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Mycoplasma arthritidis-Derived Superantigen Induces Proinflammatory Monokine Gene Expression in the THP-1 Human Monocytic Cell Line

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Soluble factors produced by *Mycoplasma arthritidis* play an important role in the pathology of arthritis in rodents, which closely resembles human rheumatoid arthritis. At least one of the products of these microorganisms, *M. arthritidis*-T cell mitogen (MAM), has biological activities in common with superantigens. These superantigens activate T cells in a Vβ-restricted fashion, and this response is strictly dependent on the presence of major histocompatibility complex (MHC) class II-positive cells. In the present study, we have examined the ability of MAM to induce proinflammatory monokine (interleukin 1β [IL-1β] and tumor necrosis factor alpha [TNF-α]) gene expression in the THP-1 monocytic cell line. Treatment of these cells (which express a very low level of HLA-DR molecules) with gamma interferon (INF-γ) induced HLA-DR, -DQ, and -DP molecules and enabled them to respond to MAM in a dose-dependent manner, resulting in an increase in the level of steady-state mRNA for IL-1β and TNF-α. Stimulation of the U937 monocytic cell line (MHC class II-negative even after INF-γ treatment) with MAM did not induce either IL-1β or TNF-α transcription. Moreover, MAM adsorption on Raji (MHC class II-positive) cells resulted in the loss of its cytokine-inducing activity to induce monokine gene expression. These findings demonstrate clearly that MAM induces monokine gene expression following interaction with MHC class II molecules. Pretreatment of INF-γ-treated THP-1 cells with the transcription inhibitor actinomycin D prevented the induction of monokine mRNA, whereas cycloheximide superinduced mRNA after stimulation with MAM. Finally, our results, obtained with protein tyrosine kinase inhibitors and antiphosphotyrosine Western blotting (immunoblotting), indicate that protein tyrosine kinase is involved in MAM-induced IL-1β and TNF-α gene expression in the THP-1 monocytic cell line. The capacity of MAM to induce proinflammatory cytokine transcription in monocytes via MHC class II molecules can be one pathway of MAM contribution to autoimmune diseases.

Several microorganisms produce substances or toxins which have different effects on cells of the immune system. Such products can modulate the host immune response and may contribute to the pathogenesis and immunopathology of various diseases. The toxins produced by diverse microorganisms including staphylococci, streptococci, and mycoplasma represent a group of antigens which are newly described as potent immunomodulatory molecules and are referred to as superantigens (22).

Recently, several investigators have proposed that superantigens might play a role in the development of autoimmune diseases (21, 31). Of special interest is the T-cell mitogen produced by *Mycoplasma arthritidis*. This microorganism is a naturally occurring arthritogen that causes chronic relapsing disease in rodents and that, histologically, closely resembles human rheumatoid arthritis (RA). *M. arthritidis* T-cell mitogen (MAM) is a soluble, hydrophobic, heat (56°C)- and acid (pH <7)-labile protein with a molecular mass ranging between 15 and 30 kDa (2, 18). Like other superantigens, MAM interacts with and potentially activates two of the main subsets of the immune system, T lymphocytes and major histocompatibility complex (MHC) class II-positive cells. It is well documented that MAM-induced activation of murine T cells is dependent on the presence of accessory cells bearing MHC class II molecules, in particular the I-Eα chain (10). The reaction of human T cells is also MHC class II dependent. An increase in the level of intracellular free Ca^{2+} in human T cells has been reported after MAM stimulation, while full activation of T cells occurs only in the presence of MHC class II-positive cells (23). However, there is some evidence that the effect of MHC class II could be partially substituted for by certain factors, such as interleukin 1 (IL-1) (4) or phorbol myristate acetate (23), which may act as a second signal. Like staphylococcal superantigens (26), polyclonal B-cell activation and immunoglobulin secretion were observed following MAM stimulation of cocultures of human B cells and MAM-responsive Th cells (42). This observation indicated the ability of this superantigen to initiate a B-Th cell collaboration and to form a superantigen bridge which acts as a signal for B cells. Recently, with the use of unfractionated human peripheral blood mononuclear cells, it has been shown that MAM induces IL-1α, IL-1β, IL-2, IL-4, IL-6, and gamma interferon (INF-γ) (35). The mechanisms involved in MAM-induced monokine production by human monocytes are not yet well defined. Nevertheless, the previous observations suggest that MAM, like staphyloccocal superantigens, acts as an inducer of signal transduction in MHC class II-positive cells.

In view of the potential role of IL-1β and TNF-α in the pathology of RA, the experiments described below were designed to study the effects of MAM on the gene expression of these monokines in human monocytic cell lines and, eventually, the intracellular signaling mechanisms involved in this induction. Our results demonstrate that stimulation of the

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INF-γ-treated human monocytic cell line THP-1 with MAM induces IL-1β and TNF-α mRNA expression. The induction of monokines occurred rapidly in a dose-dependent manner and does not require newly translated proteins. Moreover, our findings implicate protein tyrosine kinase (PTK) as an early biochemical event in MAM-induced IL-1β and TNFα mRNA expression.

MATERIALS AND METHODS

Reagents. MAM was prepared as previously described (35), and the preparation (MAM represents 20% of the protein preparation) was proven to be lipopolysaccharide (LPS)-free in a system with C3H/HeJ (MAM responder and LPS nonresponder) and C52BI/6 (MAM nonresponder and LPS responder) mice. LPS was obtained from Difco Laboratories (Detroit, Mich.). Human rINF-γ was obtained from Becton Dickinson Labware (Bedford, Mass.). Actinomycin D was purchased from Boehringer Mannheim Canada (Laval, Québec, Canada). The antiphosphotyrosine monoclonal antibody UBO5-321 was obtained from UBI (Lake Placid, N.Y.). The ECL Western blotting (immunoblotting) detection system and the horseradish peroxidase-labeled sheep anti-mouse antibody were from Amersham Canada Limited (Oakville, Ontario, Canada). Phorbol-12,13-dibutyrate (PDBu) was purchased from Sigma Chemical Co. (St. Louis, Mo.). Herbimycin A was purchased from Calbiochem-Novabiochem Corporation (La Jolla, Calif.). Genistein and cycloheximide were purchased from ICN Biochemicals Canada Ltd. (Mississauga, Ontario, Canada). RO 318220 was generously provided by D. P. Clough (Roche Products Ltd., Hertfordshire, United Kingdom). Erbstatin was a generous gift from K. Umezawa (Department of Applied Chemistry, Keio University, Yokohama, Japan). The cDNA probes for IL-1β, TNF-α, and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were obtained from American Type Culture Collection (Bethesda, Md.).

Cells. The THP-1 and U937 monocytic human cell lines were obtained from American Type Culture Collection. THP-1 cells were stimulated with INF-γ (200 U/ml) for 48 h to express MHC class II molecules. Before use, cells were washed twice with Hanks balanced salt solution and were resuspended at a concentration of 10^7/ml. The Raji cell line (MHC class II-positive) was obtained from American Type Culture Collection. The RM3 cell line (MHC class II-negative) derived from Raji (5) was a generous gift from R. P. Sékaly (Clinical Research Institute of Montreal, Montreal, Québec, Canada). All cells were maintained in RPMI 1640 medium containing 10% fetal calf serum and antibiotics and were always washed twice with Hanks balanced salt solution before use.

MAM adsorption. MAM solutions were prepared at a concentration of 1:500 in RPMI 1640 containing 10% fetal calf serum. Raji and RM3 cells were washed twice with Hanks balanced salt solution, resuspended in MAM solutions at a concentration of 10^7/ml, and then incubated at 4°C for 2 h. The cycle was repeated twice, and the adsorbed MAM solutions were collected for further experiments.

Quantification of the levels of IL-1β and TNF-α mRNAs. Stimulation conditions for each experiment are detailed in the appropriate figure legends. Cytoplasmic RNA was purified according to classical methods (36), and 10 μg of RNA was loaded onto 1% agarose gels. The RNA was then transferred onto Hybond-N filter paper and was hybridized with random primer-labeled cDNA probes for IL-1β and TNF-α. Equal loadings of RNA were confirmed by hybridization with a GAPDH cDNA probe. All washes were performed under stringent conditions. The mRNA hybridization with the two cytokine cDNA probes was visualized by autoradiography.

Tyrosine phosphorylation. INF-γ-treated THP-1 cells (1.6×10^6/100 μl) were incubated for 15 min at 37°C before stimulation with MAM. Stimulations were carried out as indicated in the figure legends. The reactions were stopped by adding 100 μl of 2× Laemmli sample buffer containing protease inhibitors (1× buffer is 62.5 mM Tris-HCl [pH 6.8], 4% sodium dodecyl sulfate [SDS], 5% β-mercaptoethanol, 8.5% glycerol, 2.5 mM orthovanadate, 10 mM paranitrophenylphosphate, 12 μg of leupeptin per ml, 12 μg of aprotinin per ml, 1.25 mM phenylmethylsulfonyl fluoride, 0.025% bromophenol blue) preheated at 95°C. The samples were boiled for 7 min and then were loaded (8×10^5 cell equivalent) onto 7.5 to 20% SDS-polyacrylamide gels (19), and proteins were transferred from the gels to polyvinylidine difluoride membranes (Immobilon-P, Millipore Corporation) as previously described (40). Nonspecific sites were blocked with 2% gelatin in Tris-buffered saline-Tween (25 mM Tris-HCl [pH 7.8], 190 mM NaCl, 0.15% Tween 20) for 1 h at 37°C. The membranes were then incubated with the monoclonal antibody UBO5-321 for 1 h at 37°C at a final concentration of 1:4,000 in fresh blocking solution. The membranes were washed three times at room temperature in Tris-buffered saline-Tween and were then incubated with horseradish peroxidase-labeled sheep anti-mouse immunoglobulin G for 1 h at 37°C. The membranes were then washed three times with Tris-buffered saline-Tween, and the phosphotyrosine bands were revealed by using the ECL Western blotting detection system.

RESULTS

MAM-induced IL-1β and TNF-α mRNA expression. THP-1 is a human monocytic cell line which expresses a very low level of HLA-DR molecules, while it is completely negative for HLA-DQ and HLA-DP. After treatment with INF-γ, the MHC class II molecule expression is markedly increased, with high expression of HLA-DR and lesser expression of HLA-DQ and -DP (43). This cell line was shown to transcribe the IL-1β gene and synthesize its protein in response to LPS and phorbol myristate acetate in a manner similar to that of peripheral blood monocytes (14). Hence, it was chosen for our stimulation assays with MAM.

THP-1 cells were treated with 200 U of INF-γ per ml for 48 h; standard fluorescence-activated cell sorter (FACS) analysis revealed maximal MHC class II molecule expression under these conditions. We designated the INF-γ-treated 99% MHC class II-positive cells MFI 117 and designated the untreated 10% MHC class II-positive cells MFI 72. INF-γ-treated and untreated cells were then stimulated with different concentrations of MAM or with LPS (as a control) and were incubated for 1 h at 37°C; the levels of steady-state mRNAs of IL-1β and TNF-α were then determined. Stimulation with MAM resulted in the induction of mRNA, for both monokines, in only INF-γ-treated cells (Fig. 1). LPS response was observed in both THP-1 populations. On its own, INF-γ did not have any detectable effect on either gene. IL-1β and TNF-α mRNA induction by MAM in THP-1 cells is dose-dependent, with a maximum induction when MAM is used at 1:100. A detailed time course experiment revealed an early peak at 1 h, followed by a significant reduction in the mRNA levels at 3 and 6 h for both IL-1β and TNF-α, respectively (Fig. 2). Comparable results were obtained when human monocytes were stimulated with MAM or with other superantigens such as SEA, SEB, and toxic shock syndrome toxin 1 (data not shown) (38).

MAM-induced IL-1β and TNF-α gene expression implicates

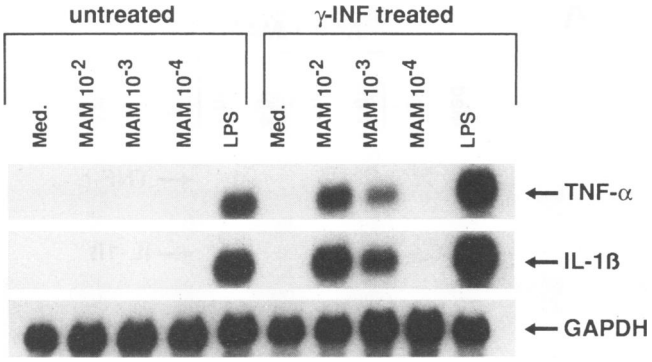

FIG. 1. MAM-induced monokine gene expression in the THP-1 human monocytic cell line. THP-1 cells were pretreated with 200 U of INF-γ per ml for 48 h to induce maximal MHC class II molecule expression. INF-γ-treated and untreated THP-1 cells were then stimulated with different concentrations of MAM or with LPS (1 μg/ml) and were incubated for 1 h at 37°C, after which total RNA was extracted and IL-1β, TNF-α, and GAPDH mRNA induction was analyzed by Northern blotting.

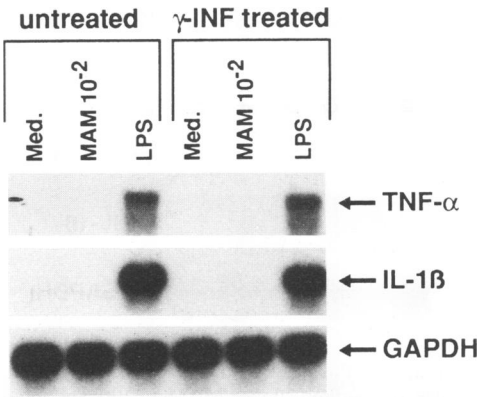

FIG. 3. Induction of IL-1β and TNF-α mRNA by MAM and LPS in the human monocytic cell line U937. U937 cells were pretreated with 200 U of INF-γ per ml for 48 h. INF-γ-treated and untreated cells were then stimulated with medium alone, MAM (1:100), or LPS (1 μg/ml). Total RNA was isolated after 1 h of incubation at 37°C, and the levels of IL-1β, TNF-α, and GAPDH mRNAs were determined by Northern analysis.

MHC class II molecules. In order to confirm that the observed IL-1β and TNF-α gene is the result of MAM activity via MHC class II molecules, we used another human monocytic cell line (U937) which is MHC class II-negative and in which the expression of these MHC class II molecules is not inducible with INF-γ (43). U937 cells were treated with 200 U of INF-γ per ml for 48 h, and FACS analysis for MHC class II expression confirmed the absence of these molecules under the same conditions used for maximal class II induction in THP-1 (data not shown). As shown in Fig. 3, IL-1β and TNF-α transcription was induced in both INF-γ-treated and untreated cells when they were stimulated by LPS, while no response was detected in either population after MAM stimulation. In addition, we adsorbed MAM on either MHC class II-positive (Raji) or MHC class II-negative (RM3) cell lines before reassessing its cytokine-inducing activity in INF-γ-treated THP-1 cells. MAM solutions (at 1:500) were prepared and were adsorbed with Raji or RM3 cells as indicated in Materials and Methods. The MAM solution adsorbed with Raji cells completely lost its capacity to induce IL-1β and TNF-α mRNA, while the solution adsorbed with RM3 retained all its activity (Fig. 4). Together, these results demonstrate clearly that the observed IL-1β and TNF-α gene expression is due to MAM cytokine-inducing activity via MHC class II molecules. Accordingly, all our experiments were carried out with INF-γ-treated THP-1 cells, which express a high level of MHC class II molecules.

The effect of cycloheximide and actinomycin D on MAM-induced monokine gene expression. To determine whether the IL-1β and TNF-α induced upon MAM treatment is protein synthesis dependent, we conducted experiments in the presence of cycloheximide, an inhibitor of peptidyltransferase. Treatment of THP-1 cells for 15 min with cycloheximide (1 μg/ml), followed by a stimulation with MAM for a further hour, showed enhanced (by at least 2×) IL-1β and TNF-α mRNA expression (Fig. 5). Cycloheximide by itself did not have any effect on cells not stimulated by MAM. This result reflects that the MAM-induced transcription of these two

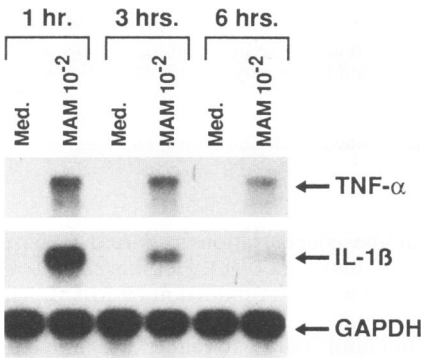

FIG. 2. Detailed time course of MAM-induced IL-β and TNF-α mRNA expression. INF-γ-treated THP-1 cells were stimulated with MAM (1:100) at 37°C. Cells were lysed at different time intervals and total RNA was analyzed by Northern blotting for IL-1β, TNF-α, and GAPDH mRNA levels.

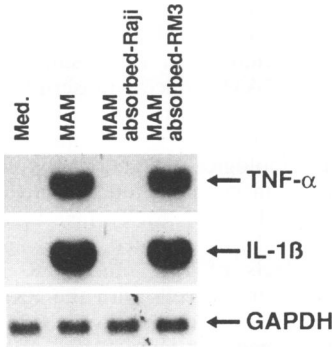

FIG. 4. Effect of MAM adsorption with an MHC class II-positive cell line on MAM mitogenic activity. MAM solutions were prepared at 1:500 and were then added to the MHC class II-positive cell line Raji (10^7 cells/ml) or to the MHC class II-negative cell line RM3 (10^7 cells/ml). After 2 h of incubation at 4°C, the supernatants were collected and the cycle was repeated twice. INF-γ-treated THP-1 cells were then stimulated with medium alone, MAM (1:500), MAM solution adsorbed with Raji cells, or MAM solution adsorbed with RM3 cells for 1 h at 37°C. Total RNA was prepared and the levels of IL-β, TNF-α, and GAPDH mRNAs were analyzed as described.

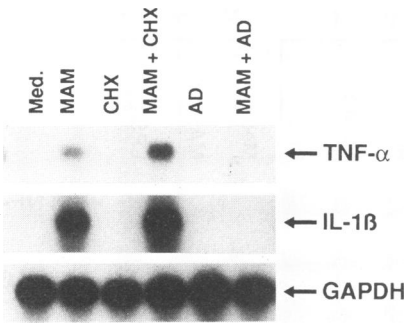

FIG. 5. The effect of actinomycin D and cycloheximide on MAM-induced IL-1β and TNF-α mRNAs. INF-γ-treated cells were cultured in the presence of medium alone, MAM (1:100), cycloheximide (1 μg/ml), MAM plus cycloheximide, actinomycin D (10 μg/ml), or MAM plus actinomycin D. After incubation for 1 h at 37°C, the cells were lysed and total RNA was isolated. The levels of IL-1β, TNF-α, and GAPDH mRNA were then determined by Northern analysis.

monokines does not require newly translated proteins. Treatment of THP-1 cells with actinomycin D, an RNA transcription inhibitor, before stimulation with MAM completely blocked the IL-1β and TNF-α gene expression. This indicates that MAM-induced monokine gene expression results from the accumulation of de novo-initiated transcripts.

The stability of MAM-induced IL-1β and TNF-α mRNA was also evaluated by using actinomycin D. THP-1 cells were stimulated with MAM for 1 h, after which actinomycin D (10 μg/ml) was added and RNA was isolated for analysis at different time intervals (Fig. 6A). The approximate half-lives of IL-1β and TNF-α mRNA, calculated from the decay curve, were 1.5 h and 1 h, respectively (Fig. 6B). Although the MAM-induced IL-1β mRNA expression decreases remarkably after 3 h of stimulation, it has a half-life slightly longer than that of TNF-α mRNA, in which a transcription could be observed for 6 h after stimulation with MAM (Fig. 2). The time course profiles of MAM-induced mRNA of these two monokines reflect a certain elongation in TNF-α mRNA induction not observed for IL-1β mRNA expression. The significance of this observation, as well as of the unusual early induction of IL-1β mRNA, is not clear.

MAM-induced expression of IL-1β and TNF-α genes is suppressed by PTK inhibitors. The results mentioned above demonstrated that MAM induces transcription of IL-1β and TNF-α genes in the human monocytic cell line THP-1 and that this response occurs via MHC class II molecules. It has been suggested that the biological effects of cross-linking of MHC class II molecules can be mediated by activation of protein kinase C (PKC) or PTK (32, 38). To investigate the signaling pathway involved in MAM-induced IL-1β and TNF-α gene expression, the effects of PKC and PTK inhibitors were examined. Pretreatment of cells with the PKC inhibitor RO 318220, at a concentration reported to be optimal (16), failed to inhibit MAM-induced IL-1β and TNF-α mRNA expression. Both monokine transcriptions induced by the PKC activator PDBu were inhibited (~64%) by RO 318220 (Fig. 7). Pretreatment of cells with genistein, a PTK inhibitor, before stimulation with MAM strongly (~92%) inhibited the induction of both IL-1β and TNF-α mRNAs (Fig. 7), while PDBu-induced transcription was moderately (30%) affected.

To further examine the involvement of PTK in MAM-induced gene expression, the effects of two other PTK inhibitors, herbimycin A and erbstatin, were studied. Both inhibitors

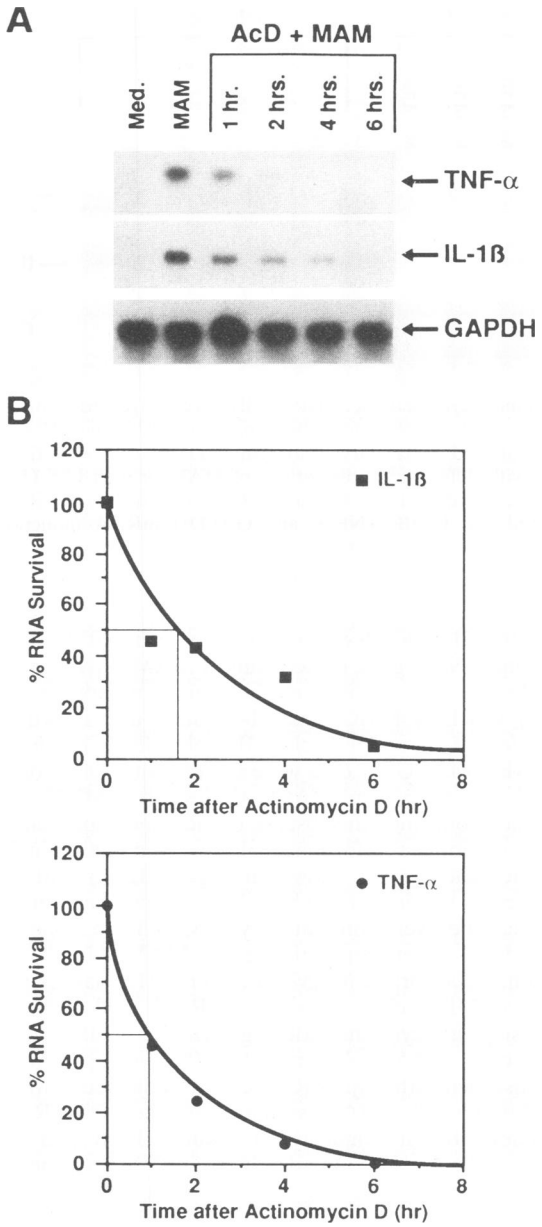

FIG. 6. Half-life of MAM-induced IL-1β and TNF-α mRNA. INF-γ-treated THP-1 cells were stimulated with MAM (1:100) for 1 h at 37°C, after which actinomycin D (10 μg/ml) was added. Cells were lysed at the indicated time intervals and total RNA was prepared. (A) The levels of IL-1β, TNF-α, and GAPDH were determined by Northern blot analysis. (B) mRNA decay curves for the data shown in panel A as determined by densitometric analysis.

were used at the concentrations reported to be optimal for PTK specific inhibition (27, 38). Herbimycin A strongly inhibited (92%) TNF-α mRNA induction by MAM, while it had a moderate effect (30% inhibition) on IL-1β mRNA induction. This PTK inhibitor failed to affect the induction of both monokines (18 and 4%, respectively) by PDBu. When cells were pretreated with erbstatin, the MAM-induced transcription of TNF-α and IL-1β was efficiently inhibited (60% and 70%, respectively), while the induction by PDBu was slightly affected (Fig. 8). These results suggest that PTKs can be

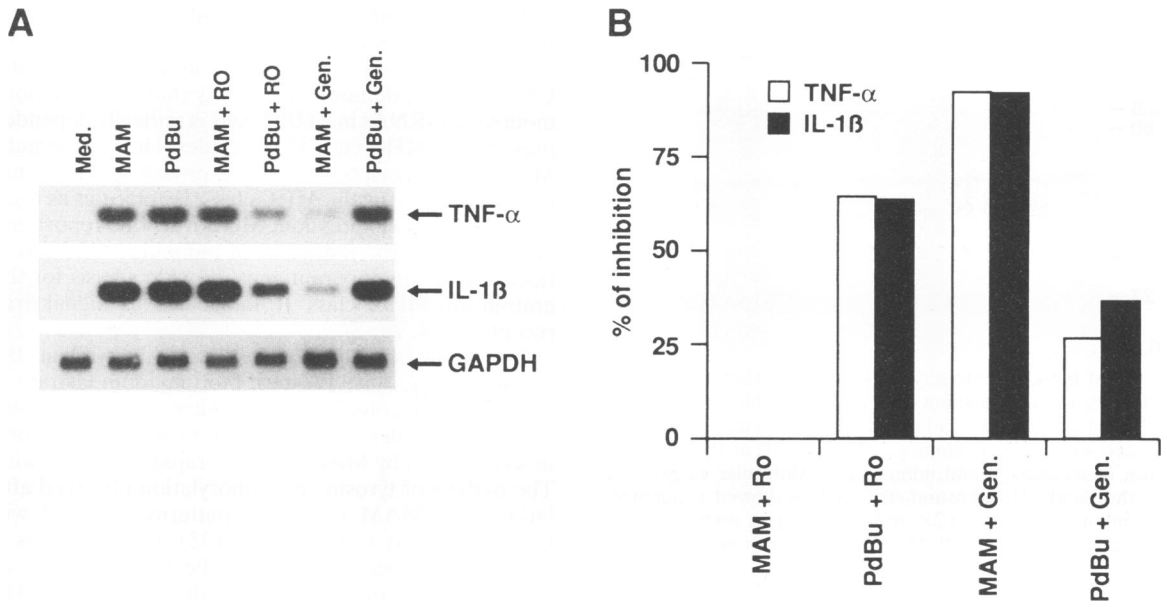

FIG. 7. Effect of PKC and PTK inhibitors on MAM-induced IL-1β and TNF-α mRNA expression. INF-γ-treated THP-1 cells were incubated with medium alone, the PKC inhibitor RO 318220 (RO) (3.3×10^{-7} M), or the PTK inhibitor genistein (Gen.) (200 μM) for 1 h at 37°C. Cell viability was verified, and then cells were stimulated with MAM (1:100) or the PKC activator phorbol-12,13-dibutyrate (100 nM) for another hour at 37°C. Total RNA was extracted and the levels of IL-1β, TNF-α, and GAPDH mRNAs were determined by Northern analysis (A). Percent inhibition of the data shown in panel A was determined by densitometric analysis (B).

involved in MAM-induced monokine gene expression in THP-1 cells. Moreover, we observed that the PTK inhibitors may affect differently the induction of IL-1β and TNF-α by MAM. This may reflect the presence of different PTK in the induction of each monokine transcription. MAM-induced IL-1β and TNF-α transcription did not seem to be mediated by the activation of the PKC isozymes inhibited by RO 318220.

MAM induces protein tyrosine phosphorylation in INF-γ-treated THP-1 cells. Our results showed the possibility of the implication of PTK as a biochemical signaling pathway of this

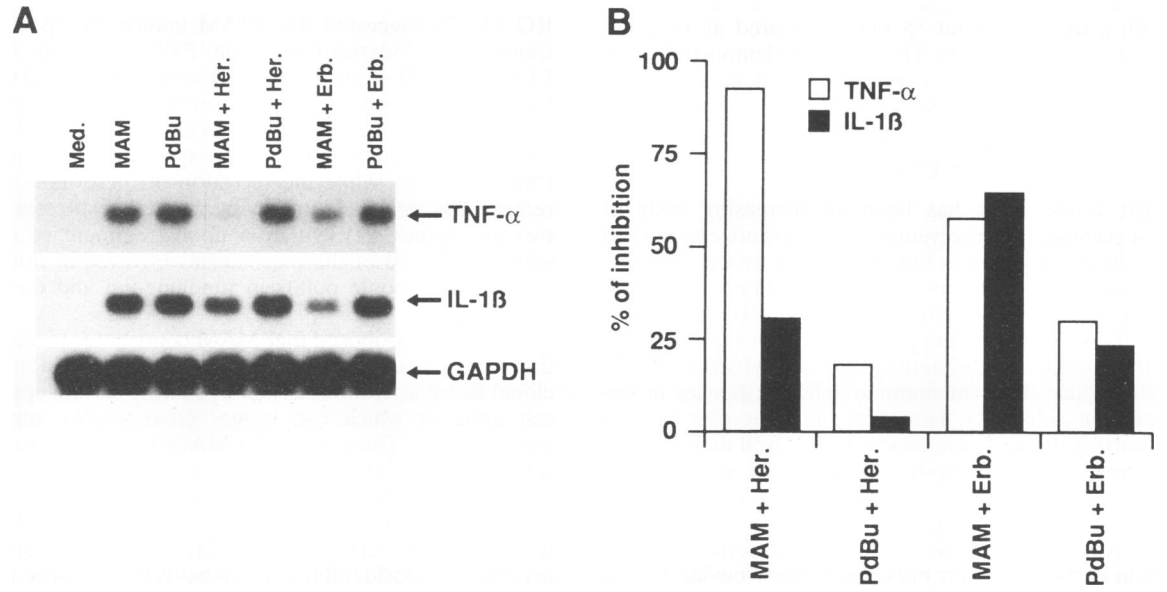

FIG. 8. Effect of herbimycin A and erbstatin (PTK inhibitors) on MAM-induced IL-1β and TNF-α mRNA expression. INF-γ-treated THP-1 cells were treated with medium alone, herbimycin A (Her.) (1 μg/ml) or erbstatin (Erb.) (5 μg/ml) for 1 h at 37°C. Cell viability was verified, and then cells were stimulated with medium alone, MAM (1:100), or PDBu (100 nM) for another hour at 37°C. Total RNA was isolated and the levels of IL-1β, TNF-α, and GAPDH mRNAs were analyzed by Northern blotting (A). Percent inhibition of the data shown in panel A was determined by densitometric analysis (B).

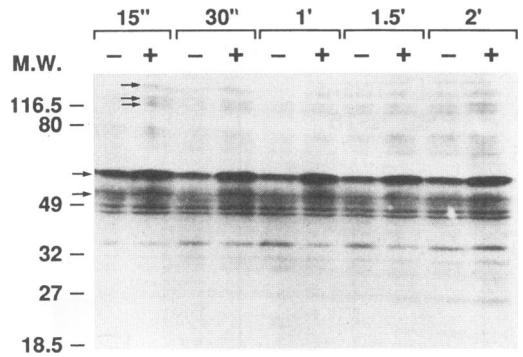

FIG. 9. MAM induces protein tyrosine phosphorylation in INF-γ-treated THP-1 cells. Antiphosphotyrosine Western blot of detergent lysates of INF-γ-treated THP-1 cells incubated for 15 min at 37°C and then stimulated with medium alone or MAM (1:20) at the indicated times (shown in seconds [″] and minutes [′]). Molecular weights are indicated in thousands. The densitometric analysis showed an augmentation in the intensity, of at least 2×, of five bands (see text), indicated by arrows. +, cells stimulated with MAM; −, cells with medium alone.

MAM activity. To further investigate this possibility, we analyzed the capacity of MAM to induce protein tyrosine phosphorylation in THP-1 cells. Cells were stimulated with MAM for various time intervals and were then lysed in detergent buffer. Lysates were analyzed by SDS-polyacrylamide gel electrophoresis followed by antiphosphotyrosine Western blotting. The densitometrical analysis showed that the addition of MAM to THP-1 cells augmented the tyrosine phosphorylation of several proteins. This response was observed as early as 15 s after the addition of MAM and persisted for about 20 min. A major protein with an approximate molecular weight of 62,000 to 66,000 was observed to be phosphorylated 15 s after the addition of MAM. Four other bands were slightly phosphorylated. Three of them, corresponding to proteins with approximate molecular masses ranging between 116 and 125 kDa, appeared as early as 15 s and persisted for 30 s, while the fourth, with a mass of about 55 kDa, appeared at 15 s and persisted for 1.5 min (Fig. 9). These results demonstrate that activation of PTK(s) can be a potential event in transmembrane signaling by MAM via MHC class II.

DISCUSSION

In recent years, there has been an increasing body of evidence suggesting the involvement of superantigens in both acute and chronic diseases in humans. MAM produced by *M. arthritidis*, associated with an autoimmune-like disorder in rodents with characteristics parallel to human RA, presents a useful tool for investigating the potential role of superantigens in the pathogenesis of autoimmune diseases. MAM has been reported to induce different immunological responses in immunocompetent cells (34). However, the molecular mechanisms permitting these responses are not yet well defined. The results of the present investigation demonstrate an important induction of IL-1β and TNF-α mRNAs in the INF-γ-treated THP-1 monocytic cell line after stimulation with MAM. These monokines play a crucial role in initiating autoimmune diseases, RA in particular. Their presence in the synovial fluid of patients with RA has been reported (28, 37). The contribution of these monokines to disease manifestation is mainly via their induction of functional alterations, in cells of various types present in the arthritic joints, resulting in the release of a panel of chemotactic mediators (1, 12, 33).

The adsorption of MAM solutions with an MHC class II-positive cell line resulted in a complete loss of monokine induction. These results, as well as those obtained with the U937 cell line, demonstrate clearly that the induction of these monokine mRNAs in THP-1 cells is critically dependent on the presence of MHC class II molecules. Hence, the induction by MAM of transcription of these proinflammatory monokines implicates, once again, MHC class II molecules as regulators of monokine gene expression. Moreover, this report and others, demonstrating the induction of monokine synthesis by *Staphylococcus aureus* superantigens, provide a basis for the consideration of MHC class II molecules as signal transducing receptors (24, 25, 7).

Our findings with PTK inhibitors, as well as those with antiphosphotyrosine Western blotting, demonstrate that PTK activation is involved in biochemical signaling through MHC class II molecules. The induction of tyrosine phosphorylation in THP-1 cells by MAM is a very rapid response (within 15 s). The pattern of tyrosine phosphorylation observed after stimulation with MAM resembles patterns obtained with cross-linked *S. aureus* superantigens (38). However, this similarity does not necessarily implicate the same routes to increase protein tyrosine phosphorylation after ligation of MHC class II molecules with the different superantigens. Moreover, the inhibition profiles of MAM-induced IL-1β and TNF-α gene expression obtained with the PTK inhibitors, each of which has a different mechanism of action, suggest that the gene expression of the two monokines may involve different PTK pathways. Identifying the kinases involved and the nature of their substrates will further clarify our understanding of PTK pathways as signaling events occurring via MHC class II. PKC has been previously implicated as an early biochemical event after the ligation of MHC class II molecules with Ia antibodies (6). Moreover, See and collaborators reported (39) that toxic shock syndrome toxin 1-induced secretion of IL-1β and TNF-α by human peripheral blood mononuclear cells is inhibited by both PKC and PTK inhibitors, and suggested that the two monokines are differentially regulated in response to induction by toxic shock syndrome toxin 1. Our data with the PKC inhibitor RO 318220 suggested that MAM induces IL-1β and TNF-α transcription independently from PKC activation. A study of LPS-induced IL-1 production and secretion showed that, while PKC is not involved in IL-1 production, it is importantly involved in its secretion (3). Hence, it is possible that MAM-induced IL-1β and TNF-α gene expression is independent of PKC activation, while the secretion of these monokines may require this signal. The study of the gene expression of these two monokines, of regulation upon treatment of monocytes with MAM, and of the mechanisms involved in this response can elucidate some points in fundamental and therapeutical research.

It has been proposed that MAM contributes to autoimmune diseases by activating autoimmune T cells, by inducing polyclonal B-cell activation (11), or by sharing certain epitopes with self antigens which can trigger cross-reactive autoimmune responses (8). The capacity of MAM to induce proinflammatory monokine transcription in monocytes can be another pathway of MAM contribution to autoimmunity, especially in the case of diseases such as RA with local inflammatory lesions. Recent reports (15, 17, 21, 30) have documented the presence of considerable overlap between Vβ usage in RA and Vβ usage in MAM-reactive T cells. Moreover, it has been shown that MAM can stimulate human cells to secrete immunoglobulin G and immunoglobulin M rheumatoid factors (13). This evidence, as well as the studies with animal models, reinforces the hypothesis of the contribution of MAM, or a

MAM-like superantigen, to the onset of RA. A recent report by Cole and collaborators (9) demonstrated that MAM can cause T-cell oligoclonal expansion in vivo. Moreover, the investigators hypothesized that, in certain instances, the profile of Vβ engaged by a superantigen may vary according to the specific MHC class II molecule or allele present; this may suggest the influence of MHC class II polymorphism on MAM-induced responses in humans. The oligoclonal expansion of $V\beta17^+$ T cells in rheumatoid synovial tissue of an RA patient has been recently reported (20). The relationship between MAM/MHC class II alleles associated with RA on the one hand and the Vβ usage in this disease on the other hand is worth investigating to elucidate the implication of MAM (or MAM-like) superantigen in RA.

The association of different autoimmune diseases with MHC class II alleles is strongly documented (41). However, the reason for and the nature of this association are still questions in autoimmunity. On the other hand, environmental factors could be considered important players in the onset of these diseases (29). In the light of evidence presented here, the novel role of MHC-class II molecules as regulators and signal-transducing molecules after their engagement with naturally occurring ligands (such as microbial superantigens) might be proposed as a link between autoimmune diseases, MHC class II, and environmental factors.

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