Unimpaired autoreactive T-cell traffic within the central nervous system during tumor necrosis factor receptor-mediated inhibition of experimental autoimmune encephalomyelitis

(multiple sclerosis)

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ABSTRACT The critical role of tumor necrosis factor (TNF) as a mediator in autoimmune inflammatory processes is evident from in vivo studies with TNF-blocking agents. However, the mechanisms by which TNF, and possibly also its homologue lymphotoxin α, contributes to development of pathology in rheumatoid arthritis and Crohn disease and in animal models like experimental autoimmune encephalomyelitis is unclear. Possibilities include regulation of vascular adhesion molecules enabling leukocyte movement into tissues or direct cytokine-mediated effector functions such as mediation of tissue damage. Here we show that administration of a TNF receptor (55 kDa)-IgG fusion protein prevented clinical signs of actively induced experimental autoimmune encephalomyelitis. Significantly, the total number of CD4+ T lymphocytes isolated from the central nervous system of clinically healthy treated versus untreated control animals was comparable. By using a CD45 congenic model of passively transferred experimental autoimmune encephalomyelitis to enable tracking of myelin basic protein-specific effector T lymphocytes, prevention of clinical signs of disease was again demonstrated in treated animals but without qualitative or quantitative diminution to the movement of autoreactive T lymphocytes to and within the central nervous system. Thus, despite the uninterrupted movement of specific T lymphocytes into the target tissue, subsequent disease development was blocked. This provides compelling evidence for a direct effector role of TNF/lymphotoxin α in autoimmune tissue damage.

Studies in animal models of autoimmune disease have implicated tumor necrosis factor (TNF)/lymphotoxin (LT) α in their pathogenesis (1–6). That TNF plays a role in a human autoimmune disease was recently demonstrated in clinical trials in which rheumatoid arthritis patients showed significant clinical improvement following treatment with cA2, a chimeric TNF-specific monoclonal antibody (mAb) (7, 8). Precisely how TNF is involved in the disease process remains ill-defined, but mechanisms could include the induction of major histocompatibility complex molecules on the surface of tissue cells in concert with other cytokines (9, 10), upregulation of adhesion molecules resulting in enhanced effector cell movement into tissue sites (11–13), and direct cytotoxicity (14, 15). Experimental evidence suggesting one action to be of overriding importance is lacking. Nevertheless, the virtual absence of inflammation after administration of anti-TNF/LT antibody, for example, in the central nervous system (CNS) in experimental autoimmune encephalomyelitis (EAE) has led to the proposal that the primary block in this disease process may be prevention of adhesion molecule upregulation (16). Likewise, EAE prevention by administration of a mAb to interleukin 12 (17) was assumed to involve modulation of integrin adhesion function based on the similarities between the kinetics of disease inhibition with anti-interleukin 12 mAbs and anti-α4β1 integrin mAbs (18).

Here we have used as a TNF antagonist a recombinant version of the 55-kDa human TNF receptor (TNFR) fused to human IgG (TNFR-IgG), a molecule shown previously to bind and efficiently inhibit mouse TNF (19). The advantages of these fusion protein reagents for experimental studies, in contrast to species-specific anti-TNF mAb, include binding of TNF from all species (there are no rat TNF-specific mAbs available) and substantially greater TNF neutralizing capacity and half-life in vivo compared with monovalent TNFR molecules (20). By using this TNF antagonist to treat rats with EAE elicited by immunization with myelin basic protein (MBP) in adjuvant, a profound inhibition of the signs of clinical disease was obtained, but inflammation into the CNS remained substantial. By employing a model of EAE that enables tracking of autoreactive T cells to the CNS, we have explored the mechanism of disease inhibition in such TNFR-IgG-treated animals. Our data provides compelling evidence that tissue damage in an autoimmune disease is attributable to a direct effector role of TNF/LT and not solely to TNF/LT-induced recruitment of immune cells to sites of inflammation.

MATERIALS AND METHODS

Animals, Cell Isolation Procedures, and Flow Cytometry. Eight- to 10-week-old female or male rats were used. Lewis and PVG-RT7 (CD45-) rats were obtained from the Animal Resources Center (Perth, Australia) or bred in local facilities (Blackburn, University of Sydney). Major histocompatibility complex identical PVG-RT7 (CD45-) rats congenic for an alternative allotype of the rat RT7/leukocyte common antigen molecule were bred and housed in the Centenary Institute animal facility. All animal procedures were approved by The Animal Care and Ethics Committee of the University of Sydney. CNS-associated leukocytes and microglia were isolated as described (21, 22). Isolated cells were stained for two- and three-color flow cytometry and analyzed by FACScan (two color) or FACStarPLUS (three color). mAbs were MRC OX1 (anti-pan-CD45), MRC OX42 (anti-CD11b/c), R73 (anti-β2 T-cell antigen receptor [TCR]), NDS 58 (anti-

Abbreviations: TNF, tumor necrosis factor; LT, lymphotoxin; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; mAb, monoclonal antibody; MBP, myelin basic protein; TNFR, TNF receptor; TNFR-IgG, TNFR-IgG fusion protein; TCR, T-cell antigen receptor; huIgG, human IgG.

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CD45b, and 8G6.1 (anti-CD45b). See references and cross-references in refs. 21 and 22.

Induction of EAE and TNFR-IgG Treatment. Actively induced disease was elicited in Lewis strain rats by injection of MBP in complete Freund's adjuvant (23), with signs of disease appearing 10–12 days after immunization. Short-term encephalitogenic MBP-specific CD4+ T-cell lines were derived from immunized PVG rats as described (24), and EAE was passively transferred to naive (nonirradiated) recipient rats after the second round of in vitro antigenic (MBP) restimulation of these T cells. At the time of transfer, the magnitude of T-cell proliferation to MBP was ~90% of that to concanavalin A, illustrating that most cells were MBP-specific. All cells exhibited a phenotype typical of activated encephalitogenic T cells [CD4+, αβ TCR+; CD25high, CD45RClow (24, 25)]. Recipients received 5–10 × 106 MBP-specific T-cell blasts intravenously, and signs of disease normally appeared by day 4. Clinical signs were scored as 0 = normal; 0.5 = weakness of distal part of tail; 1 = complete flaccid paralysis of the tail; 2 = weakness of hind legs; 3 = paralysis of hind legs; 3.5 = paralysis of hind legs plus incontinence; 4 = total paralysis of hind legs, incontinence, foreleg involvement, and weight loss ≥20%; 5 = moribund. Rats injected with MBP and adjuvant or receiving MBP-specific T cells were administered various doses of TNFR-IgG or protein A-purified human IgG as control, intraperitoneally, as detailed in Results and the legends to the table and figures.

Immunohistology. CD45b+ MBP-specific T cells were located in the CD45b+ host CNS by immunostaining 5-μm cryostat sections of CNS with anti-RT7.2 mAb (Serotec) and rabbit anti-mouse immunoglobulin-peroxidase (Dako) or biotinylated 8G6.1 mAb and streptavidin-peroxidase (Vector Laboratories), followed by diaminobenzidine substrate. Sections were lightly counterstained with hematoxylin.

RESULTS

TNFR-IgG Blocks Rat and Mouse TNF Activity in Vitro. When expressed as an IgG fusion protein, the TNFR (55 kDa) construct, designated p55-sf2 (20), effectively blocked mouse and rat TNF lytic activity, as measured in a standard WEHI-164 cell lysis assay (26). Five hundred units of recombinant mouse TNF or undiluted activated rat T-cell supernatant (no recombinant rat TNF was available for testing) killed 100% and 50% of cells, respectively, and this was blocked to 80–90% in both cases by addition of 1 mg of TNFR-IgG. Five nanograms of TNFR-IgG virtually prevented all cell lysis (data not shown).

TNFR-IgG Inhibits Actively Induced EAE Without Preventing Inflammation. Clinical signs of actively induced EAE in highly susceptible Lewis rats were ameliorated substantially by administration of TNFR-IgG (3 mg per rat) just prior to onset of disease (which occurred 11–12 days after immunization; Table 1), consistent with a recent study in mice employing this same reagent (27) and in rats in which a different form of TNFR was used (28). Only limited dose–response experiments have been performed, but they revealed that one injection of 2 mg of TNFR-IgG just before onset of disease is also effective at blocking EAE (data not shown). A smaller amount of TNFR-IgG given from the time of MBP immunization (i.e., a total of 1 mg over 10 days) did not prevent disease (data not shown). This could be due to insufficient TNFR-IgG or, alternatively, clearance by an immune response directed against the TNFR-IgG (which is of totally human sequence) in the lag time between MBP immunization and appearance of clinical signs of EAE 11–12 days later.

Subsequent isolation of CNS leukocytes and staining for cell surface markers revealed a similar pattern of infiltrating cells in treated and untreated animals. Most surprisingly, the absolute numbers of CD4+ T cells recovered from the CNS of the TNFR-IgG- and control IgG-treated groups of rats was essentially identical, and macrophage infiltrate was present in both cases (Table 1). Nevertheless, it is well known that the magnitude of an inflammatory response in the CNS does not necessarily correlate with disease severity (23, 29). As the majority of T cells in CNS inflammatory infiltrates are usually nonspecific (29), this could indicate in the present case that TNFR-IgG treatment reduced MBP-specific T-cell numbers in the CNS to a suboptimal level that was sufficient to generate a substantial non-specific (T-cell) inflammatory response but not to elicit enough tissue damage to produce clinical disease. To explore this further, a model of passively transferred EAE in CD45 congenic PVG strain rats was established to enable tracking of effector cells to the CNS.

Unimpaired T-Cell Movement into the CNS After TNFR-IgG Treatment in Passively Induced EAE. When rats are more resistant to EAE than the Lewis strain, but the PVG rats are the only ones on which a suitable congenic marker (CD45) is available. As a consequence, the EAE disease course is shorter in PVG rats compared with the Lewis strain (23), and clinical signs of passively transferred disease are less severe. Injection of 5 × 107 MBP-specific PVG+ T-cells blasts led to

Table 1. Amelioration of actively induced EAE with TNFR-IgG

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Diseased animals/total animals</th>
<th>Mean clinical score ± SD†</th>
<th>Mean day of onset ± SD</th>
<th>CNS-associated CD4+ T cells%</th>
</tr>
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<tr>
<td>huIgG</td>
<td>11/11</td>
<td>3.3 ± 0.3</td>
<td>11.2 ± 0.7</td>
<td>5.0 ± 2.8</td>
</tr>
<tr>
<td>TNFR-IgG</td>
<td>5/9</td>
<td>0.55 ± 0.6</td>
<td>11.8 ± 2.7</td>
<td>5.7 ± 2.9</td>
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Lewis rats in three separate experiments (here pooled) received intraperitoneal injections of 2 mg of human IgG (huIgG) or TNFR-IgG 8 or 9 days after immunization with MBP in adjuvant and another 1 mg on day 11, equivalent to a total of 10–15 mg/kg of body weight. *Scored positive if exhibited clinical signs of EAE at any time. †The mean peak clinical score includes all animals, not just those exhibiting signs of disease, hence the large SD in the TNFR-IgG-treated group. huIgG (control) animals were severely diseased with hind limb paralysis and incontinence. TNFR-IgG-treated rats had, at most, a flaccid tail and most were asymptomatic. ‡The mean recovery of two animals in each group was taken on days 13 and 16, when CNS-associated leukocytes from each CNS were processed separately. CD4+ T cells accounted for ~50% of the total CD45+ cells in TNFR-IgG-treated rat CNS and 30% in control rat CNS. In both cases, at least half of the remaining CD45+ cells were CD11b/c+ CD4+ macrophages or microglia.

Fig. 1. TNFR-IgG prevents passively transferred EAE in CD45 congenic rats. Groups of five PVG CD45+ recipients received 5 × 107 PVG CD45+ MBP-specific CD4+ T-cell blasts by the intravenous route (day 0) and 200 μg of TNFR-IgG (● and hatched bar) or huIgG (○ and open bars) daily intraperitoneally as indicated (†). Shown are the weights (lines) and clinical scores (bars) of a representative experiment.
Early influx of nonspecific inflammatory cells prevented by TNFR-IgG. Cells isolated from the CNS of TNFR-IgG- or control IgG-treated PVG CD45<sup>+</sup> recipient rats 4 days after receiving 5 x 10<sup>6</sup> PVG CD45<sup>+</sup> MBP-specific T cells were stained for surface markers as shown. The treatment dosage and schedule was as in Fig. 1. Population 1, which tends to exhibit some level of autofluorescence but is otherwise CD45<sup>+</sup>, is resident CNS cells, which could include neurons, astrocytes, oligodendrocytes, and others. Population 2 is resident CNS macrophages (microglia CD45<sup>low</sup>CD11b/c<sup>+</sup>). Populations 1 and 2 are present in the normal CNS and their phenotype and properties have been described in detail elsewhere (21, 22). Populations 3–6 are inflammatory cells. Shown are single animals in each case, but the results are representative of two other animals in the TNFR-IgG group and numerous control (hulgG or no treatment) animals.

Fig. 2. Early influx of nonspecific inflammatory cells prevented by TNFR-IgG. Cells isolated from the CNS of TNFR-IgG- or control IgG-treated PVG CD45<sup>+</sup> recipient rats 4 days after receiving 5 x 10<sup>6</sup> PVG CD45<sup>+</sup> MBP-specific T cells were stained for surface markers as shown. The treatment dosage and schedule was as in Fig. 1. Population 1, which tends to exhibit some level of autofluorescence but is otherwise CD45<sup>+</sup>, is resident CNS cells, which could include neurons, astrocytes, oligodendrocytes, and others. Population 2 is resident CNS macrophages (microglia CD45<sup>low</sup>CD11b/c<sup>+</sup>). Populations 1 and 2 are present in the normal CNS and their phenotype and properties have been described in detail elsewhere (21, 22). Populations 3–6 are inflammatory cells. Shown are single animals in each case, but the results are representative of two other animals in the TNFR-IgG group and numerous control (hulgG or no treatment) animals.

a reliable and predictable onset of disease in control IgG-treated rats, with weight loss beginning at day 3 and clinical signs of EAE at day 4. Two days later, the animals started to recover (Fig. 1). No clinical signs developed in TNFR-IgG-treated animals, and only a mild and transient weight loss could be detected. No relapses were observed up to 20 days after T-cell injection despite discontinuation of treatment at day 5. Our ability to assess animals over the longer term is limited by animal ethics considerations.

Analysis of donor and host-derived cells isolated from the CNS of PVG CD45-congenic TNFR-IgG-treated and mock-treated rats from the earliest signs of disease (day 4) to recovery (day 7) was highly informative. Fig. 2 shows flow cytometric plots of day 4, as these illustrate most clearly the early effects of TNFR-IgG treatment, and in Fig. 3, a quantitative analysis of cell recoveries from this and later time points is shown. Dual staining with pan-CD45 mAb [recognizing resident host CNS macrophages (microglia) and donor and host inflammatory cells] and anti-CD11b/c mAb (Fig. 2 Upper) revealed four populations (1–4; see legend for details). The salient points to note are that there are relatively more inflammatory cells in control rats compared with TNFR-IgG-treated animals (quadrants 3 and 4). However, staining for the αβ TCR shows that the majority of populations 3 and 4 in TNFR-IgG-treated rats (Fig. 2D) are T cells (population 5) while in controls (Fig. 2C), only a proportion are T cells (population 5); the remainder are non-T cells (population 6). The latter are not macrophages, since they are mostly CD11b/c<sup>-</sup> (Fig. 2A), and also are not MBP-reactive T cells that have downregulated their αβ TCR after antigenic interaction. That is, in three-color analysis (see below) all cells displaying the donor CD45 allotype in treated and untreated rats were found to express the αβ TCR at levels well above background. Preliminary studies indicate that population 6 (Fig. 2C) is a complex mixture of cells, many staining with the mAb 3.2.3, a reported natural killer cell marker (30). However, the bulk of the cells also lack CD11b/c (Fig. 2A), but it is possible that natural killer cell expression of this may depend on the activation state of the cells or their locality (for example, downregulating CD11b/c as they move into tissues). The main point to stress is that early in the disease process, there is no apparent impediment to the initial influx of T cells in the presence of TNFR-IgG, but the early nonspecific (as yet to be classified) inflammatory response has been blunted substantially.

Fig. 3. Unimpeded movement of MBP-specific T cells into the CNS occurs in the presence of TNFR-IgG. Cells were isolated from the CNS of TNFR-IgG- or control IgG-treated PVG CD45<sup>+</sup> recipient rats 4–7 days after receiving 5 x 10<sup>6</sup> (days 4 and 6) or 1 x 10<sup>7</sup> (day 7) PVG CD45<sup>+</sup> MBP-specific T cells. The treatment dosage and schedule was as in Fig. 1. CNS leukocytes were stained for markers as in Fig. 2 and additionally for the CD45 allotypes of the donor or recipient. Recoveries were estimated by a combination of viable cell counts (trypan blue exclusion) of extracted cells and flow cytometric determination of relative size (percentage) of each population by setting appropriate analysis gates. Data are from one complete time course experiment and illustrate a single animal in each case. Consistent trends were obtained in three other experiments but analyzing only one or two time points.
The origin of the CNS T-cell response in TNFR–IgG-treated rats was analyzed by three-color flow cytometry (Fig. 3), and the results revealed that the number of MBP-specific T cells in the CNS of control vs. TNFR–IgG-treated rats was very similar, particularly at day 4 but also at day 7. At the height of disease (day 6), control animals had about twice as many MBP-specific T cells as TNFR–IgG-treated rats, almost certainly representing self-recruitment as a result of tissue damage. The number of non-T inflammatory cells was generally higher in control rats, particularly evident at day 4, and this is also true for nonspecifically recruited T cells (from the host, CD45<sup>+</sup>). A higher number of MBP-specific T cells (that is, 1 x 10<sup>7</sup> cells vs. 5 x 10<sup>6</sup>, culled day 7) was administered to some animals to examine whether disease prevention by TNFR–IgG was simply an effect of reducing MBP-specific T-cell numbers below a critical threshold. This was not the case because high cell numbers were recovered (e.g., Fig. 3 Lower), and, despite a slightly increased weight loss in TNFR–IgG-treated rats, still no disease could be detected. Untreated animals, in contrast, showed more severe and extended clinical signs including incontinence and paralysis of the hind legs (up to disease score 3.5; data not shown). A consistent trend in all studies in actively or passively-induced EAE in the PVG-congenic system or in Lewis rats was the more rapid loss of T cells from the CNS of control rats during the recovery phase of disease (for example, Fig. 3, hulgG vs. TNFR–IgG at day 7). This was most evident in the MBP-specific donor T cells and could represent delayed apoptotic loss of specific T cells in the CNS, for which good evidence now exists (31). The implication is that TNFR–IgG arrests this effect in some way. Further studies with larger animal numbers are required to determine if this trend is a statistically significant one.

**Specific T-Cell Movement in the CNS Is Normal.** Potentially, the distribution of MBP-specific T cells within the CNS could affect their ability to cause damage and disease. Staining for donor MBP-specific T cells (Fig. 4) at two sites in the CNS (the medulla and spinal cord) revealed no apparent difference in cell distribution between hulgG-treated control rats with EAE (Fig. 4A) and healthy TNFR–IgG-treated control rats (Fig. 4 B and C). In particular, there is significant movement of MBP-specific T cells into the CNS parenchyma in both control (Fig. 4A) and treated rats (Fig. 4 B and C) as well as cells remaining associated with perivascular cuffs. Host (unstained) cells can be seen, particularly within and around the vasculature.

**DISCUSSION**

The application of a system to track autoreactive T cells into the CNS in EAE has enabled us to distinguish precisely between the two most likely potential mechanisms of disease inhibition by TNF-blocking agents. The first, that TNFR–IgG treatment blocked disease by preventing movement of autoreactive T cells into the CNS, was supported by a number of studies (1–3, 5) using anti-TNF antibody reagents. In particular, a recent immunohistological analysis of CNS tissue in anti-TNF-treated mice (16) revealed substantially reduced levels of vascular cell adhesion molecule 1 (VCAM-1) expression on CNS vascular endothelium. This, coupled with the fact that a4 integrin expression by autoreactive T cells is demonstrably important in T-cell movement into the CNS (18, 32), suggested that anti-TNF treatment blocked this adhesion and penetration process. Results consistent with this conclusion have also been reported in one study using soluble TNFR where complete blockade of pathological changes was noted (33). However, close examination of the data indicates that treated animals did have inflammation in the CNS, although it was reduced relative to controls. The use of TNFR–IgG here demonstrates clearly that blockade of TNF and/or LT α function and not effector T-cell movement can similarly prevent autoimmune tissue damage and expression of clinical disease. Indeed, as these studies show, early in the disease process there is no quantitative difference in the absolute number of MBP-specific T cells entering the CNS in treated compared to control animals. As disease progresses, a difference does emerge, however, in terms of both the number of specific T cells as well as the increased number of nonspecific inflammatory cells in control vs. TNFR–IgG-treated rats. There are two interpretations of this result. First, although autoreactive T cells enter the CNS, their secreted/surface TNF and/or secreted LT α-mediated effector function is blocked. Thus, the chain of downstream events producing accumulation of more MBP-specific T cells and other nonspecific inflammatory leukocytes does not get initiated or, more likely, is less efficiently elicited. The effector function of other cells that do enter is probably blocked in a similar manner. Second, while movement of T cells is not impeded, TNFR–IgG may prevent the upregulation of adhesion molecules necessary for attachment and penetration of nonspecific inflammatory cells, such as macrophages, considered important for disease development (34). This possibility cannot be excluded entirely, but in actively induced disease in TNFR–IgG-treated rats, some accumulation of CD11b/e<sup>+</sup> cells (mostly macrophages) occurs.
in addition to CD4+ T lymphocytes (see Table 1 legend). Thus, the first mechanism is the favored one.

Why the disparity between these data and those using anti-TNF antibodies? Two possible explanations are binding specificity and lytic capacity. In addition to binding soluble TNF and LT α, our preliminary results suggest that the TNFR-IgG molecule can bind the cell surface transmembrane form of TNF. The major form of surface LT, having the stoichiometry α2β2 does not bind the 55-kDa or 75-kDa TNFRs but uses an alternative receptor (35). It is possible that the anti-TNF antibodies that blocked inflammatory cell infiltration could bind to the major form of surface LT and this binding modulated inflammation. Although a possible scenario, it seems unlikely, nevertheless, that this could in itself account for the difference between the antibody and TNFR–IgG studies. A more plausible explanation is that lytic anti-TNF antibodies (raised in rabbit, hamster, etc.) may bind to the surface form of TNF or LT on activated T cells, leading to cell death. Some lytic anti-CD4 antibodies, for example, inhibit tissue-specific autoimmune inflammatory disease in this way (36, 37). Thus, the lack of adhesion molecule upregulation could reflect the fact that there are reduced numbers of activated, TNF-expressing MBP-specific T cells to elicit it and not that TNF-mediated adhesion molecule upregulation has been prevented directly. The continued ability to isolate proliferating or autoreactive T cells from lymphoid tissue of anti-TNF antibody-treated animals (2, 27) does not argue against this possibility as the transient nature of very high levels of surface TNF expression after T-cell activation (38) may render many cells resistant to lysis. Although the ability of TNFR–IgG bound to surface TNF to trigger cell lysis in vivo is not known for any species and indeed could be different between species, recent in vitro studies comparing the capacity of the TNFR–IgG used in these studies and anti-human TNF cα2 antibody to lyse targets expressing surface TNF (human) clearly indicate the potent lytic potential of cα2 (39) while TNFR–IgG is ~3 times less efficient (B.J.S., unpublished data). The localization of high numbers of MBP-specific T cells in the CNS of treated rats in the present study (Figs. 3 and 4) is consistent with this.

Because little is known about the role of LT α in autoimmune disease, and because the TNF antagonist used in this study inhibits soluble LT α as well as TNF, it is not clear whether inhibition of LT α contributes to EAE disease amelioration. In the light of promising clinical data using TNF-specific mAbs to treat two different inflammatory diseases (8, 40) and the major role of surface LT in lymphoid tissue modeling (41), it seems likely that TNF and not LT α is the primary mediator in the EAE model. This suggests, furthermore, that TNF-specific reagents may prove beneficial for patients with multiple sclerosis.

In conclusion, by virtue of the observation that EAE can be prevented by inhibiting TNF function, but without blockade of T-cell movement into tissues, these studies provide the strongest evidence yet for the role of TNF as a direct effector molecule in the induction of autoimmune pathology. Nevertheless, the actual mechanism of disease inhibition by TNFR–IgG remains unresolved, but one avenue to be explored is that in addition to blocking the effect of preformed surface/secreted TNF, this treatment also affects the further production of pathogenetically relevant cytokines (TNF as well as others) by encephalitogenic MBP-specific T cells entering the CNS.

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