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Staphylococcus aureus and Derived Exotoxins Induce Nuclear Factor κB-Like Activity in Murine Bone Marrow Macrophages

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Heat-killed gram-positive *Staphylococcus aureus* as well as *S. aureus*-derived exotoxins B and toxic shock syndrome toxin 1 can induce nuclear factor κB (NF-κB)-like activity in murine bone marrow macrophages. The induction of NF-κB-like activity in murine macrophages by *S. aureus* was as effective as induction by tumor necrosis factor alpha (TNF-α) or lipopolysaccharides (LPS) and was observed in macrophages derived from LPS-sensitive and LPS-resistant mice. Stimulation of macrophages with *S. aureus* but not with the exotoxins resulted in the accumulation of TNF-α in the culture medium. The induction of NF-κB-like activity by *S. aureus*, however, clearly preceded TNF-α secretion and was not inhibited by a neutralizing serum against TNF-α. In addition, pretreatment of macrophages with the protein synthesis inhibitor cycloheximide or dexamethasone, which prevented the secretion of TNF-α from macrophages, did not interfere with the induction of NF-κB-like activity by *S. aureus*. This findings reveal the existence of bacterial components other than LPS which can induce NF-κB-like activity in susceptible cells.

Endogenous inflammatory mediators play an important role in the pathology caused by bacterial infections. The principal toxins of gram-negative bacteria are lipopolysaccharides (LPS), which are constituents of the bacterial outer membrane (62). Many of the host responses to LPS can be attributed to macrophage-derived cytokines, such as tumor necrosis factor α (TNF-α) and interleukin-1 (IL-1) (49). Excessive amounts of these cytokines may lead to hypotension, intravascular coagulation, multisystem organ failure, and even shock (47). Individual cytokines may mediate different LPS effects. Thus, TNF-α has been shown to mediate the lethality of LPS (6, 22, 23, 59) or tissue injury (4). IL-1 may activate lymphocytes (14). Anti-IL-1 and anti-TNF-α antisera inhibited certain effects of LPS or the respective cytokines in experimental animals (6, 15). Recently, it was recognized that, in addition to LPS, gram-negative bacteria contain at least one other common component which exhibits LPS-like biological activities (21).

Although the interaction of macrophages with LPS is well characterized, less is known about the components of gram-positive bacteria, e.g., *Staphylococcus aureus*, which was used in the present study. These components are also potent activators of macrophages. For example, a previous study showed that heat-killed *S. aureus* effectively stimulates TNF-α production in murine macrophages (21). Exotoxins secreted from *S. aureus* were found to induce lymphocyte function-associated molecule 1 in monocytes, B cells, and activated T cells (48). Unlike LPS, *S. aureus*-derived exotoxins, which have significant binding affinity for major histocompatibility complex (MHC) class II molecules (17, 20, 46, 56), are strong T-cell mitogens. Complexes of *S. aureus*-derived exotoxins and class II MHC molecules on antigen-presenting cells can interact with the T-cell receptors of a much larger proportion of T cells than can antigenic peptides bound to class II MHC molecules (for a review, see reference 33).

In an attempt to characterize some of the mechanisms which underlie the activation of macrophages by *S. aureus*, we studied the activation of nuclear factor κB (NF-κB) in this event. NF-κB specifically binds to an 11-bp DNA fragment from the κ light chain enhancer or to some slight variants of that sequence (reviewed in references 27 and 40). NF-κB is involved in the transcriptional control of a variety of genes, many of which are activated during the immune response. NF-κB is involved in transcriptional activation of the κ light chain, the IL-2 receptor α gene (7, 41), and genes encoding beta interferon (61) and TNF-α (11) and in the activation of human immunodeficiency virus gene expression (19, 28, 50). Potential NF-κB binding sites are also present in the 5′-flanking regions of a number of other genes (61), in which they are also likely to be involved in the transcriptional regulation of gene expression. NF-κB consists of two different subunits: a DNA binding protein of 51 kDa and an associated 65-kDa protein (3, 36). Both subunits were recently cloned and found to be homologous to the oncogene v-*rel*, its cellular homolog c-*rel*, and the *Drosophila* maternal effect gene dorsal (8, 26, 37, 45, 51, 55). The p65 subunit serves as a receptor for inhibitory protein IκB (3, 51), which can prevent NF-κB from binding to the target sequence (1). An IκB-like activity was cloned recently as well (29). NF-κB is constitutively active in mature B cells but can be activated posttranslationally (57) in a variety of other cell types. In unstimulated cells, NF-κB is located in an inactive form in the cytoplasm, in which it is complexed to IκB (2). Various inducers, such as phorbol esters, LPS, double-stranded RNA, and certain viruses, allow NF-κB to be released from the complex and to travel to the nucleus. NF-κB can also be activated by the cytokines TNF-α, TNF-β, and IL-1 (30, 50), suggesting a mechanism for

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autostimulatory and costimulatory regulatory loops (40). Prolonged activation of NF-κB observed in LPS- and TNF-α-stimulated cells requires de novo protein synthesis (31) and is committed by enhanced levels of mRNA encoding the p51 DNA binding subunit of NF-κB (45).

Since components of *S. aureus* are some of the most potent activators of macrophages and NF-κB seems to be a vital control element in the process leading to the activation of these cells, we addressed the role of NF-κB in macrophages exposed to whole *S. aureus* and *S. aureus*-derived exotoxins.

MATERIALS AND METHODS

Mice. The LPS-responder C57BL/10ScSn and the LPS-nonresponder C57BL/10ScCr mice (12, 21) of both sexes were obtained from the breeding stock of the Max-Planck-Institut. Six-week-old animals served as donors of bone marrow cells.

Cells. Bone marrow cells were flushed from mouse femora and cultivated at a concentration of 5×10^5 cells per ml in hydrophobic Teflon film bags (Heraeus, Hanau, Germany) as described previously (18). The culture medium consisted of 70% high-glucose-level Dulbecco modified Eagle medium (GIBCO BRL GmbH, Karlsruhe, Germany) containing 10% fetal calf serum, 5% horse serum, 0.01 mM sodium pyruvate (GIBCO), 50 nM 2-mercaptoethanol (Roth, Karlsruhe, Germany), 50 U of penicillin, and 50 μg of streptomycin (Seromed, Berlin, Germany) per ml and 30% L-cell-conditioned medium (44) and was prepared as described previously (22). After 10 days of culturing, the cells were harvested by centrifugation, washed twice with phosphate-buffered saline (PBS), and resuspended in Dulbecco modified Eagle medium supplemented with 1% (vol/vol) fetal bovine serum at a concentration of 10^6 cells per ml. Ten milliliters of cell suspension was used per dish (8.6-cm diameter). After 24 h, the adherent cells were washed once and incubated with media containing various stimuli. Human promyelocytic HL60 cells were cultivated and stimulated with TNF-α as described previously (30).

Bacteria. Preparations of heat-killed *S. aureus* were obtained as described previously (21). Macrophages were stimulated with 20 to 50 μg of heat-killed bacteria per ml of culture medium.

Materials. LPS from *Salmonella minnesota* R595 (rough form) was isolated, purified, and converted to the uniform triethylamine salt form as described previously (24, 25). Recombinant murine TNF-α (1.2×10^{10} U/mg) was a gift from G. R. Adolf (Boehringer Institut für Arzneimittelforschung, Vienna, Austria). *S. aureus*-derived exotoxins B and toxic shock syndrome toxin 1 (TSST-1) were obtained from Toxin Technology Inc., Madison, Wis. Unless indicated otherwise, the macrophages were stimulated with 500 ng of LPS, 100 ng of TNF-α, and 70 μg of exotoxins per ml of culture medium. Rabbit serum raised against recombinant murine TNF-α was purchased from Genzyme Corp., Boston, Mass., and used at a 1:1,000 dilution. Dexamethasone (Sigma) and cycloheximide (Fluka, Buchs, Switzerland) were used at 1 μM and 100 μg/ml, respectively.

Preparation of nuclear extracts and EMSA. The media containing the stimulating agents were removed from the macrophages, the cells were detached from the culture dishes after 5 min of incubation at 37°C with PBS containing 1 mM EDTA and collected by centrifugation, and about 2×10^7 cells were resuspended in 500 μl of hypotonic lysis buffer A (13). After 20 min, the cells were homogenized by 20 strokes with a loose-fitting Dounce homogenizer. The cell homogenates were centrifuged for 4 min at 6,500 rpm in a Microfuge (ca. $4,000 \times g$). The supernatants (6.5k supernatants) were removed, and the pellets were extracted with four packed pellet volumes of high-salt buffer (buffer C) (13). After 60 min, the samples were centrifuged as described above. The high-salt extracts were diluted with 3 volumes of low-salt buffer (buffer D) (13) containing 1% Nonidet P-40 and were used immediately for electrophoretic mobility shift assays (EMSA) or kept frozen at −20°C. EMSA were performed as described previously (58). Five thousand counts per minute of a ^{32}P-end-labeled *Dde*I-*Hae*III fragment of the κ light chain enhancer containing the NF-κB binding site was used per assay. A restriction fragment mutated in the NF-κB binding site (aattaACTTTCC instead of GGGGACTTTCC) but otherwise identical to the wild-type fragment was used as a negative control. This mutated fragment does not bind NF-κB (39). In some experiments, a protease inhibitor cocktail consisting of 10 mM benzamidine, 100 U of aprotinin per ml, 10 μM leupeptin, 1 μM pepstatin, 1 mM *o*-phenanthroline, and 1 mM phenylmethylsulfonyl fluoride was present during nuclear extract preparation. The protease inhibitors were purchased from Fluka.

Methylation interference. Methylation interference assays were performed as described previously (31).

Measurement of TNF-α activity. TNF-α accumulation in macrophage supernatants was determined with an L929 cell cytotoxicity assay as described previously (21). The detection limit was 10 pg/ml.

RESULTS

***S. aureus* induces NF-κB-like proteins in murine macrophages.** Bone marrow macrophages from LPS-resistant C57BL/10ScSr mice (CR macrophages) and LPS-sensitive C57BL/10ScSn mice (SN macrophages) were incubated with *S. aureus* (Fig. 1, lanes 3, 5, 9, and 12). Activated κB binding factors in nuclear extracts of these cells were detected by EMSA with an oligonucleotide containing the intact NF-κB binding site of the κ light chain enhancer. To control for nonspecifically binding proteins, we used a fragment mutated in the NF-κB binding site but otherwise identical to the wild-type fragment. In addition, HL60 cells (Fig. 1, lanes 15 and 17) and CR and SN macrophages (Fig. 1, lanes 4, 6, 10, and 13) were stimulated with TNF-α, a well-known activator of transcription factor NF-κB (52). Parallel cell cultures were left untreated (Fig. 1, lanes 1, 7, 14, and 16). With nuclear extracts of TNF-α- and *S. aureus*-stimulated CR and SN macrophages, two retarded complexes, complex 1 and complex 2, were observed. Complex 1 comigrated with the authentic NF-κB–DNA complex (45) from TNF-α-stimulated HL60 cells, and complex 2 migrated somewhat faster. Both complexes were virtually undetectable in unstimulated cells and were not generated with the mutant DNA fragment. A band located between complex 2 and the free oligonucleotide was considered to be unrelated to NF-κB, because it was detected with the wild-type oligonucleotide and was even more pronounced with the mutant oligonucleotide. Neither complex 1 nor complex 2 was observed with nuclear extracts of LPS-treated CR macrophages (Fig. 1, lane 2). They were present, however, in nuclear extracts of LPS-treated SN macrophages (Fig. 1, lane 8), confirming the resistance of CR macrophages to LPS. These results indicate that DNA binding proteins which were similar or identical to NF-κB (see below) were induced upon *S. aureus* administration in mouse bone marrow macrophages. Since LPS-

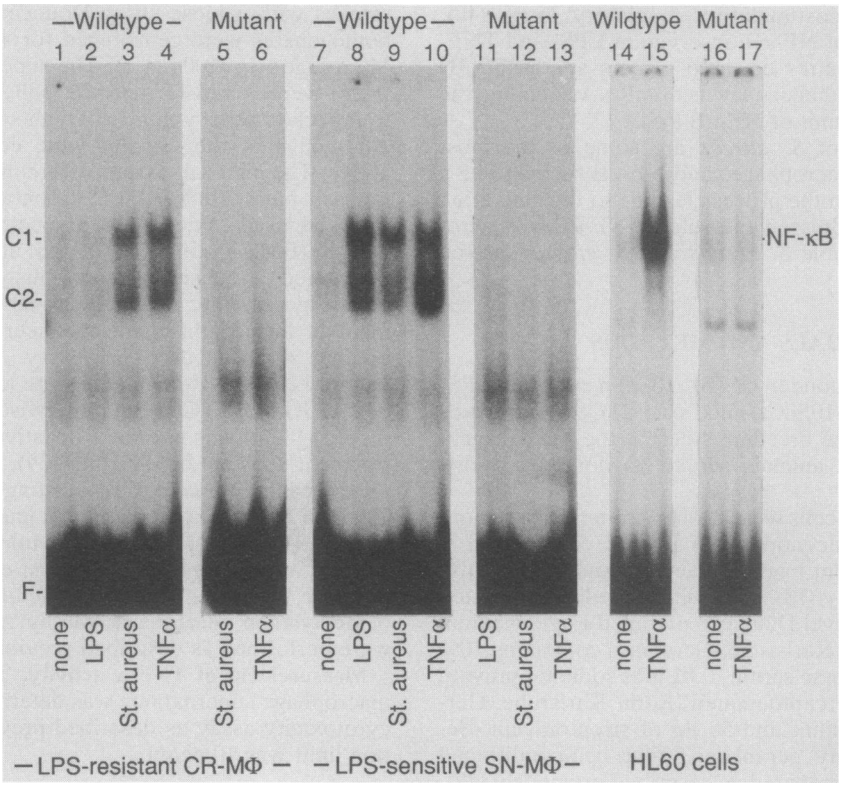

FIG. 1. *S. aureus* activation of NF-κB-like DNA binding proteins in murine macrophages. Macrophages from LPS-resistant (CR-MΦ) and LPS-sensitive (SN-MΦ) mice were stimulated for 1 h at 37°C with LPS and *S. aureus*. In addition, the macrophages and HL60 cells were stimulated with TNF-α under similar conditions. Parallel cell cultures were left untreated. NF-κB(-like) activity was measured by EMSA with an oligonucleotide that contained the wild-type binding site for NF-κB from the κ light chain (58) (Wildtype) or, as a control, an inactive mutated binding site that does not bind NF-κB (39) (Mutant). Nuclear extracts obtained from aliquots of each cell type were used. The positions of specifically retarded complexes 1 and 2 (C1 and C2) from activated macrophages, of NF-κB from HL60 cells, and of the unretarded DNA fragment (F) are indicated.

induced activation of the NF-κB-like proteins was observed in SN but not CR macrophages, the possibility that LPS contamination in the reagents or cell preparations used was responsible for NF-κB activation upon stimulation with *S. aureus* could be excluded.

***S. aureus*-induced NF-κB-like proteins from mouse macrophages are very similar to NF-κB from TNF-α-stimulated human HL60 cells.** In the experiment shown in Fig. 1, nuclear extracts were prepared in the presence of several protease inhibitors to reduce the activity of endogenous proteases. When the inhibitors were omitted, neither complex 1 nor complex 2 was generated with nuclear extracts of *S. aureus*-stimulated (Fig. 2A, lane 1) or TNF-α-stimulated (Fig. 2B, lane 1) CR macrophages. Instead, a faster-migrating complex, complex 3, was observed. When nuclear extracts were prepared from a mixture of TNF-α-stimulated CR macrophages and TNF-α-stimulated HL60 cells in the absence of protease inhibitors, only complex 3 was obtained (Fig. 2B, lane 2), whereas intact NF-κB activity was observed with nuclear extracts prepared from TNF-α-stimulated HL60 cells in the absence of macrophages (Fig. 2B, lane 3). The formation of complex 3 was not detected with the mutant oligonucleotide (Fig. 2B, lanes 4 to 6). These results reveal the presence in macrophages but not HL60 cells of proteases which can degrade NF-κB. A similar stable proteolytic fragment of NF-κB visualized as complex 3 from *S. aureus*-stimulated macrophages and TNF-α-stimulated HL60 cells was generated and specifically bound to the DNA fragment. To test whether complex 2, which was observed in addition to complex 1 with protease inhibitor-treated nuclear extracts from mouse macrophages, might result from incomplete protease inhibition, we incubated extracts of TNF-α-stimulated HL60 cells (Fig. 3C, lane 5) with nuclear extracts of unstimulated mouse macrophages (Fig. 3C, lanes 1 and 3) and reacted them with the wild-type oligonucleotide. In the absence of the protease inhibitor cocktail, only complex 3 was observed (Fig. 3C, lane 2), whereas in the presence of the protease inhibitor cocktail, only the complex of intact NF-κB and the wild-type DNA fragment could be detected (Fig. 3C, lane 4). Thus, the protease inhibitor cocktail completely inhibited the in vitro degradation of NF-κB by mouse macrophage proteases. Methylation interference assays with the upper strand of the DNA fragment containing the NF-κB binding motif revealed identical patterns of guanine contact points for NF-κB from HL60 cells and the macrophage proteolytic fragment extracted from complex 3 (Fig. 2D). In summary, protease clipping and methylation interference confirmed the similarity between NF-κB from HL60 cells and the macrophage κB binding factor. Hereafter, we call both factors NF-κB for the sake of simplicity, although we are aware that the experiments described above do not finally prove their identity.

***S. aureus*-induced NF-κB activation is not mediated by TNF-α.** TNF-α is released from mouse macrophages upon

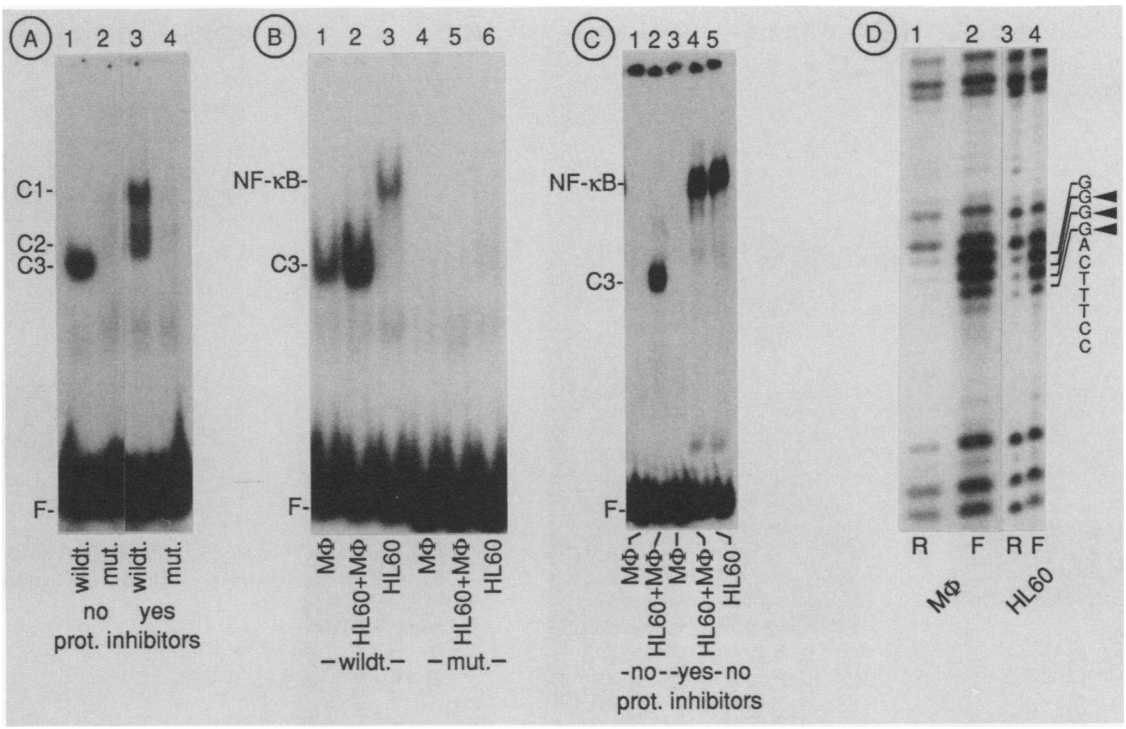

FIG. 2. Similar proteolytic fragments and identical methylation interference patterns obtained from *S. aureus*-activated NF-κB-like DNA binding proteins and authentic NF-κB. (A) Nuclear extracts of murine macrophages stimulated for 1 h at 37°C with *S. aureus* were prepared in the absence or presence of various protease inhibitors (prot. inhibitors). (B) Murine CR macrophages (MΦ), HL60 cells, and mixtures of these cell types were stimulated with TNF-α, and nuclear extracts were prepared in the absence of protease inhibitors. (C) Nuclear extracts (5 μl) from TNF-α-stimulated HL60 cells were mixed with 3 μl of the 6.5k supernatants of mouse macrophage homogenates prepared in the absence (lane 2) or presence (lane 4) of the protease inhibitor cocktail or with 3 μl of buffer D (lane 5). In addition, 3 μl of the 6.5k supernatants of mouse macrophage homogenates were mixed with 5 μl of buffer D (lanes 1 and 3). The mixtures were incubated for 1 h at 37°C. NF-κB(-like) activities were measured in the extracts by EMSA. Wild-type (wildt.) and mutant (mut.) DNA fragments were used. The positions of complexes 1, 2, and 3 (C1, C2, and C3), of NF-κB, and of the unretarded DNA fragment (F) are indicated. (D) Methylation interference assays were performed as described previously (36). Nuclear extracts of *S. aureus*-stimulated murine CR macrophages (MΦ) or partially purified NF-κB from TNF-α-treated HL60 cells prepared as described by Kawakami et al. (36) were subjected to EMSA with a partially methylated DNA fragment. Pieces of the EMSA gels containing the free DNA fragment, the DNA–NF-κB complex, or the complex of DNA and the proteolytic fragment were cut out, and the DNA was extracted and chemically degraded. Reaction products of the free DNA (F) and the DNA isolated from the retarded DNA-protein complexes (R) were applied to a sequencing gel. The positions of G residues which, upon methylation, interfered with protein binding are indicated by arrowheads.

stimulation with *S. aureus* (21). Since TNF-α can activate NF-κB in macrophages (Fig. 1), *S. aureus*-induced NF-κB activation might occur by an autocrine mechanism following TNF-α secretion. To test this possibility, we stimulated mouse macrophages with *S. aureus* in the presence or absence of an anti-TNF-α serum. At the dilution used, the antiserum was able to prevent NF-κB activation in macrophages after the administration of 1 ng of murine recombinant TNF-α per ml (Fig. 3A, lanes 2 and 3). This TNF-α concentration was much higher than the concentration that could have maximaly accumulated within 1 h of stimulation of the macrophages with *S. aureus* (see below). The anti-TNF-α serum did not interfere with *S. aureus*-induced NF-κB activation (Fig. 3A, lanes 4 and 5). Furthermore, the dependence of *S. aureus*-induced NF-κB activation on de novo protein synthesis was studied with cycloheximide (Fig. 3B). Cycloheximide induced NF-κB activity in mouse macrophages (Fig. 3B, lane 2). In nuclear extracts of macrophages treated with cycloheximide and then with *S. aureus*, enhanced levels of active NF-κB were observed, compared with those in nuclear extracts of macrophages treated with cycloheximide or *S. aureus* only (Fig. 3B, lanes 2 to 4). This result suggests that NF-κB activation by *S. aureus* did not require de novo protein synthesis. In addition, *S. aureus* was applied in the presence of dexamethasone, a well-known inhibitor of cytokine production (5, 10, 23, 35). Dexamethasone added to bone marrow macrophages 1 h prior to the administration of *S. aureus* had no effect on NF-κB activation (Fig. 3C). The amount of TNF-α, however, that was secreted into the culture supernatant of the macrophages within 6 h after the administration of *S. aureus* was substantially reduced by dexamethasone (from 3.8 to 0.1 ng/ml). Finally, whereas maximal NF-κB activation in murine bone marrow macrophages was achieved by *S. aureus* treatment in less than 1 h (Fig. 3D), no TNF-α was detectable in the cell supernatant at this time.

Bacterial exotoxins contribute to *S. aureus*-induced NF-κB activation. To extend our understanding of the interaction between *S. aureus* and NF-κB, we also studied macrophage activation by bacterial exotoxins. *S. aureus*-derived exotoxins are very potent activators of human and murine leucocytes, inducing the release of large amounts of cytokines, such as IL-2, TNF-α, and TNF-β (see Discussion). Since NF-κB is thought to be involved in the regulation of the

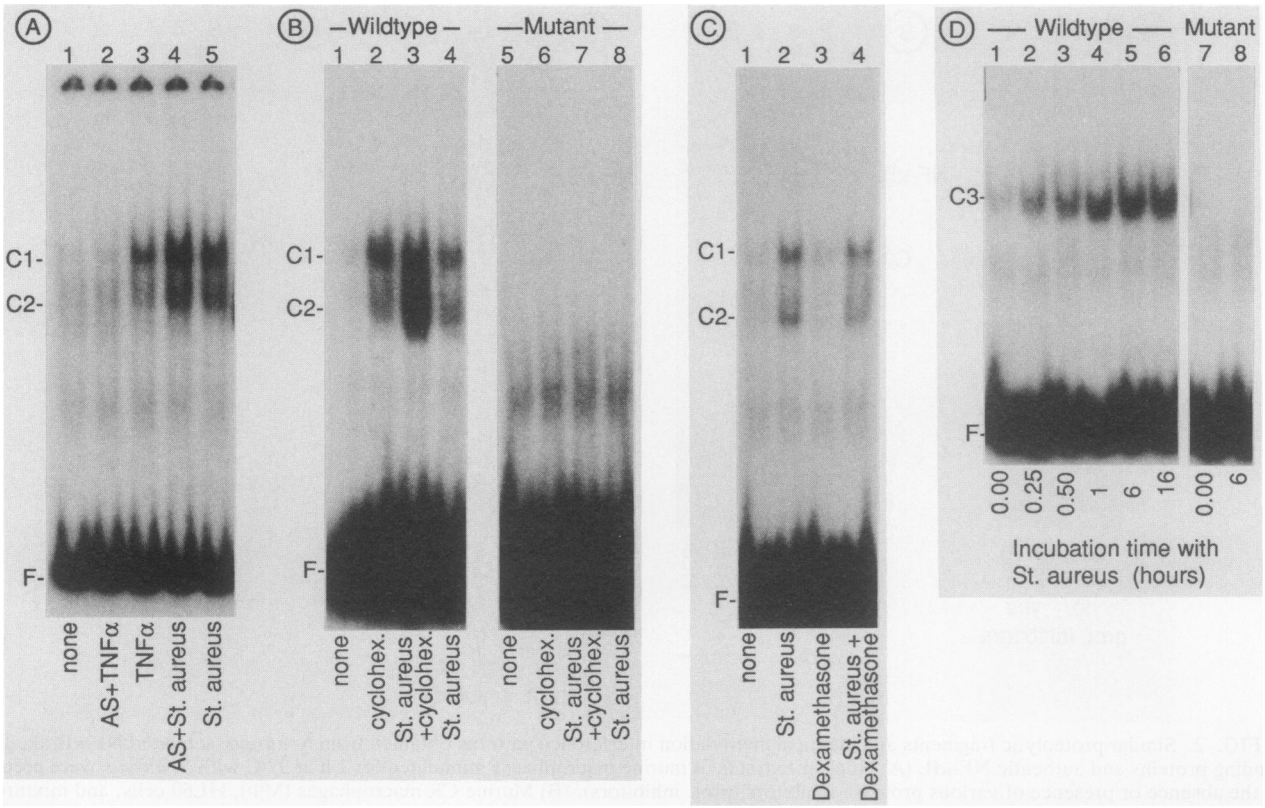

FIG. 3. *S. aureus*-induced NF-κB activation is not mediated by TNF-α. (A) Murine LPS-resistant macrophages were stimulated for 1 h with 1 ng of murine TNF-α per ml (lanes 2 and 3) or with 20 μg of *S. aureus* (lanes 4 and 5) in the absence or presence of 1:1,000-diluted anti-TNF-α serum (AS). (B) Murine LPS-resistant macrophages were treated for 1 h with cycloheximide (cyclohex.), *S. aureus* was added, and stimulation was continued for an additional hour. (C) Murine LPS-resistant macrophages were treated for 1 h with dexamethasone and then stiumlated with an *S. aureus* suspension for 6 h. From these cells and from the cells mentioned in panels A and B, nuclear extracts were prepared in the presence of protease inhibitors. (D) Murine LPS-resistant macrophages were stimulated for the indicated periods of time with *S. aureus*, and nuclear extracts were prepared in the absence of protease inhibitors. The nuclear extracts were analyzed by EMSA with an oligonucleotide containing the wild-type binding site for NF-κB (Wildtype). In panels B and D, the inactive mutated binding site (Mutant) was used as well. The positions of complexes 1, 2, and 3 (C1, C2, and C3) and of the unretarded DNA fragment (F) are indicated.

expression of the genes encoding these factors, we investigated whether the *S. aureus*-derived exotoxins could lead to NF-κB activation in murine macrophages. Within 1 h of stimulation with exotoxin B and TSST-1, NF-κB activation comparable to that obtained with whole heat-killed bacteria was observed (Fig. 4, lanes 2, 3, and 4). TNF-α activity in the culture supernatant of murine macrophages treated for 6 or 24 h with exotoxin B or TSST-1 was below the detection limit (>10 pg/ml), whereas massive secretion of TNF-α activity was observed upon stimulation with whole heat-killed bacteria (3.7 ng/ml after 6 h). Thus, the tested bacterial exotoxins represent one class of *S. aureus* components that can induce NF-κB activation. Stimulation of macrophages with these exotoxins, however, is not sufficient to induce TNF-α secretion.

DISCUSSION

In this study, we demonstrated that *S. aureus* activated DNA binding proteins in murine bone marrow macrophages that bound to a DNA fragment containing the NF-κB motif of the κ light chain enhancer, giving rise to three different DNA-protein complexes. According to the binding specificities, native proteolytic fingerprints, and methylation interference patterns, these DNA binding proteins should be closely related to NF-κB. The most retarded complex, complex 1, had an electrophoretic mobility in EMSA gels similar to that of the complex between the DNA fragment and authentic NF-κB. The protein giving rise to complex 2, which moved faster than complex 1 in EMSA gels, should also be related to NF-κB, as indicated by the binding specificity and activation profile of the protein. Complex 1 and complex 2 were converted to complex 3, which was least retarded in EMSA gels, by one or several macrophage-specific proteases, suggesting that the protein components in complexes 1 and 2 share similar structures. Whereas the protein component of complex 1 should be identical to intact mouse NF-κB, complex 2 might represent an NF-κB species that has an altered subunit composition. Alternatively, the protein component of complex 2 might be a partially degraded form of NF-κB. Such a putative degradation of NF-κB, however, would have to have occurred in intact cells, since no proteolytic activity degrading NF-κB was detected in macrophage extracts after they had been treated with the protease inhibitor cocktail. A time course determined for the in vitro degradation of NF-κB by macrophage proteases in the absence of any protease inhibitor did not indicate that the protein component of complex 2 was an intermediate of the stable degraded NF-κB species present in complex 3 (data not shown). A similar heterogeneity in

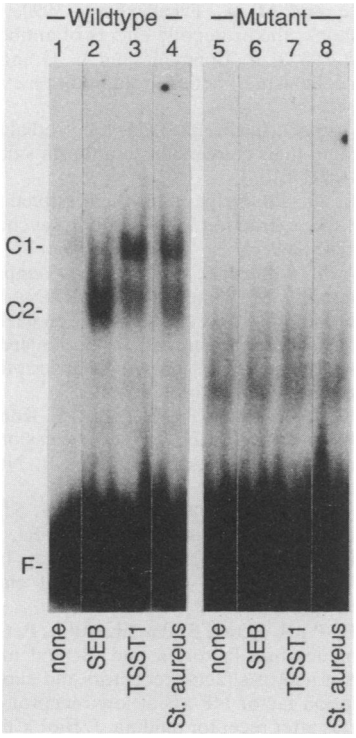

FIG. 4. Bacterial exotoxins B and TSST-1 are activators of NF-κB. Murine LPS-resistant macrophages were stimulated for 1 h with the *S. aureus*-derived exotoxins B and TSST-1. Parallel cell cultures were treated with *S. aureus* or left untreated (none). NF-κB activity was measured in nuclear extracts of the cells by EMSA with an oligonucleotide containing the wild-type binding site for NF-κB (Wildtype) or the inactive mutated binding site (Mutant). The positions of specifically retarded complexes 1 and 2 (C1 and C2) and of the unretarded DNA fragment (F) are indicated.

NF-κB binding activities was found in nuclear extracts from mouse macrophages in two recent studies (11, 28). In summary, DNA binding proteins which were very similar or even identical to NF-κB were activated in murine macrophages upon stimulation with *S. aureus*.

Since NF-κB takes part in the signal pathway of TNF-α and TNF-α is produced by macrophages after exposure to *S. aureus*, we had to rule out the possibility that TNF-α mediated *S. aureus*-induced activation of NF-κB. Four lines of evidence argue against TNF-α as a mediator. First, NF-κB activation was induced in the presence of dexamethasone, which inhibited TNF-α production. Second, an anti-TNF-α serum did not interfere with *S. aureus*-induced NF-κB activation. Third, *S. aureus*-induced NF-κB activation was enhanced in the presence of cycloheximide at concentrations inhibiting de novo protein synthesis. Cycloheximide-induced NF-κB activation was also reported by other investigators (11, 57). Fourth, NF-κB activation occurred much faster than TNF-α secretion. It should also be noted that NF-κB activation but not TNF-α secretion was observed after stimulation of murine macrophages with exotoxin B and TSST-1.

CR macrophages cannot activate NF-κB in response to LPS, whereas NF-κB activation readily occurs upon stimulation with *S. aureus* and TNF-α. This finding confirms the LPS resistance of CR macrophages, but it also demonstrates that LPS resistance is manifested early in the signal pathway of LPS, before common components are shared with the NF-κB induction pathways used by *S. aureus* and TNF-α. Mutant strains of the mouse pre-B-cell line 70Z/3 failed to express the κ light chain and were unable to activate NF-κB upon stimulation with LPS, IL-1, and 12-*O*-tetradecanoylphorbol-13-acetate (9). Therefore, signal transduction in these variant strains should be affected at a step common to these three stimulators.

S. aureus exotoxins bound to class II MHC molecules (17, 20, 46, 56) on accessory cells, e.g., monocytes, induced T cells to proliferate and to secrete cytokines, such as IL-2, gamma interferon, and TNF-α (16, 54, 60). It is a matter for debate, however, whether monocytes or macrophages can be triggered directly by the exotoxins to secrete TNF-α or TNF-β or whether the cytokine secretion that was observed in cultures of monocytes isolated from peripheral blood leucocytes (32, 34, 53) might have depended on a minor fraction of contaminating T cells possibly present in these cultures. Our data, which are in accordance with the results of Fischer and collegues (16), indicated that exotoxin B and TSST-1 did not induce TNF-α secretion from in vitro-differentiated murine macrophages. Nevertheless, the interaction of these exotoxins with these cells gave rise to an intracellular signal leading to NF-κB activation. It might be concluded from these results that class II MHC molecules present on the surface of macrophages have, in addition to their antigen-presenting function, a direct function in intracellular signaling. Accordingly, Mourad and colleagues showed that the binding of TSST-1 to class II MHC molecules activated lymphocyte function-associated molecule 1 in monocytes, B cells, and T cells and suggested a new role for class II MHC molecules as signal-transducing receptors (48). However, we cannot exclude the possibility that there are additional cell surface receptors for exotoxin B and TSST-1 on murine macrophages that can act as signal-transducing receptors mediating NF-κB activation independently from class II MHC molecules.

The components of *S. aureus* that are responsible for the effects of whole bacteria on murine macrophages should be different from exotoxin B and TSST-1, since (i) *S. aureus* exotoxins are secreted into the surrounding medium and therefore should not accumulate on the bacterial surface, and (ii) *S. aureus* was subjected to rigorous heat treatment prior to use. Proteins such as exotoxins should become denatured under these conditions. We are currently investigating the precise chemical nature of the *S. aureus* components that stimulate NF-κB activation and TNF-α secretion in murine bone marrow macrophages. In addition, we are trying to elucidate the role of class II MHC molecules in the signal transduction of the bacterial exotoxins.

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