ULTRASTRUCTURE OF ROSETTE-FORMING CELLS IN THE WALL OF AN INTESTINAL ALLOGRAFT

A. I. Lysenko and I. D. Kirpatovskii

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After total allografting, a unique phenomenon of rosette-formation was found to develop in the wall of the small intestine. The rosettes were composed of a central lymphocyte or neutrophil surrounded by plasma cells. The formation of plasma-cell rosettes (P-rosettes) is regarded as a possible indicator of the graft versus host reaction.

KEY WORDS: allografting; rosette-forming cells.

When lymphocytes are mixed with heterologous erythrocytes adhesion of the erythrocytes to the membrane of some of the lymphocytes with the formation of rosettes is observed. This phenomenon is widely used to study the surface receptors of various lymphocyte populations. T and B lymphocytes are known to differ in their functions, their specific membrane antigens, and their surface membrane receptors. To detect T lymphocytes, the reaction of spontaneous formation of E-rosettes in nonimmune and immune organisms is used [3, 4, 8]. B lymphocytes, carrying a receptor for IgG, are detected by the reaction of formation of EA-rosettes (interaction of lymphocytes with erythrocytes covered with antibodies). Lymphocytes carrying a receptor for activated complement are identified by the reaction of formation of EAC-rosettes (interaction of lymphocytes with the erythrocyte—antibody—complement complex) [1, 4].

In all the papers cited above it is a question of the ability of different types of cells to fix heterologous erythrocytes to their surface *in vitro*. According to Busch et al. [5], this phenomenon is not found in tissue slices.

The object of the present investigation was to detect and study cells forming rosettes in the wall of the small intestine.

EXPERIMENTAL METHOD

Total orthotopic autografting of the small intestine was carried out on 22 dogs, and allografting by I. D. Kirpatovskii's method [2] on 13 dogs. The animals were killed at different times: from the 4th day until 15 months after autografting and from the 4th until the 9th day after allografting; the dogs did not survive a longer period after total allografting because of the development of a tissue incompatibility reaction. The intestine of intact animals was studied as a control. Material was taken from standard regions (the proximal portion) of the small intestine, fixed in formalin and osmic acid, and mounted in appropriate medium. For light microscopy sections were stained with hematoxylin-eosin. Ultrathin sections were examined in the EMV-100L electron microscope.

EXPERIMENTAL RESULTS

Two main morphological variants of the tissue incompatibility reaction were observed in the wall of the intestinal allograft. In the first variant (7 cases) the process was confined to the lamina propria of the mucosa and consisted of a macrophagal and plasma-cell reaction. Extensive areas of the stroma of the mucosa in some cases consisted of plasma cells only. Cellular infiltration in other layers of the intestinal wall was not observed, nor was thrombosis found in its blood vessels. Electron-microscopically, distinctive rosettes were found (in 4 cases) in the mucosa; they consisted of central lymphocytes or neutrophils, surrounded by plasma cells, at different stages of maturation and secretion (Fig. 1a). Contact between

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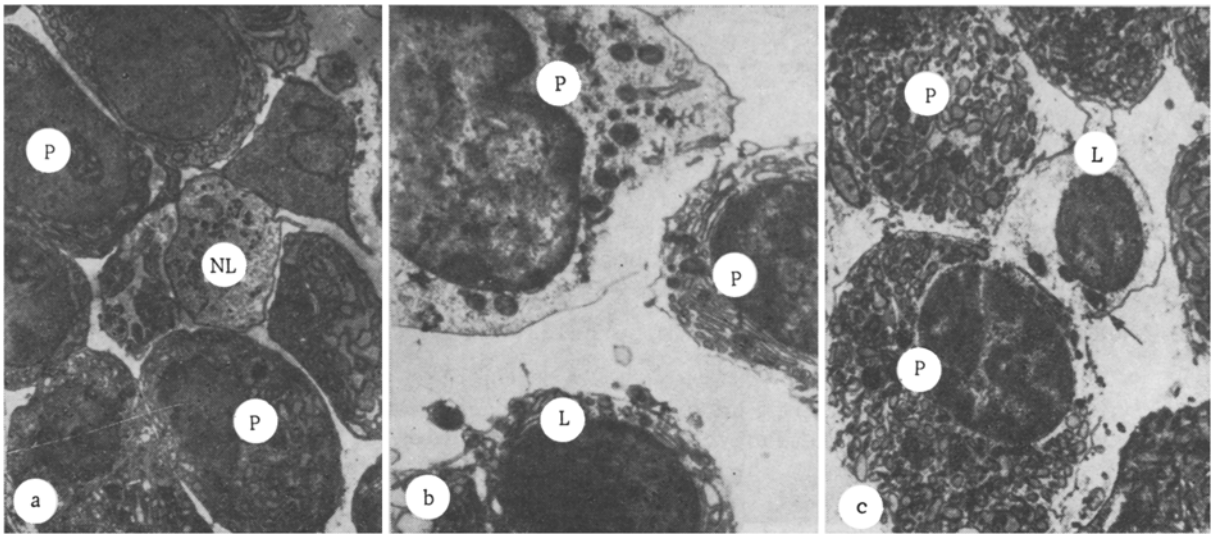

Fig. 1. Ultrastructure of rosette-forming cells in wall of intestinal allograft: a) allograft, 8 days: plasma cells (P) at various stages of maturation form rosette around neutrophilic leukocyte (NL) (7500×); b) allograft, 8 days: at point of contact between plasma cell and lymphocyte (L) cytoplasm of latter shows lysis (12,000×; c) allograft, 6 days: plasma cells forming rosette are in an active state — widening of endoplasmic reticulum, plasmatosis of cytoplasm; in regions of contact between plasma cells and lymphocyte, membrane of latter is damaged and cristae of mitochondria (arrow) are partly destroyed (10,000×).

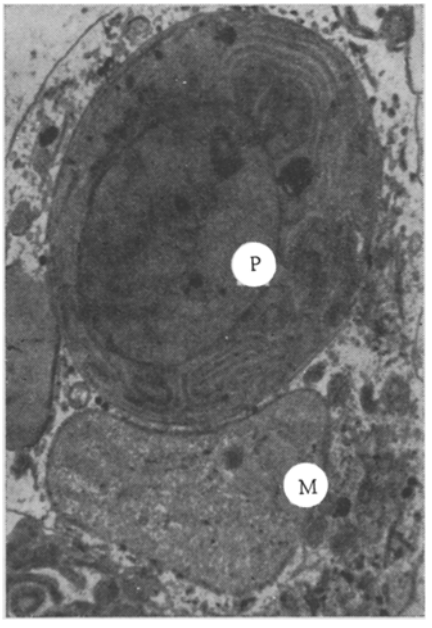

Fig. 2. Autograft, 4 days, phagocytosis of plasma cell (P) by macrophage (M); 8500×.

the central cell and the surrounding plasma cells usually was observed over wide areas of plasmalemma. The plasma cells were oriented with a narrow border of cytoplasm toward the center and a wide border externally. The structure of the contacting cells was most frequently intact, although some rosettes were seen in which the plasmalemma and the cytoplasm of the lymphocytes were undergoing lysis at the point of contact between the plasma cells and the lymphocyte (Fig. 1b).

In the second variant of the morphological picture (6 cases) sharp changes were localized in the mucosa, submucosa, and muscular coat and were characterized by infiltration of lymphocytes and plasma cells and widespread foci of destructive vasculitis. Electron-microscopically, rosettes also were found (in 3 cases) under these circumstances, and they consisted of a central lymphocyte surrounded by plasma cells. The endoplasmic reticulum in the plasma cells was considerably widened and filled with finely granular contents, and in some cases plasmatosis of the cytoplasm was well marked (Fig. 1c).

After total autografting of the small intestine, a marked macrophagal and plasma-cell reaction was observed in the stroma of the mucosa at times analogous to those of allografting. The plasma cells were in a state of active secretion and breakdown, and "superfluous" plasma cells were phagocytosed by macrophages (Fig. 2). The normal cell composition was restored 1-2 months after the operation. Plasma-cell rosettes as described in the experiments with allografting were never found.

The control study of the small intestine of intact animals showed that the cell composition of the stroma of the mucosa is distinguished by considerable morphological heterogeneity.

The phenomenon of the rosette formation can thus be observed not only *in vitro*, but also *in vivo*. It was difficult in the experiments thus described to make any quantitative evaluation of this phenomenon. It can merely be emphasized that during allografting a lymphocyte was present in the center of six of seven rosettes, and a neutrophil in the center of one rosette. In one case the rosette was formed by seven plasma cells, in one case by six cells, in two cases by five cells, and in three by three cells. By analogy with the E-rosettes, EA-rosettes, and EAC-rosettes, the rosettes consisting of plasma cells as described above in the stroma of the intestinal mucosa can reasonably be termed P-rosettes.

The physiological and immunological importance of the rosettes has not been finally explained. They are considered to reflect definite activity of the immune system of the recipient. The rosettes found in the wall of the grafted allogeneic intestine may perhaps characterize complex immune relations between the immunocompetent graft and the recipients. These relations may be formed differently, including as a graft-versus-host reaction. In particular, the work of Cohen et al. [7] has shown that if the intestine is transplanted without the use of immunosuppression, death of the dogs takes place on the 9th day; the graft, however, remains well preserved and no signs of rejection can be detected morphologically. If the graft is irradiated in a dose of 150 R before transplantation, a rejection reaction develops in the intestine, and death of the animal is observed after 9.2 days. If the graft is irradiated in a dose of 50 R, survival of the recipient is increased to 28 days and the intestine remains microscopically normal. Cohen et al. [7] concluded that, in the last group of experiments, the action of two immune systems — that of the recipient and that of the intestinal graft — was balanced.

The function of the central cells in the rosette (lymphocyte, neutrophil) and of the surrounding plasma cells evidently differs. Adhesions of plasma cells to the neutrophil or lymphocyte may reflect the binding of immunoglobulins secreted by the plasma cells of the graft, and antigen represented by the recipient's neutrophil (or lymphocyte), which has migrated into the stroma. Different cells are known to have a membrane receptor for the Fc fragment of the IgG molecule on their surface (neutrophils, monocytes, macrophages, lymphocytes, and so on). However, among the cells infiltrating allogeneic kidneys, such a receptor is possessed most frequently by cells of the lymphoid series [8]. It has been shown that T lymphocytes taken from the spleen [6] or from a focus of cellular infiltration of a grafted allogeneic heart [9], and also kidney [8], can destroy target cells in the early postoperative period. The same property is possessed by B lymphocytes carrying an Fc receptor. It may be that the morphological manifestations of the tissue incompatibility reaction observed in the present experiments after total allografting of the small intestine, as well as the formation of plasma-cell rosettes, are a reflection of this mechanism. Blocking of the Fc receptor of

the recipient's central lymphocyte (neutrophil) by immunoglobulins produced by the graft's plasma cells evidently takes place.

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DISTRIBUTION OF BONE MARROW CELLS IN THE MOUSE SKELETON

V. L. Shaposhnikov

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Details are given of the distribution of nucleated bone marrow cells in 17 parts of the skeleton of laboratory hybrid mice (CBA × C57BL) weighing 18-21 g. The content of bone marrow in the bones of the spine, head, lower limb, pelvis, upper limb, sternum, and ribs was 33.7, 19.6, 11.9, 8.2, and 9.0% respectively of the total.

KEY WORDS: distribution; bone marrow; hybrid mice.

With the appearance of many publications showing inequality of radiation loads and the need to evaluate doses falling on different parts of the bone marrow under these conditions, the distribution of bone marrow cells in the skeleton of animals of different species assumes great importance. Such data have now been published for rats [1, 7], dogs [3, 5-7], and monkeys [7]. Incomplete and contradictory data on the distribution of bone marrow cells in the skeleton of mice can be found in a few publications [2, 4, 7].

For a detailed study of the distribution of bone marrow cells in different parts and individual bones of the mouse skeleton experiments were carried out on 10 male (CBA × C57BL)F_1 hybrid mice weighing 18-21 g.

EXPERIMENTAL METHOD

The animals were killed by cutting the jugular veins. The skeletal bones were carefully freed from soft tissues and cut into small pieces with scissors. The bone marrow cells were flushed out of the bones with 5% acetic acid solution by means of a syringe for 2 min. The minced cranial bones were treated with 16 ml of 5% acetic acid solution, and in other cases 8 ml was used. The bone marrow cell suspensions were counted in a Goryaev chamber. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Data on the distribution of bone marrow cells in the bones of the mice are given in Table 1. Clearly the largest quantity of bone marrow is found in the spine, cranial bones, lower limbs, and pelvic bones. For comparison, the data of Taketa et al. [7] are given in the same table after adjustment to exclude bone marrow cells from the bones of the manus and pes. Satisfactory agreement with our own findings will be apparent. To compare the distribution

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