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Effect of Dexamethasone on Elevated Cytokine mRNA Levels in Chemical-Induced Hippocampal Injury

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An acute administration of the hippocampal toxicant trimethyltin (TMT) produced a specific pattern of neuronal necrosis in dentate granule cells with accompanying astrogliosis and initiation of a cytokine response within 24 hours. The purpose of this study was to examine the effects of the anti-inflammatory agent, dexamethasone (DEX), on the pattern of cytokine expression and neuronal degeneration occurring after an acute TMT injection. Dexamethasone (0.2 mg/kg or 10 mg/kg) was administered to 21-day-old male mice 1 hour prior to an injection of TMT hydroxide (2.5 mg/kg, i.p.). Mice receiving 0.2 mg/kg DEX received a second injection 6 hours after TMT. Twenty-four hours later, neuronal necrosis and astrogliosis were assessed and found to be similar in animals treated with TMT, either in the presence or absence of dexamethasone. Pretreatment with dexamethasone failed to prevent the neurodegeneration and astrogliosis. The TMT-induced injury response was represented in elevations of mRNA levels for the injury-associated host response genes glial fibrillary acidic protein (GFAP), EB22/5.3, and intercellular adhesion molecule-1 (ICAM-1). The combination of DEX and TMT produced increased elevation in mRNA levels for EB22/5.3 and ICAM, while GFAP levels remained the same as with TMT alone. The injury response from TMT was accompanied by elevations in mRNA levels for the cytokines tumor necrosis factor (TNF) α, TNFβ, and interleukin (IL)-1α. Treatment with dexamethasone prior to TMT resulted in significantly elevated levels of TNFα, TNFβ, and IL-1α as compared to TMT alone. These data represent the inability of glucocorticoids to downregulate the injury response in rat hippocampus following a systemic injection of TMT and suggest a stimulation and "priming" of hippocampal cells by dexamethasone. J. Neurosci. Res. 57:916–926, 1999. **Published 1999 Wiley-Liss, Inc.**[†]

Key words: trimethyltin; dexamethasone; hippocampus; GFAP; cytokines

INTRODUCTION

Immediately following injury, distinct phases of the host defense response are triggered by various antigens and consist of cell recruitment and induction of cytokines that contribute to activation of proinflammatory, immunostimulatory, and catabolic responses. Cytokines initiating these early events include interleukin-1 (IL-1) and tumor necrosis factor (TNF) which stimulate a set of chemotactive factors whose primary function is to promote recruitment of inflammatory cells. In the nervous system, glial cells become activated from injury and emit an inflammatory-like response including the release of proinflammatory cytokines. This gliotic process and associated secreted factors are thought to be detrimental to neurons (Giulian et al., 1993; Gelbard et al., 1995) and inhibit neuronal regeneration by physical or biochemical impediments (Lees, 1993; Fitch and Silver, 1997).

Glucocorticoids (GCS) are potent anti-inflammatory steroids which can inhibit both central and peripheral cytokine synthesis and action (Guyre et al., 1988; Bateman et al., 1989; Almawi et al., 1996; Cato and Wade, 1996). Anti-inflammatory actions of glucocorticoid administration are usually evident in acute injury and short-term pretreatment with drugs such as dexamethasone (DEX). While glucocorticoids have limited, if any, demonstrated effects in the uninjured brain, they have the capability to suppress many functions of activated monocyte/macrophages in the injured brain including production and release of cytokines. Dexamethasone has been reported to inhibit the release of TNFα, IL-1, and IL-6 protein (Leu et al., 1988; Zuckerman et al., 1989; Chao et al., 1992). Direct effects upon activated microglia and astrocytes have been demonstrated in vitro with inhibition of TNF protein production and release (Chao et al., 1992; Brenner et al., 1993). Such acute effects of glucocorticoids

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may be beneficial in the downregulation of cytokine secretion and the inflammatory response. In fact, administration of dexamethasone has resulted in beneficial effects in spinal cord trauma (Hall and Braughler, 1982; Kiwerski, 1993), hypoxia-ischemia (Tuor, 1997; Barks et al., 1991), irradiation (Hong et al., 1995), and bacterial meningitis (Pfister and Scheld, 1997). While dexamethasone has been shown to protect against the effects of acute brain injury, adverse effects have been reported with chronic use (Sapolsky, 1985, 1987; Sapolsky and Pulsinelli, 1985; Tombaugh, 1992; Goujon et al., 1997). Glucocorticoid effects on more progressive degenerative processes in the brain in the absence of infiltrating cells from the periphery have yet to be demonstrated.

Using the prototypic hippocampal neurotoxicant, trimethyltin, our laboratory recently reported an association of proinflammatory cytokines with a gliotic response in the hippocampus of mice in the absence of infiltrating cells (Bruccoleri et al., 1998). Within 12 hours of acute administration of trimethyltin (TMT), mRNA levels for the proinflammatory cytokines TNFα and IL-1α were significantly elevated in hippocampus. By 24 hours, morphological evidence of both neuronal degeneration and astrogliosis were prominent in the hippocampus. In the same area, there was a minimal increase in the number of ramified microglia. By 72 hours, neuronal necrosis was prominent in the dentate granule cells, as were activated ameboid microglia expressing mRNA for TNFα and IL-1α. The correlation between the increased expression of TNFα and IL-1α mRNAs and the neuropathological changes suggest a causal relationship. We examined the effects of dexamethasone on TMT-induced cytokine expression to further address the role of cytokines as a causal agent in this model of chemical-induced neurodegeneration.

MATERIALS AND METHODS

Materials

Materials were purchased as follows: trimethyltin hydroxide from Alpha Products (Danvers, MA); proteinase K from Boehringer Mannheim Corp. (Indianapolis, IN); Trizol™, oligo d(T) 12–18 Primer, and reverse transcriptase enzyme from Gibco BRL (Gaithersburg, MD); dNTPs from Perkin Elmer, Cetus (Norwalk, CT); T7 RNA polymerase from Promega (Madison, WI); RNase A and RNase T1 from Ambion (Austin, TX); phenol:chloroform:isoamyl alcohol (25:24:1); bromophenol blue and xylene cyanol FF from BioRad (Melville, NY). Hyperfilm-MP was purchased from Amersham (Buckinghamshire, England). The polyclonal antibody to glial fibrillary acidic protein (GFAP) was obtained from Dako Corp. (Carpinteria, CA); Dexamethasone, 3,3′-diaminobenzidine tetrahydrochloride (DAB), and lectin (Bandeiraea Simplicifolia BS-4) from Sigma-Aldrich Research, (St. Louis, MO); VectastainTM Elite immunohistochemistry kit from Vector Laboratories (Burlingame, CA); 32P-ATP and UTP[α-^{32}P] 3,000 Ci/mmol from New England Nuclear/Dupont (Wilmington, DE). RNase protection assay multiprobe sets for intercellular adhesion molecule-1 (ICAM-1), inducible nitric oxide synthetase (iNOS), A20, Mac-1 (Macrophage-1 antigen), EB22, and GFAP; Tumor necrosis factor - alpha and beta (TNFα, TNFβ,), interleukin -1α–1β, 2, 3, 4, 5, 6, (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6), interferon gamma (IFNγ,), and L32 were generous gifts of Dr. Iain Campbell (Scripps Research Institute, La Jolla, CA). AP-1 (22-mer; 5′-CTAGTGATGAGTCAGCCGGATC3′) oligomers was purchased from Research Genetics (Huntsville, AL), and T4 polynucleotide kinase from USB/Amersham (Cleveland, OH). All other chemicals were of reagent grade.

Animals

Twenty-one-day-old male CD1 mice (Charles River Breeding Laboratories; Raleigh, NC) were housed in a dual corridor, semi-barrier animal facility at a constant temperature (21°+2°C), humidity (50%+5%), and on a 12-hr light/dark cycle. Food (autoclaved NIH 31 rodent chow) and deionized, reverse osmotic-treated water were available ad libitum. Sentinel animals recorded negative for pathogenic bacteria, mycoplasma, viruses, ectoparasites, and endoparasites. Mice were randomly assigned to experimental groups and received either a single intraperitoneal (i.p.) injection of trimethyltin hydroxide (2.5 mg/kg body wt; TMT) or saline in a dosing volume of 4 ml/kg body wt. Within each of these groups a subset of animals received dexamethasone (0.2 mg/kg body wt in corn oil, i.p.) 1 hour prior to and 6 hours after TMT injection, and a second subset received dexamethasone (10 mg/kg) 1 hour prior to TMT. Each group had a concurrent control group injected with corn oil vehicle according to the same dosing schedules. The oil suspension was used to create a dosing model of slow release and continued elevation of dexamethasone. Based upon early studies examining the time course of TMT-induced neuropathology and elevated cytokine mRNA levels, animals were examined at 24 hours following injection. Animals were lightly anesthetized with carbon dioxide, decapitated, brains excised, the hippocampus dissected, rapidly frozen on dry ice and stored at -80°C. All experiments were conducted in compliance with a NIH/NIEHS Animal Care and Use Committee approved animal protocol.

Histopathology

Animals were sacrificed, brains bisected in the midsagittal plane, and immersion-fixed in 4% paraformal-

dehyde (PBS: 0.1 M, pH 7.2) overnight at room temperature. Brains were then dehydrated in a series of ethanol concentrations, embedded in paraffin, and 8 μm sections cut. Cellularity and neuronal necrosis were visualized by routine Hematoxylin and Eosin (H&E) staining.

Microglial cells were identified by peroxidase-labeled lectin (Bandeiraea Simplicifolia BS-I, B4; 1:10) binding using the method of Streit and Kreutzberg (1987), with slight modification. Sites containing microglia bound peroxidase-lectin conjugates were visualized by nickel-enhanced DAB substrate. Astrocytes were identified by immunohistochemistry using antibodies to GFAP. Briefly, rehydrated sections were treated with 3% H_2O_2 for 10 min to remove endogenous peroxidase activity, rinsed in PBS, and incubated with nonimmune goat serum (in 1% bovine serum albumin (BSA)/PBS) for 20 min prior to a 60-min incubation with polyclonal rabbit anti-cow GFAP (1:2,000 in 1% BSA/PBS) and a 30-min incubation with a secondary IgG antibody then VectastainTM Elite ABC for 30 min, rinsed, and stained with a DAB substrate.

RNase Protection Assays

RNase protection assays were conducted with two probe sets. One set contained probes for: ICAM-1, iNOS, A20 (a cytokine-inducible response gene), Mac-1 (Macrophage-1 antigen), EB22 (an acute-phase response gene encoding a protein homologous to human ACT (Inglis et al., 1991) activated by the host immunoinflammatory response), and GFAP (the major intermediate filament protein specific for astrocytes) as previously described by Campbell et al. (1994). A second set contained probes for: TNFα, TNFβ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IFNγ and L32 as described by Hobbs et al. (1993).

A 1-μl aliquot of an equimolar pool of plasmid templates was used for the synthesis of ^{32}[P]-labeled cRNA probe set using a T7 RNA polymerase. Briefly, a 10-μg aliquot of total RNA was hybridized with 8 μl of hybridization buffer and 4.4 × 105 cpm of ^{32}[P]-labeled probe overnight at 56°C. Individual samples of total RNA isolated from hippocampal tissue with Trizol™ reagent were digested in a 100-μl solution of RNase A and RNase T1 cocktail (1:400) and treated with 0.5 mg/ml proteinase K. Protected fragments were extracted with phenol: chloroform:isoamyl alcohol (25:24:1), ethanol-precipitated, and dissolved in 80% formamide containing bromophenol blue and xylene cyanol FF. Individual fragments were separated by 5% acrylamide/8 M Urea sequencing gel electrophoresis and visualized by autoradiography (Hyperfilm-MP). The optical density of each fragment within a lane was determined by video densitometry using an Eagle Eye II Still Video System (Stratagene, Inc., La Jolla, CA). Densitometric analysis of the captured image was performed using NIH Image 1.54 software.

Electrophoresis Mobility Shift Assay

AP-1 binding activity was determined by a modification of the gel electrophoresis DNA binding assay described by Pennypacker et al. (1994). Briefly, the AP-1 (22-mer; 5′-CTAGTGATGAGTCAGCCGGATC3′) oligomers (Research Genetics, Huntsville, AL) were labeled with ^{32}[P]-ATP (New England Nuclear/Dupont, Boston, MA) using 6–10 U of T4 polynucleotide kinase (USB/Amersham). Binding reactions (30 μl) were performed at room temperature. The reaction mixture contained 50 μg of nuclear protein in 20 mM Tris-HCl (pH 7.8), 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 5 mM dithiothreitol, 50 μg /ml bovine serum albumin, 100 μg/ml of sonicated salmon sperm DNA, 10% glycerol, and approximately 0.1 ng (2×10^5 cpm) of specific probe. Protein-DNA complexes were separated on a 5% nondenaturing polyacrylamide gel. Gels were electrophoresed at 125 V in 50 mM tris-borate containing 1 mM EDTA, dried, and placed on autoradiographic film overnight. For characterization of DNA binding activity, the nuclear protein extract was preincubated for 10 min prior to the addition of labeled probe with a 100-fold excess of unlabeled oligomers.

Statistical Analysis

The response of each mRNA was calculated relative to corresponding L32 mRNA level for 6 animals per group. Data for each mRNA transcript were analyzed by an analysis of variance (ANOVA). Subsequent independent group comparisons were conducted using a Fisher's LSD post-hoc analysis. A statistical significance level was set at $P < 0.05$.

RESULTS

Histological Alterations in the Hippocampus

Twenty-four hours after TMT administration, neuronal necrosis characterized by nuclear pyknosis, and karyolysis was evident in dentate granule cells (Fig. 1). While the localization of necrosis to the dentate was not altered, a slight decrease in the number of hyperchromatic neuronal nuclei was evident with DEX pretreatment (Fig. 1). At the same time point, an increase in GFAP immunoreactivity showing astrocyte hypertrophy was evident throughout the hippocampus (Fig. 2). Consistent with previous studies (Bruccoleri et al., 1998), morphological evidence of microglia activation as determined by lectin histochemistry was not present at this early time point. However, there was an increase in overall lectin staining in the perivascular cell lining following TMT exposure (data not shown), suggesting an increase in either the

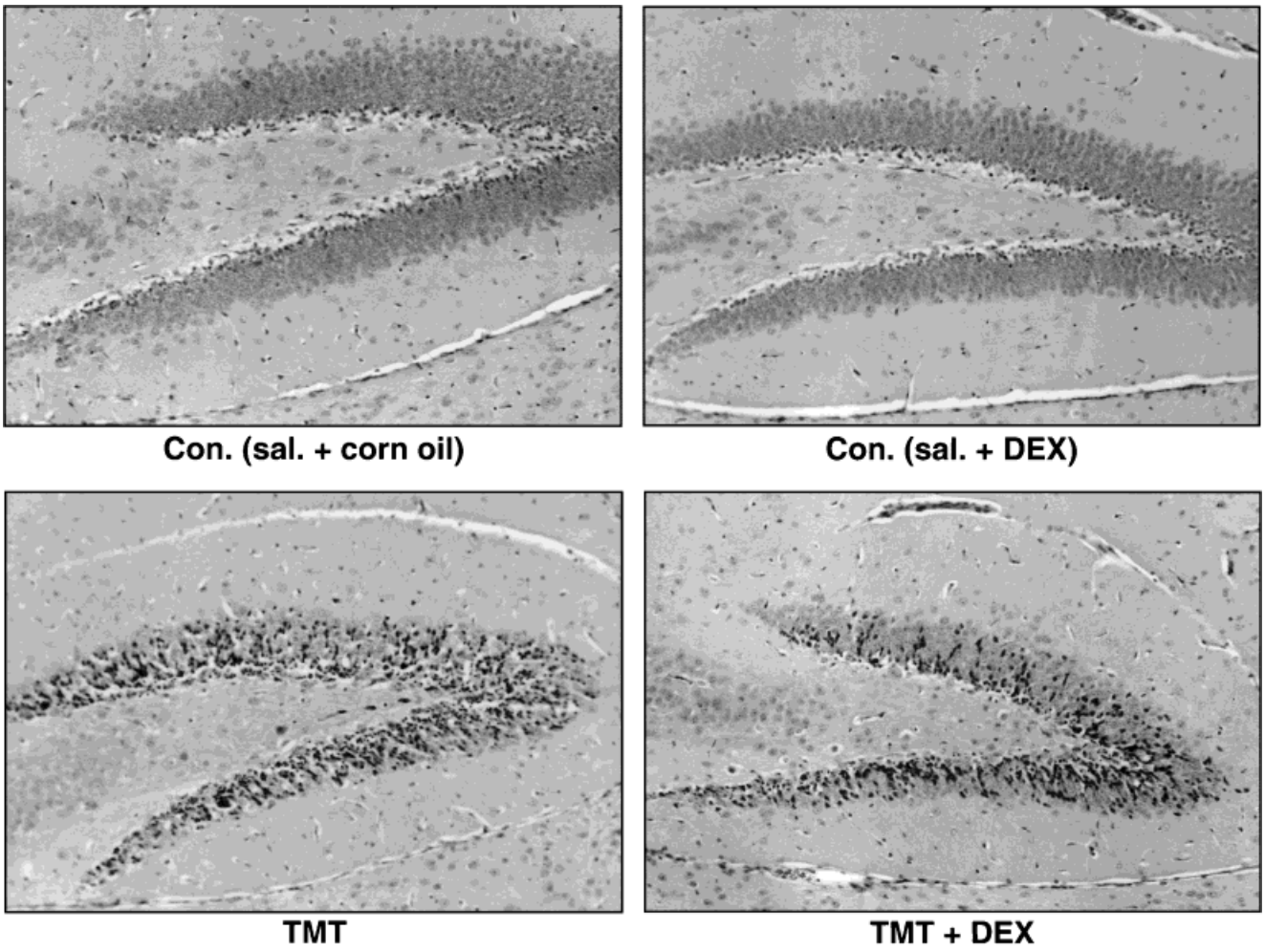

Fig. 1. Hematoxylin and Eosin staining of hippocampal dentate granule cells 24 hours following an acute intraperitoneal injection of either saline (sal) controls (Con) or trimethyltin (TMT; 2.5 mg/kg body wt) each in the presence (DEX) or absence (corn oil) of dexamethasone pretreatment. Severe neuronal necrosis induced by TMT was characterized by nuclear pyknosis and karyolysis in the dentate granule cells. Dexamethasone (DEX) pretreatment did not alter the pattern of neuronal necrosis in that necrosis continued to be localized to the dentate; however, the number of hyperchromatic neuronal nuclei appeared to be decreased. Magnification ×350.

perivascular cells or the juxtavascular microglia. Such a staining pattern was not present in animals pretreated with DEX. Thus, while this anti-inflammatory agent was able to modulate perivascular cell responses, it was unable to alter the response of resident cells of the brain or to attenuate either the hippocampal neuronal necrosis or reactivity of GFAP-positive astrocytes induced by TMT.

RNase Protection Assays

Consistent with the morphological changes in astrocytes, mRNA levels for the astrocyte specific protein GFAP were elevated by TMT ($P < 0.01$) and pretreatment with DEX showed no modulation of this effect (Figs. 3, 4). As compared to saline-treated mice, TMT exposed mice showed a 3-fold elevation in ICAM-1 mRNA level ($P < 0.05$). Pretreatment with DEX at the lower dose (0.2 mg/kg × 2) showed no modulation of the TMT-induced elevation, while the higher dose of DEX (10 mg/kg) significantly increased ($P < 0.01$) the level over that seen with TMT alone (Figs. 3, 4). TMT exposure produced no significant elevation in EB22 mRNA levels; however, the combined dosing of TMT and DEX (10 mg/kg) did produce a significant elevation ($P < 0.05$) over all other dose groups. The significant reduction ($P < 0.05$) in mRNA levels for Mac-1 in the TMT/DEX (10 mg/kg) group (Figs. 3, 4) strongly suggested that the pretreatment with DEX was successful in attenuating the generalized inflammatory response. No

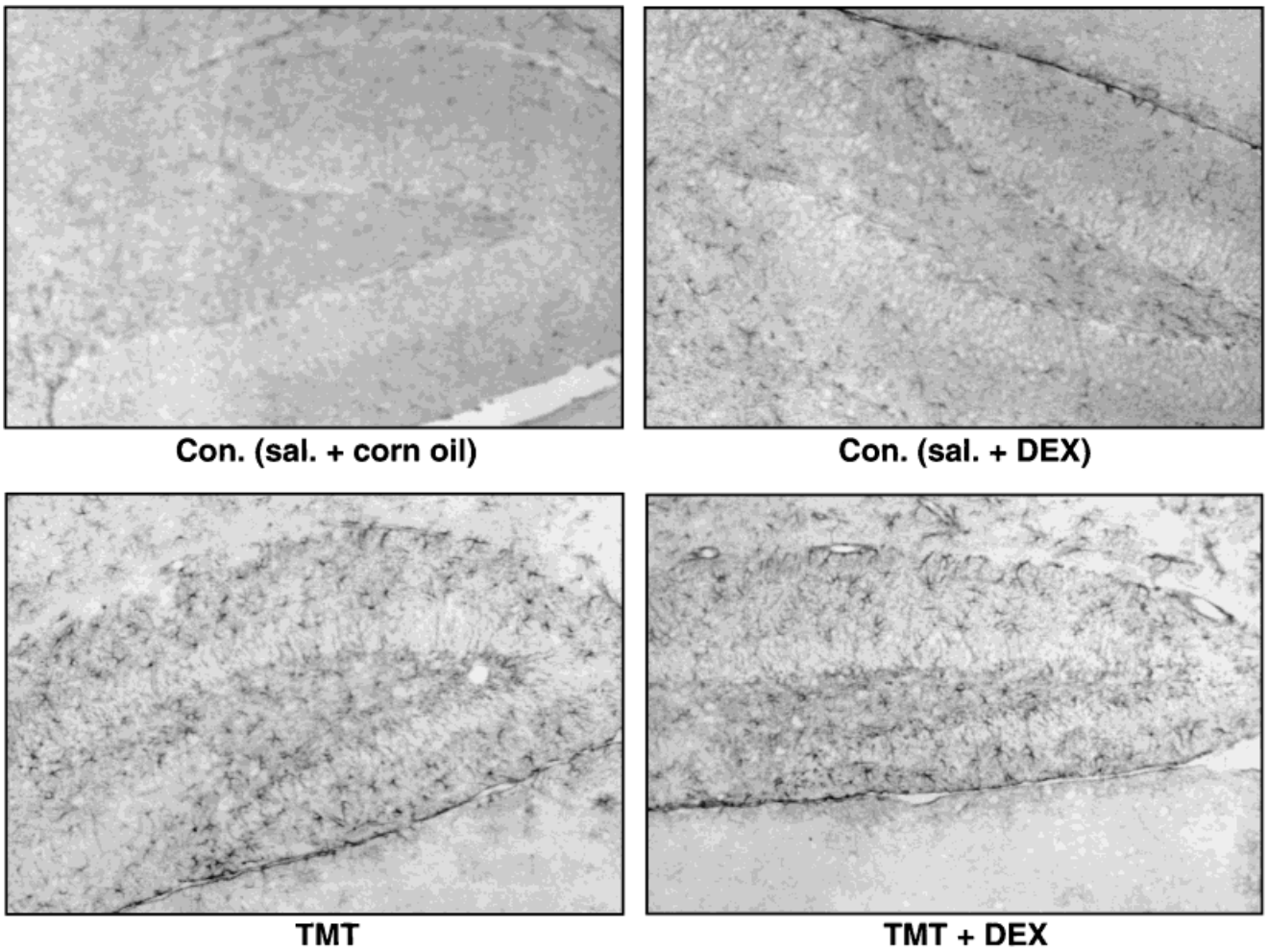

Fig. 2. Glial fibrillary acidic protein (GFAP) immunoreactivity in the hippocampal dentate region 24 hours following an acute intraperitoneal injection of either saline of TMT (2.5 mg/kg body wt) each in the presence or absence of DEX pretreatment. Astrocytes were evident throughout the hippocampus and displayed dense immunoreactive cell bodies and processes characteristic of astrocyte reactivity as compared with the thin processes evident in the same region of control tissue. Magnification ×350.

changes were seen in levels of iNOS and A20 mRNA. No changes in any of the host response mRNAs were seen with DEX alone.

Cytokine mRNA levels in the hippocampus were examined by a multi-probe ribonuclease protection assay (Figs. 5, 6). Twenty-four hours after a single i.p. injection of TMT hydroxide, TNFα and IL-1α mRNA levels were elevated by 10-fold ($P < 0.01$) and 3-fold ($P < 0.05$), respectively, relative to levels found in saline-injected animals. Dexamethasone alone had no effect on mRNA levels for any cytokine examined, but pretreatment with either dose of DEX augmented the elevation induced by TMT. The combined dosing of DEX (either dose level) and TMT resulted in an approximate 2-fold increase in TNFα; and IL-1α mRNA levels relative to the increase induced by TMT alone ($P < 0.01$; Fig. 6). TNFβ mRNA levels were elevated 2-fold by TMT exposure ($P < 0.05$) and 4-fold ($P < 0.01$) when combined with 10 mg/kg DEX (Fig. 6). Dexamethasone alone had no effect on levels of TNFβ mRNA. Consistent with previously reported findings (Bruccoleri et al., 1998), no elevations were seen in mRNA levels for IL-4, IL-2, IL-6, or IFNγ following TMT administration and pretreatment with dexamethasone did not alter this profile.

Effect of TMT and DEX on AP-1 Transcription Factors

AP-1 DNA binding activity was examined by electrophoresis mobility shift assay (EMSA). Control

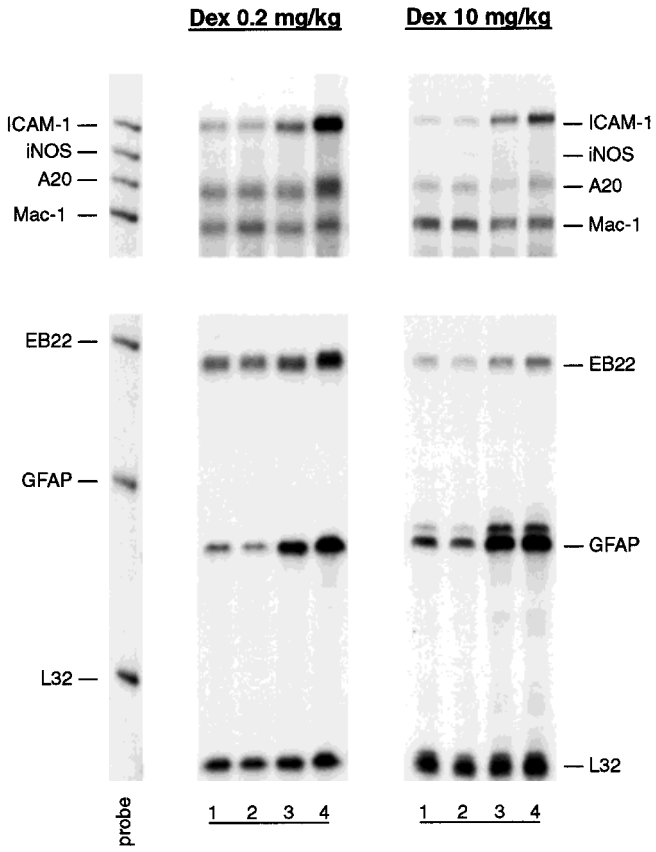

Fig. 3. Representative autoradiograph of ^{32}[P]-radiolabeled RNA protected fragments following RNase protection assay. Data represents the profile of mRNA for intercellular adhesion molecule-1 (ICAM-1), nitric oxide synthetase (iNOS), A20, macrophage-1 antigen (Mac-1), EB22, and GFAP in individual dose groups: **lane 1**, corn-oil + saline; **lane 2**, DEX + saline; **lane 3**, corn-oil + TMT (2.5 mg/kg); and **lane 4**, DEX + TMT (2.5 mg/kg) for both 0.2 mg/kg and 10 mg/kg DEX pretreatment.

mouse hippocampus showed constitutive levels of AP-1 DNA binding activity (Fig. 7, lane 1). Within 24 hours following administration of TMT, the DNA binding of AP-1 was slightly increased (Fig. 7, lane 3) compared to control mice. Animals that received DEX alone showed a significant increase in AP-1 DNA binding activity (Fig. 7, lane 2) as compared to either the control or TMT-treated mice. In agreement with the effect of the coadministration of TMT and DEX on the cytokine mRNA levels, mice that received DEX followed by a TMT injection showed the highest elevation of AP-1 DNA binding activity (Fig. 7, lane 4).

DISCUSSION

In this study, we used dexamethasone as an anti-inflammatory and immunosuppressive agent to examine the role of immune-mediated responses in the process of chemical-induced neurodegeneration. While the majority of studies have emphasized the immunosuppressive role of glucocorticoids, both immunosuppressive and immunoenhancement effects can occur (Munck and Naray-Fejes-Toth, 1994; Wilckens, 1995; Jefferies, 1994; De-Rijk and Berkenbosch, 1994). For example, the synthetic glucocorticoid, DEX, has been reported to be ineffective in suppressing the activation and proliferation of microglial cells in the optic nerve and superior colliculus following a nerve crush (Castano et al., 1996). In the present study, we showed that acute administration of the hippocampal toxicant, trimethyltin, produced a specific pattern of neuronal necrosis and that while this damage was associated with an elevation in proinflammatory cytokines, pretreatment with dexamethasone showed minimal attenuation. TMT induced an elevation in proinflammatory cytokine mRNA levels and combined treatment with dexamethasone showed even greater elevations in mRNA levels for TNFα, TNFβ and IL-1α and the injury-associated host-response genes EB22/5.3 and ICAM.

Within 24 hours, TMT produced astrocyte reactivity with a concurrent increase in the mRNA levels for the structural protein, GFAP (Bruccoleri et al., 1998). In the present study, astrocyte reactivity following TMT as indicated by immunohistochemistry and mRNA levels for GFAP was similar in both the presence or absence of DEX. These results suggest that dosing with DEX failed to modulate the astrocytic component of this injury response. This finding is consistent with a previous study by O'Callaghan et al. (1991) that demonstrated the inability of glucocorticoids to regulate the expression of GFAP in rat brain following either a stab wound or systemic injection of TMT. In an injury response of the brain, an elevation in GFAP mRNA is often accompanied by an elevation in the mRNA for the astrocyte-associated serine proteinase inhibitor, EB22/5.3. Such proteinase inhibitors can be induced by oxidative damage, cytokines, and inflammatory reactions, and serve to neutralize proteolytic enzymes released from inflammatory cells (Inglis et al., 1991; Abraham, 1992). EB22/5.3 mRNA levels were only slightly elevated by TMT; a low dose of DEX did not change this level of elevation; however, with 10 mg/kg DEX, a significant elevation was seen in the TMT-exposed hippocampus. A similar dose-related elevation of TNFβ was seen between the low and high dose of DEX.

mRNA levels for ICAM-1, a receptor for the integrins LFA-1 and Mac-1 required for leukocyte migration and extravasation (Dustin et al., 1986; Rothlein et al., 1986; Marlin and Springer, 1987; Smith et al., 1989; Carlos and Harlan, 1994), was elevated by TMT exposure. A low dose of DEX did not alter this response;

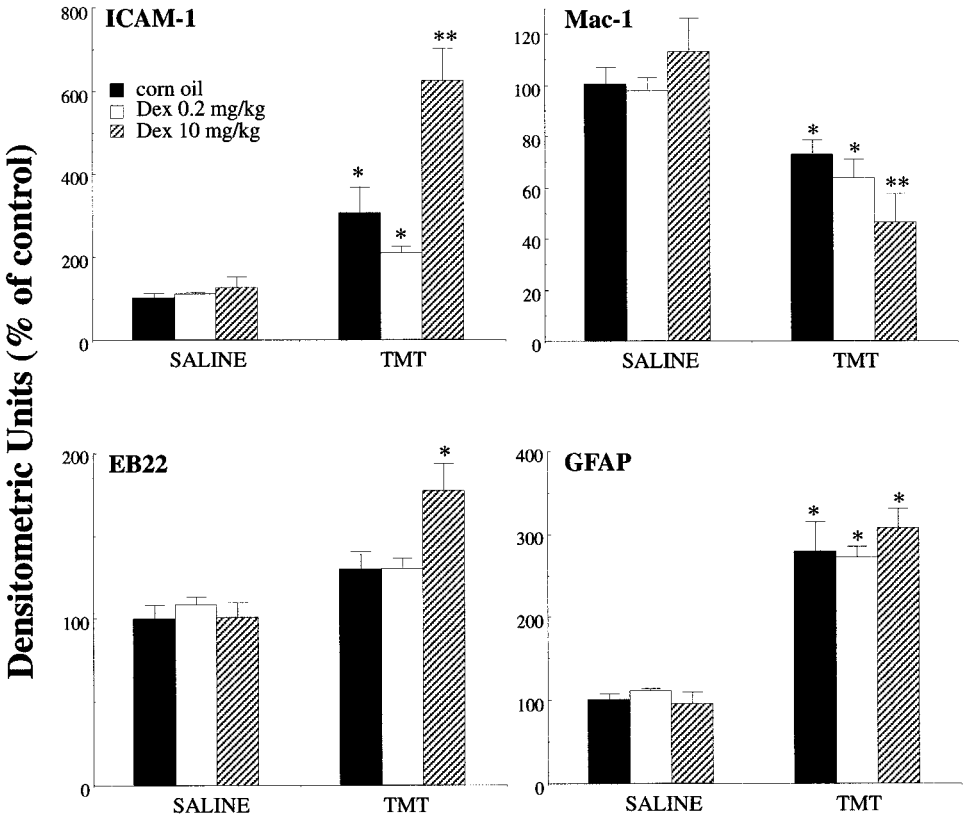

Fig. 4. Relative mRNA levels of ICAM-1, Mac-1, EB22, and GFAP in mouse hippocampus (n = 6) 24 hours following acute TMT i.p. injection (2.5 mg/kg body wt) in the presence and absence of dexamethasone pretreatment. Values represent the mean densitometric units as a percent of vehicle control group for each dosing condition. *$P < 0.05$, significant difference from vehicle control; and **$P < 0.05$, significant difference from corn oil + TMT exposure group.

however, the higher dose of DEX elevated this response substantially. While ICAM can serve as a receptor for Mac-1, the changes in Mac-1 mRNA levels did not parallel the changes in ICAM mRNA levels but instead showed a decrease with TMT/DEX. Within the central nervous system, a number of cell types can express ICAM-1 (neurons, astrocytes, microglia, and brain endothelial cells) and expression can be induced by TNFα, IL-1α, and IFNγ (Frohman et al., 1989; Shrikant et al., 1994, 1995). These cytokines have been shown to enhance expression of ICAM-1 and it may be concluded that the elevation seen with DEX/TMT is simply due to the activation of the cytokine cascade. However, the lower dose of DEX also elevated mRNA levels for TNFα and IL-1α to the same degree as the higher dose, yet had no effect on the TMT-induced elevation of ICAM-1. TNFβ was the only cytokine examined that was elevated in a similar manner as ICAM-1, suggesting a specific interaction.

One interpretation of these data from TMT-induced hippocampal injury is that DEX can interfere with the processing and production of the proinflammatory cytokine proteins. Available data suggest that many of the effects on cytokines act through both transcriptional and post-transcriptional mechanisms (Han et al., 1990, 1991). It has been reported that inhibition of cytokine expression by glucocorticoids occurs at the transcriptional level, resulting in an inhibition of cytokine mRNA expression and a parallel decrease in cytokine secretion (Boumpas et al., 1991; Amano et al., 1993; Barnes, 1998). Glucocorticoids have also been reported to antagonize the enhanced translational efficiency of TNFα mRNA that occurs with stimulation of murine monocytic cells. While these reports strongly suggest that GCS act proximally by reducing cytokine availability via transcriptional repression and/or destabilization of cytokine mRNA, other reports suggest that GCS do not alter cytokine mRNA stability or affect cytokine-mediated cellular activation (Villafuerte et al., 1995; Barnes, 1998). One possible explanation for the differing results comes from the cell culture work of Beutler et al. (1986) which demonstrated that TNF gene transcription is suppressed by 1 uM DEX at both the transcriptional and post-transcriptional level. However, once the post-transcriptional phase of TNF biosynthesis had been initiated, dexamethasone was incapable of regulating the process. The critical factor of time of exposure was also demonstrated in microglia cells stimulated with lipopolysaccharide (LPS) where simultaneous treatment with dexamethasone inhibited release of TNF, while a delay of 2 hours in dexamethasone treatment showed no inhibition of LPS stimulation (Chao et al., 1992). In vivo, LPS induction of serum TNF levels can be inhibited by DEX (10 mg/kg) if given 30 min prior to LPS, while a 24-hour pretreatment had a potentiating

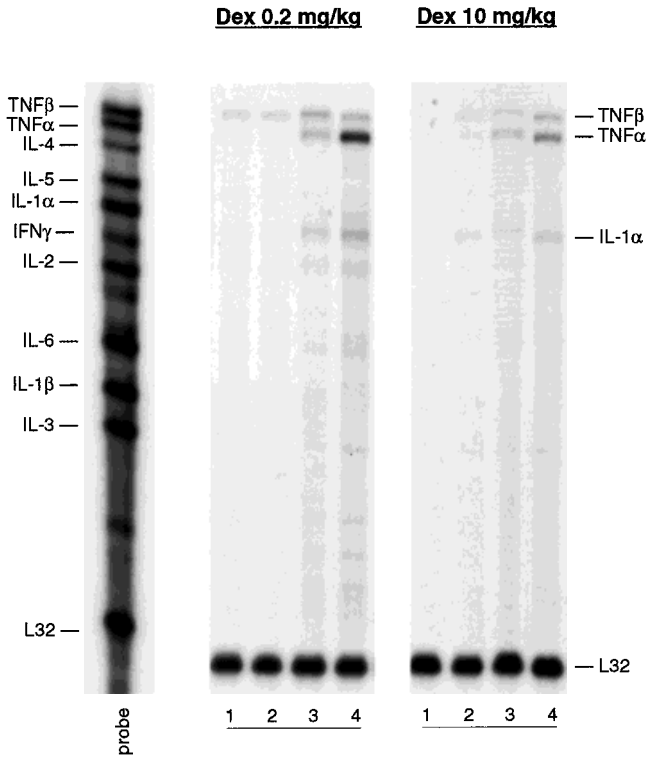

Fig. 5. Representative autoradiograph of ^{32}[P]-radiolabeled RNA protected fragments following RNase protection assay. Data represents the profile of mRNA for tumor necrosis factor (TNF)β, TNFα, and interleukin (IL)-1α, in individual dose groups: **lane 1**, corn-oil + saline; **lane 2**, DEX + saline; **lane 3**, corn-oil + TMT (2.5 mg/kg); and **lane 4**, DEX + TMT (2.5 mg/kg) for both 0.2 mg/kg and 10 mg/kg DEX pretreatment.

effect on TNF production (Barber et al., 1993; Mengozzi et al., 1994). The work of Barks et al. (1991) demonstrated that repeated treatment with dexamethasone for 3 days prior to an episode of cerebral hypoxia-ischemia in the neonatal rat reduced brain damage, while immediate pre- and post-treatment after the insult had no beneficial effect. Acute dosing with a high dose of dexamethasone immediately before neonatal unilateral cerebral hypoxia-ischemia increased mortality and brain damage in the rat (Altman et al., 1984). Data from the current study suggest that the mechanisms associated with such acute effects are associated with an increase in cytokine-mediated responses.

Some cytokines are secreted upon complete intracellular processing, while others are stored intracellularly and additional stimuli are required to trigger secretion. For example, IL-1 and TNFα mRNA can be induced by monocyte adherence and the protein produced only upon further stimulation of the cells. In many cases, the level of production induced from primed cells is at a much greater level than that which would have been induced directly (Renz et al., 1992; Hallett and Lloyds, 1995; Fathallah et al., 1998). It is possible that certain models of injury in the brain could increase the transcription of proinflammatory cytokines resulting in a ''primed'' system. The basal level of the system would then be altered and primed cells would over respond upon subsequent stimulation. This hypothesis of ''priming'' could explain the additional elevation in TNFα TNFβ and IL-1α mRNA levels with DEX.

In addition to the general cytokine-mediated effects on tissue injury, glucocorticoid receptor activation is attenuated by cytokines in immune and nonimmune tissue (Hill et al., 1986). The glucocorticoid response element (GRE) has been reported to play a role in the inhibition of cytokine expression by DEX (Knudsen et al., 1987; Kern et al., 1988; Lee et al., 1988; Kutteh et al., 1991; Amano et al., 1993; Monick et al., 1994). The work of Tanaka et al. (1997) demonstrated a cytokine-dependent regulation of glucocorticoid effects, suggesting that the inability of DEX to suppress microglial functions under pathological conditions could be related to the cytokine-induced downregulation of microglia glucocorticoid receptor expression and mineralocorticoid receptor activation. The process of priming could also be associated with a functional role for neuropeptide Y. Neuropeptide Y has been implicated in the regulation of neuronal activity both at basal functioning and during pathological hyperactivity (Vezzani et al., 1999). This peptide can be modulated by dexamethasone with treatment producing a rapid increase in synthesis and secretion from glucocorticoid sensitive neurons (Corder et al., 1988; Chen and Romsos, 1996). In the rat, TMT elevates the expression of neuropeptide Y Y2 receptors in the dentate gyrus during the initial phase of intoxication (Sadamatsu et al., 1998). While the rat model of TMT-induced neuropathology is characterized by a primary lesion in the CA3–4 pyramidal cells and not the dentate, the elevation in the Y2 receptor may be a generalized process in mediating the early responses associated with TMT-induced hippocampal damage.

The type II glucocorticoid receptors have a high affinity for the synthetic glucocorticoid, DEX (McEwen et al., 1986) and when activated, the receptor interacts with members of the Jun/Fos immediate early gene protein families to attenuate activation of the AP-1 transcription factor (Schule et al., 1990; Yang-Yen, 1990; Unlap and Jope, 1994; Wei et al., 1998). The activity in the hippocampus of DEX and cytokines is possibly mediated via the GRE that could account for the altered AP-1 binding evident in both the DEX- and the TMT-treated hippocampus. In work by Hess and Payvar (1992), it has been postulated that the glucocorticoid receptor in conjunction with some ancillary factor, possibly AP-1, binds two classes of GRE. In this model, withdrawal of glucocorticoids would result in the dissocia-

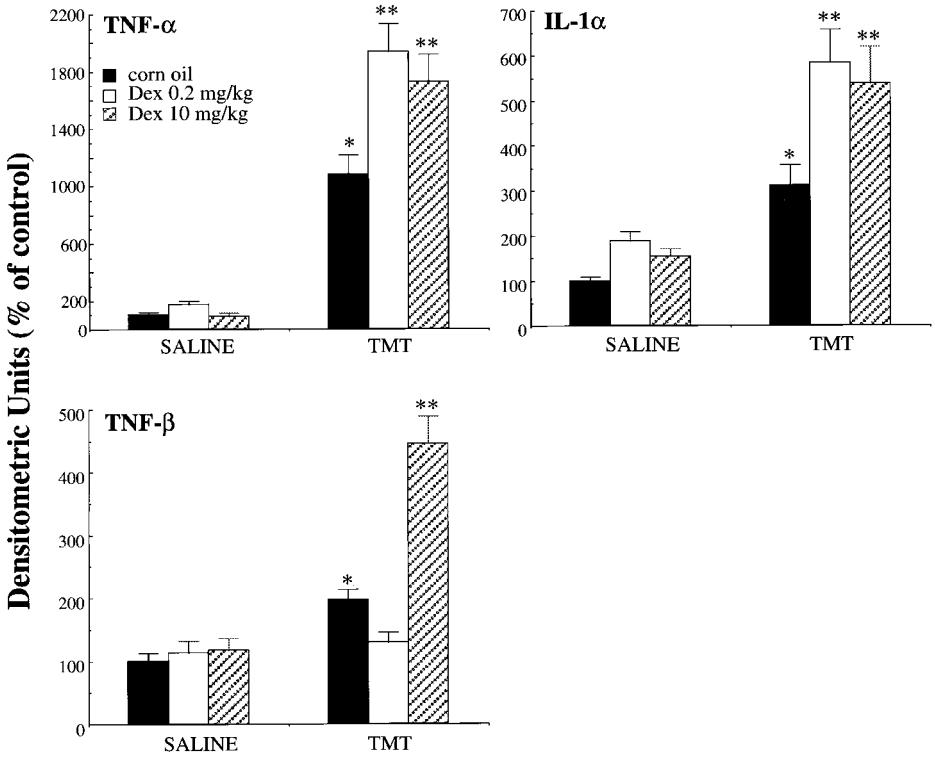

Fig. 6. Relative mRNA levels of TNFβ, TNFα, and IL-1α in mouse hippocampus (n = 6) 24 hours following acute TMT i.p. injection (2.5 mg/kg body wt) in the presence and absence of dexamethasone pretreatment. Values represent the mean densitometric units as a percent of vehicle control group for each dosing condition. $*P < 0.05$, significant difference from vehicle control; and $**P < 0.05$, significant difference from corn oil + TMT exposure group.

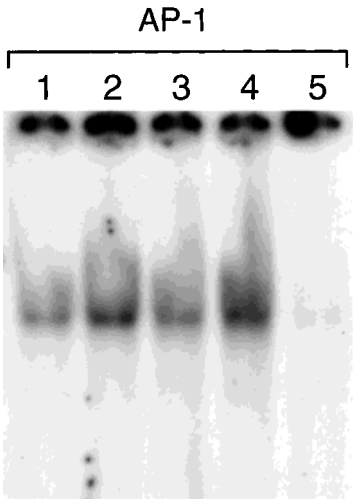

Fig. 7. Representative electrophoresis mobility shift assay gel for AP-1 DNA binding activity in the hippocampus 24 hours following saline (**lane 1**), 10 mg/kg DEX (**lane 2**), 2.5 mg/kg TMT (**lane 3**), or 10 mg/kg DEX and 2.5 mg/kg TMT (**lane 4**), negative control (**lane 5**). 12 13

tion of this ancillary factor from the glucocorticoid receptor, direct DNA binding and stimulation of transcription. This would result in exaggerated and sustained gene activation. This model offers an explanation for the DEX exposure related increase in mRNA levels for cytokines and various host-response genes induced by TMT.

Balance between activation and suppression of immune cells is critical in the host response against insult and prolonged injury. Excessive or prolonged activation may result in hypersensitivity, resulting in a more severe response to subsequent injury. In this paper, we have reported the results from studies examining the efficacy of the anti-inflammatory drug, DEX, to modulate the cytokine and glia responses induced by chemical perturbation to the hippocampus. While DEX offered no protection from the neurodegeneration, the results present interesting data with regard to both the injury-induced cytokine response and the effects of a synthetic glucocorticoid in the nervous system.

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