Activation of Toll-Like Receptor 2 in Acne Triggers Inflammatory Cytokine Responses

Jenny Kim, Maria-Teresa Ochoa, Stephan R. Krutzik, Osamu Takeuchi, Satoshi Uematsu, Annaliza J. Legaspi, Hans D. Brightbill, Diana Holland, William J. Cunliffe, Shizuo Akira, Peter A. Sieling, Paul J. Godowski and Robert L. Modlin

*J Immunol* 2002; 169:1535-1541; doi: 10.4049/jimmunol.169.3.1535

http://www.jimmunol.org/content/169/3/1535

**References**

This article cites 36 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/169/3/1535.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Activation of Toll-like Receptor 2 in Acne Triggers Inflammatory Cytokine Responses

Jenny Kim,* Maria-Teresa Ochoa,* Stephan R. Krutzik,† Osamu Takeuchi,§ Satoshi Uematsu,§ Annaliza J. Legaspi,* Hans D. Brightbill,† Diana Holland,¶ William J. Cunliffe,¶ Shizuo Akira,§ Peter A. Sieling,* Paul J. Godowski,** and Robert L. Modlin2a†‡

One of the factors that contributes to the pathogenesis of acne is Propionibacterium acnes; yet, the molecular mechanism by which P. acnes induces inflammation is not known. Recent studies have demonstrated that microbial agents trigger cytokine responses via Toll-like receptors (TLRs). We investigated whether TLR2 mediates P. acnes-induced cytokine production in acne. Transfection of TLR2 into a nonresponsive cell line was sufficient for NF-κB activation in response to P. acnes. In addition, peritoneal macrophages from wild-type, TLR6 knockout, and TLR1 knockout mice, but not TLR2 knockout mice, produced IL-6 in response to P. acnes. P. acnes also induced activation of IL-12 p40 promoter activity via TLR2. Furthermore, P. acnes induced IL-12 and IL-8 protein production by primary human monocytes and this cytokine production was inhibited by anti-TLR2 blocking Ab. Finally, in acne lesions, TLR2 was expressed on the cell surface of macrophages surrounding pilosebaceous follicles. These data suggest that P. acnes triggers inflammatory cytokine responses in acne by activation of TLR2. As such, TLR2 may provide a novel target for treatment of this common skin disease. The Journal of Immunology, 2002, 169: 1535–1541.

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.

*Division of Dermatology, †Department of Microbiology and Immunology, and ‡Microbial Diseases, Osaka University, Osaka, Japan; §Department of Dermatology, Skin Research Center, General Infirmary, University of Leeds, Leeds, United Kingdom; and ¶Genentech, South San Francisco, CA 94080.

Received for publication December 6, 2001. Accepted for publication May 17, 2002.

‡Address correspondence and reprint requests to Dr. Robert L. Modlin, Division of Dermatology, University of California, Los Angeles, School of Medicine, 52-121 Center for Health Sciences, 10833 Le Conte Avenue, Los Angeles, CA 90095. E-mail address: rmodlin@mednet.ucla.edu

©Division of Dermatology, †Department of Microbiology and Immunology, and ‡Microbial Diseases, Osaka University, Osaka, Japan; §Department of Dermatology, Skin Research Center, General Infirmary, University of Leeds, Leeds, United Kingdom; and ¶Genentech, South San Francisco, CA 94080.

Copyright © 2002 by The American Association of Immunologists, Inc.
Two-color immunofluorescence labeling in acne lesions

Cryostat sections (3–4 μm) were fixed in acetone and blocked with 10% goat serum for 30 min. Double immunofluorescence was performed by serially incubating sections with mouse anti-human CD14 or CD3 mAbs for 1 h followed by incubation with isotype-specific FITC-conjugated goat anti-mouse IgG2a (Caltag Laboratories, Burlingame, CA). Sections were then incubated with anti-TLR2 (1 μg/ml) for 1 h, followed by a tetramethylrhodamine isothiocyanate-conjugated anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL). Sections were mounted in Vectashield mounting medium (Vector Laboratories). Controls included isotype-matched irrelevant Abs.

Confocal laser microscopy

Double immunofluorescence of sections and cells was examined with a Leica-TCS-SP inverted confocal laser-scanning microscope (Heidelberg, Germany) and illuminated with 488 and 568 nm of light. Images decorated with FITC and tetramethylrhodamine isothiocyanate were recorded simultaneously through separate optical detectors with a 530-nm band-pass filter and a 590-nm long-pass filter, respectively. Pairs of images were superimposed for colocalization analysis.

Results

TLR2 is sufficient for P. acnes activation of monocytes

Previous studies have demonstrated that TLR2 mediates the response of several ligands from Gram-positive organisms. Therefore, we sought to determine whether TLR2 is sufficient for P. acnes-induced gene activation. HEK 293 cells and BaF3 cells were used because these cells do not express endogenous TLRS and are unresponsive to microbial ligands (12, 14). HEK 293 cells (expressing TLR2, CD14, and a NF-κB responsive ELAM enhancer) and BaF3 cells (expressing TLR4, CD14, MD2, and ELAM) were activated with P. acnes, M. tuberculosis 19-kDa lipoprotein, or LPS. NF-κB activation was examined because it has been shown that NF-κB activation is required for several proinflammatory cytokine promoter activities (18–21). In stable transfectants expressing TLR2 and CD14, P. acnes induced NF-κB activation (Fig. 1a). In contrast, P. acnes could not activate NF-κB in transfectants expressing TLR4, CD14, and MD2. Similar results were seen when cells were stimulated with 19-kDa lipoprotein from M. tuberculosis. In contrast, LPS activated NF-κB in stable transfectants expressing TLR4 but not TLR2. This is consistent with findings that LPS activation of cytokine production in monocytes is dependent on TLR4.

Because TLR2 cooperates with TLR6 and TLR1 in the recognition of microbial ligands (16, 17, 22), we examined the role of TLR6 and TLR1 in mediating activation by P. acnes using TLR knockout mice. Peritoneal macrophages were obtained from wild-type, TLR2–/–, TLR6–/–, and TLR1–/– mice, activated with P. acnes, and supernatants were assayed for the presence of IL-6. P. acnes induced IL-6 release from wild-type and TLR6–/– mice, but not TLR2–/– mice (Fig. 1b). P. acnes also induced IL-6 release from TLR1–/– mice (Fig. 1c). As controls, the diacylated lipopeptide mycoplasmal macrophage-activating lipopeptide-2 kDa activated wild-type and TLR1–/– mice, but not TLR2–/– or TLR6–/– mice, whereas LPS activated macrophages from all mice (data not shown). In contrast, a synthetic N-palmitoyl-S-dipalmityloylglycerol (Pam3CysK, activated wild-type mice, but TLR1–/– activation was partially inhibited as previously described (Ref. 17; data not shown). These results indicate the specificity of the response that TLR2 but not TLR4, TLR6, or TLR1 mediates P. acnes-induced cell activation.

P. acnes induces IL-12 p40 promoter activity via TLR2

IL-12 is a pivotal cytokine in activating Th1 T cell responses and is one of the major proinflammatory cytokines produced by monocytes in response to Gram-positive organisms. To determine

References

13) and TLR4 (clone HTA125; Ref. 14) was provided by P. J. Godowski (Genentech, San Francisco, CA); B351.1 (anti-CD3; Bioma, Foster City, CA); RPA-M1 (anti-CD14, Zymed Laboratories, South San Francisco, CA); NA1/34 (anti-CD1a; DAKO, Carpinteria, CA), and IgG controls (Sigma-Aldrich).

Samples from patients

Patients were clinically diagnosed with acne at the General Infirmary at Leeds (Leeds, U.K.). After informed consent was obtained, comedones and inflamed acne lesions (papules and pustules) from the upper back were biopsied under local anesthesia using a 4-mm punch. Nineteen acne samples (3 comedones, 1 pustule, and 15 papules) were obtained from sixteen different patients. To ascertain the duration of the papules, an established “mapping” technique (15) was used which allowed a reasonably accurate assessment of the duration of the lesion. Such timed biopsies were classified into four time zones (up to 6 h, from 6 to 24 h, from 24 to 48 h, and from 48 to 72 h). The biopsies were snap frozen in liquid nitrogen and stored at −70°C until sectioning.

NF-κB activation in human TLR-transfected cell lines

TLR2 negative human embryonic kidney (HEK) 293 cells were stably transfected with TLR2 and CD14 (9). Cells were plated at 1 × 10⁶ cells/well in six-well plates and transiently transfected the following day with the NF-κB responsive endothelial leukocyte adhesion molecule (ELAM) enhancer luciferase (pG3L) reporter gene (0.5 μg/ml) by the Superfect protocol at a 1:3 ratio of DNA to Superfect (Qiagen, Valencia, CA). Multiple transfectants were pooled and divided for activation with P. acnes, M. tuberculosis 19-kDa lipoprotein, or LPS for 6 h, lysed in reporter lysis buffer (Promega, Madison, WI), and used in the luciferase assay. BaF3 cells stably expressing TLR4, MD2, CD14, and an ELAM luciferase reporter gene (14) were plated at 7.5 × 10⁵ cells/well and activated with P. acnes, M. tuberculosis 19-kDa lipoprotein, or LPS. Cells were harvested 6 h after activation and used in the luciferase assay.

Responses in TLR-deficient mice

Peritoneal macrophages from TLR2–/– (11), TLR6–/– (16), and TLR1–/– (17) mice were collected 3 days after i.p. injection of 2 ml of 4% thioglycollate (Difco, Detroit, MI) and cultured in RPMI 1640 medium supplemented with 10% FCS. Cells (5 × 10⁶) were incubated in the presence of the indicated concentration of P. acnes for 24 h. Concentrations of IL-6 in the culture supernatants were measured by ELISA (R&D Systems, Minneapolis, MN). The data represent the mean ± SD of triplicate wells.

IL-12 p40 promoter activity

The murine macrophage cell line, RAW 264.7 (American Type Culture Collection), was transiently transfected with a murine IL-12 p40 promoter chloramphenicol acetyltransferase (CAT) reporter as previously described (18). TLR2 dominant negative mutant (TLR2 dn1) expression plasmids were transfected together with the IL-12 p40 promoter construct and β-galactosidase as an internal control. Transfected cells were either left unactivated or stimulated with P. acnes or M. tuberculosis 19-kDa lipoprotein for 24 h. IL-12 p40 promoter activity was measured according to CAT activity (percent chloramphenicol acetylation) with a phosphor imager for 24 h. IL-12 p40 promoter activity was measured according to CAT activity (percent chloramphenicol acetylation) with a phosphor imager (Amersham, Sunnyvale, CA). Data were normalized to a cotransfected β-galactosidase construct for transfection efficiency.

Cytokine ELISA

PBMCs were isolated from normal healthy volunteers on Ficoll-Paque gradients (Pharmacia, Piscataway, NJ) and cultured for 1 h in 1% human serum. Adherent cells were recovered and plated (1 × 10⁶ to 5 × 10⁷/well) in 96-well plates. Cells were left untreated or incubated with mouse anti-human TLR2 neutralizing mAb, mouse anti-human TLR4 neutralizing Ab, or with isotype control mouse Abs, IgG1 and IgG2a, for 30 min before stimulation with LPS (10 ng/ml), P. acnes (1:100), or 19-kDa lipoprotein (50 ng/ml). Supernatants were harvested 18 h later and assayed for IL-12 p40 and IL-8 by ELISA (BD Pharmingen, San Diego, CA). All samples were assayed in duplicate.

Immunoperoxidase staining

Cryostat sections (3–4 μm) were acetone fixed and blocked with normal horse serum before incubation with the mAbs for 60 min, followed by biotinylated horse anti-mouse IgG for 30 min. Primary Abs were visualized with the ABC Elite system (Vector Laboratories, Burlingame, CA), counterstained with hematoxylin, and mounted in aqueous dry mounting medium (Crystal Mount; Biomaedia).
whether *P. acnes*-induced IL-12 promoter activity, an IL-12 p40 promoter CAT reporter construct was transiently transfected into the murine macrophage cell line RAW 264.7. Cells were stimulated with *P. acnes* and the promoter activity was measured by CAT assay. *P. acnes* induced IL-12 p40 promoter activity in a dose-dependent manner and at a level comparable to *M. tuberculosis* 19-kDa lipoprotein (data not shown). These data suggest that *P. acnes* activates IL-12 p40 promoter activity in a TLR2-dependent mechanism.

**P. acnes induces IL-12 and IL-8 production by human adherent monocytes**

We next determined whether cytokine protein production was also dependent on TLR activation. Primary human monocytes from normal donors were stimulated with various dilutions of *P. acnes* sonicate and cytokine production was measured. IL-12 was measured given that IL-12 promoter activation by *P. acnes* occurred via TLR2. We found that *P. acnes* induced IL-12 production by monocytes in a dose-dependent manner (Fig. 3a). *P. acnes* also induced the release of IL-8, a cytokine involved in neutrophil chemotaxis (Fig. 3b). These findings were consistent in all normal donors tested (n = 3).

To determine whether the production of proinflammatory cytokines could be mediated through TLRs, monocytes from normal donors were cultured with anti-TLR2 and anti-TLR4 Abs for 30 min before stimulation with *P. acnes* sonicate. *P. acnes* induction of IL-12 production in monocytes was blocked by ~65% with the addition of anti-TLR2 Ab to the culture (Fig. 3c). In contrast, the addition of anti-TLR4 and isotype control Abs had no significant effect on IL-12 production by human monocytes, suggesting that the blocking effect was specific to TLR2. Induction of IL-12 production by monocytes upon addition of another Gram-positive

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** TLR2, but not TLR4, TLR6, or TLR1, is sufficient for induction by *P. acnes* of monocyte activation. *a*, HEK 293 cells transfected with TLR2, CD14, and the NFκB responsive E-selectin (ELAM) enhancer luciferase reporter gene and BuR3 cells stably expressing TLR4, CD14, MD-2, and an ELAM luciferase reporter gene were activated with *P. acnes*, *M. tuberculosis* 19-kDa lipoprotein, or LPS for 6 h. NFκB activity was measured by luciferase assay. Data reflect at least two independent experiments. *b*, Peritoneal macrophages were obtained from wild-type, TLR6−/−, and TLR2−/− mice, activated with *P. acnes*, and supernatants were assayed for the presence of IL-6. *c*, Peritoneal macrophages were obtained from wild-type and TLR1−/− mice, activated with *P. acnes*, and supernatants were assayed for the presence of IL-6.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** *P. acnes* induces IL-12 p40 promoter activity via TLR2. RAW 264.7 cells were transiently transfected with a murine IL-12 p40 promoter CAT reporter. Cells were also cotransfected with TLR2 dn1 (△), or with a vector control (■). Transfected cells were stimulated with *P. acnes* sonicate or *M. tuberculosis* 19-kDa lipoprotein, or left unstimulated (□) for 24 h. Activation of IL-12 p40 promoter activity was measured according to CAT activity (percent chloramphenicol acetylation) with a phosphor imager. Data reflects at least two independent experiments and are reported as a percentage of Ag-stimulated IL-12 p40 promoter activity cotransfected with a vector control. Media controls were comparable between vector control and TLR2 dn1 transfectants.
bacterial ligand, the M. tuberculosis 19-kDa lipoprotein, was also blocked with the addition of anti-TLR2 (data not shown), as demonstrated previously (12). Similarly, the production of IL-8 was also blocked by ~50% by the addition of anti-TLR2 Ab (Fig. 3d). Anti-TLR4 and isotype control Abs had no significant effect on IL-8 production. These results suggest that one mechanism by which P. acnes induces IL-12 and IL-8 production in human monocytes is by the activation of TLR2.

TLR2 is expressed on macrophages in acne lesions

We next wanted to determine whether there was any evidence for the TLR-dependent mechanism occurring at the site of the disease activity. We obtained acne biopsies from patients and analyzed the lesions for TLR expression. Immunohistochemistry labeling using a mAb specific to TLR2 (23) revealed TLR2 expression on large ovoid cells within acne lesions (Fig. 4). TLR2+ cells were detected primarily in the inflammatory infiltrate around the perifollicular/peribulbar region. Numerous CD14+ and CD3+ cells were detected in the similar area. We also detected a number of CD1a+ cells, but they were within the follicular wall and in the epidermis. Anti-CD20 and control Abs did not stain any of the cells (data not shown). All acne lesions tested (n = 19) contained TLR2+ cells whether the tissue was obtained from comedonal, papular, or pustular lesions. TLR2+ cells were not detected in normal skin biopsies (data not shown).

To determine the kinetics of TLR2 expression according to the evolution of the lesion, the frequency of TLR2-expressing cells

FIGURE 3. P. acnes induces IL-12 and IL-8 production in human adherent monocytes via TLR2. Human adherent monocytes were stimulated with P. acnes and the production of IL-12 p40 and IL-8 was determined by ELISA. A dose-response curve demonstrates the ability of P. acnes to stimulate monocytes to release IL-12 p40 (a) and IL-8 (b). Cells were coincubated with anti-TLR2, anti-TLR4, or isotype control mAbs. The IL-12 p40 (c) and IL-8 (d) release into the supernatants was determined by ELISA. The results from one representative experiment of three are shown.
was determined according to the duration of the acne lesion. In early acne lesions, up to 6 h, few TLR2+ cells were detected (Fig. 5). In lesions obtained between 12 and 24 h, TLR2+ cells were found to be more numerous around the pilosebaceous follicles. Finally, in older lesions obtained between 48 and 72 h, even greater numbers of TLR2+ cells were detected. From these experiments, we concluded that the infiltration of TLR2+ cells was an early event in the evolution of acne lesions. Furthermore, the frequency of cells expressing TLR2 proteins is up-regulated during the inflammatory process up to 48–72 h after the onset of acne lesions.

To identify the lineage of cells expressing TLR2 in acne lesions, we performed double immunofluorescence labeling and used confocal laser microscopy. The colocalization of TLR2 with CD14 and CD3 was examined because these cells appeared to be numerous in acne lesions and also localized to perifollicular areas where numerous TLR2+ cells were detected. CD14 colocalized with TLR2 on cells infiltrating around acne lesions (Fig. 6a). In contrast, although CD3+ cells were abundant in acne lesions, CD3 did not colocalize with TLR2 (Fig. 6b). These data suggest that TLR2 is expressed on cells of the monocyte/macrophage lineage in acne lesions.

**Discussion**

Recognition of microbial pathogens by the cells of the immune system triggers host defense mechanisms to combat infection and prevent disease. However, activation of these same pathways can also result in inflammation at the site of disease and subsequent tissue injury. In acne, the host response to *P. acnes* can result in the production of proinflammatory cytokines and contribute to the clinical manifestations of disease. We investigated the molecular mechanism by which *P. acnes* induces proinflammatory cytokines in monocytes and provide evidence that the *P. acnes*-induced release of cytokines is dependent on TLR2. Furthermore, TLR2+ macrophages were present in acne lesions, infiltrating around pilosebaceous follicles, and increased during the evolution of the disease. Our data suggest a novel mechanism by which *P. acnes* induces inflammation in acne by the activation of TLR2 and subsequent release of cytokines which regulate the local immune response.
Toll receptors were first identified in Drosophila as an integral part of the innate immune system and have been shown to play a crucial role in antimicrobial defense in adult flies (24, 25). Recent studies suggest that mammalian Toll homologues, TLRs, mediate responsiveness to a variety of molecular structures from microbial pathogens. The present study provides evidence that TLR2 mediates innate immune responses to P. acnes. P. acnes activated NF-kB in cell lines transfected with TLR2 but not TLR4. In addition, peritoneal macrophages from wild-type, TLR6<sup>-/-</sup>, and TLR1<sup>-/-</sup>, but not TLR2<sup>-/-</sup>, mice produced IL-6 in response to P. acnes. A role for TLR2 in mediating the response to P. acnes was further demonstrated by using a dominant negative construct of TLR2 that inhibited P. acnes induction of cytokine promoter activity. TLR2 mediated the ability of P. acnes sonicate to activate monocyte-release of IL-12 and IL-8, because cytokine induction could be blocked using an anti-TLR2 mAb. Using transgenic mice, Akira and colleagues (16, 17) have demonstrated that TLR1 associates with TLR2 and recognizes triacylated lipopeptid, but TLR6 and TLR2 interact to recognize diacylated lipopeptide. The peptidoglycan of P. acnes is distinct from most Gram-positive bacteria, containing a cross-linkage region of peptide chains with L, d-aminomupinic acid and d-alanine in which two glycine residues combine with amino and carboxyl groups of two L, d-aminomupinic acid residues (2). Previous studies have indicated that the addition of jimson lectin, which binds peptidoglycan, blocked the ability of P. acnes to induce cytokines by ~70% (3). Given our result that P. acnes induced IL-6 release from macrophages from wild-type, TLR6<sup>-/-</sup> and TLR1<sup>-/-</sup>, but not from TLR2<sup>-/-</sup> mice, it is likely that the TLR ligand in P. acnes is a peptidoglycan. Further studies will be required to identify the TLR2 ligands present in P. acnes and their role in inflammation.

The primary event in inflammatory acne involves the disruption of the follicular epithelium and colonization of the follicles with P. acnes with subsequent inflammatory reactions in the surrounding dermis. The detection of TLR2<sup>+</sup> cells in the perifollicular region provides indirect evidence that TLR2 activation contributes to the pathogenesis of acne, suggesting that these cells promote inflammatory responses at the site of the disease activity. This disease mechanism was supported by the colocalization of TLR2 with CD14, indicating its presence on cells of the monocyte/macrophage lineage. Previously, TLR2<sup>+</sup> cells have been demonstrated in tuberculoid lesions (26) but not all macrophages in lesions express TLR2, for example those in lepromatous leprosy (our unpublished observations). Furthermore, activation of TLR2 on monocytes releases proinflammatory cytokines, IL-12 and IL-8. IL-8 attracts neutrophils to the site of active lesion, and release of lysosomal enzymes by neutrophils leads to rupture of follicular epithelium and further inflammation (27). In contrast, IL-12 promotes development of Th1-mediated immune responses. Overproduction of Th1 cytokines such as IL-12 has been implicated in the development of tissue injury in certain autoimmune and inflammatory diseases (28–34). In this manner, the activation of TLR2 on monocytes and other TLRs as well as other inflammatory cells are likely involved in the pathogenesis of acne.

In addition to its primary role in combating infection, the immune system also plays a role in the pathogenesis of certain disease states. In fact, the very pathogens that the immune system is attempting to fight often play a critical role in mediating the inflammatory responses that lead to disease states. Examples of this include group A β-hemolytic streptococcus in rheumatic fever, rheumatic heart disease, and glomerulonephritis, Helicobacter pylori in gastritis and peptic ulcer disease, Chlamydia pneumoniae in atherosclerosis, and Pityrosporum oval in seborrheic dermatitis. In all of the above examples, infection by the organism itself is not the main cause of the disease, but rather the various inflammatory responses initiated by the microbial agents lead to the destruction of the host tissue. Such responses include the formation of immune complexes, the recruitment and activation of neutrophils and monocytes, the release of cytokines, and the release of degradative enzymes. P. acnes has been implicated as an important mediator of inflammation in the pathogenesis of acne. Clearly, treatment of patients with antibiotics reduces the number of P. acnes and inflammatory cells and results in clinical improvement of acne lesions (35–37). Interestingly, the inflammatory cytokine responses triggered by P. acnes and mediated by TLR2 are unlikely to have a protective role in acne. It is tempting to speculate that the release of proinflammatory cytokines mediated through TLR2 has a harmful effect in acne by promoting inflammation and tissue destruction. Given these data, TLR2 is a logical target for therapeutic intervention to block inflammatory cytokine responses in acne and other inflammatory conditions in which tissue injury is detrimental to the host.

Acknowledgments

We thank Drs. Frederick Beddington, Jennifer Gansert, and Cheryl Hertz for helpful comments and suggestions.

References

tol. 65:382.


ther. 10:387.


negative and gram-positive bacterial cell wall components. Immunity 11:443.


14. Shimazaki, R., S. Akashi, H. Ogata, Y. Nagai, S. Fukuromie, K. Miyake, and
M. Kimoto. 1999. MD-2, a molecule that confers lipopolysaccharide responsive-


