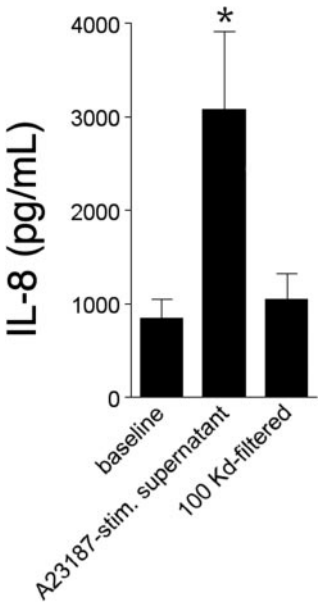

FIGURE 6. IL-8 secretion by BEAS-2B cells in baseline conditions and upon overnight incubation with the supernatant of unstimulated monocytes/macrophages or of A23187-stimulated monocytes/macrophages. *, $p < 0.05$ compared with baseline; $n = 3$.

Effect of MP originated by monocytes/macrophages on the expression of other inflammatory mediators by airway epithelial cells

We then sought to determine whether the stimulatory effects of MP were restricted to IL-8, or were part of a broader proinflammatory response. To this end, we investigated the expression of MCP-1, a member of the CC chemokine family, and of ICAM-1, a member of the Ig-like family of adhesion molecules.

Fig. 7 shows that MCP-1 expression by A549 cells is increased significantly by incubation with the supernatant of A23187-stimulated monocytes/macrophages. The effect is inhibited by ultrafiltration (Fig. 7) and ultracentrifugation (data not shown). RT-PCR shows a parallel between protein expression and mRNA (Fig. 5*B*).

Direct stimulation of A549 cells with A23187 does not modulate MCP-1 expression (data not shown).

Similar results are obtained when surface expression of the adhesion molecule, ICAM-1, is measured (Fig. 8). Again, RT-PCR shows a parallel between protein expression and mRNA (Fig. 5*C*).

Effects of histamine on MP production by monocytes/macrophages

To investigate whether agonists known to be involved in inflammatory reactions in humans could elicit a response similar to that elicited by A23187, we stimulated monocytes/macrophages with histamine. Fig. 9 shows that the supernatant of monocytes/macrophages stimulated with histamine up-regulates MCP-1 expression by A549 cells. The effect is abolished by filtration. Direct stimulation of the A549 cells with histamine does not cause any detectable up-regulation of MCP-1 expression (data not shown). Similar results were obtained for IL-8, albeit to a lesser degree compared with the effect of the supernatant of A23187-stimulated monocytes/macrophages (data not shown).

Discussion

The airway epithelium participates actively in the defense against airborne pathogens, as well as in the orchestration of the inflammatory reactions that characterize virtually all pulmonary diseases. The recruitment of blood-borne leukocytes into the airways represents a critical step in these processes and requires the combined action of chemokines and cell-cell adhesion molecules, including IL-8, MCP-1, and ICAM-1. We investigated whether monocyte/macrophage-derived MP have the potential to contribute to the

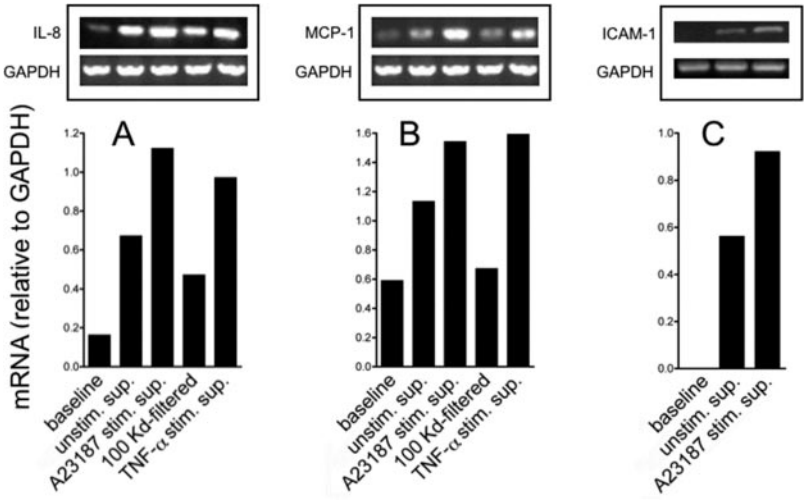

FIGURE 5. Semiquantitative analysis of IL-8 (*A*), MCP-1 (*B*), and ICAM-1 (*C*) mRNA levels by A549 in different culture conditions, assessed by RT-PCR (labels as in Fig. 3).

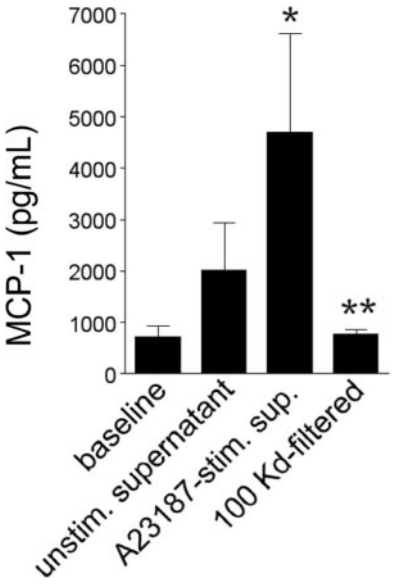

FIGURE 7. MCP-1 secretion by A549 cells in baseline conditions and upon overnight incubation with the supernatant of untreated monocytes/macrophages, or of A23187 stimulated monocytes/macrophages, or with the supernatant of A23187-stimulated monocytes/macrophages after filtration through an ultrafilter with a cut-off of 100 KD. *, $p < 0.05$ compared with baseline; **, $p < 0.05$ compared with unfiltered supernatant; $n = 4$.

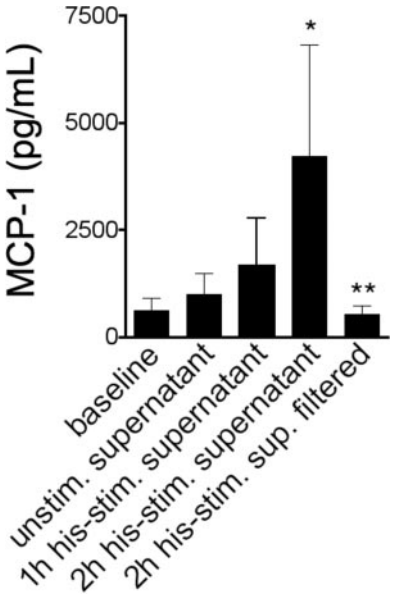

FIGURE 9. MCP-1 secretion by A549 cells in baseline conditions and upon overnight incubation with the supernatant of untreated monocytes/macrophages, or of monocytes/macrophages stimulated for 1 or 2 h with histamine, or with the supernatant of monocytes/macrophages stimulated for 2 h with histamine after filtration through a 0.22-μm filter; *, $p < 0.05$ compared with baseline; **, $p < 0.05$ compared with unfiltered supernatant; $n = 5$.

innate immune properties or the airway epithelium by modulating the synthesis of these molecules. Our data show that monocytes/macrophages can be induced to generate MP with a proinflammatory potential for human airways through their ability to up-regulate IL-8, MCP-1, and ICAM-1 synthesis by bronchial and alveolar epithelial cells.

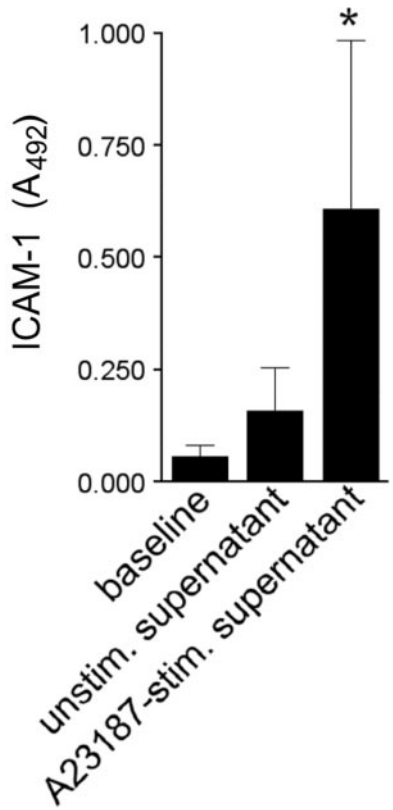

FIGURE 8. ICAM-1 expression by A549 cells in baseline conditions and upon overnight incubation with the supernatant of unstimulated monocytes/macrophages or of A23187-stimulated monocytes/macrophages. *, $p < 0.05$ compared with baseline; $n = 4$.

Activation of fluorescently labeled monocytes/macrophages with the calcium ionophore A23187 induces the shedding of fragments that can be detected by FACS and retain their fluorescent lipid intercalating label, thus confirming that they are derived from cell membranes. The effect is dose dependent. FACS analysis shows fluorescent events in two distinct regions. Filtration of the medium through a 0.22-μm filter causes the disappearance of one of these regions, whereas the other (R1) remains virtually unmodified. The conditioned medium of A23187-stimulated cells up-regulates the synthesis of proinflammatory mediators by airway epithelial cells measured at both the protein and mRNA level. The biological activity of the medium is not affected significantly by filtration. This observation suggests that most of the biological activity is associated with events in R1. On the contrary, ultrafiltration through a filter with a cut-off of 100 kDa (which did not affect the stimulatory properties of the cytokine TNF-α, used as a control) and ultracentrifugation of the material totally abolish the biological activity; furthermore, following ultracentrifugation, such activity can be reconstituted by resuspension of the pellet. These data confirm that particulate matter, rather than soluble molecules, is responsible for the biological activity. Control experiments in which A23187 was added directly to airway epithelial cells showed negligible effects on cytokine production, thus ruling out the possibility that calcium ionophore that might have remained in the MP fraction due to its lipid solubility is directly responsible for the effect. Because A23187 is not a physiological mediator, some of the experiments were also performed with agonists known to be involved in inflammatory responses in humans. Stimulation of monocytes/macrophages with histamine caused the generation of particulate matter capable of modulating IL-8 and MCP-1 expression by airway epithelial cells. Preliminary experiments suggest that histamine requires longer incubations compared

with A23187; additional studies will be required to investigate whether these differences reflect different activation pathways.

Over the past several years, numerous studies have confirmed that MP derived from different cells exert physiologically relevant effects. Monocytes have been shown to generate MP with a procoagulant potential. This activity has at least two components: first, negatively charged phospholipids, mainly phosphatidylserine, essential for the assembly of the blood clotting enzymatic complexes and normally limited to the inner leaflet of the resting cell membrane, are exposed on the outer leaflet of MP membranes. Second, tissue factor (TF), an integral membrane protein that represents the trigger of the so-called extrinsic pathway of blood coagulation, is transferred from the parental cell to the MP membrane (3). More recently, in vivo experiments have shown that MP carry simultaneously on their membrane both TF and an adhesion receptor for P-selectin, P-selectin glycoprotein ligand-1 (8). A new model for the initiation of blood coagulation has been proposed in which circulating MP are recruited to the site of vascular damage and platelet activation, where P-selectin is overexpressed, via the interaction with P-selectin glycoprotein ligand-1, and deliver the procoagulant potential of TF and phosphatidylserine (5). Finally, ex vivo data show that MP derived from platelets, leukocytes, and endothelial cells are present in human plasma and that their number increases in clinical conditions in which the coagulation system has been activated (14).

Several authors have investigated the potential role of leukocyte-derived MP in innate immunity and inflammation. Hess et al. (15) have shown, for example, that fMLP-activated PMN release MP that express a selected set of proteins such as complement receptor 1, myeloperoxidase, and elastase and that may therefore represent a functional unit with antimicrobial properties. PMN-derived MP have been shown to possess both pro- and anti-inflammatory properties, depending on the experimental model. As mentioned before, MP derived from fMLP-stimulated PMN upregulate IL-6 and IL-8 synthesis by human endothelial cells (9). By contrast, MP obtained in a similar fashion down-modulate cellular activation in human macrophages as assessed by the reduction of TNF-α, IL-8, and IL-10 synthesis by zymosan-activated cells (16). Ex vivo data confirm a potential role for MP in inflammatory diseases in humans. The MP number has been shown to be increased in the synovial fluid of inflamed joints of patients with rheumatoid arthritis (17); a potential link between the presence of such MP and the inflammation that represents the hallmark of the disease has been provided by the demonstration of the induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts upon incubation with T cell- and monocyte-derived MP (18), as well as with autologous synovial MP (19). Whether MP are also present in other body compartments besides plasma and joints is still under investigation. Of particular interest in the context of the present study is the demonstration of the presence of leukocyte-derived MP in the airway epithelium lining fluid in humans (20).

In conclusion, we demonstrate that MP derived from human monocytes/macrophages have the potential to sustain the innate immunity of the airway epithelium, as well as to contribute to the pathogenesis of inflammatory disease of the lungs characterized by increased expression of chemokines and adhesion molecules. Additional studies will be necessary to investigate whether this potential mechanism plays an actual role in pathological conditions in humans.

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Disclosures

The authors have no financial conflict of interest.

References

1. Morel, O., F. Toti, B. Hugel, and J. M. Freyssinet. 2004. Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr. Opin. Hematol.* 11: 156–164.
2. Sims, P., and T. Wiedmer. 1986. Repolarization of the membrane potential of blood platelets after complement damage: evidence for a Ca^{++}-dependent exocytotic elimination of C5b-9 pores. *Blood* 68: 556–561.
3. Satta, N., F. Toti, O. Feugeas, A. Bohbot, J. Dachary-Prigent, V. Eschwege, H. Hedman, and J. Freyssinet. 1994. Monocyte vesiculation is a possible mechanism for dissemination of membrane-associated procoagulant activities and adhesion molecules after stimulation by lipopolysaccharide. *J. Immunol.* 153: 3245–3255.
4. Gasser, O., C. Hess, S. Miot, C. Deon, J. C. Sanchez, and J. A. Schifferli. 2003. Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. *Exp. Cell Res.* 285: 243–257.
5. Celi, A., R. Lorenzet, B. C. Furie, and B. Furie. 2004. Microparticles and a P-selectin-mediated pathway of blood coagulation. *Dis. Markers* 20: 347–352.
6. Gasser, O., C. Hess, S. Miot, C. Deon, J.-C. Sanchez, and J. A. Schifferli. 2003. Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. *Exp. Cell Res.* 285: 243–257.
7. Gilbert, G., P. Sims, T. Wiedmer, B. Furie, B. Furie, and S. Shattil. 1991. Platelet-derived microparticles express high affinity receptors for factor VIII. *J. Biol. Chem.* 266: 17261–17268.
8. Falati, S., Q. Liu, P. Gross, G. Merrill-Skoloff, J. Chou, E. Vandendries, A. Celi, K. Croce, B. C. Furie, and B. Furie. 2003. Accumulation of tissue factor into developing thrombi in vivo is dependent upon microparticle P-selectin glycoprotein ligand 1 and platelet P-selectin. *J. Exp. Med.* 197: 1585–1598.
9. Mesri, M., and D. C. Altieri. 1998. Endothelial cell activation by leukocyte microparticles. *J. Immunol.* 161: 4382–4387.
10. Bals, R., and P. S. Hiemstra. 2004. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur. Respir. J.* 23: 327–333.
11. Wagner, J. G., and R. A. Roth. 2000. Neutrophil migration mechanisms, with an emphasis on the pulmonary vasculature. *Pharmacol. Rev.* 52: 349–374.
12. Pettersen, C. A., and K. B. Adler. 2002. Airways inflammation and COPD: epithelial-neutrophil interactions. *Chest* 121: 142S–150S.
13. Celi, A., S. Cianchetti, S. Petruzzelli, S. Carnevali, F. Baliva, and C. Giuntini. 1999. ICAM-1-independent adhesion of neutrophils to phorbol ester-stimulated human airway epithelial cells. *Am. J. Physiol.* 277: L465–L471.
14. VanWijk, M. J., E. VanBavel, A. Sturk, and R. Nieuwland. 2003. Microparticles in cardiovascular diseases. *Cardiovasc. Res.* 59: 277–287.
15. Hess, C., S. Sadallah, A. Hefti, R. Landmann, and J.-A. Schifferli. 1999. Ectosomes released by human neutrophils are specialized functional units. *J. Immunol.* 163: 4564–4573.
16. Gasser, O., and J. A. Schifferli. 2004. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood* 104: 2543–2548.
17. Berckmans, R. J., R. Nieuwland, P. P. Tak, A. N. Boing, F. P. Romijn, M. C. Kraan, F. C. Breedveld, C. E. Hack, and A. Sturk. 2002. Cell-derived microparticles in synovial fluid from inflamed arthritic joints support coagulation exclusively via a factor VII-dependent mechanism. *Arthritis Rheum.* 46: 2857–2866.
18. Distler, J. H., A. Jungel, L. C. Huber, C. A. Seemayer, C. F. Reich III, R. E. Gay, B. A. Michel, A. Fontana, S. Gay, D. S. Pisetsky, and O. Distler. 2005. The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. *Proc. Natl. Acad. Sci. USA* 102: 2892–2897.
19. Berckmans, R. J., R. Nieuwland, M. C. Kraan, M. C. Schaap, D. Pots, T. J. Smeets, A. Sturk, and P. P. Tak. 2005. Synovial microparticles from arthritic patients modulate chemokine and cytokine release by synoviocytes. *Arthritis Res. Ther.* 7: R536–R544.
20. Hess, C., S. Sadallah, A. Hefti, R. Landmann, and J. A. Schifferli. 1999. Ectosomes released by human neutrophils are specialized functional units. *J. Immunol.* 163: 4564–4573.