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Anti-CD14 Monoclonal Antibodies Inhibit the Production of Tumor Necrosis Factor Alpha and Interleukin-10 by Human Monocytes Stimulated with Killed and Live *Haemophilus influenzae* or *Streptococcus pneumoniae* Organisms

A. MARCELINE VAN FURTH,[1,2] ELS M. VERHARD-SEIJMONSBERGEN,[1] JAN A. M. LANGERMANS,[1,3] JAAP T. VAN DISSEL,[1] AND RALPH VAN FURTH[1]*

Department of Infectious Diseases, Leiden University Medical Center, 2300 RC Leiden,[1] Department of Pediatrics, Free University Hospital, 1007 MB Amsterdam,[2] and Department of Parasitology, Biomedical Primate Research Centre, 2288 GJ Rijswijk,[3] The Netherlands

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In previous studies, we have shown that intact, heat-killed, gram-negative bacteria (GNB) and gram-positive bacteria (GPB) can stimulate the production of various proinflammatory and anti-inflammatory cytokines. The objective of the present study was to investigate whether the production of tumor necrosis factor alpha (TNF) and interleukin-10 (IL-10) by human monocytes stimulated by intact heat-killed or live *Haemophilus influenzae* or *Streptococcus pneumoniae* is mediated by CD14. Two anti-CD14 monoclonal antibodies (MAbs) were used to study the interaction between human monocytes and bacteria; lipopolysaccharide (LPS) was used to validate the effect of anti-CD14 MAb. MAb 18E12 decreased significantly TNF and IL-10 production upon stimulation with LPS or heat-killed bacteria and TNF production during stimulation by live bacteria. MAb My-4 decreased production of TNF and IL-10 by monocytes stimulated with LPS, IL-10 but not TNF production upon stimulation with heat-killed *H. influenzae*, and production of neither TNF nor IL-10 upon stimulation with *S. pneumoniae*. Together, these results led to the conclusion that CD14 is involved in the recognition and stimulation of human monocytes by intact GNB and GPB. Consequentially, the option for adjunctive treatment of severe infections with anti-CD14 MAb is postulated.

CD14 is a 55-kDa glycoprotein which binds lipopolysaccharide (LPS) and initiates cell activation (27). This receptor is abundantly present on the cell membrane (mCD14) of monocytes and macrophages and at low density on polymorphonuclear leukocytes or as a soluble protein (sCD14) in human serum and urine (1, 27).

Binding of LPS to CD14 is enhanced by LPS-binding protein (LBP), a 60-kDa acute-phase protein formed in the liver, which is present in human serum (27). LBP, which binds LPS stoichiometrically and transfers the LPS-LBP complex to mCD14 (12, 27), acts as a shuttle to transfer LPS to the cell membrane. LBP lowers the threshold for the stimulatory concentration of LPS and enhances the effects of LPS on the induction of cytokines by monocytes (7, 13, 15, 27). Blockade of mCD14 with monoclonal antibodies (MAbs) has been shown to inhibit LPS-induced synthesis of tumor necrosis factor alpha (TNF) and interleukin-1 (IL-1) by monocytes and macrophages (4, 6, 14, 19, 27).

CD14 is a glycosylphosphatidylinositol-linked protein that does not transfer the cell membrane (27). The existence of a transmembrane molecule that functions as signal transducer upon LPS binding by CD14 has been postulated (12, 27). Probably Toll-like receptor 2, a transmembrane protein, is this signaling protein since upon stimulation by LPS-LBP, it activates NF-κB and the expression of NF-κB-controlled genes which encode cytokine (2, 35).

Peptidoglycan and lipoteichoic acid, cell wall components of gram-positive bacteria (GPB), can also stimulate the production of cytokines by human monocytes via CD14 (3, 5, 21, 33, 34). This has also been reported for lipoarabinomannan of *Mycobacterium tuberculosis* (36), rhamnose-glucose polymers from *Streptococcus mutans* (25), and manuronic acid polymers from *Pseudomonas* species (11).

We demonstrated previously that intact, heat-killed GPB and gram-negative bacteria (GNB) induce the production of various proinflammatory cytokines, such as IL-1 and TNF, and the anti-inflammatory cytokine IL-10 by human monocytes (28, 29). The objective of the present study was to determine whether the production of TNF and IL-10 by monocytes stimulated with killed or live *Haemophilus influenzae* and *Streptococcus pneumoniae* is mediated via mCD14.

MATERIALS AND METHODS

Microorganisms. *H. influenzae* type b (strain 760705) was cultured at 37°C in Mueller-Hinton broth (MH) containing 4% factor V and 0.08% factor X for 18 h. During culture, the capsule remained present on the bacteria, as confirmed by L. van Alphen (Academic Medical Center, Amsterdam, The Netherlands) (8). Next, *H. influenzae* was diluted 1 to 10 in MH, incubated at 37°C for 2 h, and then diluted in pyrogen-free saline to concentrations appropriate for the experiment. *S. pneumoniae* (serotype 6) was cultured at 37°C in brain heart infusion broth (BHI) supplemented with 1% bovine serum for 18 h. Next, the bacteria were diluted 1 to 10 in BHI, incubated at 37°C for 2 h, and then diluted in pyrogen-free saline to the appropriate concentrations. To assess the effect of anti-CD14 MAb or polymyxin B on the growth of bacteria, cultures were prepared after incubation of bacteria with monocytes. To prepare suspensions of heat-killed bacteria, *H. influenzae* and *S. pneumoniae* were cultured for 18 h at 37°C in MH or BHI, respectively, collected by centrifugation for 10 min at 3,000 × *g*, washed twice with pyrogen-free saline, killed by incubation at 70°C for 1 h, and suspended at appropriate concentrations.

MAbs. The anti-CD14 MAb 18E12 (immunoglobulin G1 [IgG1]; courtesy of P. S. Tobias, The Scripps Research Institute, La Jolla, Calif.) and MAb My-4 (IgG2b) (courtesy of R. R. Schumann, Max-Delbrück Centrum für Molekulare Medizin, Berlin, Germany) were used at concentrations of 5 (18E12) and 12.5 (My-4) μg of protein/ml. In preliminary experiments, these concentrations were

* Corresponding author. Mailing address: Laan van Old Poelgeest 44, 23412 NL Oegstgeest, The Netherlands. Phone: 31 71 5173093. Fax: 31 71 5156606. E-mail: RvanFurth@Thuisnet.LeidenUniv.NL.

TABLE 1. Effect of anti-CD14 MAb 18E12 on production of TNF and IL-10 by monocytes stimulated by LPS, *H. influenzae*, or *S. pneumoniae*[a]

Stimulus	Concn/ml	Mean concn (pg/ml) ± SD (n = 3–6)			
		TNF		IL-10	
		Control MAb	18E12	Control MAb	18E12
LPS	10 ng	515 ± 338	272 ± 230[b] (47[c])	753 ± 362	591 ± 323[b] (22)
	5 ng	1,021 ± 1,061	309 ± 340[b] (70)	678 ± 348	329 ± 121[b] (52)
	1 ng	940 ± 961	88 ± 96[b] (91)	350 ± 149	79 ± 62[b] (77)
H. influenzae	10^6	1,009 ± 771	563 ± 472[b] (44)	1,026 ± 864	770 ± 664[b] (25)
	5×10^5	643 ± 607	384 ± 301[b] (40)	898 ± 772	663 ± 462[b] (26)
	10^5	223 ± 186	129 ± 115[b] (42)	231 ± 214	34 ± 27[b] (85)
S. pneumoniae	10^6	932 ± 1,133	582 ± 805[b] (38)	792 ± 347	630 ± 356 (21)
	5×10^5	742 ± 792	638 ± 961 (14)	1,048 ± 698	441 ± 243[b] (58)

[a] Adherent monocytes were stimulated for 24 h with LPS, heat-killed *H. influenzae*, or heat-killed *S. pneumoniae* in the presence of MAb 18E12 or the corresponding control MAb.
[b] Cytokine production by LPS- or bacterium-stimulated monocytes in the presence of anti-CD14 MAb significantly ($P < 0.05$) less than with the corresponding control MAb.
[c] Percent inhibition.

shown to be optimal. As controls, similar concentrations of corresponding MAb FK40 (IgG1) (courtesy of F. Koning, Department of Immunohematology and Bloodbank, University Hospital, Leiden, The Netherlands), directed against an irrelevant surface molecule on human monocytes (20), or anti-ELAM-1 MAb BB11 (IgG2b) (courtesy R. Lobb, Biogen, Cambridge, Mass.) were used.

Isolation of monocytes. Monocytes were isolated from buffy coats of blood from healthy donors by differential centrifugation on Ficoll-Isopaque gradients (ρ = 1.077 kg/liter; Pharmacia, Uppsala, Sweden). The interphase layer contained 85 to 95% monocytes, 7 to 12% lymphocytes, and less than 4% granulocytes. Cell viability exceeded 98%, as determined by trypan blue dye exclusion. The suspension of mononuclear cells was washed three times with phosphate-buffered saline containing heparin (0.5 U/ml) and suspended at a concentration of 10^6 monocytes/ml in RPMI 1640 (Gibco BRL, Paisley, Scotland) containing penicillin (100 U/ml), streptomycin (50 μg/ml), and 10% heat-inactivated fetal calf serum (Flow Laboratories, Irvine, Scotland), hereafter called medium. Fetal calf serum contains biologically active LBP, as demonstrated by the transfer of fluorescein isothiocyanate-labeled LPS to human monocytes (14) (kindly determined by N. Lamping and R. R. Schumann, Berlin, Germany).

Stimulation of cytokine release. One-milliliter aliquots of a cell suspension containing 10^6 monocytes/ml were incubated in 24-well tissue culture plates (Costar, Cambridge, Mass.) for 1 h at 37°C and 5% CO_2. Thereafter, the nonadherent cells were removed by washing and 1 ml of fresh medium was added. The adherent population consisted of 95% ± 2% monocytes (28). When live bacteria were used to stimulate, no antibiotics were added to the medium. Monocytes were preincubated with anti-CD14 or control MAb for 20 min at 4°C, not washed; next, a suspension of heat-killed or live bacteria or LPS (*Escherichia coli* O111:B4 LPS; Difco Laboratories, Detroit, Mich.) was added, and the incubation was continued for 4 or 24 h at 37°C at 5% CO_2. Thereafter, the supernatant was centrifuged (10 min, 1,500 × g) to remove the bacteria; the resulting supernatant was collected and used to quantify the cytokines under study.

Measurement of cytokines. The concentration of TNF in the culture supernatant was measured by enzyme-linked immunosorbent assay (ELISA) (BPRC, Rijswijk, The Netherlands) as described elsewhere (29). The concentration of IL-10 was measured by ELISA (Pharmingen, San Diego, Calif.), as instructed by the manufacturer, using a capture anti-human IL-10 MAb (JES3-9D7) at a concentration of 0.1 μg per well and a biotinylated anti-IL-10 antibody (JES3-12G8) at a concentration of 0.1 μg per well, as described elsewhere (29). Tetramethylbenzidine was used as the substrate; after termination of the reaction, the absorbance was read at 450 nm.

Endotoxin measurement. Endotoxin was determined by the *Limulus* amebocyte lysate assay (Coatest endotoxin; Chromogenix, Mölndal, Sweden); the lower limit of detection was 3 pg/ml.

Statistical analysis. Since the amounts of TNF and IL-10 produced by monocytes from different donors varied, in each experiment the cytokine release determined in the presence of anti-CD14 MAb was always combined with the release in the presence of the appropriate control MAb. The results are expressed as mean values and standard deviations. The difference of the effect of anti-CD14 MAb and control MAb was analyzed by the paired two-tailed sample t test. The level of significance was set at 0.05.

RESULTS

Effect of anti-CD14 MAb 18E12 on the production of TNF and IL-10 by human monocytes stimulated by LPS. LPS was used as a reference to evaluate the effect of anti-CD14 MAb in the assay used in this study. The inhibitory effect of anti-CD14 MAb on the LPS-induced production of TNF and IL-10 by adherent monocytes during 24 h was dose dependent. The greatest inhibition of cytokine production was achieved when 1.0 ng of LPS per ml was used to stimulate monocytes; with 10 ng of LPS per ml a smaller but still significant inhibition was achieved (Table 1). Anti-CD14 MAb did not affect the production of TNF and IL-10 by unstimulated monocytes (data not shown), and the control MAb FK40 did not affect the LPS-induced production of TNF and IL-10 by LPS-stimulated monocytes (data not shown).

Effect of anti-CD14 MAb 18E12 on the production of TNF and IL-10 by human monocytes stimulated by heat-killed *H. influenzae*. The production of TNF by monocytes stimulated by heat-killed *H. influenzae* during 4 h was dependent on the concentration of bacteria (with 10^6 of bacteria per ml, 960 pg of TNF per ml; with 5×10^5 bacteria per ml, 535 pg of TNF per ml; with 10^5 bacteria per ml, 400 pg of TNF per ml; with 5×10^4 bacteria per ml, 301 pg of TNF per ml). Stimulation of monocytes with 1×10^6 to 5×10^4 heat-killed bacteria per ml in the presence of anti-CD14 MAb for 4 h resulted in a significant (45 to 65%) decrease in TNF production (data not shown).

Stimulation of monocytes with heat-killed *H. influenzae* for 24 h resulted also in a bacterium concentration-dependent production of TNF (Table 1). Incubation of monocytes stimulated with heat-killed *H. influenzae* in the presence of anti-CD14 gave a significant (about 40%) reduction in TNF production, independent of the concentration of bacteria used (Table 1). Control MAb FK40 inhibited the production of TNF slightly (8%) but not significantly (data not shown).

The production of IL-10 by monocytes incubated with heat-killed *H. influenzae* has been determined after 24 h of incubation, since after a shorter incubation period IL-10 is not detectable in the supernatant (29). Incubation of adherent monocytes with heat-killed *H. influenzae* resulted in a bacterium concentration-dependent production of IL-10, which was

TABLE 2. Effect of anti-CD14 MAb on production of TNF by monocytes stimulated by live *H. influenzae* or *S. pneumoniae*[a]

MAb	Mean concn (pg/ml) ± SD (n = 4)	
	H. influenzae	*S. pneumoniae*
18E12	103 ± 72[b]	192 ± 148[b]
FK40	429 ± 250	323 ± 250
None	472 ± 306	402 ± 279

[a] Adherent monocytes were stimulated for 24 h with live *H. influenzae* (2.0×10^3/ml) or live *S. pneumoniae* (5.0×10^3/ml) in the presence of anti-CD14 MAb 18E12, control MAb FK40, or no MAb.

[b] TNF production by bacterium-stimulated monocytes in the presence of anti-CD14 MAb significantly ($P < 0.05$) less than with control MAb or with no MAb.

significantly reduced in the presence of anti-CD14 MAb (Table 1). During incubation with 10^5 bacteria per ml, the inhibition was much more prominent (85%) than with 5×10^5 or 1×10^6 bacteria per ml (both about 25%). Control MAb FK40 did not affect the production of IL-10 by monocytes during incubation with heat-killed *H. influenzae* (data not shown).

Effect of anti-CD14 MAb 18E12 on the production of TNF and IL-10 by human monocytes stimulated by heat-killed *S. pneumoniae*. Stimulation of adherent monocytes with heat-killed *S. pneumoniae* for 24 h resulted in bacterium concentration-dependent TNF and IL-10 production (with 5×10^6 bacteria per ml, 5,314 pg of TNF and 1,758 pg of IL-10 per ml) (Table 1). Anti-CD14 MAb inhibited TNF production by adherent monocytes significantly (38%) upon stimulation with 10^6 heat-killed bacteria per ml and to a lesser extent (14%) with 5×10^5 bacteria per ml (Table 1). Control MAb FK40 had no effect on TNF production by monocytes stimulated with killed *S. pneumoniae* (data not shown). The suspensions of heat-killed *S. pneumoniae* did not contain endotoxin.

The production of IL-10 by adherent monocytes stimulated with 10^6 heat-killed *S. pneumoniae* bacteria per ml was decreased (21%) in the presence of anti-CD14 MAb (Table 1); the inhibitory effect of anti-CD14 MAb was more prominent (58%) and significant when monocytes were stimulated with 5×10^5 bacteria per ml (Table 1). The control MAb FK40 did not affect the production of IL-10 (data not shown).

Effect of anti-CD14 MAb 18E12 on the production of TNF by human monocytes induced by live *H. influenzae* or live *S. pneumoniae*. Since anti-CD14 inhibited significantly the release of TNF by adherent monocytes stimulated with heat-killed bacteria, we studied whether similar mechanisms are involved in the cytokine production induced by live bacteria. Stimulation of adherent monocytes with live *H. influenzae* led to the production of TNF, which was significantly reduced when anti-CD14 MAb was present during the stimulation (Table 2). During incubation of *H. influenzae* with monocytes, the number of bacteria increased from 2.0×10^3/ml initially to 4.0×10^5/ml after 4 h. Anti-CD14 MAb had no effect on the growth of *H. influenzae* (data not shown).

Stimulation of adherent monocytes with live *S. pneumoniae* induced also the production of TNF, which was inhibited significantly in the presence of anti-CD14 MAb and slightly but not significantly by the control MAb FK40. When these experiments were performed in the presence of polymyxin B, the amounts of TNF produced by monocytes stimulated with live *S. pneumoniae* were similar (data not shown). This finding demonstrates that no contamination with LPS had occurred, which was confirmed by the *Limulus* amebocyte assay. The number of *S. pneumoniae* organisms increased from 5.0×10^3 to 2.0×10^7/ml during the 4 h of incubation in the presence of monocytes; anti-CD14 MAb or polymyxin B had no effect on the growth of *S. pneumoniae* (data not shown).

The production of IL-10 upon stimulation by live bacteria could not be studied, since 24 h of incubation is required before this cytokine can be detected (29); during this period, the number of bacteria had increased to numbers that affected the viability of the monocytes.

Effect of anti-CD14 MAb My-4 on the production of TNF and IL-10 by monocytes stimulated by LPS, heat-killed *H. influenzae*, or heat-killed *S. pneumoniae*. To investigate whether another anti-CD14 MAb also inhibits the production of cytokines by monocytes stimulated by heat-killed bacteria, the effect of MAb My-4 was compared with the effect of MAb 18E12. Anti-CD14 My-4 did not affect the production of TNF and IL-10 by unstimulated monocytes (data not shown). The production of TNF by monocytes stimulated by LPS was significantly inhibited by MAbs 18E12 and My-4 (Table 3). When monocytes were stimulated with *H. influenzae*, both MAb 18E12 and MAb My-4 inhibited the production of TNF (Table 3); only the effect of MAb 18E12 was statistically significant. TNF production by monocytes stimulated by *S. pneumoniae* was inhibited significantly by MAb 18E12 (Table 3) but increased in the presence of MAb My-4.

The production of IL-10 by monocytes stimulated by LPS or *H. influenzae* was significantly inhibited by MAbs 18E12 and My-4 (Table 4). Only MAb 18E12 inhibited significantly the IL-10 production by monocytes stimulated by *S. pneumoniae*; MAb My-4 had no effect (Table 4).

DISCUSSION

The main conclusion to be drawn from this study is that the production of TNF and IL-10 by monocytes stimulated with intact *H. influenzae* or *S. pneumoniae*, either live or killed, is mediated in part by the interaction with CD14 on monocytes. This conclusion is based on the following observations. (i) Monocytes stimulated with heat-killed *H. influenzae* or *S. pneu-*

TABLE 3. Comparison of the effect of anti-CD14 MAbs 18E12 and My-4 on the production of TNF by monocytes stimulated with LPS, *H. influenzae*, or *S. pneumoniae*[a]

MAb	LPS			*H. influenzae*			*S. pneumoniae*		
	n	TNF concn (pg/ml)	P	n	TNF concn (pg/ml)	P	n	TNF concn (pg/ml)	P
Control	7	567 ± 252		11	1,772 ± 989		8	938 ± 848	
18E12	7	158 ± 188	0.011	11	1,012 ± 541	0.026	8	513 ± 612	0.004
Control	5	717 ± 289		7	2,751 ± 848		7	2,225 ± 824	
My-4	5	185 ± 140	0.005	7	1,675 ± 1,478	0.206	7	3,132 ± 1,356	0.039

[a] Adherent monocytes were stimulated with LPS (10 ng/ml), heat-killed *H. influenzae* (10^6 organisms/ml), or heat-killed *S. pneumoniae* (10^6 organisms/ml) for 24 h in the presence of the indicated MAb.

TABLE 4. Comparison of effects of anti-CD14 MAbs 18E12 and My-4 on the production of IL-10 by monocytes stimulated with LPS, *H. influenzae*, or *S. pneumoniae*[a]

MAb	LPS			*H. influenzae*			*S. pneumoniae*		
	n	IL-10 concn (pg/ml)	*P*	*n*	IL-10 concn (pg/ml)	*P*	*n*	IL-10 concn (pg/ml)	*P*
Control	10	442 ± 267		9	1,443 ± 1,138		9	820 ± 666	
18E12	10	94 ± 64	0.001	9	753 ± 413	0.044	9	588 ± 575	0.038
Control	7	440 ± 254		7	1,334 ± 530		9	1,033 ± 693	
My-4	7	91 ± 79	0.004	7	732 ± 429	0.033	9	1,255 ± 1,315	0.447

[a] Adherent monocytes were stimulated with LPS (10 ng/ml), heat-killed *H. influenzae* (10^6 organisms/ml), or heat-killed *S. pneumoniae* (10^6 organisms/ml) for 24 h in the presence of the indicated MAb.

moniae form TNF and IL-10, the amount being dependent on the concentration of bacteria used as the stimulus. In essence, the concentration-dependent effect found for bacteria was similar to that found for LPS, which was used to validate the effect of anti-CD14 MAb used in this study. (ii) When monocytes stimulated with heat-killed *H. influenzae* or *S. pneumoniae* were cultured in the presence of anti-CD14 MAb, the production of both cytokines was lower than in cultures with control MAb or in the absence of MAb. The degree of the inhibitory effect is dependent on the concentration of bacteria used as the stimulus. (iii) When live bacteria were used as the stimulus, a similar inhibitory effect of anti-CD14 MAb was observed for the production of TNF; the production of IL-10 could not be studied because the bacteria multiplied too much during the 24-h stimulation needed to obtain a detectable concentration of IL-10 (29).

The inhibitory effect of the two anti-CD14 MAbs on the production of TNF and IL-10 varied. Can this be explained by their functional characteristics (31)? MAb 18E12 inhibits (90%) LPS activation of cells without inhibiting (11%) the binding of LPS to CD14, and MAb My-4 inhibits both binding of LPS to CD14 (99%) and LPS activation of cells via CD14 (90%).

The results obtained with MAb 18E12, i.e., the inhibition of both TNF and IL-10 production during stimulation of monocytes with LPS, *H. influenzae*, or *S. pneumoniae*, can be explained by reduced activation of monocytes during interaction of these stimuli with CD14. The inhibitory effect of MAb My-4 on the TNF and IL-10 production by LPS- and *H. influenzae*-stimulated monocytes can be due to impaired binding of these stimuli to CD14 and/or decreased activation of monocytes via CD14; the reduction of TNF production by *H. influenzae*-stimulated monocytes was not significant, probably because of the large donor-dependent variability of the results. MAb My-4 did not inhibit the cytokine production by *S. pneumoniae*-stimulated monocytes; this MAb also does not inhibit TNF production by monocytes stimulated with purified whole cell walls of GPB (5, 16). Binding of *S. pneumoniae* and these cell walls to a different region of CD14 than MAb My-4 can explain why this MAb is not effective.

The site of CD14 that interacts with intact bacteria is not known. The region of CD14 that recognizes and binds LPS has been determined recently (24, 26, 32), and most likely GNB bind via LPS at their surface to the same site of CD14. Whether LBP is required for the binding of intact GNB to CD14 and subsequent signal transduction, as shown for purified LPS (12, 27), is not known. A recent study showed that intact GNB can bind to membrane-bound and soluble CD14 in the presence of serum (17), which indicates that LPS incorporated into the membrane of GNB binds LBP and can interact with CD14. This supports our finding that the production of TNF and IL-10 by monocytes, induced by intact live and heat-killed *H. influenzae* organisms in the presence of serum that contains biologically active LBP, can be inhibited by anti-CD14 MAb. Binding of intact GPB to monocytes and the stimulation of cytokine production via CD14 could be mediated by peptidoglycan and lipoteichoic acid at the surface of the bacteria.

Structural similarities between the cell envelopes of GNB and GPB (23, 33) could explain why both kinds of bacteria interact with CD14, which is concluded from the inhibition of cytokine production by monocytes by anti-CD14 MAb. Total inhibition of cytokine production has not been achieved with anti-CD14 MAb, which could imply that LPS and intact bacteria utilize also other binding sites on monocytes for the stimulation of cytokines. For example, LPS can bind to β_2-leukocyte integrins (CD11/CD18) and activate cells (12); LPS, peptidoglycan, and lipoteichoic acid bind also to other sites on monocytes, such as a 70-kDa protein on human monocytes, which is shown to be cell-bound albumin (9, 10, 22), and to the scavenger receptor (12). However, both cell-bound albumin and the scavenger receptor do not function as signaling receptor upon ligand binding (12).

Do our findings have clinical implications? The present study demonstrated for the first time that intact heat-killed and live *H. influenzae* and *S. pneumoniae* interact with CD14 and stimulate the cytokine production, i.e., TNF and IL-10, by human peripheral blood monocytes, which become exudate macrophages in infected tissues (30). We observed that stimulation of monocytes with an optimal concentration of bacteria, i.e., 10^6 *H. influenzae* organisms, gave a larger production of TNF than stimulation with the optimal concentration (10 ng/ml) of purified LPS. A similar difference has been reported for *E. coli*- and LPS-stimulated human leukocytes (18). Apparently intact GNB or GPB are more powerful stimuli for cytokine production by monocytes than shed bacterial components. Thus, it is conceivable that exudate macrophages in infected tissues, upon interaction of intact bacteria, produce cytokines that are involved in the initial clinical manifestations. In view of this hypothesis and our findings, adjunctive treatment of severe infections with anti-CD14 MAb might be more effective than treatment with anticytokine MAb.

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