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Soluble TNFR1 inhibits the development of experimental autoimmune neuritis by modulating blood–nerve-barrier permeability and inflammation ☆

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Abstract

The role of TNFα/LTα during EAN induced by active immunization with peripheral nerve myelin was examined by administering a recombinant soluble chimeric form of human TNF receptor 1 (TNFR1-IgG). TNFα and LTα do not directly contribute to neurological deficit during EAN since treatment with TNFR1-IgG after onset failed to alter the course of disease. Prophylaxis with a single dose of TNFR1-IgG delayed the onset of EAN and was accompanied initially by inhibition of blood–nerve-barrier permeability and inflammation. Subsequently, the number of infiltrating macrophages and blood–nerve-barrier permeability increased but the disease symptoms remained mild for five days (on average a limp tail) after which severe EAN developed. The antibody titer to peripheral nerve myelin was unaltered by prophylaxis with TNFR1-IgG. The markedly altered tempo of disease onset after TNFR1-IgG prophylaxis indicates that TNFα and/or LTα have a key role in the development of blood–nerve-barrier permeability and the coupling of macrophage activation and recruitment to peripheral nerve pathology during EAN.

Keywords: EAN; TNF; Lymphotoxin; Macrophage; Demyelination; Blood–nerve-barrier

1. Introduction

Experimental autoimmune neuritis (EAN) has histopathological, electrophysiological and clinical similarities to the Guillain Barre Syndrome (GBS) and therefore is used as an animal model to understand the pathogenesis of GBS (Asbury et al., 1969). During EAN animals develop an acute bilateral ascending limb weakness with concurrent inflammation, demyelination and nerve conduction changes in the peripheral nervous system.

In recent years there have been a number of studies examining the kinetics and function of cytokines, chemokines and other growth factors during the course of myelin induced EAN in the Lewis rat. There are a plethora of these molecules elaborated during the generation, execution and resolution of the inflammatory response and they have manifold functions including regulating the differentiation, migration and viability of T and B lymphocytes and macrophages critically involved in the pathophysiology of disease. Kinetic studies have employed PCR and in situ hybridization to examine the expression of mRNA for various chemokines and cytokines. Maximal expression of the cytokines TNFα, LTα, IL-1β, IFNγ and the chemokines MIP-1α and MIP-1β was observed at disease onset whereas IL-12 correlated with recovery (Zhu et al., 1997; Gillen et al., 1998; Kieseier et al., 2000). Manipulation, via functional blockade or depletion using the systemic administration of specific antibodies confirmed a pro-inflammatory role for Interferon gamma (Hartung et al., 1990; Strigard et al., 1989), Tumor Necrosis Factor alpha (Stoll et al., 1993) and

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MIP-1α (Zou et al., 1999b) during EAN. Conversely, prophylactic or therapeutic administration of the Type 1 Interferon's, IL-4, IL-10 and IFNβ reduced the severity of actively induced EAN demonstrating a potential immunosuppressive role for these cytokines during EAN (Gregorian et al., 1994; Bai et al., 1997; Deretzi et al., 1999; Jung et al., 1994; Vriesendorp et al., 1996; Zou et al., 1999a).

The current study was conducted to assess the effect of administering a recombinant p55 TNF receptor protein (TNFR1-IgG) to Lewis rats with EAN induced actively by immunization with peripheral nerve myelin. Membrane associated TNF, soluble TNF and soluble lymphotoxin alpha (LT-α), are ligands for TNFR1 (Korner and Sedgwick, 1996).

2. Materials and methods

2.1. Experimental animals

Male Lewis rats (300 g–400 g) and female Lewis rats (190–220 g) were bred and housed in a non-SPF animal facility (BAH, Sydney University, Australia). All procedures were conducted in accordance with protocols approved by the Animal Care and Ethics Committee of the University of Sydney.

2.2. Anesthesia

All invasive procedures were performed under pentobarbitone (Nembutal®, Lyppard, Australia), administered by intraperitoneal injection, anesthesia. A dose of 30 mg/kg was used for female rats and a dose of 60 mg/kg was used for male rats.

2.3. Preparation of antigens

Bovine and rat PNM were purified from intradural roots as previously described (Harvey et al., 1987).

2.4. Induction of EAN by immunization with PNM

EAN was induced in male and female Lewis rats by immunization in each hind footpad with 50 μl of a 1:1 saline: IFA (Sigma, St. Louis, MO, USA) emulsion containing 0.25 mg H37RA *Mycobacterium tuberculosis* (DIFCO Laboratories, Inc., Detroit, MI, USA) and 1.5 mg of lyophilized bovine PNM. Adjuvant (CFA) control rats were immunized with the same emulsion but the myelin was omitted.

2.5. Scoring of EAN symptoms

Rats were observed every one to two days for signs of disease and were scored as follows: 1, limp tail; 2, paraparesis; 3, tetraparesis; 4, tetraparesis and difficulty in breathing. Intermediate symptoms were assigned a score of 0.5, 1.5, 2.5 and 3.5. The clinical score for each rat over the course of EAN from the day of immunization to recovery was added together and used to calculate the total clinical score to compare the overall severity of disease between treated and control rats.

2.6. Administration of the TNF receptor 1-IgG fusion protein

The TNF receptor 1-IgG1 fusion protein was a generous gift from Dr Bernie Scallon (Centocor, PA, USA). In the treated group, each rat was injected intravenously with 5 mg of the TNF receptor 1 fusion protein two days after onset of disease. In separate experiments each rat was injected intravenously with 5 mg of the TNF receptor 1 fusion protein one or two days prior to the onset of disease (day 9 or 8 post-immunization respectively). Control rats for each experiment were injected intravenously with an equivalent quantity of human IgG purified from blood bank sera by protein A affinity chromatography.

2.7. Neurophysiological measurements

Neurophysiological studies were performed prior to immunization and then at regular intervals after immunization. Sensory nerve conduction was assessed using spinal somatosensory evoked potentials (SSEP) (Wietholter and Hulser, 1985). Using a Medelec MS92b neurophysiology machine and paired needle electrodes inserted at the ankle the sciatic nerve was stimulated with supra-maximal rectangular pulses of 0.05 ms duration and the resulting SSEP were recorded from needle electrodes inserted subcutaneously at the dorsal junction of the thirteenth thoracic and first lumbar vertebrae. The averaging function on the MS92b was used to record twenty SSEP and the mean peak latency and amplitude was recorded for the left and right side.

2.8. Immunohistochemistry

Rats receiving 5 mg of the p55 fusion protein or IgG one day before the expected onset of disease (and five controls) were used for immunohistochemical examination. Five or six rats were used for each time point. Under anesthesia, a small incision was made in the right ventricle and rats were perfused through the left ventricle with 150 ml phosphate buffered saline. After laminectomy the lumbar nerve roots were removed and embedded in O.C.T. compound (Miles, IN, USA). 5 μm transverse sections were cut, air dried, fixed in 95% ethanol for 10 min, washed in PBS and blocked with PBS + 10% FCS for 10 min. After a second wash in PBS, incubation with ED1 tissue culture supernatant diluted 1/2 in PBS + 10% normal rat serum and 10% fetal calf serum was performed for 1 h. Sections were then washed again and incubated with Horseradish peroxidase-labeled rabbit anti-mouse immunoglobulin (DAKO, Denmark) diluted 1/100 in PBS + 10% normal rat serum and 10% fetal calf serum for 1 h. After a fourth wash sections were incubated with