Passive Transfer of Adjuvant-induced Arthritis and Allergic Encephalomyelitis in Rats using Thoracic Duct Lymphocytes

SUSPENSIONS of cells derived from lymph nodes or the spleen have been used to transfer adjuvant-induced arthritis[1,2] and allergic encephalomyelitis[3,4] in rats. We have now successfully transferred both these experimental diseases in rats using cells obtained from the thoracic duct.

Highly inbred Wistar rats (Lewis strain), obtained from Microbiological Associates (Walkersville, Maryland), were used. Arthritis was induced in donor animals by the intradermal injection into one hind foot of 0·05 ml. of a suspension of finely ground, heat-killed *Myocobact. tuberculosis H-37* (Difco Laboratories, Detroit, Michigan) in mineral oil (10 mg/ml.). Allergic encephalomyelitis was induced in donor animals by the injection into each inguinal lymph node[5] of about 0·005 ml. of a water-in-oil emulsion containing in the aqueous phase a 25 per cent homogenate of guinea-pig spinal cord and, in the oil phase, 0·5 mg/ml. heat-killed *Mycobact. tuberculosis* (strains PN, DT and C) and 5 per cent (v/v) 'Arlacel *A*' (Atlas Chemical Industries, Wilmington, Delaware).

Thoracic duct lymph was obtained by the conventional technique[6] from male animals weighing 180–220 g. Cell-free lymph was returned to these donor animals by slow infusion through a polythene cannula inserted into a left leg vein. Animals were restrained in a Bollman cage[7] throughout the period of lymph collection and cell transfer, with unrestricted access to food and to water containing 0·4 per cent (w/v) sodium chloride and some sucrose. Lymph from each animal was collected over a period of 25 or 50 h, following cannulation of the thoracic duct, into flasks containing 1 ml. of Hanks solution with added heparin (10 IU) and penicillin G (0·5 mg buffered potassium salt) kept at room temperature; these flasks were changed at 2·5 h intervals. Cells were isolated from each 2·5 h lymph collection (from 3 to 12 ml. in volume) by centrifugation at room temperature at 180–200*g* for 5 min. The bulk of the supernatant fluid (more than 80 per cent of volume) was re-infused, together with added penicillin (1–2 mg), into the appropriate animal donor. The cells were resuspended in 0·5 ml. homologous lymph for infusion through a cannulated leg vein into normal Lewis rats of either sex, weighing 200–250 g. Usually the resuspended cells from four donors were combined for infusion into a single recipient. Large numbers of erythrocytes were frequently present in the cell fraction collected and transferred during the first 6–10 h after cannulation of the thoracic duct. Thereafter the erythrocyte contamination was minimal so that the 25–50 h collections were composed of 80 per cent or more mononuclear cells. Control animals received 2 ml. of pooled cell-free lymph from the same animal donors (contributing the cells) by slow intravenous infusion at 2·5 h intervals for 25 h.

In six out of seven consecutive experiments, animals receiving cells from donors which had been injected with the adjuvant 9 or 10 days previously, developed some of the external symptoms of adjuvant arthritis within 4 days. Florid disease was consistently seen by 10 days from the time of transfer. The recipients suffered lung, spleen and ear lesions, reduction in serum albumen, marked increase in the plasma inflammation units[8] and articular and periarticular inflammation in at least three paws and sometimes the tail. These signs closely resembled those seen in adjuvant-inoculated rats with fully developed disease[8,9]. Concurrent recipients which had received lymph only (no cells) did not develop any disease. The arthritis was successfully transferred with thoracic duct lymphocytes from male donors to both male and female recipients and with both the first 25 h cell collection ($0·9–1·4 \times 10^9$ leucocytes/four donors) and with the subsequent 25–50 h collection ($0·6–0·9 \times 10^9$ leucocytes/four donors). The disease was not transferred with cells taken from donors receiving the adjuvant 7 or fewer days previously or from donors which had received only mineral oil (in place of adjuvant) 9 days previously. Recipients of cells from the "seventh day" (post-adjuvant) donors did, however, exhibit higher "plasma inflammation units"[8] than the corresponding controls which received lymph from the same donors.

Arthritis could be transferred as late as the fifteenth day after injecting adjuvant into the donors, when these donors themselves had fully developed all of the signs of the disease. Recipients with the disease continued to show symptoms (including retarded weight gain in males) for at least 4 weeks after cell transfer, when observations were discontinued.

Encephalomyelitis was also successfully transferred with thoracic duct lymphocytes, using four donors per recipient and $0·6–0·8 \times 10^9$ cells/25 h collection, beginning on the ninth day after injection of the encephalitogen. Recipients began to lose weight on the second day after transfer and their tails and hind limbs were partially or totally paralysed on the fourth day. They regained these functions by the eighth day and their basal weight (that at the time of transfer) by the eleventh day after transfer. Cell transfer on the seventh day (post-encephalitogen) induced only a transient loss of weight and no other overt symptoms.

We conclude that a certain population of lymphocytes, contained within the blood-lymph circulation and present in sufficient number 7 days or more after the injection of adjuvant or encephalitogen, is capable of transferring these two experimental autoallergic/hypersensitivity diseases. While previous studies[1–4] had indicated that lymphoid tissue cells, present in either nodes or spleen, could transfer these diseases, they did not exclude the participation of cells other than lymphocytes present in these tissues.

As a partial corollary to these studies, we have invariably found that the donor animals from which thoracic duct lymphocytes were taken on or after the eighth day after the adjuvant injection, subsequently suffered much less severe arthritic symptoms than the control animals (which had been injected with adjuvant and then sham-operated 8 or more days later—in other words, stressed but not depleted of lymphocytes)[10]. Animals donating lymphocytes for the successful transfer of encephalomyelitis, however, still suffered paralysis as severe as that of the unoperated control animals, though the appearance of symptoms was sometimes delayed 1 or 2 days.

We thank Dr B. B. Newbould for his help in initiating allergic encephalomyelitis in these experiments. This work was supported by grants from the US National Institutes of Health.

DARLING J. WHITEHOUSE
M. W. WHITEHOUSE
C. M. PEARSON

Division of Rheumatology,
Department of Medicine,
University of California School of Medicine,
Los Angeles, California 90024.

Received August 18, 1969.

1 Waksman, B. H., and Wennersten, C., *Intern. Arch. Allergy*, **23**, 129 (1963).
2 Pearson, C. M., and Wood, F. D., *J. Exp. Med.*, **120**, 547 (1964).
3 Paterson, P. Y., *J. Exp. Med.*, **111**, 119 (1960).
4 Levine, S., and Wenk, E. J., *Amer. J. Path.*, **47**, 61 (1965).
5 Newbould, B. B., *Immunology*, **9**, 613 (1965).
6 Bollman, J. L., Cain, J. C., Grindlay, J. H., and Van Hook, E., *J. Lab. Clin. Med.*, **33**, 1349 (1948).
7 Bollman, J. L., and Van Hook, E., *J. Lab. Clin. Med.*, **33**, 1348 (1948).
8 Glenn, E. M., and Kooyers, W. M., *Life Sci.*, **5**, 619 (1966).
9 Pearson, C. M., and Wood, F. D., *Amer. J. Path.*, **42**, 73 (1963).
10 Whitehouse, M. W., and Whitehouse, D. J., *Arth. Rheum.*, **11**, 519 (1968).

© 1969 Nature Publishing Group