CD14 Is a Cell-activating Receptor for Bacterial Peptidoglycan*  

(Received for publication, June 5, 1996, and in revised form, July 2, 1996)

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The hypothesis that CD14 (an endotoxin receptor present on macrophages and neutrophils) acts as a cell-activating receptor for bacterial peptidoglycan was tested using mouse 70Z/3 cells transfected with human CD14. 70Z/3 cells transfected with an empty vector were unresponsive to insoluble and soluble peptidoglycan, as well as to low concentrations of endotoxin. 70Z/3-CD14 cells were responsive to both insoluble and soluble peptidoglycan, as well as to low concentrations of endotoxin, as measured by the expression of surface IgM, activation of NF-κB, and degradation of IκB-α. Peptidoglycan also induced activation of NF-κB and degradation of IκB-α in macrophage RAW264.7 cells. These peptidoglycan-induced effects (in contrast to endotoxin-induced effects) were not inhibited by polymyxin B. Both peptidoglycan- and endotoxin-induced activation of NF-κB were inhibited by anti-CD14 mAb. The N-terminal 151 amino acids of CD14 were sufficient for acquisition of full responsiveness to both peptidoglycan and endotoxin, but CD14 deletion mutants lacking four small regions within the N-terminal 65 amino acids showed differentially diminished responses to peptidoglycan and endotoxin. These results identify CD14 as the functional receptor for peptidoglycan and demonstrate that similar, but not identical sequences in the N-terminal 65-amino acid region of CD14 are critical for the NF-κB and IgM responses to both peptidoglycan and endotoxin.

Peptidoglycan (PGN) is a polymer of alternating GlcNAc and MurNAc cross-linked by short peptides, present in the cell walls of all bacteria. PGN is especially abundant in Gram-positive bacteria, in which it accounts for half of the mass of cell walls of Gram-negative bacteria, can reproduce most of the clinical manifestations of bacterial infections, including fever, acute-phase response, inflammation, septic shock, leukocytosis, sleepiness, malaise, abscess formation, and arthritis. Most of these effects are due to the release of cytokines and other mediators from macrophages and other cells (2, 5–9).

It is well established that CD14, a glycosylphosphatidylinositol (GPI)-linked 55-kDa protein present on the surface of macrophages and polymorphonuclear leukocytes, serves as a functional cell surface receptor for LPS (10–15). The binding of LPS to CD14 is facilitated by the LPS-binding protein present in plasma (10–15). The cellular receptor for PGN, however, is unknown (16).

Because of the similarity of the biologic effects of PGN and LPS (2, 5–9), we decided to test the hypothesis that CD14 may serve as a receptor for both LPS and PGN. Our recent results showed that activation of CD14-positive monocytes by PGN (17) and binding of PGN to CD14-positive monocytes were inhibited by anti-CD14 monoclonal antibodies and LPS partial structures, such as an LPS antagonist, compound 406. These results strongly indicated that CD14 serves as a cell-activating receptor not only for LPS but also for PGN.

Here we further test the hypothesis that CD14 is a functional PGN receptor, using mouse 70Z/3 cells stably expressing recombinant human CD14 (70Z/3-hCD14 transfectants). 70Z/3 cells are CD14-negative immature B cells that do not express surface IgM (sIgM) and are unresponsive to PGN and responsive only to high concentrations of LPS. 70Z/3-hCD14 transfectants express GPI-bound hCD14 and become highly responsive to low concentrations of LPS (19), and the N-terminal 151-amino acid fragment of hCD14 serves as a fully functional LPS receptor (20). Stimulation of these cells results in activation of nuclear factor-κB (NF-κB) and differentiation into more mature B cells that express sIgM (19, 20). NF-κB is a ubiquitous transcription factor that is sequestered in the cytoplasm bound to its inhibitory protein, IκB (21, 22). NF-κB activation involves degradation of IκB in the cytoplasm and translocation of NF-κB into the nucleus (21, 22). In this study, we used the above criteria (translocation of NF-κB into the nucleus, degradation of IκB-α in the cytoplasm, and expression of sIgM) to measure activation of 70Z/3-hCD14 cells by PGN and LPS.

EXPERIMENTAL PROCEDURES

Materials—PGN (insoluble preparation) was isolated from the cell walls of Staphylococcus aureus 845 and sonicated for 30 min (7). sPGN (soluble polymeric PGN) was purified by affinity chromatography from culture supernatants of S. aureus Rb grown in the presence of penicillin (7, 23). PGN and sPGN contained <24 and <12 pg of endotoxin/mg determined by the Limulus lysate assay (23). Streptococcus mitis cell walls were purified as described (24), and mycobacterial lipoarabinomannan (LAM, lipoarabinomannan; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; ReLPS, LPS from S. minnesota Re 595; sIgM, surface IgM; sPGN, soluble PGN; RSV, Rous sarcoma virus; mAb, monoclonal antibody.

*This work was supported by the United States Public Health Service Grants AI28797 and GM37696 from the National Institutes of Health, by a Grant 1RG-161-J from the American Cancer Society, and by the Research Service of the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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1 The abbreviations used are: PGN, peptidoglycan; DAF, decay-accelerating factor; GPI, glycosylphosphatidylinositol; LAM, lipoarabinomannan; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; ReLPS, LPS from S. minnesota Re 595; sIgM, surface IgM; sPGN, soluble PGN; RSV, Rous sarcoma virus; mAb, monoclonal antibody.
mannan (LAM) (25) was a gift from Dr. P. J. Brennan. LPS from *Salmonella minnesota* Re 595 (ReLPS, a minimal naturally occurring endotoxic structure of LPS) and all other chemicals were from Sigma, unless otherwise indicated.

**Cells—** Murine macrophage RAW264.7 cell line was cultured in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum (8). Mouse 70Z/3 transfectants were cultured in RPMI 1640 with 10 mM Hepes, 5 × 10⁻⁵ M 2-mercaptoethanol, 7.5% fetal calf serum, and 500 μg/ml G418 (19).

**Expression of hCD14, hCD14(1–151), and hCD14 Deletion Mutants in 70Z/3 Cells—** Stable 70Z/3 transfectants expressing GPI-bound hCD14 or GPI-bound full-length hCD14 fused to the C-terminal region (the GPI anchor) of the decay-accelerating factor (DAF) or hCD14 truncated at amino acid 151 and fused to the C-terminal region DAF were generated as described (19, 20). hCD14 single, double, and quadruple deletion mutants, lacking one, two, or four 4–5 amino acid long regions within the N-terminal 65 amino acids (see Fig. 8 in “Results”), fused to the C-terminal region DAF were generated as described (19, 20). hCD14 single, double, and quadruple deletion mutants, lacking one, two, or four 4–5 amino acid long regions within the N-terminal 65 amino acids (see Fig. 8 in “Results”), fused to the C-terminal region DAF (13), were subcloned into the pRc/RSV vector and transfected into the 70Z/3 cells using Lipofectamine (13, 20). Stable 70Z/3 transfectants expressing these mutated CD14-DAF constructs were selected with 1 mg/ml G418. Expression of hCD14 in 70Z/3 transfectants was measured by flow cytometry and confirmed by immunoblotting (20). 70Z/3 cells transfected with an empty vector (70Z/3-RSV) (20) were used as control.

**Stimulation of Surface IgM Expression in 70Z/3 Cells—** Parental or transfected 70Z/3 cells were cultured for 24 h without or with the stimulants indicated under “Results,” and their responses were measured by flow cytometry, expressed as the mean channel number from two determinations (19).

**Activation of NF-κB and Electrophoretic Mobility Shift Assay—** Cells were cultured at 0.35–0.4 × 10⁶/ml in 24-well plates (2 ml/well) for 18–20 h, activated with the stimulants indicated under “Results,” and rinsed with ice-cold phosphate-buffered saline. In some experiments, cells were incubated with 10 μg/ml anti-hCD14 MY4 mAb or the isotype control MPC-11 mAb (Coulter Immunology, Hialeah, FL) for 30 min at 37 °C before stimulation. Nuclear and cytoplasmic extracts (26) were prepared using 0.6% Nonidet P-40 (27). Five μg of nuclear protein was incubated for 20 min at 22 °C with ³²P-labeled oligonucleotide containing the NF-κB binding site at the −510 position of the tumor necrosis factor-α gene (28), electrophoresed on a 5% non-denaturing polyacrylamide gel (27), and the DNA-protein complexes were visualized by autoradiography. The specificity of binding was determined by adding an excess of unlabelled NF-κB oligonucleotide or nonspecific 12-O-tetradecanoyl-phorbol-13-acetate response element oligonucleotide that does not contain binding sites for NF-κB (29) to the reaction mixture for 10 min before the addition of labeled NF-κB oligonucleotide.

**IκB-α Degradation—** Equal amounts of cytoplasmic extracts were separated on 10% SDS-polyacrylamide gels and immunoblotted with anti-human IκB-α antibodies (Upstate Biotechnology Inc., Lake Placid, NY) (30) and detected by the ECL system (8).

**RESULTS**

**70Z/3 Cells Expressing CD14 Respond to PGN—** To test the hypothesis that CD14 acts as a PGN receptor, we compared the responsiveness to insoluble PGN and soluble polymeric PGN (sPGN) of 70Z/3-hCD14 transfectants and 70Z/3-RSV cells (transfected with an empty vector).

Untransfected 70Z/3 cells (not shown) or 70Z/3-RSV cells were unresponsive to the cell wall components from other bacteria, because CD14 was recently proposed to mediate responsiveness to various bacterial cell wall components by acting as a “pattern recognition receptor” (31). Untransfected 70Z/3 cells (not shown) or 70Z/3-RSV cells were unresponsive to the cell walls from a Gram-positive bacterial species.
bacterium, *S. mitis*, and to mycobacterial LAM (Fig. 1). 70Z/3-hCD14 transfectants responded both to *S. mitis* cell walls and, as expected (31), to LAM with a dose-response similar to the PGN dose-response (Fig. 1).

Since LPS induces CD14-mediated activation of NF-κB (19, 20), we next determined if PGN and sPGN also induced activation of NF-κB in macrophages, which are natural PGN target cells (5, 7). sPGN induced rapid concentration-dependent activation of NF-κB transcription factor in mouse macrophage RAW264.7 cells, with the maximum activation occurring in 20 min (Fig. 2A). The maximum ReLPS-induced activation occurred in 10 min (Fig. 2A). In the groups without the stimulant, there was no NF-κB activation throughout the entire 120 min (0 and 20 min are only shown).

Activation of NF-κB was accompanied by transient degradation of its inhibitory protein, IκB-α, in the cytoplasm, and followed similar kinetics and dose-response pattern (Fig. 2B). In the groups without the stimulant, there was no IκB-α degradation throughout the entire 40 min.

To determine if PGN-induced activation of NF-κB is mediated through CD14 and to further confirm that CD14 acts as a functional PGN receptor, we then determined if 70Z/3-hCD14 transfectants show NF-κB responsiveness to sPGN and PGN.

Neither sPGN, nor PGN or ReLPS activated NF-κB in 70Z/3-RSV cells, whereas, in 70Z/3-hCD14 cells, sPGN, PGN, and ReLPS induced dose-dependent activation of NF-κB (Figs. 3A and 4). The time kinetics for the three stimulants was different: sPGN-induced NF-κB appeared in 20 min and continued to increase up to 80 min, PGN-induced NF-κB appeared in 60 min and continued to increase up to 240 min, and ReLPS-induced NF-κB appeared in 5 min and peaked in 10 min (Figs. 3A and 4). The difference in the kinetics of activation of NF-κB by PGN, sPGN, and ReLPS may reflect the difference in the binding kinetics and the molecular size and physical form (soluble versus insoluble) of these preparations. In the groups without the stimulant, there was no NF-κB activation throughout the entire 240 min.

Translocation of activated NF-κB into the nucleus in 70Z/3-hCD14 cells was accompanied by transient degradation of IκB-α in the cytoplasm that followed similar kinetics and dose-response pattern (Fig. 3B). In the groups without the stimulant, there was no IκB-α degradation throughout the entire 80 min. There was no degradation of IκB-α in the cytoplasm of 70Z/3-RSV cells stimulated with sPGN or ReLPS (Fig. 3B).

The specificity of binding to NF-κB sites in both RAW264.7 and 70Z/3-hCD14 extracts was confirmed using excess of non-

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**FIG. 2.** sPGN induces NF-κB activation and IκB-α degradation in RAW264.7 macrophage cell line. A, cells were stimulated with 10 μg/ml sPGN or 1 ng/ml ReLPS for the indicated periods of time or with the indicated concentrations of sPGN for 30 min, and activated NF-κB in nuclear extracts was assayed by the gel shift assay; −E, no nuclear extract added; −P, no radioactive probe added. B, cytoplasmic extracts from cells stimulated as in A were subjected to SDS-polyacrylamide gel electrophoresis and blotted with anti-IκB-α antibodies. C, specificity of binding to κB sites was demonstrated using excess amounts of unlabeled specific (κB) and nonspecific (12-O-tetradecanoylphorbol-13-acetate response element, TRE) oligonucleotides. Similar results were obtained in three experiments.
labeled specific and nonspecific oligonucleotide sequences (Figs. 2C and 3C).

Activation of NF-κB by sPGN and degradation of IκB-α in both RAW264.7 and 70Z/3-hCD14 cells were not inhibited by polymyxin B (a polypeptide antibiotic that binds to LPS and inhibits its biologic effects, Refs. 8 and 32), in contrast to ReLPS-induced NF-κB activation and degradation of IκB-α (Fig. 5). Similarly, activation of NF-κB by PGN in 70Z/3-hCD14 cells was not inhibited by polymyxin B. These results confirm that the sPGN- and PGN-induced effects were not due to contaminations of these preparations by endotoxin. Both sPGN- and ReLPS-induced activation of NF-κB in 70Z/3-hCD14 cells was inhibited by anti-hCD14 MY4 mAb, but not by an isotype control MPC-11 mAb (Fig. 6).

Similar, but Not Identical Regions of CD14 Are Involved in PGN- and LPS-induced Cell Activation—Our next experiments were designed to determine if the same regions of the CD14 molecule are involved in the sPGN-, PGN-, and LPS-induced cell activation.

We have recently shown that the N-terminal 151-amino acid fragment of CD14 is a functional cellular LPS receptor (20). To determine if the same CD14 fragment is also a functional PGN receptor, we used 70Z/3-hCD14(1–151)-DAF transfectants, i.e. 70Z/3 cells expressing less than half of the recombinant human CD14, amino acids 1–151, fused to the C-terminal region of DAF (20). Fusion of the C-terminal region of DAF to CD14,
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The next experiments were performed to more precisely define the region of CD14 that is involved in PGN-induced cell activation. We have generated a series of 4–5-amino acid long deletion mutants within the N-terminal 65 amino acids of human CD14 (20), and for this study, we have stably expressed these four single, one double, and one quadruple deletion mutants (fused to the C-terminal region of DAF) in 70Z/3 cells (Fig. 8). The various deletion mutations reduced to a different extent the responses (measured by expression of sIgM) to PGN and sPGN, i.e., the quadruple mutant (with all four sequences deleted) showed the lowest sIgM response (reduced by 88–90%), and the single and double deletion mutants showed the sIgM responses reduced by 45–75% (Fig. 8). These mutations also reduced the ReLPS-induced sIgM responses, i.e., the response of the quadruple mutant was almost completely abolished (reduced by 91–96%), and the responses of other single and double deletion mutants were reduced by 20–90% (Fig. 8). Deletion of the AVEVE region had the single greatest effect on LPS-induced sIgM expression.

The expression of CD14 in all transfectants was verified by flow cytometry (20), and differences in the expression of CD14 did not account for the differences in the responsiveness of the transfectants to the stimulants (because they were not large enough and they did not correlate with the differences in responsiveness). Moreover, differences in CD14 expression also did not account for the differences between the responsiveness to PGN and LPS, because each PGN and LPS experiment was done on the same day with the same batch of cells, and consistently similar results were obtained in five experiments.

To determine which regions of CD14 are needed for sPGN- and PGN-induced activation of NF-κB, we then assayed for NF-κB activation in 70Z/3 transfectants expressing the same single, double, or quadruple 4–5-amino acid long deletion mutants within the N-terminal 65 amino acids of hCD14.

The various deletion mutants showed a similar pattern of NF-κB activation in response to sPGN and PGN (Fig. 9). The quadruple mutant lost all responsiveness, the ΔAVEVE and ΔPQPD single deletion mutants showed severely diminished responses, the ΔDPRQY and ΔDDED single deletion mutants showed not as severely reduced responses, and the double deletion mutant ΔDDED,ΔPQPD responded almost as well as cells expressing unmutated CD14 (Fig. 9). These results demonstrate that deletion of all four sequences is needed to abolish NF-κB responses to sPGN and PGN, and identify the sequences AVEVE and PQPD as the single most critical sequences needed for full NF-κB activation by sPGN and PGN.

In ReLPS-stimulated cells, the quadruple deletion mutant, the double ΔDDED,ΔPQPD deletion mutant, and the single ΔAVEVE and ΔPQPD deletion mutants all showed completely or almost completely abolished NF-κB responses, and single ΔDDED and ΔDPRQY mutants showed diminished NF-κB responses, compared to the responses of cells expressing unmutated CD14 (Fig. 9). These results demonstrate that a single deletion of the AVEVE or PQPD sequence can abolish NF-κB activation by ReLPS. By contrast, sPGN- or PGN-induced NF-κB response is only partially diminished by these single deletions, and quadruple deletion of all the sequences studied is needed to abolish sPGN- or PGN-induced NF-κB response.

These data indicate that specific deletion mutations within the N-terminal 65 amino acids of CD14 can totally abolish the responsiveness to PGN, sPGN, and ReLPS, and that similar, but not identical sequences in this N-terminal region of CD14 are critical for the NF-κB responses to PGN and sPGN than to ReLPS.

CD14(1–151) fragment, or CD14 deletion mutants (see below) does not affect its function as an LPS receptor, but enables GPI-linked cell surface expression of truncated CD14 (13, 20, 33).

70Z/3-hCD14(1–151) cells fully responded to both PGN and ReLPS with the synthesis of surface IgM (Fig. 7). The 151-amino acid fragment of hCD14 was also sufficient for full NF-κB activation by both sPGN and PGN, as well as LPS (Fig. 9, below). These data further confirm that CD14 is a functional receptor for PGN, and demonstrate that both the binding and activating domains for both PGN and LPS are located in the N-terminal 151 amino acids of the CD14 molecule.

Fig. 5. sPGN- and PGN-induced NF-κB activation and IκB-α degradation in 70Z/3-hCD14 and RAW264.7 cells are not inhibited by polymyxin B. Cells were stimulated with 10 μg/ml sPGN, 200 μg/ml PGN, or 1 ng/ml ReLPS in the absence (−) or presence (+) of 5 μg/ml polymyxin B. Nuclear extracts and cytoplasmic extracts were assayed for NF-κB activation and IκB-α degradation, respectively, as described in the legend to Fig. 2. Similar results were obtained in two experiments.

Fig. 6. sPGN-induced NF-κB activation in 70Z/3-hCD14 cells is inhibited by anti-CD14 mAbs. Cells were stimulated with sPGN or ReLPS in the absence (−) or presence (+) of 10 μg/ml MY4 anti-hCD14 mAb or isotype control MPC-11 mAb, and nuclear extracts were assayed for NF-κB activation as in Fig. 2. Similar results were obtained in two experiments.
Our results strongly support the hypothesis that CD14 is a functional receptor for both insoluble and soluble staphylococcal PGN, as well as for PGN-containing streptococcal cell walls. They also demonstrate that less than half of membrane CD14 (the N-terminal 151 amino acids) is sufficient for full function of CD14 as a cell-activating PGN receptor, and that the sequences that are most critical for the receptor function of CD14 are located within the N-terminal 65 amino acid region.

These results support the hypothesis proposed earlier by us (17) and other investigators (31, 34) that CD14 functions not only as an LPS receptor, but also as a receptor for PGN and other bacterial cell wall components. The domain of CD14 that is critical for its function as a receptor for both PGN and LPS is located within the N-terminal 65-amino acid region. The specific amino acid sequences responsible for the function of CD14 as a receptor for both PGN and LPS are similar, but not identical. In agreement with the previous studies (13) we confirmed that the AVEVE and PQPD sequences are most crucial for the responsiveness to LPS and that their deletion can almost totally abolish or severely diminish the function of CD14 as an LPS receptor. Single deletions of these sequences only partially diminish the responsiveness to PGN, and deletion of

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T. N. Kirkland, unpublished observations.

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FIG. 7. Expression of the N-terminal 151 amino acids of hCD14 in 70Z/3 cells is sufficient for sIgM responsiveness to PGN. The responsiveness of 70Z/3-hCD14-DAF and 70Z/3-hCD14(1–151)-DAF transfectants to PGN and ReLPS was measured as described in the legend to Fig. 1. The results are means of three experiments with the range shown by the bars (the range is not shown if it was smaller then the size of the data points).

FIG. 8. 70Z/3 cells expressing deletion mutants of hCD14 lose sIgM responsiveness to PGN, sPGN, and ReLPS. Amino acids in the N-terminal region of hCD14 shown in bold type in brackets were deleted, and the indicated single, double, and quadruple (QUAD) mutants (fused to the C-terminal region of DAF) were stably expressed in 70Z/3 cells. Cells expressing the unmutated hCD14-DAF or transfected with an empty vector (RSV) were used as a positive and negative control, respectively. The responsiveness of cells to PGN (30 μg/ml), sPGN (10 μg/ml), and ReLPS (0.1 ng/ml) was then measured as described in the legend to Fig. 1. The values (sIgM channel number) for the positive control (unmutated CD14) stimulated with PGN, sPGN, or ReLPS were 35.5, 97.5, and 94, respectively. The results are means of two determinations, and are from one of five similar experiments.

FIG. 9. The N-terminal 151 amino acids of hCD14 are sufficient for sPGN- and PGN-induced activation of NF-κB, and specific mutations within the N-terminal 65 amino acids abolish NF-κB responsiveness to sPGN and PGN. 70Z/3 cells expressing hCD14-DAF, hCD14(1–151)-DAF, indicated deletion mutants within the N-terminal 65 residues of hCD14-DAF (see legend to Fig. 8), or vector alone (RSV), were stimulated with 10 μg/ml sPGN for 40 min, 200 μg/ml PGN for 240 min, or 1 ng/ml ReLPS for 10 min. Nuclear extracts were assayed for NF-κB activation as described in the legend to Fig. 2. Similar results were obtained in four experiments with sPGN and ReLPS and two experiments with PGN.
all four studied sequences (DDED, PQPD, DPRQY, and AVEVE) is required to abolish the responsiveness to PGN.

The differences between the responses of CD14 mutants to PGN and LPS were consistently observed in several experiments, and they may be due to fine differences in the binding of PGN and LPS to CD14 and/or due to the differences in the requirement for LBP. LBP greatly enhances the responses of CD14-positive cells to low concentrations of LPS (10–15, 17, 19), whereas, LBP does not enhance the responses of human (17) or rabbit (35) macrophages or 70Z/3-hCD14 transfectants3 to PGN. These differences may also be due to the post-binding events that are responsible for signal transduction from the receptor and cell activation. The latter notion is supported by our recent discovery that three families of mitogen-activated protein kinases are differentially activated by PGN and LPS in mouse macrophages (36). PGN strongly activates ERK1 and ERK2, moderately activates JNK, and weakly activates p38 mitogen-activated protein kinases, in contrast to LPS, which strongly activates all of these kinases (36).

The effects of deletion mutations on NF-κB activation (Fig. 9) were usually more severe and clear-cut than the effects on sIgM expression (Fig. 8), e.g. a single ΔPQPD deletion was sufficient to abrogate NF-κB, but not sIgM responses to LPS. These differences most likely reflect the differences in the assay systems and in the requirements for sIgM expression and NF-κB activation, i.e. long stimulation period and likely activation of multiple signal transduction molecules that are required for induction of sIgM. For example, induction of the κ chains in 70Z/3 cells requires activation of at least two transcription factors, NF-κB and OTF-2 (37).

The sequences in CD14 that are most critical for the responsiveness to LPS and PGN are hydrophilic and they would be expected to be exposed on the surface of CD14, and thus be available for binding of ligands. These results are consistent with the proposal that LPS binds to the hydrophilic region(s) of CD14 (38). Comparing the structure of PGN and lipid A (the active part of LPS) (17) indicates that the only similar parts of the molecules are the (GlcNAc-MurNAc)1 backbone of PGN and the (GlcNAc)2 part of lipid A. Because of the hydrophobic nature of the fatty acids in lipid A, in aqueous solutions, lipid A forms aggregates with hydrophilic (GlcNAc)2 exposed on the surface and available for binding to CD14. Therefore, it appears that polysaccharide portions of PGN and lipid A may be involved in binding to CD14.

In addition to identifying CD14 as a PGN receptor, our results also demonstrate for the first time that PGN induces activation of transcription factor NF-κB and degradation of IκB-α in both macrophages and in 70Z/3-CD14 transfectants, and expression of surface IgM in 70Z/3-CD14 transfectants. These results extend a previous observation of NF-κB activation in human monocytes by a low molecular weight anhydro monomer derivative of PGN (18).

Acknowledgments—We are grateful to Patrick J. Brennan for providing LAM and to Frances Muller for technical assistance.

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