IMMUNOMORPHOLOGICAL STUDY OF INDUCED REJECTION OF A SKIN AUTOGRAFT

V. V. Serov, R. P. Ogurtsov, and Yu. N. Zubzhitskii

UDC 612.79:612.6.02.017.1

The results of morphological, immunoluminescence and serologic studies of the phenomenon of induced rejection of a skin autograft in CC57BR mice sensitized with streptococcal S vaccine, possessing common antigens with the tissues of these animals, are compared in this paper.

The induced rejection of the autograft and rejection of a skin homograft are shown to be identical, and participation of immunologic mechanisms in the phenomenon of induced rejection is demonstrated.

* * *

For technical reasons, the previously established fact of rejection of a skin autograft in CC57BR mice sensitized with vaccines possessing common antigens with the tissues of the experimental animals [1-3] has proved difficult to investigate immunologically. Nevertheless, by means of the complement fixation reaction (CFR) in the cold, some idea has been obtained of the dynamics of circulating antibodies against microbial inducing vaccines.

The results of a morphological study of grafts and of the surrounding tissues are compared in this paper with luminescence-microscopic data on detection of fixed γ-globulins in these same autografts. In addition, accumulation of fixed globulins in the graft and surrounding tissue was compared with the dynamics of antibodies against inducing microbial and isologous skin antigen against the background of the autografting procedure.

EXPERIMENTAL MATERIAL AND METHOD

Experiments were carried out on 60 CC57BR mice 2, 4, 6, and 8 days after operation [1-3]. At each time grafts of five control mice (uninduced rejection) were studied. The usual histological methods and histochemical methods of staining were used and the material was fixed in alcohol and formalin and embedded in paraffin wax. Antibodies were investigated in sera of each separate group of mice from the 5th until the 22nd day after injection of streptococcal S vaccine. At each time a mixture of sera from 4 or 5 animals was studied in the CFR in the cold with corresponding antigens [3]. Sections for luminescence microscopy were cut on a cryostat at −20° and fixed with cold acetone. Luminescence microscopy was performed in short-wave blue light (filters SzS-7, RS-1, T2N) and RF-3 film was used for photography. The usual methods of work with luminescent antibodies were adopted.

EXPERIMENTAL RESULTS

At morphological examination of the skin autograft in mice of the control group 2 days after operation foci of necrosis were found at the edges of the graft with a leukocytic demarcation barrier at the periphery (Fig. 1a). Loose connective tissue of the graft bed was diffusely infiltrated with lymphocytes and histiocytes and was edematous (Fig. 1b).

Four days after the operation the necrosis had extended to the whole of the surface of the graft. The demarcation zone of inflammation was increased. Beneath the areas of necrosis, stratified squamous epithelium was "creeping" from the skin surrounding the graft. The graft was surrounded by loose connective tissue in which fibroblasts, histiocytes, and polymorphs were predominant (Fig. 1c).

Department of Pathological Anatomy, I. M. Sechenov First Moscow Medical Institute; Department of Immunology and Microbiology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. (Presented by Active Member of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 3, pp. 90-95, March, 1969. Original article submitted June 27, 1968.

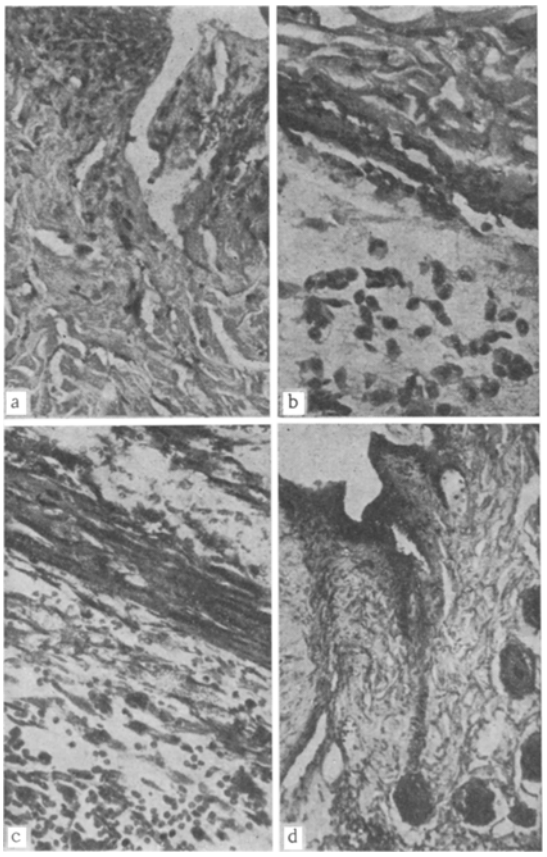

Fig. 1. Morphology of skin autograft of control animals. a) Focus of necrosis at edge of graft with demarcation barrier of inflammation at periphery (2 days after operation), Hematoxylin-eosin, 200×; b) loose connective tissue of graft bed edematous and infiltrated with leukocytes and histiocytes (2 days after operation), Hematoxylin-eosin, 200×; c) granulation tissue rich in polymorphs visible in graft bed (4 days after operation), Brachet, 200×; d) graft has taken: graft surrounded by fibrous connective tissue with fat cells and foci of infiltration with lymphocytes and histiocytes. Surface epithelium continuous (6 days after operation). Hematoxylin-eosin, 100×.

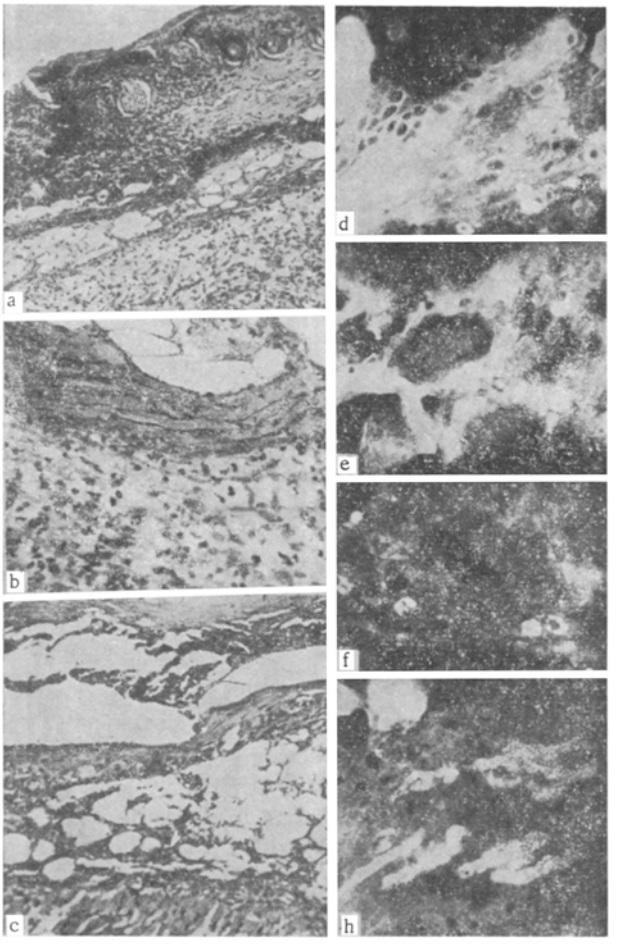

Fig. 2. Morphology of skin autograft of experimental animals. a) Massive areas of necrosis of graft surrounded by leukocytic barrier (4 days after operation). Hematoxylin-eosin, 100×; b) graft surrounded by granulations and infiltrated with monocytes, plasma cells, and neutrophils. Muscle fibers of deep layers of dermis necrotic (5 days after operation). Picrofuchsin, 200×; c) necrotic graft completely rejected. Graft bed covered with epithelium (8 days after operation). Hematoxylin-eosin, 100×; d) fixed γ-globulin in epidermis of autograft (4th day after operation). Coons' direct method, 400×; e) fixed γ-globulin on collagen fibers of dermis (4th day after operation); g) fixed γ-globulin on disintegrating muscle fibers (6th day after operation); f) specific luminescence of cells infiltrating into graft bed (6th day after operation). Coons' direct method, 400×.

Six days after the operation the graft appeared to have taken. The surface epithelium was continuous. Where the graft was in contact with the surrounding skin, and also in the bed of the graft fibrous connective tissue with fat cells and moderate infiltration with histiocytes and lymphocytes could be seen (Fig. 1d).

On the 8th day after the operation the picture was one of complete taking of the graft, although its epidermis and dermis were atrophic and the deep layers of the dermis were replaced by adipose tissue. The skin appendages were calcified. The graft bed consisted of coarse fibrous connective tissue.

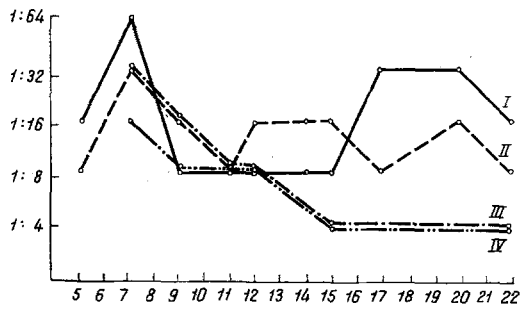

Fig. 3. Dynamics of antibodies against skin antigen and streptococcal S vaccine. I) Titer of antibodies against streptococcal S vaccine in animals without autografting; II) titer of antibodies against isologous skin antigen in animals without grafting; III) titer of antibodies against isologous skin antigen with autografting; IV) titer of antibodies against streptococcal S vaccine in animals with skin grafting.

Morphological investigation of the skin autograft of animals of the experimental group after 2 days revealed massive areas of superficial necrosis of the graft surrounded by a leukocytic barrier. At the border with the surrounding skin the full thickness of the graft was necrotic and the leukocytic demarcation barrier was particularly well defined. The deep layers of the graft were edematous, and its collagen fibers were swollen and orthochromatic. The dermis surrounding the graft also was edematous, and the small blood vessels were surrounded by cuffs of lymphocytes and histiocytes. The small vessels were thrombosed. The reaction of the loose connective tissue of the graft bed was severe, consisting mainly of infiltration by monocytes into the deep layers of the graft.

Four days after the operation the full thickness of the graft was necrotic at its border and infiltrated with leukocytes (Fig. 2a). Its central portions were also in a state of focal necrosis, in which bundles of collagen fibers appeared to have melted and died without any reaction to the necrosis. Both at its borders and where it faced the bed the rejected graft was surrounded by granulations consisting of maturing fibroblasts infiltrated with polymorphs and containing hemorrhagic foci.

On the 6th day the complete graft was necrotic and infiltrated with polymorphs both from the side facing the bed and from the borders. Mature granulations infiltrated with monocytes, plasma cells, and neutrophils were situated next to the demarcation barrier of inflammation. Over a wide area these granulations were covered with a layer of stratified squamous epithelium, apparently sliding in beneath the rejected graft. Muscle fibers of the deep layers of the dermis were in a state of cloudy swelling (Fig. 2b).

After 8 days the shrunken necrotic masses of the graft were completely rejected. Beneath them a layer of epithelium could be seen covering the former bed. Over a considerable distance it consisted only of adipose tissue, in direct contact with the muscular layer of the dermis (Fig. 2c).

Comparison of the observed histological changes with the results of treatment of the sections with luminescent antibodies (labeled with fluorescein isothiocyanate) against mouse γ-globulin (AMG), and also using the method of luminescent antibodies with complement, yielded the following results. In both the control and the experimental series, diffuse luminescence was found in sections of the autografts 2 days after the operation in association with AMG, the brightness of the luminescence being much greater in sections from the experimental animals. Rinsing the sections in buffered physiological saline (pH 7.2) before staining practically removed all the fluoroescence, indicating the absence of specific fixation of the detected globulin with structures in the skin. The negative reaction with complement suggests that the detected globulin resulted from protein infiltration of the graft on the 2nd day after operation. Later, starting on the 4th day after grafting, the appearance of fixed γ-globulin, not eluted in buffered physiological saline at pH 7.2-7.4, could be observed on the following skin structures: the cell membranes of the epidermis (Fig. 2d), collagen fibers of the graft dermis (Fig. 2e), and disintegrating muscle cells of the dermis (Fig. 2g) near the graft bed. Furthermore, in different layers of the skin, especially the epithelium, and also in the infiltrated zone of the graft bed, cells resembling plasma cells containing γ-globulin were found (Fig. 2d and f). As a rule the neutrophils exhibited nonspecific luminescence.

At later stages (5th-6th day) specifically bound γ-globulin was seen particularly clearly in foci of disorganization of collagen fibers and disintegrating muscle cells of the dermis.

In control sections (skin of a sensitized animal without grafting, skin graft of an unsensitized animal) treated with heterologous serum and also with normal labeled rabbit serum, specific luminescence was absent. Destruction of the autograft thus takes place simultaneously with an increase in the content of fixed globulin in the skin structures. However, this still does not allow a causal connection to be established between rejection of the graft and deposition of γ-globulin.

It is interesting to compare the dynamics of serum antibodies against skin and "inducing" (streptococcal S) antigens with the development of fixation of γ-globulin in the graft and its bed. As Fig. 3 shows, the first peak of circulating antibodies against skin antigen occurred on the 7th day after the last injection of streptococcal S vaccine, when no fixation of globulin has appeared (1st-2nd day after operation). Fixed globulin was found in the period from the 3rd to the 6th days after transplantation (12th day after the last injection of antigen), which did not coincide with the fall in antibody titers in the experimental animals. The titers of serum antibodies fell in the experimental mice from the 14th-15th day after vaccination, when the graft had already been rejected (8th-9th day after transplantation) and, consequently, the fall in titers of humoral antibodies was perhaps connected with their neutralization by circulating antigens (coming from the graft) or by microbial inducing vaccines. The results suggest that the fixed antibodies and the circulating antibodies detectable by the CFR are different in nature.

This investigation revealed the identity of the morphological characteristics of induced rejection of an autograft with rejection of a homograft, thus suggesting the participation of immunologic mechanisms in the phenomenon of induced rejection of the autograft.

LITERATURE CITED

1. Yu. N. Zubzhitskii and R. P. Ogurtsov, in: Transactions of the Leningrad Division of the All-Union Society of Microbiologists [in Russian] (1967), p. 16.
2. Yu. N. Zubzhitskii and R. P. Ogurtsov, Vestn. Dermatol., No. 3, 23 (1968).
3. Yu. N. Zubzhitskii and R. P. Ogurtsov, Byull. Éksperim. Biol. i Med., No. 4, 107 (1968).