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Serum Concentrations of Lipopolysaccharide Activity–Modulating Proteins during Tuberculosis

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Lipopolysaccharide (LPS) is the principal stimulator of host defense against gram-negative bacteria. LPS-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), and soluble CD14 (sCD14) bind LPS and regulate its toxicity. Lipoarabinomannan, a cell wall component of *Mycobacterium tuberculosis*, resembles LPS with respect to induction of inflammatory responses through recognition by LBP and sCD14. LBP, BPI, and sCD14 were measured in serum of 124 patients with tuberculosis in various stages of disease, in persons who had been in close contact with patients with contagious pulmonary tuberculosis, and in healthy controls. Levels of these LPS toxicity–regulating proteins were elevated in patients with active tuberculosis compared with those in contacts and controls and declined during treatment. The levels of LBP and sCD14 were higher in patients with fever and anorexia. LPS-regulating proteins may play a role in host defense during tuberculosis, presumably through interaction with lipoarabinomannan.

It has been estimated that one-third of the world population is infected with *Mycobacterium tuberculosis*. In industrialized countries, the number of tuberculosis cases has failed to decline [1]. Host defense mechanisms during tuberculosis and pathways by which *M. tuberculosis* induces inflammatory responses are incompletely understood.

Lipopolysaccharide (LPS) is the principal stimulator of host defense against gram-negative bacteria. The biologic availability of LPS is regulated by a number of serum proteins, including LPS-binding protein (LBP), bactericidal/permeability-increasing protein (BPI), and soluble CD14 (sCD14) [2]. LBP facilitates the binding of LPS to CD14, a glycoprotein expressed on monocytes and neutrophils that is essential for the induction of an inflammatory response to LPS. BPI is a protein secreted by the azurophilic granules of neutrophils that binds and neutralizes LPS. LPS activity can be further regulated by sCD14, the extracellular domain of cell-bound CD14, which can either enable LPS to activate cell types that lack membrane CD14 or inhibit LPS effects on CD14-expressing cell types by competition for the binding of LPS with cell-associated CD14 [2].

Lipoarabinomannan (LAM) is a lipid glycoprotein cell wall component of *M. tuberculosis* that has been implicated as a major factor in the induction of cytokine release during tuberculosis [3, 4]. LAM shares many physiochemical properties with LPS and uses LBP, cell-associated CD14, and sCD14 in a manner similar to that of LPS to exert inflammatory effects on cells [3–6]. Hence, it is conceivable that serum proteins involved in the regulation of LPS activity also play a role in the regulation of the inflammatory response during tuberculosis by interference with the bioavailability of LAM. Therefore, in the present study, we determined serum concentrations of LBP, BPI, and sCD14 in patients with tuberculosis before, during, and after antituberculous treatment.

Methods

Patient groups. Patient groups have been described elsewhere [7]. Sera were obtained from 82 patients with active, culture-proven tuberculosis. Of these patients, 32% were female. Mean age was 35 years (range, 15–86). Of them, 46 had pulmonary tuberculosis and 36 had extrapulmonary tuberculosis. Extrapulmonary sites included lymph nodes (*n* = 8), pleura (*n* = 12), bone and joints (*n* = 6), soft tissue (*n* = 2), meninges (*n* = 3), and gastrointestinal tract (*n* = 2). In 3 patients, disease was disseminated. Sera were also obtained from 15 patients with tuberculosis who had received therapy for at least 2 weeks but had not yet completed therapy at the time of blood sampling, from 16 patients who had completed therapy at least 1 month and not more than 1 year before blood sampling, and from 11 patients who had completed therapy at least...
1 year and not more than 2 years before blood sampling. Since analysis of the latter 2 groups revealed no differences (data not shown), their results were combined. Of these 124 patients, 66 attended the Academic Medical Center and 58 the Municipal Health Service in Amsterdam. There was no significant difference in ethnic origin between patient groups, which comprised European (43%), Asian (24%), African (17%), and South American (16%) patients. Records of all patients with active tuberculosis were reviewed, and clinical data such as fever (rectal temperature >38°C) and anorexia (gross loss of appetite and weight loss) were scored. Fourteen patients were human immunodeficiency virus (HIV)-seropositive and 67 patients either were HIV-seronegative or had no antibodies to HIV measured. Since no differences were found between HIV-seropositive and -seronegative patients and patients with an unknown HIV status (data not shown), all patient data were combined.

Control groups. Sera were obtained from 16 persons who had been in close contact with patients with contagious pulmonary tuberculosis; 1 person was tuberculin skin test–positive and 15 were tuberculin nonresponders. Close contacts were all recruited from the municipal health service during contact research and did not differ from patient groups in age and ethnic origin. Sera were also obtained from 10 healthy controls, matching in sex and age, all of whom were skin test–negative.

Assays. Sera were collected after centrifugation and stored at −20°C until measurements. All assays were done in duplicate. LBP was measured by ELISA as described previously [8], using polyclonal rabbit anti-human LBP (5 μg/mL) as capturing antibody, biotinylated polyclonal rabbit anti-human LBP as labeling antibody, and recombinant LBP as standard. BPI was measured with an ELISA as described [8], using monoclonal anti-human antibody (3 μg/mL) as capturing antibody, biotinylated polyclonal rabbit anti-human BPI IgG as detecting antibody, and recombinant human BPI as standard. sCD14 was measured by an ELISA according to the instructions of the manufacturer (Biosource Europe, Fleurus, Belgium). Detection limits of assays were 781 pg/mL (LBP), 391 pg/mL (BPI), and 2 ng/mL (sCD14).

Statistical analysis. All values are presented as median (range). Comparisons between groups were made using the Wilcoxon rank sum test for unmatched samples. Two-sided P < .05 was considered significant.

Results

LBP. Serum LBP concentrations did not differ between patients with pulmonary and extrapulmonary tuberculosis (85.1 μg/mL [range, 12.3–411.0] and 71.5 μg/mL [range, 25.4–334.0], respectively; not significant). Patients with active tuberculosis had higher levels (78.6 μg/mL [range, 12.3–3340.0]) than did patients during therapy (32.7 μg/mL [range, 8.8–187.0]; P = .001), patients who had completed therapy (29.0 μg/mL [range, 10.6–268.0]; P < .005), close contacts (40.4 μg/mL [range, 12.2–150.0]; P < .05), and controls (12.6 μg/mL [range, 6.4–60.4]; P < .001) (figure 1). In patients with active tuberculosis who had fever or anorexia, LBP was significantly higher.
raised compared with levels in patients with a normal temperature or without anorexia (table 1).

**BPI.** Serum BPI concentrations did not differ between patients with pulmonary and extrapulmonary tuberculosis (5.9 ng/mL [range, 0.4–51.5] and 8.3 ng/mL [range, 0.4–123.0], respectively; not significant). Patients with active tuberculosis had higher BPI levels (6.7 ng/mL [range, <0.4–123.0]) than did close contacts (3.9 ng/mL [range, <0.4–0.5]; P = .05) and controls (1.8 ng/mL [range, 0.5–8.0]; P < .005). The median serum levels of BPI in patients who had completed therapy (4.8 ng/mL [range, 0.4–32.9]) and in close contacts were raised compared with those in controls (P < .05 and P = .05, respectively). There was no difference in BPI between patients with and without clinical symptoms (table 1).

**sCD14.** Serum sCD14 was significantly higher in extrapulmonary tuberculosis than in pulmonary tuberculosis (7.2 µg/mL [range, 3.2–16.2] and 5.8 µg/mL [range, 2.1–13.6], respectively; P < .05). All patient groups had significantly higher levels of sCD14 than did close contacts, but levels of sCD14 of patients during therapy did not differ from those in controls. Median serum sCD14 concentration in patients with active tuberculosis (pulmonary or extrapulmonary) was 6.0 µg/mL (range, 2.1–16.2), which was significantly higher than in patients during therapy (4.8 µg/mL [range, 2.0–9.0]; P = .01), in patients who had completed therapy (3.4 µg/mL [range, 2.0–9.4]; P < .001), in close contacts (2.6 µg/mL [range, 2.0–4.0]; P < .001), and in healthy controls (3.5 µg/mL [range, 2.0–6.4]; P < .001). The median serum level of sCD14 in patients with active tuberculosis who had fever was significantly raised compared with that in patients with a normal temperature (table 1).

**Discussion**

The host immune response to tuberculosis is at least in part initiated by stimulation of inflammatory cells by the mycobacterial cell wall component LAM. Proteins identified as regulators of LPS activity appear critically involved in the cellular response to LAM. We determined the serum concentrations of LBP, BPI, and sCD14 in patients with various manifestations and stages of tuberculosis. All three proteins were elevated during active tuberculosis and declined during treatment.

Increased serum concentrations of LBP have been reported previously in patients with sepsis and healthy humans injected with LPS [8, 9]. Our finding of elevated concentrations of LBP in patients with active tuberculosis may have relevance for the host reaction to tuberculosis. Indeed, LAM induces production of tumor necrosis factor and interleukin-1β by the monocyte/macrophage cell line THP-1 via a CD14-dependent mechanism, a process that is greatly enhanced by LBP [3, 4]. Thus, elevated levels of LBP may facilitate inflammatory reactions in patients with tuberculosis. In accordance, LBP levels were higher in patients with fever and/or anorexia. Since LBP has also been found in bronchoalveolar lavage fluids of healthy humans and patients with lung injury [10], it is likely that LBP also influences LAM bioavailability in lungs during pulmonary tuberculosis.

BPI is a neutrophil degranulation product that exerts bactericidal effects on gram-negative bacteria and neutralizes LPS activity in vitro and in vivo [2]. As in patients with sepsis and in volunteers to whom endotoxin had been administered [8, 11], serum BPI concentrations were higher in patients with active tuberculosis, albeit no differences were found between patients with and without fever or anorexia. Further studies are needed to determine whether BPI can influence LAM bioactivity in a manner similar to that of LPS, although this seems likely considering the >40% amino acid sequence homology between LBP and BPI and the shared properties of LPS and LAM with respect to LBP/CD14 interactions [2–6].

Previous studies have documented elevated serum concentrations of sCD14 in patients with sepsis [8]. Also, high levels of sCD14 have been found in bronchoalveolar lavage fluid in patients with lung disorders, including tuberculosis [12, 13]. Our study expands these findings to elevated serum concentrations of sCD14 in patients with active tuberculosis. sCD14 enables responses to LPS by cells with little or no membrane-bound CD14 [2]. Of interest, patients with extrapulmonary tuberculosis had higher levels of sCD14 than did patients with pulmonary tuberculosis. This may indicate a decreased sensitivity to LAM of cell types in extrapulmonary sites compared with alveolar cells, but further studies on regulation of LAM bioactivity by sCD14 during tuberculosis are needed.

We here report that LBP, BPI, and sCD14 are elevated in serum of patients with active tuberculosis and decrease during

| Table 1. Association of lipopolysaccharide-binding protein (LBP), bactericidal/permeability-increasing protein, and soluble (s) CD14 with clinical symptoms during active tuberculosis. |
|--------------------------------------------------|-----------------|-------------------------------------------------|-----------------|-----------------|-----------------|
|                     | Fever           | Anorexia                                      |
|--------------------------------------------------|-----------------|-------------------------------------------------|-----------------|-----------------|-----------------|
| LBP (µg/mL)        | Yes             | No                                            | Yes             | No              |
| 92.3 (18.9–3340.0) | 59.9 (13.0–411.0) | 91.2 (18.5–3340.0) | 64.7 (12.3–670.0) |
| BPI (ng/mL)        | 6.6 (0.4–123.0)  | 6.8 (0.4–123.0) | 5.5 (0.4–51.5) | 6.94 (0.4–123.0) |
| sCD14 (µg/mL)      | 7.8 (2.1–16.2)  | 5.4 (2.7–8.5) | 6.5 (3.1–16.2) | 5.8 (2.1–15.3) |

NOTE. Data are median (range) of 82 patients with active tuberculosis. Fever was defined as rectal temperature >38°C; anorexia as gross loss of appetite and weight loss.

*P < .05 for difference between absence and presence of fever or anorexia.
treatment. Whether these proteins have an essential role in mounting an inflammatory response during tuberculosis remains to be determined.

References