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Tissue resident macrophages are sufficient for demyelination during peripheral nerve myelin induced experimental autoimmune neuritis?

Jude Matthew Taylor[1]

Neurology Laboratory, University of Sydney, 2006 Sydney, Australia

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ABSTRACT

The contribution of resident endoneurial tissue macrophages versus recruited monocyte derived macrophages to demyelination and disease during Experimental Autoimmune Neuritis (EAN) was investigated using passive transfer of peripheral nerve myelin (PNM) specific serum antibodies or adoptive co-transfer of PNM specific T and B cells from EAN donors to leukopenic and normal hosts.

Passive transfer of PNM specific serum antibodies or adoptive co-transfer of myelin specific T and B cells into leukopenic recipients resulted in a moderate reduction in nerve conduction block or in the disease severity compared to the normal recipients. This was despite at least a 95% decrease in the number of circulating mononuclear cells during the development of nerve conduction block and disease and a 50% reduction in the number of infiltrating endoneurial macrophages in the nerve lesions of the leukopenic recipients.

These observations suggest that during EAN in Lewis rats actively induced by immunization with peripheral nerve myelin, phagocytic macrophages originating from the resident endoneurial population may be sufficient to engage in demyelination initiated by anti-myelin antibodies in this model.

1. Introduction

Experimental autoimmune neuritis (EAN) induced by immunizing rodents with whole peripheral nerve myelin (PNM), purified PNM proteins or glycolipids, encompasses a diverse range of neuropathologies and acute motor-sensory symptoms that vary depending on the antigen and host species used. Distinct forms of EAN serve to model the different subtypes of the Guillain-Barre Syndrome (GBS) based on the respective clinical and pathological similarities. Thus the classical EAN model using whole PNM as the antigen induces co-operative T and B cell autoimmunity, inflammation and demyelination in the peripheral nerves and is commonly used to model the Acute Inflammatory Demyelinating Polyneuropathy (AIDP) variant of GBS (Toyka and Heininger, 1987). Gangliosides have also been used as antigens in rabbits and immunization with GD1a or GM1 induces a slowly progressive, non-inflammatory axonopathy mediated by anti-ganglioside autoantibodies (Nagai et al., 1976; Yuki et al., 2001) that resemble the Acute Motor Axonal Neuropathy (AMAN) variant of GBS.

Lewis rats immunized with PNM develop an acute bilateral ascending limb weakness with concurrent inflammation, demyelination and nerve conduction block and dispersion in the peripheral nervous system (Harvey and Pollard, 1992; Taylor and Pollard, 2003). The pathogenesis of this form of EAN requires specific T and B cell immunity to PNM proteins (Taylor and Pollard, 2001), high serum antibody titers to PNM proteins (Archelos et al., 1993; Koehler et al., 1996) and immunoglobulin can be detected in the endoneurium of the nerve roots just prior to the onset of disease (Rosen et al., 1990a). Macrophages and infiltrating T cells comprise the bulk of the inflammatory population in the nerve roots (Fujioka et al., 2000) and demyelination is intimately associated with endoneurial macrophages (Lampert, 1969; Muller et al., 2006; Stevens et al., 1989). Pharmacological methods to systemically deplete or modulate macrophage activity have clearly demonstrated the importance of macrophages to the disease process during EAN (Hartung and Toyka, 1990). However, to date there have been no interventional studies to directly assess how dependent the process of demyelination, initiated by anti-PNM IgG/IgM antibodies and complement, is on nerve resident tissue macrophages versus recruited monocyte derived macrophages or the macrophage receptors that engage in this process.

Normal peripheral nerves contain resident monocyte derived macrophages that continually turn over and a small subpopulation of true tissue macrophages that are both involved in the pathogenesis of peripheral nerve disease (Muller et al., 2010). During an inflammatory

Abbreviations: AIDP, Acute Inflammatory Demyelinating Neuropathy; BNB, blood-nerve-barrier; CFA, complete freunds adjuvant; CMAP, compound muscle action potentials; EAN, experimental autoimmune neuritis; IV, intravenous; LNC, lymph node cells; OVA, ovalbumin; PI, post-injection; PNM, peripheral nerve myelin; SSEP, samatosensory evoked potential; WBI, whole body irradiation

E-mail address: jude.taylor@scu.edu.au.

[1] Current address: M.1.24, Southern Cross University, Hogbin Drive, 2450 Coffs Harbour, Australia.

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response infiltrating tissue macrophages can also differentiate from recruited circulating monocytes that are released from the bone marrow or deployed from stores in the spleen (Italiani and Boraschi, 2017; Swirski et al., 2009).

In the current study whole body irradiation induced leukopenia and adoptive transfer was used to determine the relative contribution of peripheral nerve resident macrophages and recruited monocyte derived macrophages to the neuropathology of EAN induced by immunization with PNM.

2. Materials and methods

2.1. Animals

Lewis rats were bred and housed in a 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 or surgical procedures were performed under pentobarbitone sodium (Lyppard, Australia) anesthesia. An intraperitoneal 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 PNM and rat PNM were purified from intradural roots (Harvey et al., 1987). Purified ovalbumin was purchased from Sigma.

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 50μl of a 1:1 saline:IFA (Sigma) emulsion containing 0.25 mg H37RA *Mycobacterium tuberculosis* (DIFCO) and 2.5 mg of lyophilized bovine PNM. Control 'CFA' rats were immunized in the same manner but the PNM was omitted from the emulsion. Control 'OVA' rats were immunized in the same manner with 2.5 mg of ovalbumin in the emulsion.

2.5. Preparation of lymph node cells for intraneural injection

Eight days following immunization with PNM or CFA the popliteal lymph nodes were excised. A lymph node cell suspension was prepared by gentle grinding between the frosted ends of two glass slides. The lymph node cells were washed and resuspended at a final concentration of 5×10^6 cells/10 μl in RPMI 1640/10% FCS for intraneural injection.

2.6. Preparation of sera for intraneural injection

Blood was collected by cardiac puncture from rats immunized with bovine PNM at two time points after immunization: the day of onset of disease (day 13 PI), and at the height of disease (day 20 PI). After clotting the sera were pooled and frozen at − 20 °C until use. At the same time points sera were prepared from control CFA immunized rats.

2.7. Preparation of immunoglobulin for passive transfer

Blood was collected by cardiac puncture from rats immunized with bovine PNM. Serum was pooled and stored at − 20 °C. At the same time serum was prepared from control CFA immunized rats. The whole immunoglobulin fraction was purified from the EAN and CFA sera by serial precipitation with 30% and 50% saturated ammonium sulfate (Tatum, 1993). EAN immunoglobulin or control CFA immunoglubulin was dissolved in PBS (equivalent to approximately half the donor volume of sera), dialyzed against 0.9% NaCl for overnight, and 4 ml was injected IV to each recipient rat via the tail vein at day 3 after the intravenous injection of 1×10^6 OVA $CD4^+$ T cells and intraneural injection of OVA in PBS.

2.8. Preparation of T cells

OVA-specific $CD4^+$ T cell lines were prepared as described previously (Taylor and Pollard, 2001). T cells were re-suspended at a final concentration 1×10^6 cells/ml in RPMI 1640/10% FCS for intravenous injection.

2.9. Whole body irradiation to induce leukopenia

Leukopenia was induced by whole body irradiation with 6 Gy 3 days prior to injection and on the day of injection from a ^{60}Co source (Sedgwick et al., 1987). Recipients (whole body irradiated with a combined dose of 12 Gy) were maintained on acid water, 18 mM HCl, pH 2.3, starting one week before irradiation and had daily changes of cages after irradiation. A control cohort of recipients receiving PNM activated LNC from EAN donors were irradiated with a non-ablative 4 Gy dose on the day of injection.

2.10. Flow cytometry

Whole blood: blood was collected via tail vein venepuncture into a K3EDTA vacutainer containing CTAD (Becton Dickinson) and 1 mM Hoechst 33,342 dye (Molecular Probes) was added to a final concentration of 50um. After incubation for 15 min at 37 °C 5ul of blood was removed and added to 1 ml in 1 mM HBBS (Sigma) for flow cytometry. A Becton Dickinson LSR cytometer was used to acquire 1 million total events, Hoerchst stained nucleated leukocytes were gated from red blood cells and platelets and differentiated on the basis of forward scatter and side scatter. The number of mononuclear cells per million red blood cell events was calculated. Red blood cell counts were determined independently (Sysmex 3000, Roche) and divided by the baseline count to correct the mononuclear cell count as all the rats became anemic after serial whole body irradiation.

2.11. Intraneural injection of lymph node cells, whole serum or OVA

The posterior tibial branch of the sciatic nerve was exposed through an incision in the lateral thigh from the sciatic notch to the popliteal fossa. Ten microliters of RPMI 1640/10% FCS containing 5×10^6 peripheral nerve myelin sensitized lymph node cells was injected slowly into the endoneurium using an operating microscope, a 50 μl microsyringe and a 30 gauge needle. The contra-lateral nerve or the left sciatic nerve of a separate group of rats was injected with control cells: 5×10^6 CFA sensitized lymph node cells. For systemic injection of EAN IgG and IgM following OVA-specific $CD4^+$ T cells rats were first injected bilaterally at baseline with 10 μl of OVA (1 mg/ml). Whole EAN or CFA sera were injected using the same method; 10 μl of EAN or CFA sera were supplemented with 2.5 μl of freshly reconstituted Guinea Pig complement (ICN Biomedicals).

2.12. Electrophysiological measurements

Electrophysiological studies were performed prior to intraneural injection and then at regular intervals after injection. Sciatic nerve motor conduction was measured by examining the amplitude of the evoked compound muscle action potentials (CMAP) recorded from the feet. Using a Medelec MS92b neurophysiology machine and paired needle electrodes inserted at the sciatic notch or the ankle the sciatic nerve was stimulated with supra-maximal rectangular pulses of 0.05 ms duration and the resulting CMAP was recorded from needle electrodes placed subcutaneously over the dorsal foot muscles. In the intraneural