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Immunospecific inhibition of nerve conduction by T lymphocytes reactive to basic protein of myelin

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Experimental autoimmune encephalomyelitis (EAE) induced by immunization to the basic protein of central nervous system myelin (BP) is a paralytic disease in which T lymphocytes attack the individual's own central nervous system[1]. As the target is in white matter, EAE has been considered an experimental model of some aspects of human disease such as multiple sclerosis. To investigate whether autoimmune T lymphocytes could produce paralysis, we studied the effects on the electrophysiology of isolated nerves produced by T-lymphocyte lines reactive specifically to BP or other antigens. We now report that propagation of action potentials evoked by electrical stimulation was blocked by incubating optic nerves with specific anti-BP T cells. This blockade could be reversed for up to two hours by removing the anti-BP line cells from the optic nerve. The anti-BP line cells had no effect on conduction along allogeneic optic nerves or syngeneic peripheral nerves. This indicates that disruption of the function of myelin in neuroimmunological disease may result from an immunologically specific interaction between autoimmune T lymphocytes and myelin antigens.

We have succeeded in isolating and growing as long-term cell lines T lymphocytes reactive to BP or to other antigens[2-4]. Anti-BP T cells were found to mediate EAE within several days of intravenous inoculation into naive recipient rats. The rats developed paralysis and showed the same perivascular inflammatory cell infiltration as found in EAE induced by active immunization to BP in complete Freund's adjuvant. Moreover, anti-BP line cells, attenuated by treatment with mitomycin C or irradiation, could be used to vaccinate rats against subsequent induction of active EAE by immunization[5,6].

To investigate whether anti-BP T lymphocytes could disrupt nerve conduction, we constructed a chamber in which it was possible to observe conduction in an isolated nerve in the presence of test lymphocytes (Fig. 1). Each end of a section of nerve was aspirated into the tip of a suction electrode that could be used either for stimulation or recording. Propagation of action potentials was studied in a single set of axons before, during and after incubation of the nerve with T cells.

We used T cells from Lewis rat-derived lines directed against BP or against the purified protein derivative (PPD) of *Mycobacterium tuberculosis*[2-4], and assayed their effects on optic nerves containing BP of central myelin or on sciatic nerves containing an immunologically distinct peripheral myelin[7]. Nerves were obtained from Lewis rats syngeneic to the Lewis line cells, or from BN strain rats whose major histocompatibility complex (MHC) genes differ from those of Lewis rats[8]. Before use, it was necessary to activate each population of T-lymphocyte line cells by incubation for 72 h with its specific antigen, either BP or PPD, in the presence of irradiated (1,500 R) thymus cells from normal syngeneic rats as antigen-presenting accessory cells[2-4,9]. The large majority of irradiated accessory cells had died after 72 h and about 80–90% of the remaining cells were T lymphoblasts. The cells were washed by centrifugation and resuspended for testing in fresh medium that did not contain either BP or PPD antigens.

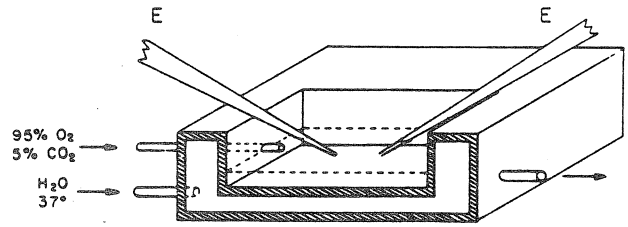

Fig. 1 Chamber for study of physiology of an isolated nerve. Segments of optic or sciatic nerves of 5 mm were obtained from decapitated rats and suspended in the chamber by aspirating each end into a suction electrode (E). The chamber was bathed in H_2O kept at a temperature of 37 °C and exposed to a constant flow of 95% O_2 and 5% CO_2. The nerve was immersed just under the surface of the Eagle's tissue culture medium. The portions of nerve within the suction electrodes were not exposed to the medium in the bath into which the lymphocytes were introduced during testing. Hence stimulating and recording conditions remained the same during the experiments. Cells were added to the medium at a concentration of 2×10^5 per ml. Three days before testing, the cells were activated by incubation with their specific antigen, either BP of central myelin or PPD, in the presence of irradiated (1,500 R) syngeneic thymocytes as accessory cells[2-4].

Blockade of action potentials by incubation of Lewis optic nerve with Lewis anti-BP T cells is illustrated in Fig. 2. Figure 2*a* shows a stepwise increase in the amplitude of stimuli; the compound action potential in optic nerve generated by stimuli of increasing voltage intensities before incubation with line cells. The two superimposed traces shown in Fig. 2*b* were obtained before (upper) and after (lower) incubation of the nerve with line cells for 40 min. The marked decrease in the amplitude of the induced action potential suggests a reduction in the number of functioning axons is caused by incubation with anti-BP T cells. This blockade could not be explained by an increase in the firing threshold because it was not reversed by increasing the intensity of the stimulus (Fig. 2*c*). Figure 2*d–f* show that the blockade produced by incubation with anti-BP T cells was reversible. The control response of the optic nerve to various stimulus intensities before incubation is illustrated in Fig. 2*d*, after incubation in Fig. 2*e*, and after removal of the anti-BP line cells in Fig. 2*f*. The blockade was not reversible after incubation of optic nerves with anti-BP T cells for longer than 2 h.

The immunological specificity of the blockade is shown in Fig. 3. It can be seen that conduction was affected by incubating Lewis optic nerves with anti-BP T cells (Fig. 3*a*) but not with anti-PPD line cells (Fig. 3*b*). Conduction was not affected in BN optic nerves incubated with Lewis anti-BP cells (Fig. 3*c*), nor in Lewis sciatic nerves incubated with Lewis anti-BP cells (Fig. 3*d*). Blockade was not produced by cell-free culture medium collected from anti-BP or anti-PPD cell lines that had been incubated with their specific antigen in the presence of irradiated antigen-presenting cells (not shown).

The immunospecificity of the interaction suggests that blockade of the nerve impulse required the recognition by the anti-BP T lymphocytes of the BP target antigen together with strain-specific self markers. We found that anti-BP lymphocytes proliferated *in vitro* when presented BP by accessory cells syngeneic at the MHC but did not respond to BP presented by accessory cells with MHC genes of BN origin[4]. Associative recognition of MHC together with target antigen appears to be a general property of effector T lymphocytes[10] and appears to be occurring here.

At present we do not know whether the line cells recognize BP together with self-MHC on antigen presenting cells or on

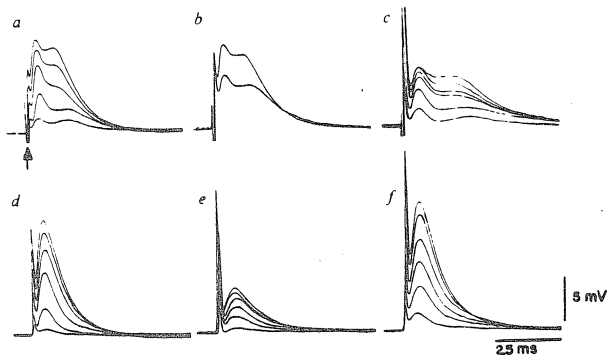

Fig. 2 Blockade of action potential along optic nerve by anti-BP line cells. Anti-BP T-cell lines were raised, maintained, and activated by incubation with BP in the presence of irradiated (1,500 R) syngeneic thymus cells as described[4]. The line cells (2×10^5 per ml) were incubated with Lewis optic nerves for 40 min and the optic nerve was stimulated by 0.05 ms pulses of various amplitudes produced by an isolated stimulator (Devices, MK IV, London). The compound action potential was recorded using a differential amplifier connected to a digital oscilloscope (Nicollat) and an *x*–*y* recorder. The arrow indicates a stimulus artefact. *a*, Before incubation with the line cells using stimuli of 0.5, 1, 2, 3 and 4 V. *b*, Recording before and after 40 min of incubation with line cells using a stimulus of 3 V. *c*, Multiple stimuli made after incubation with anti-BP line cells at intensities 1, 2, 3, 4, 5 and 6 V. *d*, Recording from a different optic nerve before incubation with anti-BP line cells using stimuli of 0.25, 0.5, 1, 1.5, 2 and 2.5 V. *e*, The response after 60 min of incubation using stimuli of 1, 2, 3, 4, 5 and 6 V, and *f*, after the line cells had been washed out of the chamber using 0.5, 1, 1.5, 2, 2.5 and 3 V.

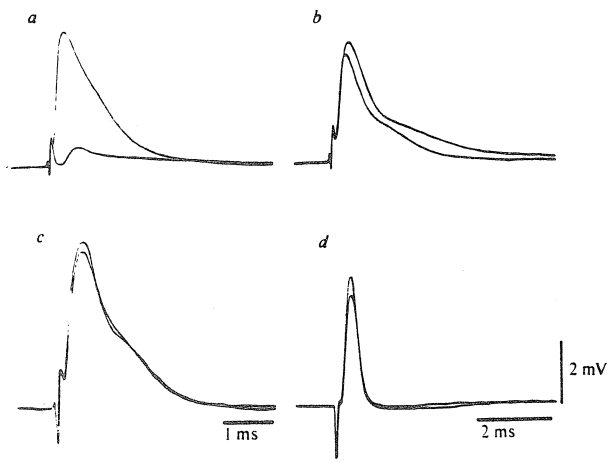

Fig. 3 Immunological specificity of blockade of nerve conduction. The preparations were as described in the legend to Figs 1 and 2. The upper curve was recorded before incubation and the lower curve after incubation with T cells. *a*, The effect of incubating a Lewis optic nerve with syngeneic anti-BP line cells. *b*, The effect of Lewis anti-PPD line cells on a Lewis optic nerve. *c*, The effect of Lewis anti-BP line cells on an optic nerve originating from an allogeneic BN rat. *d*, The effect of anti-BP line cells on syngeneic sciatic nerve.

the optic nerve itself. Before exposure to the nerves, the anti-BP line had been activated by incubation with BP in the presence of syngeneic accessory cells, yet the BN optic nerve and the Lewis sciatic nerve were not affected. Thus, dual recognition of BP and self-MHC mediated by the accessory cells before incubation with the nerve was not sufficient for the T-cell line to interrupt the nerve impulse. This argues that the inhibitory effect may require recognition of both BP and MHC gene products on the nerve itself. MHC gene products have not been demonstrated on optic nerves, but it is possible that they were expressed there because murine brain cells have been shown to express Ia antigens[11] and HLA-DR antigens appear to be present on human glial cells[12]. The specificity of inhibition of nerve conduction also argues against the possibility that macrophage-like accessory cells were the mediators of the inhibition of nerve conduction. Such macrophage-like cells were present in the control cultures of the activated anti-PPD T cells incubated with optic nerve, and in the cultures of activated anti-BP T cells incubated with either syngeneic peripheral nerve or allogeneic optic nerve. Yet no inhibition of conduction was seen.

Previous studies have claimed that sera obtained from patients with multiple sclerosis or animals with EAE can affect the electrical activity of cultured nervous tissues[13,14]. Evidence that nerve conduction can be blocked by antibodies to components of myelin was obtained in studies using antiserum to galactocerebroside[15]. However, it is highly unlikely that our lines of immunoglobulin-negative T lymphocytes[4] could have secreted antibodies that blocked nerve conduction. Demyelination of nerves produced by sensitized lymphocytes has been observed in tissue culture[16]. The present system extends these investigations as we have shown an immunologically specific and reversible blockade of nerve conduction. Two general processes could be responsible for blockade of conduction in myelinated nerve fibres; changes in the current generating mechanisms due to changes in channel properties or ion gradients[17], and alteration in fibre geometry due to disruption of myelin[18]. Although the mechanism has yet to be explored, it is likely that conduction block was initiated by changes in myelin, the site of the BP target antigen.

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1. Paterson, P. Y. in *Autoimmunity, Genetics, Immunology, Virology and Clinical Aspects* (ed. Talal, N.) 643–692 (Academic, New York, 1977).
2. Ben-Nun, A., Wekerle, H. & Cohen, I. R. *Eur. J. Immun.* **11**, 195–199 (1981).
3. Ben-Nun, A. & Cohen, I. R. *J. Immun.* **128**, 1450–1457 (1982).
4. Ben-Nun, A. & Cohen, I. R. *J. Immun.* **129**, 303–308 (1982).
5. Ben-Nun, A., Wekerle, H. & Cohen, I. R. *Nature* **292**, 60–61 (1981).
6. Ben-Nun, A. & Cohen, I. R. *Eur. J. Immun.* **11**, 949–852 (1981).
7. Brostoff, S. W., Karkhanis, Y. D., Carlo, D. J., Reuter, W. & Eylar, E. H. *Brain Res.* **86**, 449–458 (1975).
8. Altman, P. C. & Katz, D. D. *Inbred and Genetically Defined Strains of Laboratory Animals. Pt I*, 316 (FASEB, Bethesda, 1979).
9. Naparstek, Y. *et al. Eur. J. Immun.* (in the press).
10. Bevan, M. *Nature* **269**, 417–418 (1977).
11. Ting, J. P. Y., Shigekawa, B. L., Linthicum, D. S., Weiner, L. P. & Freilinger, J. A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3170–3174 (1981).
12. Carrel, S., De Tribolet, N. & Gross, N. *Eur. J. Immun.* **12**, 354–357 (1982).
13. Bornstein, M. B. & Crain, S. M. *Science* **148**, 1242–1244 (1965).
14. Lumsden, C. E., Howard, L., Aparicio, S. R. & Bradbury, M. *Brain Res.* **93**, 283–299 (1975).
15. LaFontaine, S., Rasminsky, M., Saida, T. & Sumner, A. J. *J. Physiol., Lond.*, **323**, 287–306 (1982).
16. Arnason, B. G. W., Winkiker, G. F. & Hadler, N. M. *Lab. Invest.* **21**, 1–10 (1969).
17. Huxley, A. F. *Ann. N.Y. Acad. Sci.* **81**, 221–246 (1959).
18. Rasminsky, M. in *Physiology and Pathobiology of Axons* (ed. Waxman, S. G.) 361–376 (Raven, New York, 1978).

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