ANTIGEN-BINDING CELLS IN THE LYMPH GLANDS AND SPLEEN DURING SKIN GRAFTING IN RATS

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Skin from Wistar rats was grafted on to rats of line AVN. A whole extract was prepared from the donors' skin and conjugated with fluorescein isothiocyanate. The conjugate was used to stain impressions and films of a cell suspension of the regional lymph glands and spleen of the recipient rats. On the fourth day after grafting, lymphocytes specifically binding the antigens of the donor's skin were found in the specimens. Typical antigen-binding plasma cells were found during the later stage of the graft rejection reaction.

Experiments have shown that sensitized lymphocytes are a significant factor in the graft rejection reaction [3, 4]. The mechanisms of cellular immunity of this type are not clear. Some workers consider that the receptors of lymphocytes responsible for the identification of essential antigens are immunoglobulins or structures related to them [2, 6, 8, 12]. The view is held that the action of sensitized lymphocytes in vivo cannot be attributed entirely to the presence of immunoglobulins or their fragments in them [1, 2, 5, 7].

The dynamics of appearance of antigen-binding lymphocytes in the lymph glands and spleen was studied in the course of the graft rejection reaction.

EXPERIMENTAL METHOD

Rats of line AVN weighing about 250 g were used as recipients in skin grafting experiments. Wistar albino rats were the donors.

The recipient rats were anesthetized with nembutal, with ether for maintenance. After disinfection of an area of skin on the dorsum of the recipient a graft measuring 2.0 × 1.5 cm was removed and the exposed surface was covered with a skin graft of the same size and shape taken from the donor. The graft was fixed with four sutures and the whole area irrigated with nebacetin.

Preparation of the skin extract and its conjugation with fluorescein isothiocyanate (FITC) were carried out as follows. The skin of each donor was freed from hair and the subjacent fatty and muscular tissue, cut up into small pieces, and carefully ground in a mortar with the addition of quartz sand and physiological saline. The resulting material was centrifuged at 5,000 rpm for 15 min to remove large particles, after which the supernatant was centrifuged twice at 15,000 rpm for 15 min at 4°C. The protein concentration in the skin extract thus obtained was determined by Lowry's method and adjusted to 12 mg/ml by the addition of buffered physiological saline (0.15 M NaCl, 0.01 M phosphate, pH 7.5). Conjugation with FITC was carried out by the methods of McKinney et al. [10], and The and Feltkamp [14]. The unbound dye was removed by dialysis against buffered physiological saline for 24 h followed by filtration through Sephadex G-50. The resulting conjugates were absorbed with liver powder or with a thick suspension of spleen cells of normal animals.

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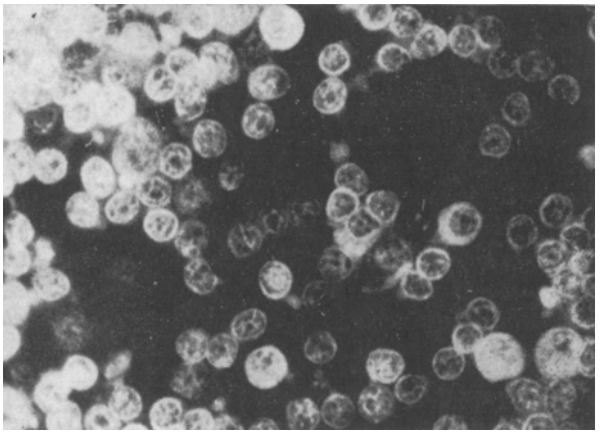

Fig. 1. Fluorescent lymphocytes (impression from lymph gland; 320×).

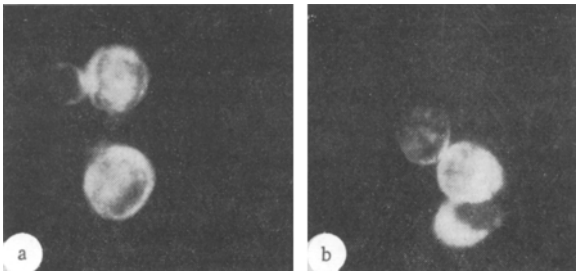

Fig. 2. Fluorescent large lymphocytes with abundant cytoplasm (a) and plasma cells (b), 800×.

From the fourth day until the end of the second week after grafting, impressions and films of the cell suspension were prepared from the regional lymph glands (brachial and axillary) and the spleen of the recipient animals. These were fixed in absolute methanol for 10 min, dried in air at 37°C for 30 min, rinsed with buffered physiological saline, and stained with the corresponding conjugate prepared from the extract of the donor's skin for 30 min in a moist chamber at room temperature. After staining, the preparations were rinsed for 10 min with buffered physiological saline.

The following controls were used: 1) impressions and films of a suspension of lymph gland and spleen cells of normal rats and rats receiving an injection of triple vaccine (against diphtheria, tetanus, and whooping-cough; "DIFTETKOK-NIEM"), in a dose of 0.3 ml into the forepaws, stained with conjugated extract of donor's skin; 2) analogous preparations made from the lymph glands and spleen of experimental animals, stained with conjugates not related to the tested model (goat anti-rabbit γ-globulin and rabbit antiserum against E. coli 0111); 3) the same types of preparations from material obtained from experimental animals and incubated before staining with unconjugated extract of the skin of the corresponding donor for 45 min in a moist chamber at room temperature (a test of the suppression of fluorescence).

Observations were made with the ML-2 microscope.

EXPERIMENTAL RESULTS

On the fourth to fifth day after grafting, darkening of the graft was observed, and by the 10th day it resembled a dry scab with elevated edges.

On the fourth day after grafting, many cells of the same shape and size as small lymphocytes (Fig. 1), and whose membranes exhibited specific fluorescence, were observed in the regional lymph glands of the grafted animals. No fluorescence of the nucleus was observed, and for this reason the cells looked like rings. The number of these cells increased until the 10th day, and parallel with this increase larger lymphocytes with a considerable quantity of cytoplasm, together with characteristic plasma cells with fluorescent cytoplasm appeared (Fig. 2). The last two types of cells were particularly numerous by the 10th-14th day after grafting. Lymphocytes binding the labeled antigen were found after the sixth day of grafting in films taken from the spleen, and their number increased on the following days when, besides small lymphocytes, a considerable number of large lymphocytes and plasma cells appeared in the specimens.

Fluorescence of the types of cells described was not observed in cases in which another type of conjugated protein was used instead of the corresponding conjugated skin. Preliminary treatment of the preparations with conjugated skin extract considerably reduced the fluorescence.

In specimens obtained from normal rats, animals receiving triple vaccine, and experimental rats individual cells were observed with intensive fluorescence on staining not only with conjugated skin extract, but also with the other conjugates. These cells differed morphologically from lymphocytes and plasma cells. They had an abundant, granular cytoplasm, a large, centrally situated nucleus, and they were larger than the plasma cell. The cause of this fluorescence has not yet been explained.

The results of this investigation show that in the early stage of the graft rejection response cells of the lymphoid series bound with antigens of the graft can be found in the regional lymph glands. Not until a later stage were cells of the large lymphocyte and plasma cell types, with similar affinity, observed. Antigen-binding cells in the spleen appeared rather later, when the process of graft rejection had become much more marked. These observations agree with those of other workers who perform their experiments on mice and used different methods of detecting sensitized lymphocytes. For example, Micklem et al. [11] found an increase in the number of cells forming rosettes with the donor's erythrocytes after the fourth day of transplantation, whereas humoral antibodies were not found until the eighth day. Hildeman [9], who used Jerne's method, found cells which formed cytotoxic antibodies by the second to third day after transplantation, and they appeared sooner in the regional lymph glands than in the spleen. Irrespective of the method used, these results are evidence of the same phenomenon: the formation of antigen-binding cells [11]. A method similar to that used in the present investigation to study antibody-binding lymphocytes was used by Scheiffarth et al. [13], but on a different model and with a technique of indirect immunofluorescence.

The distribution of the fluorescence observed on the surface of the lymphocytes in these experiments agrees with the fact established previously [2, 12] that the membrane of these cells contains immunoglobulins which can be found by immunofluorescence. These results do not explain the character of the receptors for the antigen [6, 8, 12] or the method by means of which the sensitized lymphocyte performs its function in vivo [1, 2, 5, 7]. The use of a conjugate obtained from whole skin extract appears justified, bearing in mind the results obtained by Brondz [5], who showed that sensitized lymphocytes exert their action in vivo in the presence of all or nearly all the antigens found in the graft. The method used in the present investigation may prove useful for the investigation of immunocompetent cells during the graft rejection reaction or in other phenomena of cellular immunity.

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