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Research Article

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Microglial Production of TNF-α Is Induced by Activated T Lymphocytes

Involvement of VLA-4 and Inhibition by Interferonβ-1b

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Abstract

TNF-α is a proinflammatory cytokine involved in many inflammatory conditions such as Crohn's disease, rheumatoid arthritis, cachexia, AIDS, and multiple sclerosis (MS). TNF-α is produced mainly by cells of the macrophage lineage, which includes microglia in the central nervous system. Here, we describe a mechanism through which TNF-α is generated by microglia. We show that activated human T lymphocytes induce the microglial production of TNF-α, and that is attenuated by a functional blocking antibody to CD49d, the alpha chain of the VLA-4 integrin on T cells. We also report that interferonβ-1b (IFNβ-1b), a drug that alleviates symptoms in MS, downregulates the expression of CD49d and reduces TNF-α production, mechanisms which can help account for its efficacy in MS. (*J. Clin. Invest.* 1997. 100:604–612.) Key words: cytokine • EAE • glia • macrophage • multiple sclerosis

Introduction

Multiple sclerosis (MS)[1] is a chronic inflammatory disease of the central nervous system (CNS). The infiltration of immune cells, such as T lymphocytes and macrophages, into the brain triggers a cascade of immunological reactions, regulated by cytokines, which lead to demyelination and loss of neurological functions (1, 2). An important regulator of these events is the potent proinflammatory cytokine tumor necrosis factor alpha (TNF-α). TNF-α can influence lymphocyte trafficking across endothelium by upregulating the expression of various adhesion molecules involved in this process (3), and it is implicated in the process of demyelination. Indeed, TNF-α directly induces in vitro the apoptotic death of the myelin-producing cells in the brain, the oligodendrocytes (4–6), and intravitreal injection of TNF-α causes demyelination of mouse optic nerve axons (7). The level of TNF-α is found to be elevated in the serum, cerebrospinal fluid, and brain lesions of MS patients, and is correlated with the disease activity (8–11). TNF-α is also implicated in the pathogenecity of an inflammatory disease of the CNS in mice, experimental allergic encephalomyelitis (EAE), often used as a model for MS. The administration of antibodies to TNF-α or soluble TNF-α receptors prevents the transfer of EAE and abrogates autoimmune demyelination (12–14). Moreover, during TNF-α receptor–mediated inhibition of EAE, lymphocyte trafficking into the CNS becomes impaired (15). Finally, transgenic mice overexpressing TNF-α in the CNS develop CNS inflammation and Wallerian degeneration (16).

Interferon beta-1b (IFNβ-1b), a recombinant and modified form of natural human IFNβ, is a drug used in the treatment for MS. Recent clinical trials have demonstrated that IFNβ-1b decreases the number of relapses in relapsing-remitting MS, and that it also reduces the frequency of lesion formation detected by magnetic resonance imaging (MRI) (17–19). In EAE, the systemic administration of IFNβ prevents the development of the disease (20), and the adoptive transfer of EAE can be inhibited when myelin-basic protein–(MBP) specific lymphocytes are pretreated with IFNβ (21). Interestingly, IFNβ-1b has been shown recently to decrease peripheral blood mononuclear cell production of TNF-α in MS patients (22).

The mechanism by which TNF-α is generated in the MS brain is unclear, but it is known that the resident CNS macrophage, the microglia, is an important source (9, 23). Because most of the T lymphocytes infiltrating the MS brains are activated (24), we investigated whether, and how, the interaction of activated T-lymphocytes with microglia in vitro can generate TNF-α, and whether IFNβ-1b can impact upon this interaction. This paper demonstrates that activated T lymphocytes can indeed induce the microglial production of TNF-α through a mechanism that appears to involve VLA-4, and that IFNβ-1b affects this interaction to help account for its efficacy in MS.

Methods

Cell cultures and treatment. Adult human microglia, of over 95% purity, were cultured from resected brain specimens of patients undergoing surgery to treat intractable epilepsy, as described previously (25, 26). Mononuclear cells were obtained from peripheral blood of healthy individuals or from epileptic patients, and were activated with an anti-CD3 antibody, OKT3 (1 ng/ml), for 72 h. The purity of T cells at the end of this activation period was > 90%, consisting of roughly equal quantities of $CD4^+$ and $CD8^+$ T cells (27). 2.5×10^4 microglial cells were plated per well of a 96-well tissue-culture plate. Activated T cells (5.0×10^4) were then added to the microglia culture for a period of 24 h coculture, after which the culture medium was collected for TNF-α measurements (see below). In some experiments, the medium conditioned by activated T lymphocytes for 72 h was added to microglia. We have determined previously that this activated T cell supernatant contains at least 100 U/ml of IFNγ (28). In other experi-

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1. *Abbreviations used in this paper:* CNS, central nervous system; EAE, encephalomyelitis; LFA-1 leukocyte function antigen-1; MS, multiple sclerosis; RT-PCR, reverse transcriptase-PCR; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

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ments, activated T cells were placed in cell culture inserts (Becton Dickinson, Lincoln Park, NJ), which were subsequently placed in close proximity to, but not contacting, the microglia.

Where noted, cells were treated with a recombinant form of human IFNβ, IFNβ-1b (provided by Berlex Laboratories, Richmond, CA, with a specific activity of 32×10^6 IU/mg of protein); this was the same preparation that has been demonstrated to have therapeutic efficacy in MS patients (17–19). Activated T cells, or microglia, were treated with IFNβ-1b for 72 h before their coculture with microglia or T cells respectively. 3 h before this coculture, another dose of IFNβ-1b was administered. Cells were then collected, washed, and used in coculture. Because IFNβ-1b has an antimitotic effect, equal numbers of control or IFNβ-1b treated activated T cells were added to microglia. To further control for this antiproliferative action, some T cells preparations were exposed to recombinant human IFNγ (Boehringer Mannheim Biochemica, Laval, Quebec). Finally, activated T cells (5×10^4) were also pretreated with mouse antihuman CD49d (Clone HP2/1; Serotec, Mississauga, Ontario), anti-LFA–1a (CD11a) (Becton Dickinson, Mississauga, Ontario), or purified mouse IgG1 (isotype control) antibody (Chemicon International, Inc., Temecula, CA), for 1 h, before they were cocultured with microglia.

TNF-α measurements. The level of TNF-α protein found in cell culture conditioned medium was measured using an ELISA kit (Cedarlane Labs Ltd., Hornby, Ontario), following detailed instructions by the manufacturer.

Reverse transcriptase-PCR (RT-PCR). Total RNA was extracted using TRIZOL reagent (GIBCO BRL, Burlington, Ontario) and mRNA levels of TNF-α and actin were determined using semi-quantitative RT-PCR. This method, including the primer sequences (obtained from Sheldon Biotechnology, Montreal), has been described in detail previously (29).

Immunohistochemistry. Live microglial cells (2.5×10^4), on polylysine-coated cover slips, were incubated with mouse antihuman vascular cell adhesion molecule-1 (VCAM-1) (1:100 dilution; Chemicon International, Inc.) or HHG (26) (the diluting medium of the antibody used as a control) for 1 h. Biotinylated antimouse immunoglobulin was then applied, and the signal was amplified using the ABC amplification kit (Vector Laboratories Inc., Burlingame, CA), followed by an incubation with FITC-conjugated streptavidin (1:100 dilution; Jackson ImmunoResearch Labs, Inc., West Grove, PA) for 1 h. Cells were then fixed for 10 min with 4% paraformaldehyde, and viewed using an immunofluorescence microscope.

Western blot. A primary antibody to CD49d, PS/2 (30, 31), was purified from cultured medium conditioned by the hybridoma cell line, CRL-1911 (American Type Culture Collection, Rockville, MD). 11 μg of protein extracts obtained from activated T cells that were either treated or not treated with 100 IU/ml IFNβ-1b was loaded onto gels and Western blot analysis were performed as described previously (32). The signal was amplified using the Vistra Fluorescence Western blotting kit (Amersham, Sunnyvale, CA). Protein expression levels were quantified using a Fluorimager (Molecular Dynamics, Sunnyvale, CA).

Flow cytometry. T cells were incubated with one of four antibodies (PS/2 and HP2/1 described above, anti-VLAα4–FITC from Serotec or anti-VLAα4–PE from Becton Dickinson) for 30 min and incubated at 4°C as described previously (27). After labeling with a secondary antibody (for PS/2 and HP2/1 only), stained cells were analyzed with an argon laser FACS® equipped with Consort 30 and Lysys II software; data was collected on 5,000 cells per condition.

Cross-linking of VCAM-1 on microglia. Adherent microglial cells were treated with 100 IU/ml of recombinant human IFNγ (Boehringer Mannheim, Ontario, Canada) for 24 h to maximize the number of VCAM-1 molecules expressed at the surface of the microglia. Cells were then treated with 10 μg/ml of the primary antibody, anti–VCAM-1 (Chemicon International, Inc.), for 10 min. Cells were washed twice with the culture medium to remove the excess antibody before being treated with a secondary antibody, goat-anti–mouse $F(ab)_2$ (20 μg/ml), used as a cross-linker. 6 h later, total RNA was extracted from cells for RT-PCR of TNF-α transcript levels.

Results

Activated T lymphocytes promote the production of TNF-α by microglia in a cell contact–dependent manner. Adult human microglial cells in culture can assume various morphologies (25), but the majority tend to be bipolar (elongated) (Fig. 1). In coculture with activated T cells, microglia becomes amoeboid (rounded) in appearance, a morphological transformation that is suggestive of an increased activation state (33). The change in morphology of microglia after coculture with activated T cells is apparent by 4 h, and is most marked at 24 h. Another feature of T cell–microglia coculture is that activated T cells

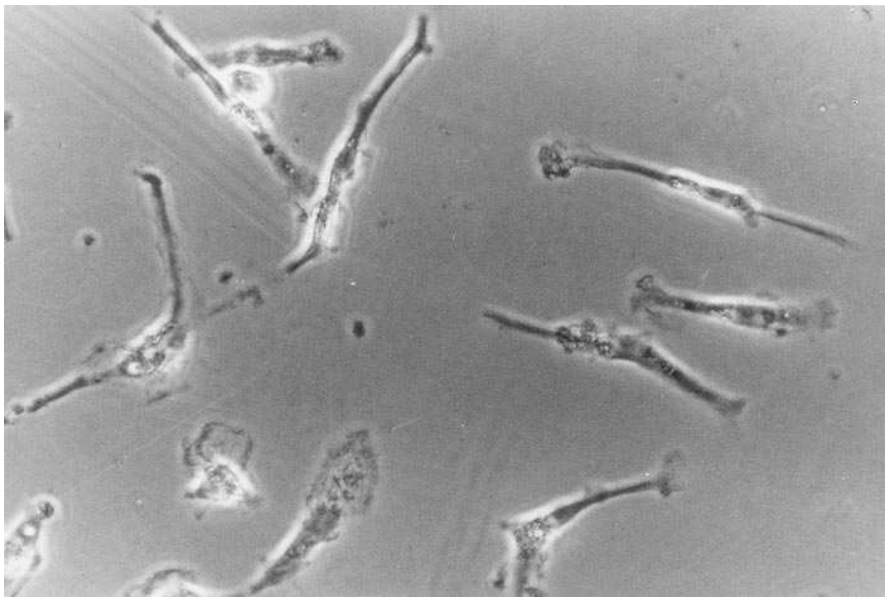


Figure 1. Phase contrast micrograph shows morphological appearance of microglia used in this study. ×500.

tend to clump and aggregate around microglial cells (results not shown).

When activated T cells were co-incubated with microglia for 24 h, the resultant conditioned medium, when assayed for TNF-α protein levels, contained significant amounts of TNF-α when compared to microglia or T cells by themselves (Fig. 2 *A*). To ascertain whether the TNF-α production was mediated by soluble factors or direct microglia–T cell surface interactions, the conditioned medium from activated T cells was added to microglia cultures; very little TNF-α production resulted, indicating that soluble factors were unlikely to be involved. This was supported by cell culture insert experiments where microglia cells were incubated in close proximity to, but not contacting, the T cells. Minimal TNF-α was generated under this condition (Fig. 2 *A*). These results suggest that T cell–microglia contact was necessary for the generation of TNF-α.

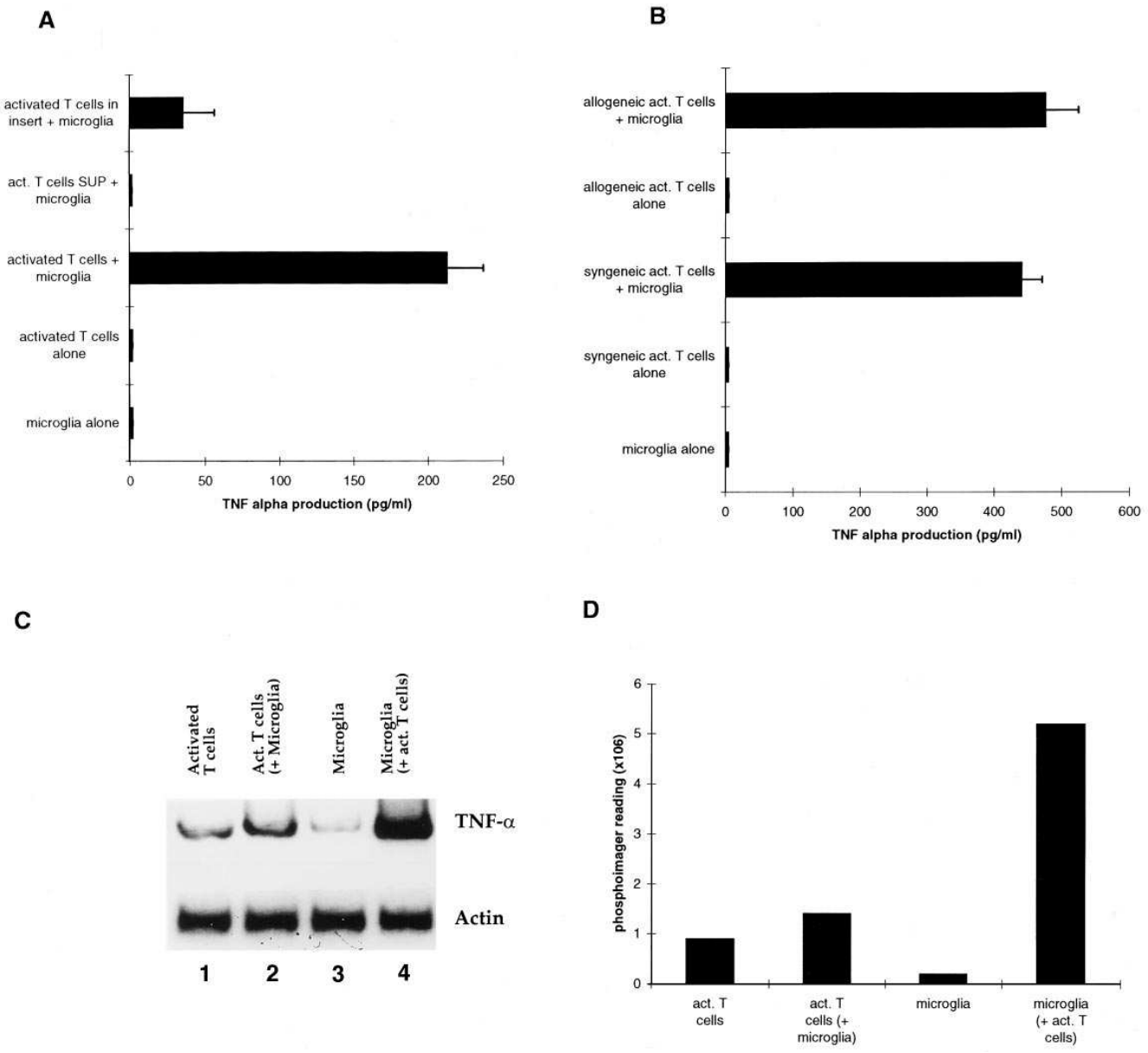
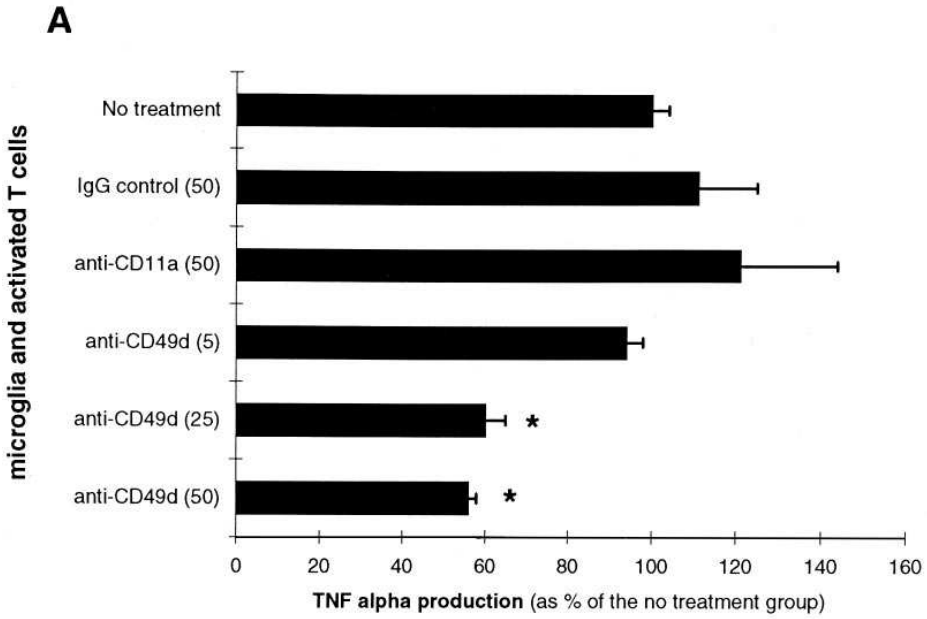


Figure 2. The coculture of activated T cells with microglia is a potent promoter of TNF-α production. (*A*) While microglia or activated T lymphocytes in isolation secrete negligible amounts of TNF-α into the culture medium, their coculture for 24 h resulted in substantial TNF-α production. This result (mean±SEM of triplicates), shown by a representative series of experiments in *A*, has been observed in eight other separate series, using human microglia and T cell populations from different donors. The TNF-a produced by T cell–microglia coculture cannot be reproduced if activated T cell supernatant (*SUP*), rather than cells, were exposed to microglia, or if T cells were contained within cell culture inserts in close proximity to, but not contacting, microglia. (*B*) Both allogeneic or syngeneic activated T cells induce the production of TNF-α by microglia, and to approximately the same extent. This result was also repeated when another series of allogeneic or syngeneic T cell–microglia cocultures was used. (*C*) The RNA isolated from activated T cells (lane *1*) or microglia (lane *3*) contains detectable amounts of TNF-α transcripts. The level of TNF-α mRNA in microglia increases significantly after their coculture with activated T cells (lane *4*); TNF-α mRNA elevates only modestly in T cells after their incubation with microglia (lane *2*). The level of actin transcripts shows equal loading for each sample. The relative level of the TNF-α mRNA is quantitated using a phosphoimager and plotted in *D*.

The cell surface molecules encoded by the major histocompatibility complex (MHC) gene clusters are crucial for antigen presentation to T cells and their activation and are the principal determinants of graft rejection. To determine whether the interaction between activated T cells and microglia is MHC restricted, i.e., that it involves MHC molecules, we incubated syngeneic (T cells and microglia were from the same donor) or allogeneic (T cells and microglia were from different donors) activated T cells with microglia. Both T cell types elicited the production of TNF-α (Fig. 2 *B*), indicating that the generation of TNF-α was not MHC restricted. Stout and colleagues (34, 35) have also reported that activated T cells can provide antigen-nonspecific, MHC-nonrestricted cognate signals that induce TNF-α production by IFNγ-primed peripheral macrophages.

T cells and microglia are both potential sources of TNF-α (Fig. 2 *C*). To determine which cell type, or both, is the principal source of TNF-α in the T cell–microglia cocultures, we took advantage of the fact that activated T cells, unlike microglia, are loosely adherent during their initial period of coculture with microglia. 6 h after the addition of activated T cells to microglia, the loosely adherent T cells were removed by several washes of culture medium and collected. Microscopy confirmed the removal of T cells from the adherent microglia. When the RNA of both cell populations was analyzed, the level of transcript for TNF-α was dramatically increased in the



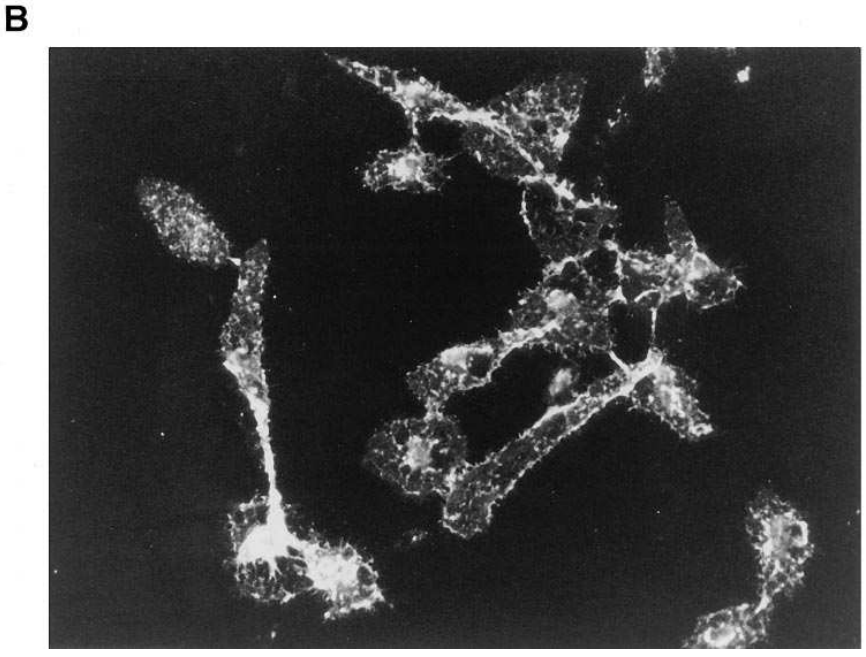


Figure 3. Activated T cells pretreated with a neutralizing antibody (HP2/1 clone) against the α chain of VLA-4 (CD49d or α4) are less able to promote the microglial production of TNF-α. (*A*) Levels of TNF-α in the cell culture supernatant when activated T cells are treated with different concentrations of anti-CD49d (concentrations in parentheses in μg/ml) before being exposed to microglia. Values are mean of triplicate analyses±SEM, and are normalized to the no treatment sample. Note that 50 μg/ml of an IgG isotype control, or an anti-LFA antibody (anti-CD11a), did not affect TNF-α levels, in contrast to the anti-CD49d treatment. $*P < 0.05$ compared to IgG control (one way ANOVA with Duncan's multiple comparisons). (*B*) Immunoreactivity of VCAM-1 of microglia, to confirm that this ligand for T cell integrin is present on the surface of microglia.

microglia fraction, but not in the T cell fraction (Fig. 2, *C* and *D*). We conclude that microglia were the major source of TNF-α in T cell–microglia cocultures.

TNF-α production in microglia–T cell cocultures is dependent on VLA-4. To elucidate the identity of the cell surface molecules involved in the interactions between microglia and activated T lymphocytes in producing TNF-α, we studied the contribution of an integrin found on T lymphocytes, the very late antigen-4 (VLA-4, or α4β1 integrin). VLA-4 and its ligand, VCAM- 1, found on microglia (Fig. 3 *B*), are expressed at a higher level than normal in lesions of MS, as are other adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) (8). Monoclonal antibodies against VLA-4 can successfully prevent, suppress, or reverse the development of EAE in rats (36) or guinea pigs (37). In addition, the expression of VLA-4 on myelin-specific T cell clones is associated with encephalitogenecity in EAE (38, 39). Therefore, we treated activated T lymphocytes with a neutralizing antibody against CD49d, the α chain of VLA-4, and found that it attenuated the subsequent secretion of TNF-α in T cell–microglia cocultures in a dose-dependent fashion (Fig. 3 *A*). In contrast, an antibody to leukocyte function antigen-1 (LFA-1), another integrin found on T cells, did not affect TNF-α production. We should note, however, that high concentrations of the VLA-4 antibody (25 or 50 μg/ml) were required to reduce TNF-α production. Nonetheless, we should also note that 50 μg/ml of the LFA-1 antibody, or the isotype control, did not affect TNF-α levels at all (Fig. 3 *A*), suggesting the specificity of the reduction of TNF-α production when VLA-4 is blocked.

What is the interacting partner of VLA-4 on microglia? VCAM-1 is an obvious candidate since microglia cells express this adhesion molecule (Fig. 3 *B*). To test the involvement of VCAM-1, we addressed whether the cross-linking of VCAM-1 on the surface of microglia was sufficient to generate TNF-α. Fig. 4 demonstrates that the addition of $F(ab)_2$ goat anti–mouse Ig fragment alone enhanced TNF-α production by microglia, likely because of nonspecific interaction with microglia. An isotype control for the VCAM-1 antibody also elicited TNF-α transcription to similar level as that for the $F(ab)_2$ group alone, and this is likely due to the $F(ab)_2$ Ig fragment. However, in the presence of the VCAM-1 antibody and the $F(ab)_2$ Ig fragment, TNF-α mRNA level was clearly elevated over all control groups. Thus, the cross-linking of VCAM-1 is sufficient to induce signaling for the TNF-α production. Previously, the cross-linking of a related adhesion molecule, ICAM-1, on a rheumatoid synovial cell line, was reported to induce the transcription of IL-1β (40). Hence, adhesion molecules can function not only as adhesive substrates, but also as transducer of signals for cytokine production.

On balance, the findings suggest that the production of TNF-α by microglia involves VLA-4 and its ligand, VCAM-1, on microglia.

IFNβ-1b inhibits TNF-α production. Because human IFNβ can downregulate the expression of VLA-4 on human peripheral blood monocytes (41), we examined whether IFNβ-1b inhibits the production of TNF-α in T cell–microglia cocultures through its effect on T lymphocytes. Fig. 5 *A* demonstrates that the pretreatment of T cells with IFNβ-1b reduced the production of TNF-α in a dose-dependent fashion by T cells when they were cocultured with microglia. Pretreatment of microglial cells with IFNβ-1b before their exposure to T lymphocytes also resulted in a slight diminution of TNF-α production,

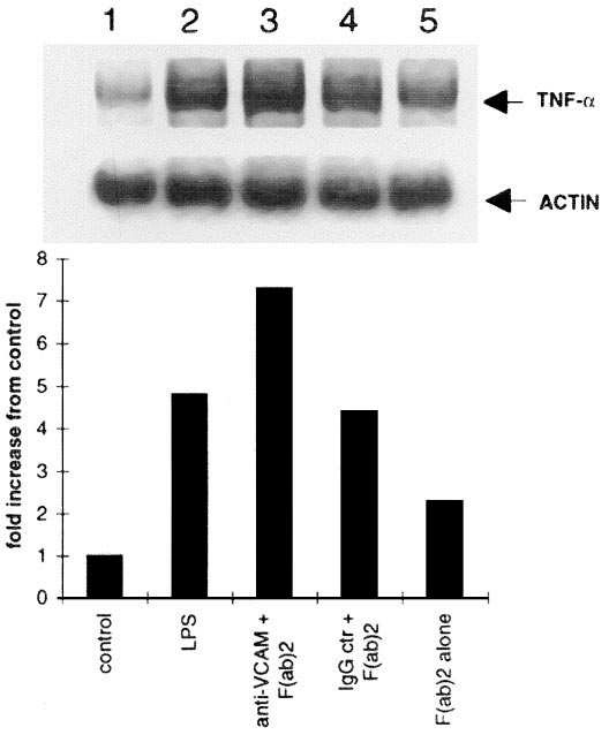


Figure 4. The cross-linking of VCAM-1 on the surface of microglia by an anti–VCAM-1 antibody and an $F(ab)_2$ Ig fragment results in increased TNF-α transcript level. Microglia were exposed to the various conditions listed here as described in the text, and RT-PCR for TNF-α and actin mRNA was performed. Representative blots are shown and the level of transcripts for TNF-α or actin in each treatment group was obtained using a phosphoimager. The ratio of TNF-α to actin mRNA level for each group was obtained, and the ratio was then expressed as a percentage of untreated controls (lane *1*), with the level of untreated control being set at onefold. LPS (5 μg/ml) was used as a positive control to stimulate TNF-α production (lane *2*). Note that $F(ab)_2$ fragment alone (lane *5*) or IgG isotype control plus $F(ab)_2$ (lane *4*) stimulated TNF-α transcription, likely the result of nonspecific interaction of this fragment with microglia. Nonetheless, in the presence of anti–VCAM-1 and $F(ab)_2$ fragment (lane *3*) TNF-α was clearly increased.

but this effect was less marked than when T cells were pretreated with IFNβ-1b.

Interestingly, the effect of IFNβ-1b on T cell–microglia cocultures is not selective to TNF-α, since IL-6 level is also diminished by IFNβ-1b (content of IL-6 in activated T cells and microglia cocultures = 3,140 pg/ml; in the presence of 100 IU/ml IFNβ-1b, IL-6 level = 2,000 pg/ml).

It is known that IFNβ is an antimitotic agent for T lymphocytes, but three factors rule out the antiproliferative effect of IFNβ-1b as being responsible for the decrease in TNF-α production (42, 43). First, after the treatment with different concentrations of IFNβ-1b, cell numbers were counted and equal numbers of T cells from each treatment group and controls were then co-incubated with microglia to obtain the results presented here. Second, IFNβ-1b at 1,000 IU/ml decreased the rate of proliferation of activated T cells by 50% at best (data not shown), while the reduction of TNF-α production at this IFNβ-1b concentration was consistently > 90% (Fig. 5 *A*).

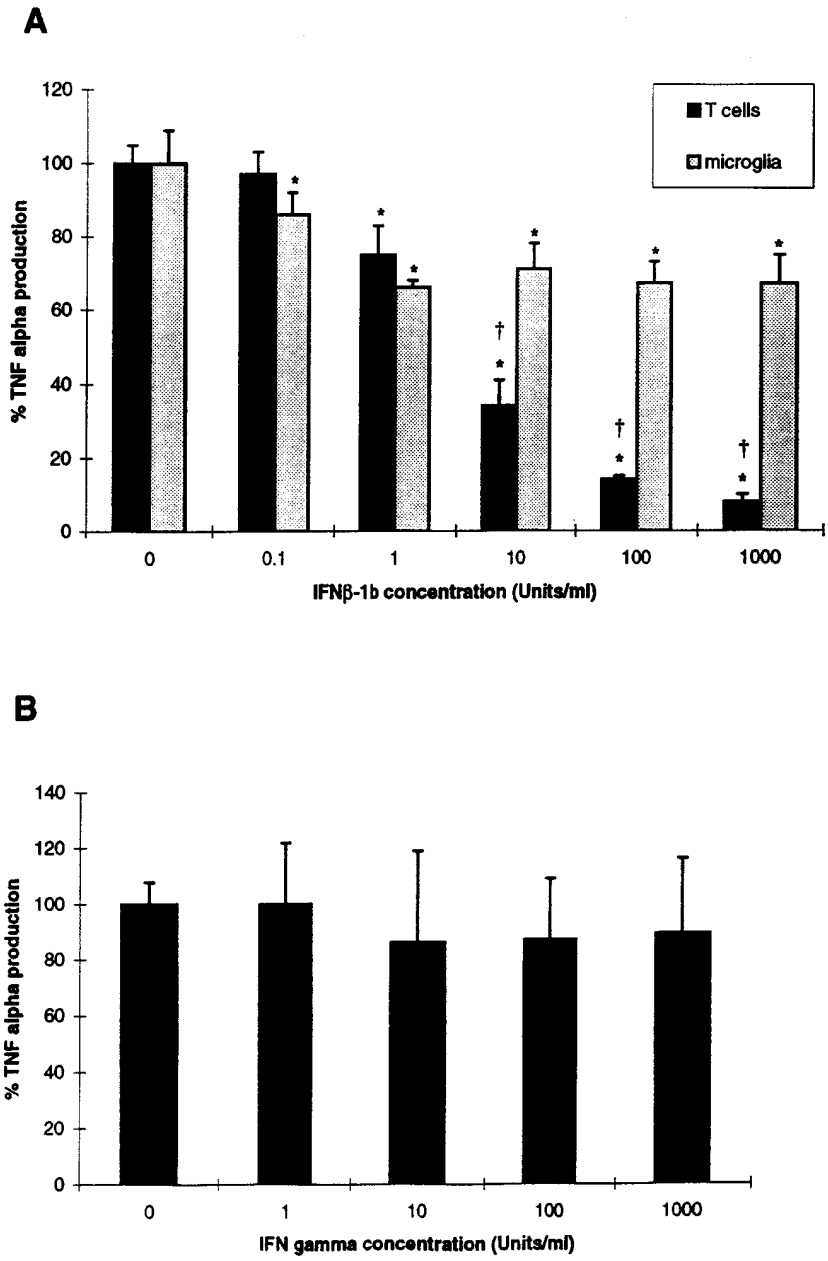


Figure 5. IFNβ-1b reduces the production of TNF-α in T cell–microglia coculture. (*A*) The pretreatment of activated T cells with different concentrations of IFNβ-1b (ranging from 0.1 to 1000 IU/ml) reduces the production of TNF-α in subsequent T cell–microglia cocultures. When microglia, but not T cells, are pretreated with IFNβ-1b, the effect on TNF-α production is less marked. $^{*}P < 0.05$ compared to their respective controls (i.e., 0 IU/ml IFNβ) (one way ANOVA with Duncan's multiple comparisons). $^{\dagger}P < 0.001$ compared between T cells and microglia after the same concentration of IFNb-1b (Student's *t* test). (*B*) The pretreatment of activated T cells with different concentrations of IFNγ (1–1000 IU/ml) does not affect the production of TNF-α in subsequent T cell–microglia cocultures. All values in this figure are mean±SEM of at least three samples, and have been normalized to untreated controls.

And third, the pretreatment of T lymphocytes with another human IFN type, IFNγ, which also reduces T cell proliferation (44) (49% decrease in [^{3}H]thymidine uptake at 100 IU/ml in our study), did not affect the TNF-α production (Fig. 5 *B*).

Effects of IFNβ-1b on CD49d. To determine whether the decreased TNF-α production after the treatment of T cells with IFNβ-1b or anti-CD49d were mechanistically related, two approaches were taken. First, we determined whether there would be a synergistic effect, implying different pathways, when T cells were treated with both IFNβ-1b and anti-CD49d before their exposure to microglia. Second, we examined whether the treatment of T cells with IFNβ-1b would lead to a reduction of CD49d expression. Fig. 6 *A* reveals that while the treatment of T cells with IFNβ-1b or anti-CD49d alone reduced TNF-α production to the same extent, their cotreatment did not further reduce TNF-α levels. Furthermore, Western blot analysis demonstrates that the total cellular level of the 80 kD CD49d is lower in T lymphocytes treated with IFNβ-1b than that in the control cells (Fig. 6 *B*), supporting the postulate that the mechanism by which IFNβ-1b decreases TNF-α production is by regulating VLA-4 on T cells. Notably, however, IFNβ-1b did not affect the cell surface level of α4 integrin as assessed by flow cytometry. Using four different antibodies to VLA-4 (PS/2 and HP2/1 described above, anti-VLAα4–FITC from Serotec, and anti-VLAα4–PE from Becton Dickinson), the mean intensity fluorescence of control T cells for cell surface (i.e., nonfixed cells) α4 integrin did not differ from that of IFNβ-1b treated cells (results not shown).

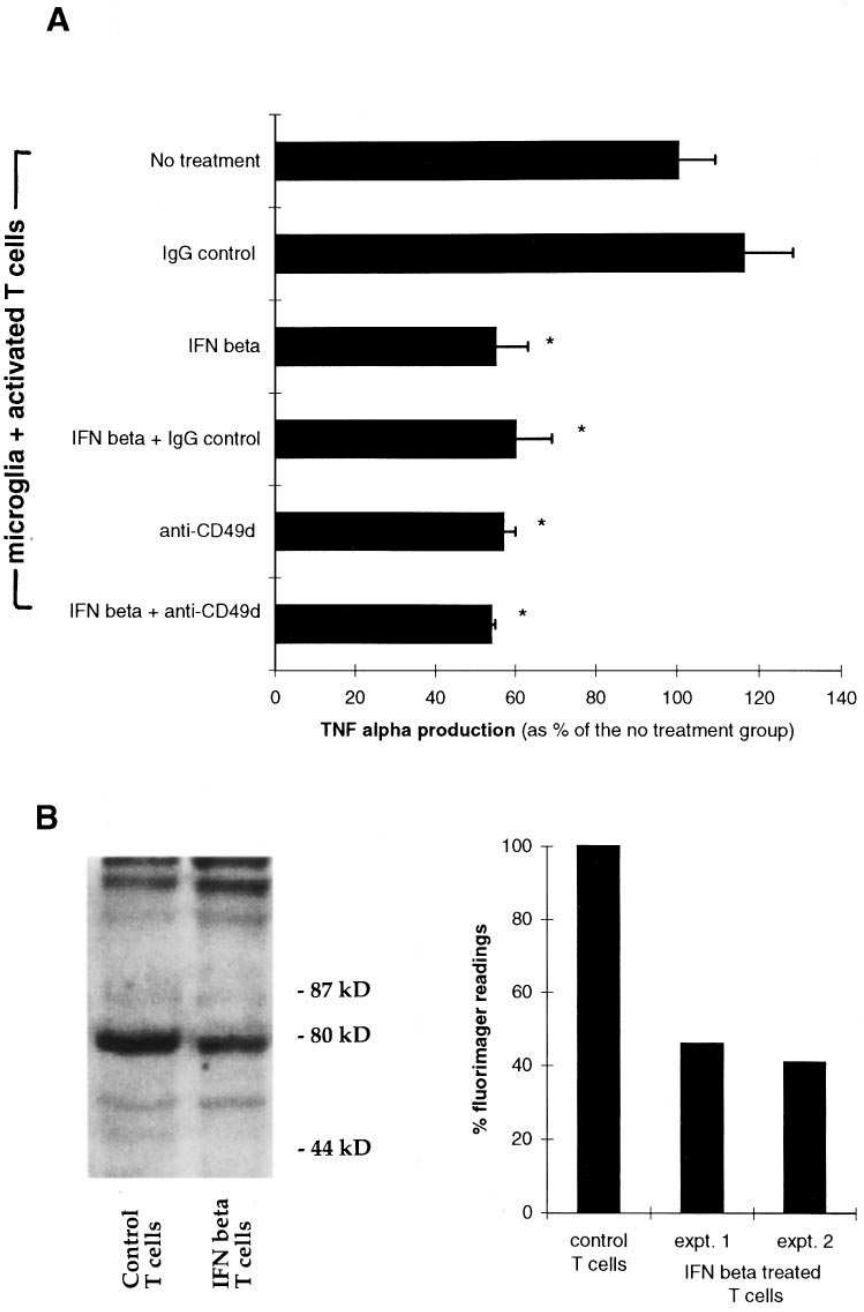


Figure 6. IFNβ-1b reduces TNF-α production by downregulating the expression level of the α chain of VLA-4, CD49d. (*A*) The co-administration of IFNβ-1b (100 IU/ml) with anti-CD49d (50 mg/ml) does not result in a synergistic effect on TNF-α production. Values are mean±SEM of triplicate cultures, normalized to the no treatment group. $*P < 0.05$ compared to the no treatment group (one way ANOVA with Duncan's multiple comparisons). TNF-α production by IFNβ-1b treated T cells was not statistically different from that obtained in the presence of anti-CD49d alone, or IFNβ + anti-CD49d. (*B*) The protein expression level of the α chain of the VLA-4 integrin, CD49d, in cellular extracts for activated T cells is reduced, compared to control, when T cells (IFNβ T cells) were treated for 72 h with IFNβ-1b (100 IU/ml).

Discussion

The infiltration of T cells into the CNS is considered a key event in the pathogenesis of MS or EAE. In EAE, chronologic studies have demonstrated that antigen-specific T cells home to the CNS early in the immune response, presumably aided by chemotactic gradients provided by chemokines, and localize to the perivascular space (45–47). After the initial wave of antigen-specific T lymphocytes, there is an enhanced recruitment of a large number of nonantigen-specific T cells which traverse into the CNS parenchyma (45–47); indeed, the later arriving T cells need not even be in activated state (48). Of interest, the antigen-specific T cells in adoptive transfer EAE experiments constitute a minority population (< 2% of the total cell infiltrate), and furthermore, the clinical signs of EAE correlate temporally with the arrival of the nonantigen-specific T cells (47).

The entry of T cells into the parenchyma of the CNS places them in close proximity to microglia, the resident macrophage of the CNS, and a source of many inflammatory cytokines, including TNF-α. Of note, TNF-α, as mentioned previously, can be toxic to oligodendrocytes and can produce demyelination. Understanding the mechanism by which TNF-α is generated within the CNS can thus impact upon the rational treatment of inflammatory demyelinating diseases that include MS.

How is TNF-α generated by the microglia cells? Stimula-

tion by soluble molecules, such as lipopolysaccharide (LPS) or IFNγ is one mode (49), although LPS has not been demonstrated to have a physiological relevance in MS, and the adult human microglia tends to be a poor source of TNF-α in response to IFNγ (50). In this manuscript, we demonstrate that the contact of microglia with T cells is another mechanism of TNF-α production by microglia. Our results suggest that the T lymphocyte uses VLA-4 integrin to interact with microglia to induce TNF-α production. Specificity is revealed by the inability of a functional blocking antibody to LFA-1, another T cell integrin, to affect TNF-α production by T cell–microglia interactions. It is likely that other membrane molecules are involved in this process since the inhibition of TNF-α production by anti-CD49d was incomplete and required high concentrations. Other candidates include the membrane-bound TNF-α (35) and CD40 (51, 52) which have been suggested to be important in the T cell–mediated activation of peripheral macrophages. The ligand for VLA-4 on microglia appears to be VCAM-1, and the cross-linking of VCAM-1 alone by an antibody is sufficient to trigger TNF-α transcription.

The finding that the TNF-α generated in T cell–microglia coculture requires that VLA-4 has relevance to the MS and EAE disease processes. In animal experiments, the treatment with a monoclonal antibody to VLA-4 prevents the development, or suppresses and reverses, the clinical signs of EAE (36, 37). Furthermore, the expression of VLA-4 on proteolipid protein-specific (38), or myelin basic protein-specific (53), T cell clones has been associated with encephalitogenecity. T cell clones that express low levels of α4 integrins are nonencephalogenic (54).

Further significance of the finding that the VLA-4 integrin on T cells may regulate TNF-α expression by microglia relates to the drug IFNβ-1b. Since the initial reports in 1993 (18, 19), several studies, using either IFNβ-1b or other forms of human IFNβ, have shown that these interferons decrease the number of relapses and MRI-detected lesions in relapsing/remitting MS patients (43, 55–57). Despite the clinical advances, however, the precise mechanism(s) by which IFNβ-1b is effective in the treatment of MS has remained unclear. Suggested modes of action refer primarily to systemic immune mediation (18, 58) and include an effect of IFNβ-1b in decreasing T cell reactivity or its synthesis of IFNγ (41, 42), attenuating antigen presentation to T cells, improving the suppressor function of T lymphocytes (59), or modifying the humoral immune response (60). We reported recently that IFNβ-1b decreases the T cell production of the matrix-degrading protease, matrix metalloproteinase-9 (MMP-9), with the resultant reduced capacity of T cells to proteolytically remodel and infiltrate across barriers (27).

Our current results have uncovered another mechanism by which IFNβ-1b may be efficient in MS. In this regard, IFNβ-1b attenuates the ability of T cells to stimulate the production of the oligodendrocyte-toxic cytokine, TNF-α. With respect to whether the mechanism of IFNβ-1b involves the T cell VLA-4 integrin, the results are unclear. Although the cellular level of the α4 chain of VLA-4 is reduced by IFNβ-1b as determined by Western blot analyses, the cell surface level of α4 was not affected as shown by flow cytometry. Besides decreasing intracellular α4 level, it is possible that IFNβ-1b might have affected the affinity state of the VLA-4 integrin, which was not reflected by the cell surface flow cytometry results. For VLA-4 and other integrins, the switch from a low to a high affinity state on the cell membrane, with an associated increase in cellular function, can occur without alterations of the level of that integrin (for review see reference 61). That the affinity state, and function, of the VLA-4 integrin could have been affected by IFNβ-1b is supported by the findings of the lack of synergy of IFNβ-1b with the functional α4 integrin blocking antibody in affecting TNF-α production (Fig. 6 *A*).

In summary, IFNβ-1b reduces the production of TNF-α in T cell/microglia coculture. The results are relevant to MS where T cells infiltrate into the CNS to be in close proximity to microglia, and where TNF-α is known be proinflammatory and to be toxic to oligodendrocytes. Finally, while the focus of this work has been MS, the results have relevance to other disease states where T cell–macrophage/microglia interactions may occur and where TNF-α is produced to be pathogenic; these disorders include Crohn's disease, rheumatoid arthritis, cancer, and even AIDS (62).

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