Concurrent Upregulation of Urokinase Plasminogen Activator Receptor and CD11b during Tuberculosis and Experimental Endotoxemia

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Patients with tuberculosis had higher expression of monocyte urokinase receptor (uPAR) and CD11b than controls. In vitro, lipoarabinomannan and lipopolysaccharide (LPS) from Escherichia coli shared the ability to enhance uPAR and CD11b expression on monocytes and granulocytes. In healthy volunteers, LPS induced increases in monocyte and granulocyte uPAR and CD11b.

The receptor for urokinase plasminogen activator (uPAR; also called CD87) can act as an adhesion receptor, and in vitro chemotaxis of monocytes and granulocytes at least in part depends on the expression of uPAR (2, 5). uPAR is a glycosylphosphatidylinositol-linked receptor without a transmembrane domain; it therefore lacks a direct link to the cell interior. However, uPAR can form functional complexes with complement receptor 3 (CR3, CD11b/CD18) on monocytes (13, 14) and granulocytes (4, 16), thus enabling signal transduction for both the monocytes and the granulocytes and facilitating the adhesive capacity of the granulocytes. In addition, uPAR gene-deficient mice demonstrated a reduced β-integrin-mediated leukocyte recruitment to inflamed areas in vivo (10). Hence, the ability of uPAR to interact with CR3 seems important for adhesion and migration of monocytes and granulocytes. Recently, it was reported that intravenous injection of lipopolysaccharide (LPS) into healthy humans was associated with an upregulation of monocyte uPAR expression, while the effect on granulocyte uPAR expression was inconsistent (3). Thus far, the expression of the uPAR-CR3 complex during infections in vivo has not been studied. Therefore, we found it of interest to measure cellular uPAR and CD11b expression in patients with tuberculosis (TB). In addition, the capacity of lipoarabinomannan (LAM; derived from Mycobacterium tuberculosis) to influence cellular uPAR and CD11b expression was compared with the effect of LPS, and the model of human experimental endotoxemia was used to further study the kinetics of uPAR and CD11b expression during inflammation in vivo.

All studies were approved by the institutional research and ethics committees, and written informed consent was obtained from all subjects. Blood was obtained from eight patients (six male and two female) with active, culture-proven TB attending the Academic Medical Center (n = 5), the Sint Lucas Hospital (n = 2), and the Municipal Health Center (n = 1) in Amsterdam, The Netherlands. The age (mean ± standard error) of TB patients was (32 ± 4 years) and did not differ from that of healthy controls (29 ± 2 years; four male and four female). Four of the patients had pulmonary TB, and four had extrapulmonary TB. Extrapulmonary sites included pleural (n = 2), soft tissue (n = 1), and gastrointestinal tract (n = 1) sites. Three TB patients were human immunodeficiency virus seropositive and were treated with antiretroviral therapy. None of the TB patients took immunosuppressive drugs. Six patients had fever (rectal temperature > 38°C). Blood for fluorescence...

FIG. 1. Expression of uPAR and CD11b on peripheral blood monocytes and granulocytes of patients with active TB and healthy controls. Horizontal lines represent medians. MCF, mean channel fluorescence; NS, not significant.
activated cell sorter analysis was drawn prior to administration of antituberculous medication. On the same day a patient was analyzed, blood was also obtained from a healthy control. After collection, blood was immediately prepared for fluorescence-activated cell sorter analysis (Calibrite; Becton Dickinson, San Jose, Calif.). For in vitro experiments, blood was collected aseptically from six healthy subjects and diluted 1:1 with RPMI 1640 (BioWhittaker, Verviers, Belgium), to which LAM (1 mg/ml, prepared from M. tuberculosis strain H37Rv, provided by J. T. Belisle [Fort Collins, Colo.]) or LPS (10 ng/ml, from Escherichia coli serotype O111; B4; Sigma, [St Louis, Mo.,] was added, and incubated at 37°C for 6 h. The LAM preparation contained 21.6 pg of LPS per mg of LAM as determined by the Limulus test. LPS potentially contaminating the LAM dose used (1 mg of LAM may contain 21.6 pg of LPS) was insufficient to influence uPAR or CD11b expression (data not shown). In addition, eight healthy male subjects, ages 24 ± 2 years (mean ± standard error), received a bolus intravenous injection of LPS (from E. coli, lot G; U.S. Pharmacopeia, Rockville, Md.) at a dose of 4 ng/kg of body weight. Venous blood samples were obtained directly before and 6 h after injection of LPS. (The timing of blood sampling was based on a previous study, in which the effect of intravenous LPS on cellular uPAR expression in humans in vivo was determined [3]). Blood was put on ice immediately, and erythrocytes were lysed with bicarbonate-buffered ammonium chloride solution (pH 7.4). After centrifugation, 10⁶ leukocytes were resuspended in phosphate-buffered saline containing EDTA (100 mM), sodium azide (0.1%), and bovine serum albumin (5%). Blood of patients was incubated with fluorescein isothiocyanate-labeled mouse anti-human uPAR monoclonal antibody (clone VIM-5; Instruchemie, Hilversum, The Netherlands) or with mouse anti-human CD11b (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, CLB, Amsterdam, The Netherlands), followed by a fluorescein isothiocyanate-labeled F(ab')₂, goat-anti-mouse antibody (Zymed, San Francisco, Calif.). A murine isotype-matched antibody was used to control for aspecific staining (immunoglobulin G1; Becton Dickinson & Co., Rutherford, NJ.). Blood samples from the in vitro experiments and blood samples from the volunteers receiving LPS were incubated with phycoerythrin-labeled mouse anti-human CD11b or mouse anti-human uPAR (both from Pharmingen, San Diego, Calif.). Aspecific staining was controlled for by incubation of cells with phycoerythrin-labeled mouse immunoglobulin G1 (Coulter Immunotech. Marseilles, France). Data are presented as the difference between mean cell fluorescence intensities of specifically and nonspecifically stained cells. Data were analyzed using the Wilcoxon test. A P value of <0.05 was considered statistically significant.

Patients with active TB had increased expression of both uPAR and CD11b on circulating monocytes compared to healthy controls (P < 0.05) (Fig. 1). Although granulocyte uPAR and granulocyte CD11b expression tended to be higher in patients than in controls, the differences did not reach sta-
LPS resulted in a strong upregulation of monocyte uPAR expression during inflammation in vivo. Administration of experimental endotoxemia model to study uPAR and CD11b upregulate cellular uPAR and CD11b, we next used the human

tions are very similar (7, 11). Having established that LPS can
LPS and LAM effects on many different inflammatory reac-
tent. Stimulation of whole blood with
ulocytes, although granulocyte upregulation was less promi-
expression of uPAR and CD11b on both monocytes and gran-
ocytes in terms of uPAR upregulation upon stimulation. This work was supported by grants from the “Mr. Willem Bakhuys Roozeboom” Foundation to N. P. Juiffermans.

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