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*J Immunol* 2002; 169:795-801; doi: 10.4049/jimmunol.169.2.795

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Rapid Chromatin Remodeling of Toll-Like Receptor 2 Promoter During Infection of Macrophages with Mycobacterium avium

Tianyi Wang,* William P. Lafuse, † Kiyoshi Takeda, ‡ Shizuo Akira, ‡ and Bruce S. Zwilling²*†

We have previously reported that NF-κB and stimulating factor 1 elements within the proximal mouse Toll-like receptor 2 (TLR2) promoter region are required for the transcriptional activation of TLR2 expression following infection with Mycobacterium avium. In the present study, we found that a rapid increase in both DNase I sensitivity and restriction enzyme accessibility at the TLR2 promoter region occurred following infection with M. avium. Increase in restriction enzyme accessibility at the TLR2 promoter region covering the NF-κB and stimulating factor 1 elements was associated with the induction of TLR2 expression at the mRNA level. Furthermore, the increase in restriction enzyme accessibility at the TLR2 promoter region did not appear to result from binding of NF-κB, but rather depended on a TLR2-myeloid differentiation factor 88 signaling pathway. Together our results indicate that chromatin remodeling occurs at TLR2 promoter region following infection with M. avium, allowing the access of transcription factors to initiate the transcription of TLR2. The Journal of Immunology, 2002, 169: 795–801.

Toll is a type I transmembrane receptor in Drosophila that is involved in dorsal-ventral patterning in larvae and in the induction of an antifungal response in adult flies (1–3). Activation of the Toll receptor by its ligand Spatzle results in the interaction and stimulation of several signaling molecules that are homologous to proteins involved in NF-κB activation by the IL-1R in mammalian cells. Medzhitov et al. (4) cloned a human receptor, homologous to Toll, which participates in activation of cells during both innate and adaptive immune responses. Ten members of the human Toll-like receptor (TLR)³ family have been identified (5). At least some of these are involved in mediating NF-κB activation following the interaction of macrophages with different bacterial pathogens or bacterial cell wall components (6–8). TLR4 has been shown to mediate the response of macrophages to LPS (9, 10). The hyporesponsiveness of C3H/HeJ mice (Lpsd) to LPS is the result of a missense mutation in TLR4 (11, 12). TLR2 has been implicated in the activation of NF-κB following interaction of macrophages with LPS, lipoarabinomannan from mycobacterial species, and lipoteichoic acids from the cell walls of Gram-positive bacteria (13–16). We have previously reported that the transcription of TLR2 promoter was induced following infection of macrophages with Mycobacterium avium. We also found that two NF-κB and stimulating factor 1 (Sp1) sites are required for the full induction of TLR2 transcription following infection with M. avium.

Genes in eukaryotic cells are packed into chromatin. The primary repeating units of chromatin are nucleosomes (17). Formation of nucleosomes and other higher order chromatin structures can render the DNA inaccessible to transcription factors and RNA polymerases. The repressive chromatin structure must be remodeled to allow transcription to occur. Studies at the Saccharomyces cerevisiae Pho5, HIV, and murine mammary tumor virus (MMTV) promoters have indicated that nucleosomes contribute important regulatory functions for genes that are rapidly induced in response to extracellular signals. The Pho5 promoter is contained within positioned nucleosomes. Induction with low phosphate results in dephosphorylation of activator Pho4, which binds a DNase I-hypersensitive region flanked by the promoter, ultimately allowing DNA binding and the selective remodeling of four nucleosomes in the vicinity of the promoter (18, 19). Nucleosomes are positioned downstream of the start site of the nucleosome-free HIV-1 promoter (20). During T cell activation, transcription factors bind the promoter, leading to the remodeling of the downstream nucleosome and the initiation of the transcription. Finally, the glucocorticoid-inducible MMTV promoter is contained within a nucleosome array, with one nucleosome spanning the glucocorticoid receptor binding sites (21, 22). Addition of ligand selectively remodels this nucleosome via the interaction with a remodeling protein, BRG1, facilitating the binding of additional factors required for transcription (23).

A recent report by Weinmann et al. (24) further supports the hypothesis that nucleosome remodeling is important for inducible transcription in mammalian cells. High resolution micrococcal nuclease analyses revealed that a positioned nucleosome, nucleosome 1, spanned the IL-12 promoter, while three additional positioned nucleosomes were found further upstream. Upon activation, nucleosome 1 was rapidly and selectively remodeled in a protein synthesis-dependent manner. These results suggested that remodeling complexes are selectively targeted to a single, promoter-encompassing nucleosome.
To investigate whether the induction of TLR2 transcription also correlates with chromatin remodeling, we determined whether infection with *M. avium* resulted in an alteration in DNase I sensitivity or restriction enzyme accessibility. We found that infection with *M. avium* resulted in an increase in restriction enzyme accessibility and in sensitivity to digestion with DNase I within the TLR2 promoter. The chromatin change required TLR2 and myeloid differentiation factor 88 (MyD88), but not new protein synthesis. We interpret our findings as indicating that chromatin remodeling has occurred at the TLR2 promoter following infection with *M. avium*.

**Materials and Methods**

**Cell lines and reagents**

The murine alveolar macrophage cell line AMJ2-C8 (ATCC CRL-2455) was a generous gift from M. J. Fenton (Boston University, Boston, MA). Another murine macrophage cell line P388D1 (CCL-46) was obtained from American Type Culture Collection (Manassas, VA). Cells were cultured in IMDM (Life Technologies, Grand Island, NY) supplemented with 10% FBS. Each vial contained 2.5 × 10^5 cell/ml. When used in experiments, cells were washed with PBS before treatment. Monolayers were infected with *M. avium* (ATCC 35712) at an 8:1 bacteria-macrophage ratio for 30 min or (at specified concentration) was added, and the reaction (total volume 100 μl) was incubated at room temperature for 2, 5, and 10 min. For restriction enzyme digestion, cell nuclei were resuspended in ice-cold Nonident P-40 lysis buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5% Nonident P-40, 0.15 mM spermine, and 0.5 mM spermidine) (17) and incubated on ice for 5 min. Nuclei were centrifuged at 1000 rpm, followed by washing in the respective digestion buffer (without CaCl₂).

**Nuclease digestions and isolation of genomic DNA**

For DNase I hypersensitivity assay, cell nuclei were resuspended in buffer A (100 mM NaCl, 50 mM Tris, pH 8.0, 3 mM MgCl₂, 0.15 mM spermine, and 0.5 mM spermidine) (17) supplemented with CaCl₂ (1 mM). DNase I (at specified concentration) was added, and the reaction (total volume 100 μl) was incubated at 37°C for 2 min.

**Micrococcal nuclease (Mnase) digestion** proceeded as described by Carey and Smale (17) with minor modifications. Briefly, cell nuclei were resuspended in Mnase digestion buffer (10 mM Tris, pH 7.4, 15 mM NaCl, 60 mM KCl, 0.15 mM spermine, and 0.5 mM spermidine) containing Mnase (stock solution = 25 U/ml) was added, and the reaction mixture was incubated at room temperature for 2, 5, and 10 min. The reaction was terminated by addition of 80 μl Mnase digestion buffer and 20 μl stop buffer (100 mM EDTA and 10 mM EGTA).

**For restriction enzyme digestion**, cell nuclei were washed in restriction enzyme buffer (10 mM Tris, pH 7.4, 50 mM NaCl, 10 mM MgCl₂, 0.2 mM EDTA, 0.2 mM EGTA, 1 mM 2-ME, 0.15 mM spermine, and 0.5 mM spermidine) (24). Nuclei were resuspended in the specific buffer for restriction enzyme digestion (50 μl). Digestion reactions were initiated with 2 μl restriction enzyme and incubated at 37°C for 10 min.

After digestion with nucleases, the total volume of reaction was brought to 200 μl by adding ddH₂O. The reaction was stopped, and genomic DNA was isolated using the DNeasy Tissue kit from Qiagen (Valencia, CA), according to the manufacturer’s instructions.

**Southern blot**

Purified genomic DNA (15–25 μg) was incubated overnight at 37°C with an excess of restriction enzyme SSP1. The DNA was then cleaned by phenol-chloroform extraction and analyzed on a 1% agarose gel, followed by transfer and hybridization with a [32P]dCTP-radioabeled probe specific for the TLR2 promoter. The prehybridization and hybridization process was done within 3 h using the Stratagene (La Jolla, CA) QuickHyb hybridization buffer, according to the manufacturer’s instruction. Films were exposed for 2–18 h before development.

**Nuclear extracts and EMSA**

Nuclear extracts were prepared as described by Hussain et al. (26). Protein concentration was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL). Nuclear extracts were either assayed immediately or stored at −70°C until further use. The oligonucleotide used in the EMSA was: NF-κB 5'-ACACCTGGGGAATTCCCACACG-3'. Oligonucleotide was end labeled with [γ-32P]ATP using T4 polynucleotide kinase. The binding reactions were conducted using the gel shift assay core system from Promega (Madison, WI). Binding reactions contained 2–5 μg nuclear extract and 20,000 cpm radioabeled oligonucleotide in 10 mM Tris-HCl, pH 7.5, 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 1 μg poly(dIdC), and 4% glycerol. Binding reactions were electrophoresed at 4°C in 5% native polyacrylamide gels using 0.5× Tris-borate buffer, followed by autoradiography.

**RNase protection assay**

Total RNA was extracted using the RNeasy kit (Ambion, Austin, TX). RNase protection assays (RPA) were performed using reagents from BD PharMingen (San Diego, CA), as previously described (27). Probe templates for mouse TLR2 and GAPDH were made in this laboratory and have been previously described (27). A total of 10 μg RNA was used for each RPA. Reactions were run on 6% polyacrylamide/8 M urea sequencing gels, followed by autoradiography.

**Transient transfection**

A total of 1 μg plasmid expressing NF-κB p65 was transfected into C8 cells with 4 μl lipofectamine plus 6 μl lipofectamine. After 24 h, the cell nuclei were isolated for restriction enzyme accessibility determination or RNA was isolated for RPA. The transfection has been proved effective when a luciferase reporter gene was used (25). To measure luciferase activity, 15 ng pCMV-NF-κB p65 or pCMV alone was cotransfected into C8 cells with 0.2 μg 326-bp TLR2 promoter-driven luciferase reporter. Forty-eight hours after transfection, the cells were lysed and luciferase was measured, as described before (25).

**Results**

*Increase in DNase I sensitivity and restriction enzyme accessibility at the murine TLR2 promoter following infection with M. avium*.

To investigate the active element in TLR2 promoter region, DNase I hypersensitivity of the chromosomal region covering TLR2 gene was measured by Southern blot. Genomic DNA was isolated and digested overnight with SSP1, followed by separation on a 1% agarose gel. Southern blot was conducted using the probe derived from PstI-SSP1 region of mouse TLR2 gene. The arrow indicates the band that appeared following infection, labeled as “H.” Incubation of the nuclei from infected cells with of DNase I for 8 min completely digested the DNA. The results are representative of three independent experiments.

**FIGURE 1.** Infection of *M. avium* resulted in the appearance of a DNase I-hypersensitive band at TLR2 promoter. Nuclei from infected or uninfected C8 cells were digested with 25 U DNase I for specified time. Genomic DNA was isolated and digested overnight with SSP1, followed by separation on a 1% agarose gel. Southern blot was conducted using the probe derived from PstI-SSP1 region of mouse TLR2 gene. The arrow indicates the band that appeared following infection, labeled as “H.” Incubation of the nuclei from infected cells with of DNase I for 8 min completely digested the DNA. The results are representative of three independent experiments.
FIGURE 2. Restriction enzyme accessibility increases at TLR2 promoter region following infection with *M. avium*. Nuclei from infected or uninfected C8 cells were digested with specified restriction enzymes (20 U) for 10 min at 37°C. Genomic DNA was isolated and then completely digested with SSP1 overnight. Southern blot was performed using the probe derived from *Pst*-SSP1 region of mouse TLR2 gene. The upper band represents the fragment derived from in vitro digestion by SSP1, while the lower band represents the fragment derived from digestion by EcoRI or HaeII or EcoI 109. The intensity of the band representing nuclear cleavage or in vitro cleavage product was determined by scanning using software Sigma Pro (SPSS, Chicago, IL.). The ratio of nuclear cleavage vs in vitro cleavage is provided in which a band representing restriction enzyme accessibility correlates with the activation of TLR2, we compared the expression of TLR2 at the mRNA level in two different macrophage cell lines, P388D1 and C8. C8 cells express a relatively high level of TLR2, while TLR2 mRNA was not detectable in P388D1 cells under the same conditions (Fig. 4A). The chromatin from P388D1 cells was much more resistant to digestion with EcoRI and HaeII than the chromatin from C8 cells (Fig. 4B). Furthermore, infection with *M. avium* induced TLR2 at mRNA level in C8 cells, but failed to do so in P388D1 cells (Fig. 4A). Infection with *M. avium* led to an increase in restriction enzyme sensitivity of the chromatin from C8 cells, but not P388D1 cells (Fig. 4B).

**Increased restriction enzyme accessibility is independent of NF-κB binding**

The EcoRI cuts the NF-κB binding site, which is necessary for the induction of TLR2 transcription by LPS stimulation or infection with *M. avium* (25, 28). To investigate the role of NF-κB in increasing the restriction enzyme accessibility of TLR2 promoter region covering NF-κB site and Sp1 sites, two approaches were used. First, we tested whether the overexpression of NF-κB p65 can result in the increased restriction enzyme accessibility at TLR2 promoter. Overexpression of NF-κB p65 induced the 326-bp TLR2 promoter-driven luciferase expression (Fig. 5A), but failed.
to increase the sensitivity to EcoRI and HaeII of the endogenous TLR2 promoter (Fig. 5B). Additionally, overexpression of NF-κB p65 did not lead to an induction of endogenous TLR2 expression (Fig. 5C). Another approach was to determine whether blocking of NF-κB binding to TLR2 promoter can abolish the increased restriction enzyme accessibility following infection with M. avium. Pyrrolidine dithiocarbamate (PDTC), an antioxidant, has been reported to block the binding of NF-κB to numerous genes (29). The results in Fig. 6A show that PDTC blocked the binding of NF-κB to the TLR2 promoter. PDTC also suppressed the induction of TLR2 following infection. However, it failed to block the increase in restriction enzyme accessibility following infection with M. avium (Fig. 6B).

To further characterize the increased restriction enzyme accessibility, its kinetics and requirement for de novo protein synthesis were evaluated. The results in Fig. 7 show that substantial restriction enzyme accessibility occurred 30 min after infection and was not affected by protein synthesis inhibitor, cycloheximide.

**Increased restriction enzyme accessibility of the TLR2 promoter is TLR2 and MyD88 dependent**

Activation of macrophages upon infection with M. avium requires the activation of TLR2 signaling pathways. This signaling pathway also requires the participation of an adapter protein, MyD88. To determine whether the TLR2-MyD88 signaling is essential for the increase in the restriction enzyme accessibility within the TLR2 promoter in response to M. avium infection, peritoneal macrophages from TLR2-/- mice and MyD88-/- mice and the control C57BL/6 mice were analyzed. As a control, we also analyzed the response of macrophages from TLR2-/- mice following stimulation with LPS. The results in Fig. 8 show that restriction enzyme accessibility did not increase in macrophages from TLR2-/- mice and MyD88-/- mice following infection with M. avium. In a parallel experiment, restriction enzyme accessibility increased in macrophages from the wild-type C57BL/6 mice following infection with M. avium. Restriction enzyme accessibility also increased in macrophages from TLR2-/- treated with LPS. These results suggest that the increase in restriction enzyme accessibility of the TLR2 promoter following M. avium infection appears to require
the TLR2-MyD88 signaling pathway. The ability of LPS stimulation to induce the restriction enzyme accessibility at TLR2 promoter region in cells from TLR2/H11002/H11002 mice (Fig. 8, lanes 3 and 4) rules out the possibility that the failure of M. avium infection to increase the restriction enzyme accessibility at TLR2 promoter was due to the changes of TLR2 gene locus in TLR2/H11002/H11002 mice. The promoter region of the TLR2/H11002/H11002 mice is intact (30).

Discussion
Previously, we have reported that the expression of TLR2 was induced following infection of macrophages with M. avium. We also analyzed the promoter region of mouse TLR2 and found that both NF-κB and Sp1 elements were required for the induction of

FIGURE 6. Binding of NF-κB is not required for an increase in restriction enzyme accessibility to occur at the TLR2 promoter region. A, PDTC at specified concentration was added to medium 1 h before infection. Nuclear protein was then extracted. EMSA was carried using a probe derived from the NF-κB site of TLR2 promoter. Increasing concentrations of PDTC inhibited binding of NF-κB. B, RPA was conducted to analyze the induction of TLR2 mRNA following infection. PDTC (200 μM) suppressed the induction of TLR2 mRNA following infection with M. avium. C, PDTC (200 μM)-pretreated cells were infected with M. avium for 30 min. Restriction enzyme accessibility assay was performed, as described above. The numbers below the blot indicated the ratio of nuclear cleavage vs in vitro cleavage. PDTC did not inhibit the increase in restriction enzyme accessibility. The results are representatives of three independent experiments.

FIGURE 7. Characterization of restriction enzyme accessibility following infection with M. avium at TLR2 promoter. A, C8 cells were infected with M. avium for specified times, and the nuclei were isolated for digestion with restriction enzymes. Southern blot was then conducted as described. The numbers below the blot indicated the ratio of nuclear cleavage vs in vitro cleavage. B, Protein synthesis inhibitor cycloheximide (10 μg/ml) was added into medium 1 h before infection with M. avium. Southern blot was conducted following restriction enzyme digestion. The results are representatives of three independent experiments.

FIGURE 8. Increase in restriction enzyme accessibility of TLR2 promoter following infection with M. avium is TLR2-MyD88 dependent. A, Thioglycolate-elicited cells from C57BL/6, TLR2−/−, and MyD88−/− mice were infected with M. avium for 30 min. Restriction enzyme accessibility was assessed, as described in Materials and Methods. Lane 1, HaeII; lane 2, EcoRI; lane 3, HaeII; lane 4, EcoRI; Lanes 1 and 2, Uninfected samples; lanes 3 and 4, infected samples. The promoter region of TLR2 knockout mice is intact, although the coding region of TLR2 in these mice was knocked out. Again the numbers below the blot indicated the ratio of nuclear cleavage vs in vitro cleavage. B, The thioglycolate-elicited cells from the TLR2−/− mice were stimulated with 1 μg/ml LPS (Escherichia coli 055:B5) for 30 min. Lanes 1, 3, and 5, Digested with EcoRI; lanes 2, 4, and 6, digested with HaeII. Lanes 1 and 2, M. avium infected; lanes 3 and 4, LPS stimulated; lanes 5 and 6, infected samples. The results are representative of three independent experiments.
TLR2 transcription following infection (25). In this study, we found an inducible DNase I-hypersensitive site around the NF-κB site of TLR2 promoter following infection with M. avium, indicating that this region may undergo chromatin remodeling. By determining the restriction enzyme accessibility, we were able to map the site that undergoes chromatin remodeling to the elements in which NF-κB and Sp1 bind. This observation supports our previous results using a transient transfection system: both NF-κB and Sp1 elements are required for the full induction of the TLR2 promoter. Although the restriction enzyme accessibility increased at NF-κB and Sp1 site after infection, the inefficient cleavage at Eco 109 site may indicate that this region is not undergoing a chromatin remodeling following infection.

Not all promoters are assembled into nucleosomes and remodeled during transcriptional activation. Only the first nucleosome was remodeled during activation of both the IL-12 and the MMTV promoters (24). In contrast, analysis of Pho5 and HIV-1 promoters showed that at least one binding site for an inducible activator was in a nucleosome-free region (18, 20). To determine whether the TLR2 promoter region is incorporated into nucleosomes before activation, the sensitivity of chromatin, including TLR2 promoter region, to MNase was determined. The appearance of a DNA ladder after digestion with MNase indicates that the TLR2 promoter is assembled into a nucleosome array.

The regulation of gene expression in mammalian cells is not only restricted to the control of transcriptional, posttranscriptional, translational, or posttranslational events. Chromatin remodeling is another target for controlling the gene expression. Chromatin remodeling has been shown to be important for IL-4 gene activation (31). We observed that an increase in restriction enzyme accessibility following infection with M. avium, within the TLR2 promoter, correlated with the induction of TLR2 expression. P388D1 cells express almost no detectable levels of TLR2 mRNA. The chromatin from these cells was more resistant to restriction enzyme digestion. In contrast, C8 cells expressed a relatively high sensitivity of nucleosomes compared to the endogenous TLR2. The chromatin from these cells was more resistant to restriction enzyme digestion. In contrast, P388D1 cells express almost no detectable levels of TLR2 mRNA. The chromatin from these cells was more resistant to restriction enzyme digestion. In contrast, C8 cells expressed a relatively high sensitivity of nucleosomes compared to the endogenous TLR2.

Our results demonstrate that the TLR2 promoter region becomes more susceptible for DNase I or restriction enzyme digestion following infection with M. avium. The increase in sensitivity to DNase I or restriction enzymes digestion correlated with induction of the TLR2 expression. Both M. avium (TLR2 agonist) and LPS (TLR4 agonist) that induce TLR2 expression resulted in an increase in restriction enzyme accessibility. The increased restriction enzyme accessibility induced following infection with M. avium was TLR2-MyD88 dependent, but not NF-κB dependent. Thus, we propose that chromatin remodeling occurs within TLR2 promoter following infection with M. avium. The chromatin remodeling may occur before binding of NF-κB to TLR2 promoter, following LPS stimulation, did not require NF-κB (34).

The activation of TLR2 signaling involves TLR2-MyD88-NF-κB-dependent pathways (30, 35). Recently, other pathways were reported to become activated following TLR2 activation (36). The increase in restriction enzyme accessibility within the TLR2 promoter following infection was TLR2-MyD88 dependent and did not require new protein synthesis. Because NF-κB is neither sufficient nor necessary for the chromatin remodeling of TLR2 promoter, different signals may mediate the chromatin remodeling of TLR2 promoter following infection with M. avium.

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Acknowledgments
We thank Dr. A. Satoskar (Ohio State University) and Dr. S. Smale (University of California, Los Angeles, CA) for helpful discussion. We also thank Liz Abraham for her technical assistance.

References


