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Rapid Chromatin Remodeling of Toll-Like Receptor 2 Promoter During Infection of Macrophages with *Mycobacterium avium*¹

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*⁸) to LPS is the result of a missense mutation in TLR4 (11, 12). TLR2 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*⁸) 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 remodelled to allow transcription to occur. Studies at the *Saccharomyces cerevisiae* Pho5, HIV, and marine mammor 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.

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¹Department of Microbiology, College of Biological Sciences, and *Department of Molecular Virology, Immunology and Medical Genetics, College of Medicine and Public Health, Ohio State University, Columbus, OH 43210; and *Department of Host Defense, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

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2 Address correspondence and reprint requests to Dr. Bruce S. Zwilling, Department of Microbiology, 484 W. 12th Avenue, Ohio State University, Columbus, OH 43210. E-mail address: zwilling.1@osu.edu

3 Abbreviations used in this paper: TLR, Toll-like receptor; MMTV, murine mammary tumor virus; MMTV, micrococcal nuclease; MyD88, myeloid differentiation factor 88; PTDC, pyrrolidine dithiocarbamate; RPA, RNase protection assay; Sp1, stimulating factor 1.
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 1 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 1 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 AM2-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 (HyClone Laboratories, Logan, UT), 1% penicillin, and streptomycin.

The expression and regulation of TLR2 by AM2-C8 cells were the same as those previously described by us for J774.1 macrophage cell line (25).

**Primary macrophages**

C57BL/6 mice (Charles River Breeding Laboratories, Wilmington, MA) and TLR2−/− and MyD88−/− (generous gifts from S. Akira, Osaka University, Osaka, Japan) mice were injected i.p. with thioglycolate. Three days later, the peritoneal exudate cells were isolated and allowed to adhere to the tissue culture plates. After overnight incubation, the 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 overnight (16). Nonidet P-40, 0.15 mM spermine, and 0.5 mM spermidine) (17) and in digestion buffer containing 10 mM Tris, pH 7.4, 15 mM NaCl, 3 mM MgCl₂, 0.5% Nonidet 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).

**Preparation of nuclei**

Cells grown to confluence were removed by scraping and centrifuged. Cells were washed once with ice-cold PBS and resuspended in ice-cold Nonidet P-40 lysis buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5% Nonidet P-40, 0.15 mM spermine, and 0.5 mM spermidine) (17) and incubated in 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 1 hypersensitivity assay, cell nuclei were resuspended in buffer A (100 mM NaCl, 50 mM Tris, pH 8.0, 1 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 CaCl₂ (1 mM). Mnase (stock solution = 25 U/μl) 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-radio labeled 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 cpmp radiolabeled 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 RNAqueous 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 325-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 1 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 1 hypersensitivity of the chromosomal region covering TLR2 gene

![FIGURE 1](http://www.jimmunol.org/)
was measured. The results in Fig. 1 show that a band appeared when cells were infected with *M. avium*. The size of this band is consistent with it being the part of the TLR2 promoter region that includes the NF-κB and Sp1 elements. The DNA was completely digested by DNase 1 within 8 min in the *M. avium*-infected cells.

DNase 1 hypersensitivity is not precise, and it is difficult to determine the exact region in which DNase 1 digestion occurs. Therefore, we measured restriction enzyme accessibility to more precisely map the chromosomal site. C8 cell nuclei were treated with a limiting concentration of an appropriate restriction enzyme (*Eco*RI, *Hae*II, and *Eco*I 109). The cleaved genomic DNA was then purified and digested to completion in vitro with a different restriction enzyme (SSP1) that recognizes sites on either side of the *Eco*RI, *Hae*II, and *Eco*I 109 restriction sites (Fig. 2). To normalize the reaction, the results are also presented as the ratio of nuclear cleavage to in vitro cleavage. An increase in the ratio upon cell activation would suggest that a nucleosome becomes more accessible to nuclear cleavage and thus remodels. C8 cell activation led to a strong and reproducible increase in nuclear cleavage by *Eco*RI, *Hae*II, but not *Eco*I 109 (Fig. 2). Nuclear cleavage at the *Eco*RI site increased by 5-fold, while the nuclear cleavage at the *Hae*II site increased 3-fold after infection. The nuclear cleavage at the *Eco*I 109 site remained unchanged following infection. The exact position of the nucleosome, as presented in Fig. 2, is hypothetical and remains to be defined.

To demonstrate that the TLR2 promoter region may be assembled into nucleosomes, the isolated nuclei were digested with MNase. MNase digests genomic DNA at the internucleosomal region, resulting in a DNA ladder when separated on an agarose gel, which may be detected when using a specific probe for TLR2. The results in Fig. 3 indicate that upon digestion with MNase, a DNA ladder appeared, while MNase digested naked DNA much more efficiently and the DNA ladder was not observed. These data suggest that TLR2 promoter region is incorporated into nucleosomes.

**Increased restriction enzyme accessibility correlates with the induction of TLR2 expression**

Increased restriction enzyme accessibility occurs before activation of some genes. To test whether an increase in 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 *Eco*RI and *Hae*II 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 *Eco*RI 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. 8B, 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 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.](http://www.jimmunol.org/)

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

![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.](http://www.jimmunol.org/)
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 EcoRI 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 level of TLR2, and its chromatin was easily digested by the restriction enzymes. The restriction enzyme accessibility assays shown in Figs. 4b, 6c, 7, and 8 appear to indicate that there may be additional cleavage sites for *Hae*II and *Eco*RI. However, the probe used for these studies encompassed nucleotides −292 to +32, i.e., the region containing the restriction sites for both *Eco*RI and *Hae*II (see Fig. 2). In contrast, the probe used to detect the increase in restriction enzyme accessibility for the experiments shown in Figs. 2 and 5 was remote from this site and between restriction sites *Pst*I and SSP1, as shown in Fig. 2. Therefore, only one nuclear cleavage fragment was apparent following restriction enzyme digestion. In contrast, two nuclear cleavage fragments are visible when the probe spanning both the *Eco*RI and *Hae*II sites was used because it overlaps both cleavage fragments.

Overexpression of NF-κB p65 led to an increased transcription of a TLR2 promoter-driven luciferase reporter vector cotransfected into P388D1 cells (our unpublished results). However, infection of P388D1 cells with *M. avium* did not induce TLR2 expression, or chromatin remodeling at TLR2 promoter. These cells may be defective in the signaling pathway, which is necessary for the chromatin remodeling to occur at the TLR2 promoter region, thus preventing the promoter region from being accessible for the transcription factors.

Chromatin remodeling occurred within GM-CSF promoter, and it is dependent upon an intact NF-κB and Sp1 elements (32). NF-κB and Sp1 have also been shown to be associated with chromatin remodeling of the HIV long terminal repeat promoter (33). In the present study, the role of NF-κB in chromatin remodeling was investigated. Overexpression of NF-κB p65 did not increase restriction enzyme accessibility. Thus, activation of NF-κB alone is not sufficient to lead to the chromatin remodeling within the TLR2 promoter region. In contrast, we have previously reported that overexpression of NF-κB p65 alone was sufficient to activate a TLR2 promoter-driven reporter construct transiently transfected into macrophage cell lines (25). Transiently transfected plasmids are not assembled into chromatin; thus, the activation of transcription of the reporter construct is not dependent upon chromatin remodeling. In the present study, exogenous NF-κB failed to increase the restriction enzyme accessibility of the endogenous TLR2 promoter; neither did it induce the expression of endogenous TLR2.

NF-κB did not appear to be necessary for the chromatin remodeling. PDTC inhibited the binding of NF-κB to TLR2 promoter and also suppressed the induction of TLR2 at the mRNA level following infection, while it did not have any effect on the chromatin remodeling. Taken together, these results suggest that the chromatin remodeling within TLR2 promoter following infection with *M. avium* is NF-κB independent. Alternatively, chromatin remodeling may occur before the binding of NF-κB to TLR2 promoter. Similarly, chromatin remodeling at IL-12 p40 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*.

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 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 through TLR2-MyD88 pathway, thus allowing transcription factor NF-κB to interact with TLR2 promoter to initiate transcription.

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**References**


