

Aggravation of experimental autoimmune neuritis in TNF- α receptor 1 deficient mice

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Abstract

The role of tumor necrosis factor (TNF)- α and its receptors in the pathogenesis of experimental autoimmune neuritis (EAN) induced by P0 peptide 180–199 in TNFR1 (p55) deficient (TNFR1^{-/-}) mice was investigated. Compared to wild type EAN mice, TNFR1^{-/-} EAN mice developed significantly more severe clinical signs, in parallel with enhanced numbers of inflammatory infiltrating cells in peripheral nerves and splenic P0-reactive T cell proliferation, as well as *increased* obviously MHC class II and CCR3 expression on the macrophages in the cauda equina. Our data indicated that TNF- α might have anti-inflammatory effect preventing the development of EAN in this mouse model.

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1. Introduction

Experimental autoimmune neuritis (EAN) is an animal model of the Guillain-Barré syndrome (GBS) in humans, a demyelinating autoimmune disease of the peripheral nervous system (PNS) (Zhu et al., 1998; Gold et al., 2000).

One key mediator of regulatory processes in autoimmune demyelinating diseases is tumor necrosis factor- α (TNF- α), which is produced mainly by activated macrophages and T cells. TNF- α was initially characterized as having anti-tumor activity but was later found to exert pleiotropic, often contradictory, biological activities (Vassalli, 1992; Zhu et al., 1997a; Eugster et al., 1999; Kassiotis and Kollias, 2001; Bao et al., 2003). Its functions are mediated through two receptors, TNFR1 (p55) and

TNFR2 (p75), both of which are expressed on multiple cell types, especially on the surfaces of activated CD4 and CD8 positive T cell subsets (Ware et al., 1991). The majority of biological responses classically attributed to TNF- α are mediated by TNFR1 (Bao et al., 2003). The pathogenic mechanisms of TNF- α in inflammatory and autoimmune diseases include modulation of MHC class II (MHCII) expression and activation of macrophage (Jasinski et al., 1995) as well as the impairment of permeability of the blood–nerve barrier (BNB) (Jasinski et al., 1995). TNF- α has also been regarded as an active mediator of tissue destruction in EAN (Zhu et al., 1997a,b; Zou et al., 2000; Zhu et al., 2003, 2004).

However, despite its harmful pro-inflammatory activities, TNF- α may have beneficial functions in ischemia and autoimmunity by suppressing immune activities (Liu et al., 1998; Cope et al., 1997; Kassiotis and Kollias, 2001; McDevitt et al., 2002). TNF- α has been widely studied in experimental autoimmune encephalomyelitis (EAE), an EAN-like analogous disease of the central nervous system (CNS). Some reports have demonstrated a delayed and reduced clinical disease in

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TNF- α deficient mice (Korner et al., 1997), while other studies have shown that TNF-deficient mice are either equally susceptible or even more susceptible to EAE than controls (Frei et al., 1997; Liu et al., 1998). This ambiguity regarding the role of TNF also extends to other spontaneous and experimental models of autoimmunity, such as diabetes and collagen-induced arthritis.

High levels of TNF- α exist in sera of EAN rats and GBS patients (Exley et al., 1994); up-regulated TNF- α mRNA expression was found at the height of EAN course (Zhu et al., 1997a), and phosphatidylserine suppressed EAN due to down-regulating TNF- α production (Maeda et al., 1994). Still little is known about whether TNF- α is involved in regulating T cell activation or in the traffic and function of macrophages in EAN. One way to approach these questions is the use of TNF- α receptor deficient (TNFR1 $^{-/-}$) animals. Here, we have used such mice to study the role of TNF- α and its receptors in EAN. Unexpectedly, TNF- α seemed to play an anti-inflammatory role in EAN as demonstrated by more severe clinical signs, enhanced P0 peptide 180–199 specific T cell proliferation and expansion, and increased infiltrating cells in sciatic nerves and cauda equina, as well as MHCII and CCR3 expression on macrophages of cauda equina in TNFR1 $^{-/-}$ mice.

2. Materials and methods

2.1. Animals

TNFR1 $^{-/-}$ mice and corresponding wild type C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, Me) and bred at the animal facilities of Huddinge Hospital, Karolinska Institute, Stockholm, Sweden.

2.2. Antigen preparation

The neuritogenic P0 protein peptide corresponding to amino acids 180–199 of the rat PNS myelin P0 protein was synthesized by solid-phase stepwise elongation using a Tecan/Syro peptide synthesizer (Multisynth, Bochum, Germany).

2.3. Induction of EAN and assessment of clinical signs

A total of 96 TNFR1 $^{-/-}$ and 95 wild type male mice, 5–6 weeks old, weighing 16–20 g were used in the present study. For induction of EAN, mice were immunized twice with a one week interval by subcutaneous injection into the back with 120 μ g of P0 peptide 180–199 and 0.5 mg of *Mycobacterium tuberculosis* (strain H 37 RA; Difco, Detroit, MI, USA) emulsified in 25 μ l saline and 25 μ l Freund's incomplete adjuvant (FIA) (Chemicon Inc., Temecula, CA). The FIA + *M. tuberculosis* mixture is referred to as Freund's complete adjuvant (FCA). All mice received 400, 200, and 200 ng pertussis toxin (Sigma, St. Louis, MO, USA) by intravenous injection on days -1, 0 and 3 post immunization (p.i.), respectively. Using a blinded protocol, two to four different examiners assessed clinical scores of EAN immediately before immunization (day 0) and thereafter every other day until day

24 p.i. EAN was scored as follows: 0, normal; 1, flaccid tail, decreased tone in whole tail or mild limb weakness; 2, severe limb weakness or mild hind limb paralysis; 3, moderate hind limb paraparesis; 4, severe hind limb paralysis; 5, severe tetraparesis.

2.4. Histopathological assessment

Five mice from each group were sacrificed on day 24 p.i., i.e. at the height of clinical EAN, and used as the source of sciatic nerve segments, which were excised close to the lumbar spinal cord. The segments of sciatic nerves were dissected, fixed in 4% formaldehyde and embedded in paraffin. Multiple longitudinal sections (5 to 6 mm slices) (4 sections for each mouse) of sciatic nerves were stained with haematoxylin–eosin for evaluation of the extent of mononuclear cell (MNC) infiltration. Tissue areas (2–3 fields per section) were measured by image analysis and the numbers of inflammatory cells counted at $\times 20$ magnification. The results were averaged and expressed as cells per mm² tissue section.

2.5. Preparation of single Schwann cell (SC) suspensions

On day 24 p.i., the sciatic nerves of 15 mice from each group were collected as described in the above. Segments of sciatic nerves close to lumbar spinal cord from sacrificed mice were mixed with 0.4% collagenase and 0.25% Trypsin at 37 °C for 30 min and dissociated, grinded as well as passed through a 70 μ m nylon mesh. Single Schwann cell (SC) suspensions were washed three times with phosphate-buffered saline (PBS).

2.6. Isolation of mononuclear cells (MNC) from spleens

Spleens from six mice of each group, sacrificed on day 24 p.i., were removed under aseptic conditions. Single cell suspensions of MNC from individual mice were prepared. The cells were washed three times in culture medium before being suspended at 2×10^6 MNC/ml. The culture medium consisted of RPMI 1640 (supplemented with 25 mM HEPES and L-glutamine) (Gibco Invitrogen, Karlsruhe, Germany), 50 IU/ml penicillin, 60 mg/ml streptomycin (Gibco, Paisley, UK), and 10% fetal bovine serum (FBS) (GibcoBRL, Germany).

2.7. MNC and proliferation assay

MNC suspended in 200 μ l portions were cultured in triplicates in round-bottomed 96-well polystyrene microtiter plates (Nunc, Copenhagen, Denmark) at a cell density of 2×10^6 cells/ml in a humidified atmosphere with 5% CO₂ at 37 °C. For lymphocyte stimulation, 10 μ l of either P0 180–199 peptide (final concentration 10 μ g/ml) or 10 μ l of phytohemagglutinin (PHA, final concentration 20 μ g/ml) (Difco) were added. These concentrations had optimal stimulatory effects as assessed in preliminary experiments. After 60 h of incubation, cells were pulsed with ³H-methylthymidine (1 Ci/well, Amersham, Little Chalfont, UK) and cultured for an additional 12 h. Cells were harvested onto glass fiber filters (Titertek, Skatron, Lierbyen,

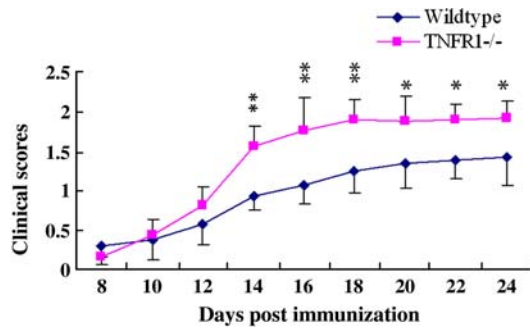


Fig. 1. Effect of TNFR1 deficiency on the outcome of EAN. The wild type mice ($n=22$) and TNFR1^{-/-} mice ($n=23$) were immunized with P0 peptide 180–199 in Freund's complete adjuvant (FCA) twice, at day 0 and day 7, and monitored for the development of clinical EAN. The results are presented as mean values \pm SD of clinical scores. * $P<0.05$ ** $P<0.01$. Data are representative of four separate experiments.

Norway). ³H-thymidine incorporation was measured in a liquid β -scintillation counter, and results were expressed as counts per minute (cpm).

2.8. Enumeration of IFN- γ and IL-4 secreting cells in the spleen

A solid-phase enzyme-linked immunospot assay (ELISPOT) was used to detect single cells that secreted IFN- γ and IL-4 upon antigen stimulation (Zou et al., 2000). Briefly, nitrocellulose-bottomed plates (Microtiter-HAM, Millipore, Bedford, UK) were coated with 100 μ l of anti-rat IFN- γ and anti-rat IL-4 monoclonal antibodies (mAbs) (from the Central Laboratory Animal Institute (CLAI), Utrecht, The Netherlands)), overnight at 4 °C. Then 200 μ l containing 4×10^5 MNC (6 mice for each group) were added in duplicate with either medium alone (control cultures without antigen) or 10 μ l aliquots of P0 peptide 180–199 (final concentration of 10 mg/ml) or PHA (final concentration of 20 mg/ml) (Difco). After 48 h culture, secreted and bound IFN- γ and IL-4 were visualized by sequential application of rabbit anti-rat IFN- γ polyclonal antibody and rabbit anti-rat IL-4 polyclonal antibody (CLAI), respectively, followed by biotinylated swine anti-rabbit IgG (Sigma) and then avidin–biotin peroxidase complex (ABC

Table 1
Survey of inflammatory cell infiltrations in sciatic nerves of EAN mice on day 24 p.i.

EAN groups	Numbers of mice	Mean \pm SD inflammatory cells/mm ²	<i>P</i> value
Wild type	5	79.8 \pm 9.4	
TNFR1 ^{-/-}	5	124.4 \pm 14.8	$P<0.01$

n = number of rats.

Inflammatory cell infiltrations in sciatic nerves of the two EAN groups (4 sections for each mouse) were counted and the results were averaged and expressed as cells per mm² PNS nerve sections. Tissue areas (2–3 fields per section) were measured by image analysis. Values indicate mean numbers of cellular infiltration \pm SD in groups of five mice on day 24 p.i. *P* value refers to comparisons between wild type and TNFR1^{-/-} mice. Two independent experiments gave similar results.

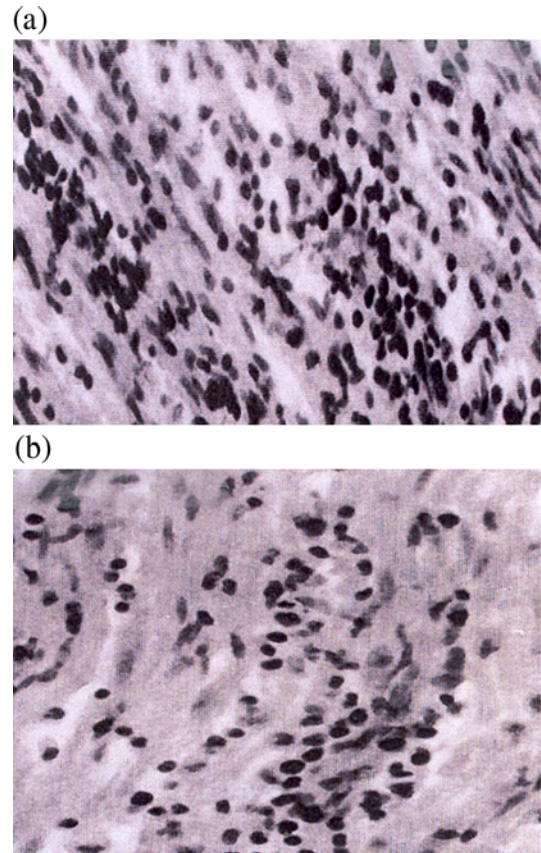


Fig. 2. Histopathological analysis of the PNS during EAN. Sciatic nerve sections from TNFR1^{-/-} and wild type mice immunized with P0 peptide 180–199 were collected on day 24 p.i. (five mice per group). Haematoxylin–eosin staining shows an increase of inflammatory infiltrates in sciatic nerve section of TNFR1^{-/-} mouse (a) compared to that of wild type mice (b) ($\times 400$).

Vectastain Elite Kit, Vector). After peroxidase staining, the red–brown immunospots corresponding to the cells that had secreted IFN- γ or IL-4 were counted in a dissection microscope. Results were expressed as numbers of spots per 10^6 splenic MNC.

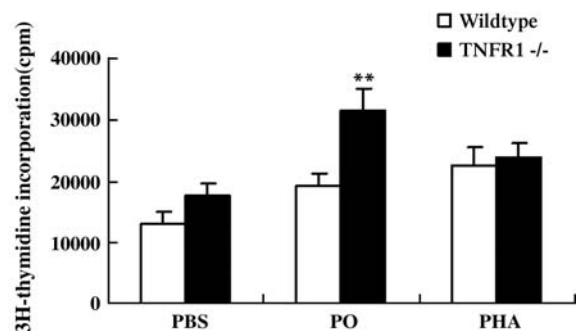


Fig. 3. Antigen-specific proliferative responses of spleen cells. Mice were immunized with P0 peptide 180–199 plus FCA twice, at day 0 and day 7 p.i. Spleen cells from wild type and TNFR1^{-/-} mice were harvested on day 24 p.i. and tested for their proliferation in response to *in vitro* re-stimulation with P0 peptide 180–199 and PHA or PBS as controls. Mean values and SEM of ³H-thymidine incorporation are indicated (six mice per group). Asterisks refer to comparisons between TNFR1^{-/-} and wild type mice. ** $P<0.01$. Two independent experiments gave similar results.

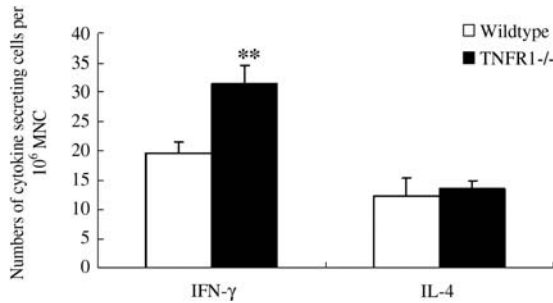


Fig. 4. The numbers of cytokine secreting cells by spleen cell from wild type and TNFR1^{-/-} mice immunized with P0 peptide 180–199. Spleen cells were harvested on day 24 p.i. and tested for the numbers of IFN- γ and IL-4 secreting cells in response to in vitro re-stimulation with P0 peptide 180–199 by ELISPOT assay. The results are expressed as the mean values \pm SD (six mice per group). Statistical evaluation compared two groups. ** P <0.01. One representative experiment of two is shown.

2.9. Flow cytometry analysis

Surface phenotypes of the infiltrating cells of the cauda equina were identified by immunofluorescence. Briefly, after dissociation and enrichment by Percoll gradient, infiltrating cells from cauda equina suspended in 50 ml 1% bovine serum

albumin (BSA) in PBS were mixed with the following PE or FITC-conjugated anti-mouse monoclonal antibodies (mAb): anti-mouse F4/80, anti-mouse MHC II (Serotec, Oxford, UK), anti-mouse chemokine receptor 3 (CCR3) (R and D Systems Inc. Oxon, UK), at saturating titers for 30 min at 4 °C. For intracellular cytokine staining, cells were fixed in 2% paraformaldehyde for 20 min, then washed twice with PBS–BSA and 0.5% saponin1 PBS–BSA followed by the addition of anti-mouse TNF- α and anti-mouse interferon (IFN)- γ Mab (BD Biosciences Pharmingen, San Jose, CA) for 30 min at 4 °C. FITC- and PE-conjugated isotype antibodies (both from Serotec) were used as negative controls. Cells were subsequently washed twice with PBS–BSA, re-suspended in PBS–BSA containing 1% paraformaldehyde, stored at 4 °C until flow cytometric analysis. The mean fluorescence intensity (MFI) or percentage of positive cells of F4/80⁺ macrophages or infiltrating cells was analyzed by a FACSCalibur cytometer using CellQuest software (Becton Dickinson, San Jose, CA).

For detection of SC apoptosis, the isolated SC were incubated with rabbit anti cow S-100 antibody and followed staining by APC-goat anti rabbit IgG (both from BD) with FITC-Annexin V and PI (Sigma), or PE–Fas (BD). The percentages of expression of Fas and Annexin-V on gated S-100 positive SC were analyzed by a FACSCalibur cytometer using CellQuest software (Becton).

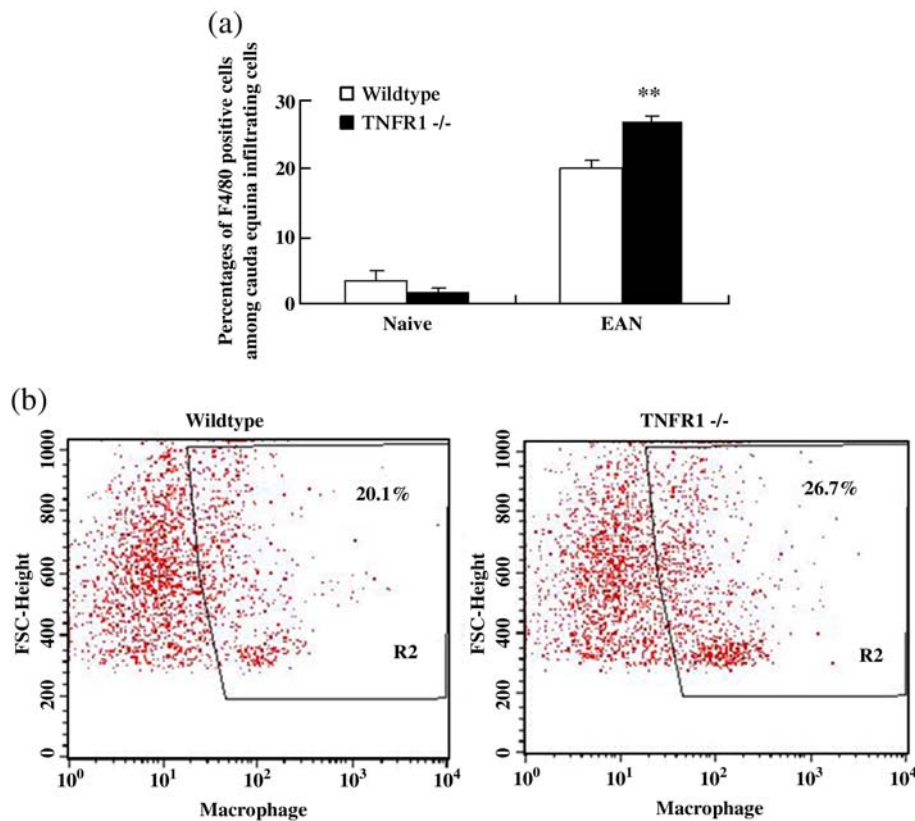


Fig. 5. FACS analysis of cells expressing F4/80 among the infiltrating cells in the cauda equina within both naïve and EAN wild type as well as TNFR1^{-/-} mice (five mice per group) (a) Mean percentages of F4/80⁺ macrophages and SEMs are indicated. Asterisks refer to comparisons between TNFR1^{-/-} mice and wild type controls. ** P <0.01. (b) FACS analysis of infiltrating macrophages in the cauda equina of EAN wild type and TNFR1^{-/-} mice. Numbers refer to the positive percentages from three experiments.

2.10. Statistics

Differences between two groups were tested by using Student's *t*-test. Differences among more than two groups were tested by using one-factor analysis of variance (ANOVA). The level of significance was set at $P < 0.05$.

3. Results

3.1. More severe clinical signs in *TNFR1*^{-/-} EAN mice

All *TNFR1*^{-/-} and wild type mice developed clinical signs of EAN on days 8–10 after inoculation with P0 peptide 180–199 and FCA. However, the clinical signs of the disease were more severe in *TNFR1*^{-/-} mice than in wild type mice (Fig. 1). In *TNFR1*^{-/-} mice, the peak clinical score was 1.9 ± 0.6 and the average day of onset was 10.2 ± 1.1 , while in wild type mice the peak clinical

score was 1.5 ± 0.3 and the average day of onset was 9.4 ± 0.7 . Differences in clinical scores were statistically significant while comparing the two groups from day 14 p.i. and onwards.

3.2. Enhanced inflammatory cell infiltrations into the PNS in *TNFR1*^{-/-} EAN mice

The entry of inflammatory cells into the PNS is a critical step in the development of EAN. To examine the influence of *TNFR1* deficiency on recruitment of inflammatory cells to the PNS, we performed histopathological study at the height of the clinical course of EAN (day 24 p.i.). The data of histopathological evaluation are presented in Table 1. The results revealed more significantly pronounced inflammatory infiltrate composed of macrophages and lymphocytes in sciatic nerve sections in *TNFR1*^{-/-} mice (Fig. 2a; $P < 0.01$ when compared with the wild type mice, see Table 1) than in wild type mice (Fig. 2b).

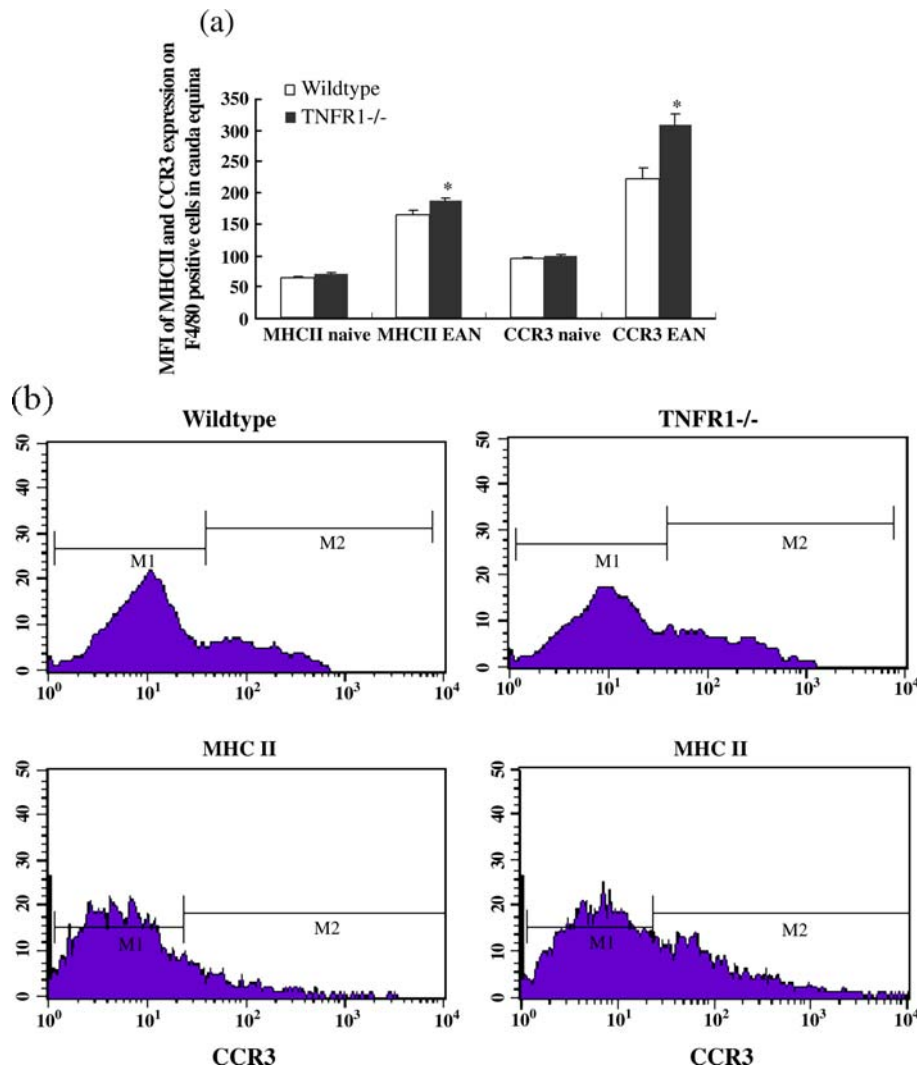


Fig. 6. MFI of MHCII and CCR3 on F4/80⁺ macrophages in the cauda equina on day 24 p.i., which was analyzed by setting gate as F4/80 positive population of cells. MHCII and CCR3 on F4/80⁺ macrophages were increased on *TNFR1*^{-/-} EAN mice as compared to wild type EAN mice. While in naive group, there was no significant difference between *TNFR1*^{-/-} and wild type groups. (a) Mean values and SEM are indicated (five mice per group). Asterisks refer to comparisons between *TNFR1*^{-/-} and wild type mice. ($*P < 0.05$). (b) The relevant histograms are presented. Two independent experiments gave similar results.

3.3. Increased T cell priming response and Th1 cytokine production in TNFR1^{-/-} EAN mice

To obtain further insight into the mechanisms of the increased severity of EAN in TNFR1^{-/-} mice and to search for an effect of TNFR1 on antigen-specific T cell activation and expansion as well as Th1/Th2 responses in EAN, we examined spleen cells from TNFR1^{-/-} and wild type EAN mice at the height of their disease course (on day 24 p.i.). When tested for proliferation in response to re-stimulation with P0 peptide 180–199 and PHA in vitro, cells from TNFR1^{-/-} EAN mice ($n=6$, in each experiment) reached significantly greater numbers after P0 peptide 180–199 stimulation than did their wild type EAN mice ($n=6$, $P<0.01$), whereas control and PHA-induced T cell proliferation was not significantly different between the two groups (Fig. 3). Thus, the CD4 T cells had undergone excessive priming in TNFR1^{-/-} EAN mice.

We also tested Th1 and Th2 cytokine production by quantified the cytokine-secreting cells by ELISPOT assay. Clearly, the TNFR1^{-/-} EAN mice produced greater frequency of IFN- γ -producing Th1 splenic lymphocytes in response to re-stimulation with P0 peptide 180–199 than did wild type mice (Fig. 4). However, there was no difference between the two groups with respect to numbers of IL-4-producing cells. Thus, CD4⁺ T cells apparently received excessive priming for a Th1 effector cell response in the periphery and re-direction from a Th2 towards a Th1 phenotype. This indicates an important role for TNFR1 in antigen-specific T cell activation and expansion.

3.4. Increased macrophage infiltration as well as MHCII and CCR3 expression in TNFR1^{-/-} EAN mice

Since the cauda equina is the most easily and earliest affected tissue and macrophages represent a large proportion of the infiltrating cells in EAN mice, infiltrating macrophages (F4/80⁺ cells) as well as MHCII and CCR3 expression on macrophages were assessed by FACS analysis on day 24 p.i. For comparison, naive mice and EAN mice on a wild type or TNFR1^{-/-} background were also investigated. Infiltrating cells in the cauda equina were enriched by Percoll gradient and identified via light scatter analysis as a homogeneous population (data not shown). Among infiltrating cells, the percentage of F4/80⁺ macrophages was higher in TNFR1^{-/-} EAN mice than in wild type EAN mice (Fig. 5a and b). The MFIs of MHCII and CCR3 on F4/80⁺ macrophages derived from a gated F4/80 positive population of cells were also increased in TNFR1^{-/-} EAN mice as compared to wild type EAN mice (Fig. 6a and b). However, as expected, TNF- α expression was significantly lower in infiltrating cells of the cauda equina in TNFR1^{-/-} EAN mice compared to wild type EAN mice (Fig. 7a and b), whereas IFN- γ expression was similar in both groups (data not shown). In naive mice, F4/80⁺ macrophages possessed less than 5% of resident cells in the cauda equina (Fig. 5a), and the expression of MHCII and CCR3 molecules on these cells was 2.5- to 4-fold lower than those in both groups of mice with EAN (Fig. 6a). The levels of TNF- α and IFN- γ was significantly lower in naive TNFR1^{-/-} mice than in naive wild type mice,

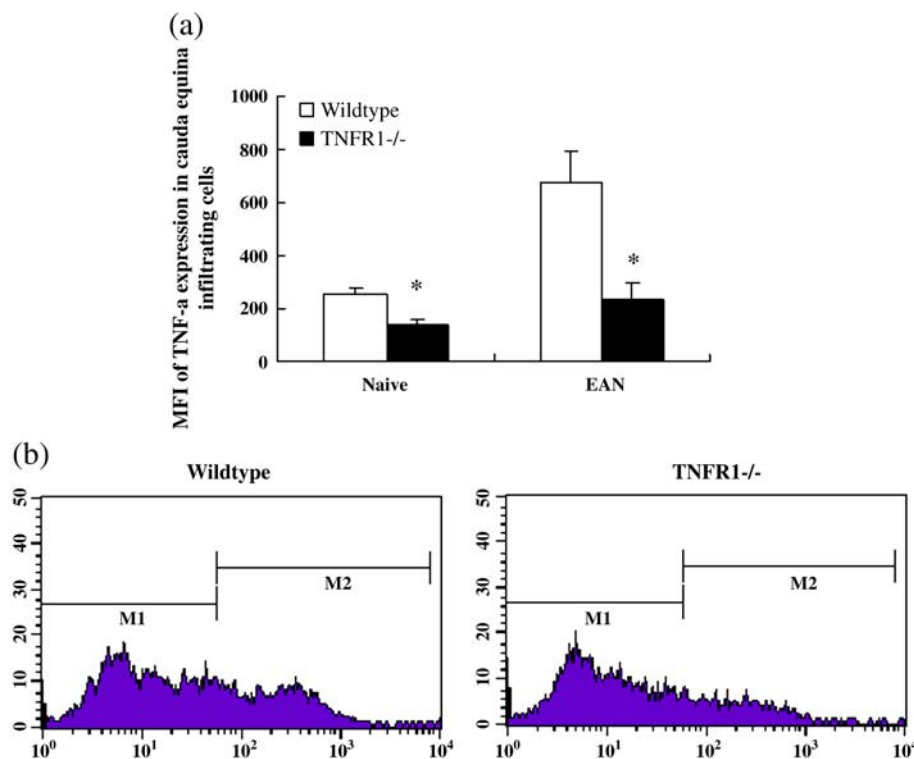


Fig. 7. FACS analysis of MFI of TNF- α expression on infiltrating cells in the cauda equina of naïve and EAN wild type and TNFR1^{-/-} mice. (a) Mean values and SEMs are indicated (five mice per group). Asterisks refer to comparisons between TNFR1^{-/-} mice and wild type controls. * $P<0.05$. (b) The relevant histograms are presented. Two independent experiments gave similar results.

although generally less than in the EAN mice (Fig. 7a for TNF- α ; data not shown for IFN- γ).

3.5. TNFR1 deficiency did not affect on SC apoptosis in the sciatic nerve

The percentages of Fas and Annexin-V expression on gated S-100 positive SCs of TNFR1 $^{-/-}$ mice was similar to those of wild type mice (data not shown). The result indicated that TNFR1 deficiency did not affect on the SC apoptosis in the target tissue during the acute phase of EAN.

4. Discussion

Using the model of EAN established in TNFR1 $^{-/-}$ mice, we investigated the roles of TNF- α and its receptor I in autoimmune-mediated demyelination. Our results showed that TNFR1 $^{-/-}$ EAN mice developed significantly severe clinical signs of disease, in parallel with enhanced P0-reactive T cell proliferation and Th1 cytokine (IFN- γ) production of spleen cells, as well as severe inflammation in peripheral nerves and more activated macrophages and lower level of TNF- α in the cauda equina as compared to wild type mice. Thus, TNF- α may have an anti-inflammatory effect preventing the development of EAN in this mouse model. In addition, we delineated several potential mechanisms by which TNF- α and TNFR1 exert the protective role in EAN.

EAN is a T cell mediated autoimmune disease. TNFR1 $^{-/-}$ mice showed significantly enhanced P0-induced T cell proliferation, suggested an important role of TNFR1 in suppressing specific T cell response and expansion in EAN, therefore, TNFR1 deficiency could contribute to induce severe of clinical disease of EAN. Additionally, TNF- α may even protect from autoimmune demyelination and promote remyelination in EAN, since it induces myelin basic protein transcription via NF κ B (Huang et al., 2002) and enhances oligodendrocyte progenitor cell proliferation and remyelination (Arnett et al., 2001). Moreover, the effects of TNF- α may differ dependent on timing, i.e. early proinflammatory activity may be followed by late immunosuppressive activity (Liu et al., 1998; Korner et al., 1997).

The potential mechanisms include also T helper cell shift from a Th1-to Th2-like phenotype and attenuation of T cell receptor signalling, induction of functional immunological tolerance and T cell apoptosis (Zheng et al., 1995). In EAN, Th1 cytokines predominate and mediate inflammatory damage, whereas Th2 cytokines have been associated with remissions and recovery from disease (Zhu et al., 1997a,b, 1998). The Th1 cytokines, IFN- γ and IL-12, have all been linked to disease expression in EAN (Zhu et al., 1998; Bao et al., 2002). In the present study, TNFR1 $^{-/-}$ mice increased IFN- γ production in the spleen cells. Convincing evidence asserts that a sufficiently high level of IFN- γ producing cells in blood, lymph nodes and PNS tissue roughly parallels the presence of clinical EAN, consistent with an inflammatory role of Th1-promoting cytokines in the pathogenesis of EAN (Zhu et al., 1998). However, the specific contribution of TNF- α signalling through the TNF receptors to the pathogenesis of inflammatory and auto-

immune disease remains to be investigated. Yang et al. (2001) found that CD4 $^{+}$ T cells showed a higher sensitivity to TNF- α receptor-mediated apoptosis than CD8 $^{+}$ T cells. Moreover, inhibition experiments showed that, in EAN TNF- α participated in T cell apoptosis in the inflammatory lesions of the sciatic nerve and liver (Weishaupt et al., 2000), and the levels of TNF- α increased during high-dose antigen therapy (McFarland et al., 1995).

Additionally, repeated injections of recombinant human TNF- α reversed the disease severity in animals with a TNF- α $^{-/-}$ background, a selective role for signalling by the TNFR1 in disease protection can be inferred, since human TNF- α binds and signals through murine TNFR1, but not TNFR2 (Liu et al., 1998). Our findings in TNFR1 $^{-/-}$ EAN mice also indicate that TNF- α through a TNFR1-dependent mechanism can inhibit T cell proliferation. The protective effect of TNFR1 in the present study is being exerted at both levels of the effector immune response and the target tissue, but dominantly at the level of the effector immune response, since TNFR1 deficiency enhanced P0-reactive T cell proliferation, expansion and Th1 cytokine production of spleen cells, but not inducing SC apoptosis in the acute phase of EAN.

Finally, a great deal of evidence shows that chemokines and their receptors play a critical role in autoimmune diseases by orchestrating the migration of inflammatory cells (Duan et al., 2004). In our present study, CCR3 expression on macrophages of the cauda equina in TNFR1 $^{-/-}$ EAN mice was clearly increased compared to that of wild type controls, which might partly account for the enhanced inflammation in the PNS of the TNFR1 $^{-/-}$ mice, which also contributed to the enhanced clinical disease. Increased levels of chemokines or their receptors were also evident in other animal models of TNF- α or TNFR1 deficient mice (Olleros et al., 2002).

In summary, our studies indicate that TNF- α , despite its proinflammatory activities, may also play an anti-inflammatory role, mediated mainly through TNFR1 in EAN. Our results define a previously unrecognized role for TNF- α and its receptors in EAN and call for caution in the use of anti-TNF- α therapeutics in autoimmune diseases.

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