PATHOLOGY

Tumour Transplantation: a Simple Technique

Numerous techniques of tumour transplantation have been used since Hanau¹ established the transplantability of animal tumours in 1889. One of us reported a technique using a curved surgical needle in 1963 (refs. 2 and 3).

We have now developed an even simpler method of tumour transplantation which makes use of a modified shoemaker's sewing machine needle with the tip ground down almost to the eye (Fig. 1). A 1-3 cm tumour is suitable for transplanting purposes. The donor animal is anaesthetized and immobilized on a cork board. The shoemaker's needle is plunged once into the unexposed tumour, removed and inserted horizontally into the unprepared thigh of the unanaesthetized recipient. As the needle is withdrawn a finger is pressed firmly against the thigh. The five or ten thousand tumour cells that were initially caught in the eye of the needle remain in the recipient. One donor tumour can be used for twenty or more recipients.

Fig. 1. *a*, Shoemaker's needle, ground down. *b*, Tip of needle empty ready for plunging into donor tumour. *c*, Tip of needle full after being plunged into donor tumour. *d*, Tip of needle relatively empty after withdrawal from recipient thigh.

A C_3H rhabdomyosarcoma transplanted using this technique becomes evident in two or three weeks in almost 100 per cent of the recipients.

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¹ Hanau, A., *Kongr. inn. Med.* (8 Kongr.), 289 (1889).

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IMMUNOLOGY

Failure of Spleen Cells from Immunologically Tolerant Mice to form Antibody Plaques to Sheep Erythrocytes in Agar Gel

DURING the past decade there have been numerous investigations concerned with establishment of specific immunological tolerance or unresponsiveness to either viable tissues or to a variety of non-living antigens, such as soluble serum proteins, bacterial products, and mammalian or avian erythrocytes^{1,2}. Most investigators believe that tolerance is due to a central inhibition of antibody formation and not to masking or binding of antibody by excess antigen³.

Acquired tolerance to non-living antigens is often demonstrated by lack of an expected immune response, such as failure to demonstrate circulating antibodies following immunization, failure to induce anaphylactic shock or an Arthus reaction, or failure to establish delayed or immediate skin reactivity following attempted sensitization. It has not been unequivocally established in such investigations whether or not the lack of demonstrable immunological activity or suppressed antibody titres is caused by absence of antibody-forming cells, or due to formation of low concentrations of antibody by relatively normal numbers of immunologically competent cells.

Fluorescent antibody investigations by Sercarz and Coons^{3,4} and cell transfer work by Brooke and Karnowsky⁵ have demonstrated an absence of cells forming specific antibody to a soluble serum protein antigen or to pneumococcal polysaccharide in tolerant adult mice. Others have also reported failure to transfer specific antibody-forming activity with lymphoid cells from tolerant donors⁶⁻⁹. This communication is concerned with investigations utilizing the 'antibody plaque' technique of Jerne¹⁰ to demonstrate directly the relative absence of haemolysin-forming cells in young mice receiving throughout early life tolerance-inducing injections of sheep red blood cells.

For these experiments, litters of new-born NIH albino mice were injected intraperitoneally with 0.05-0.1 ml. of a 50 per cent suspension of washed sheep red blood cells twice a week from birth to shortly before testing. As controls, litter mates were injected either with saline or left untreated. At 6-8 weeks of age, the mice were challenged intravenously with 0.1 ml. of a 20 per cent suspension of sheep erythrocytes. The mice were bled from the retro-orbital plexus before and after immunization to obtain serum specimens for determination of serum haemolysins to sheep erythrocytes. At varying intervals after challenge immunization, individual mice were killed, spleens obtained, and cell suspensions prepared⁹. Viability was determined by trypan blue stain, and cell counts were obtained with a haemocytometer. A 0.1 ml. of a suspension of cells containing 10×10^6 to 5×10^5 nucleated cells per ml. was used for pouring agar plates essentially as described by Jerne *et al.*^{10,11}. In brief, 0.1 ml. of the test spleen cell suspension was added to 2.0 ml. of melted 0.7 per cent Noble agar containing 0.1 ml. of a 10 per cent suspension of freshly washed sheep erythrocytes and 1.0 mg DEAE. This mixture was carefully layered over a previously prepared 3-mm thick layer of agar in a 100-mm diameter Petri plate. After the upper cell-agar layer had solidified, the plates were incubated for 1 h at 37° C. The plates were then treated with 5.0 ml. of a 1:10 dilution of fresh guinea-pig complement and incubated for an additional 30 min at 37° C. Following this incubation, zones of haemolysis could be readily seen against the light pink background of unlysed erythrocytes, indicating lymphoid cells which had secreted haemolysins to sheep red blood cells^{10,11}.

Table 1 indicates the results observed after plating lymphoid cell suspensions obtained from mice immun-