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TECHNICAL MANUSCRIPT 475

RETICULAR CELL RESPONSE IN THE RABBIT APPENDIX TO AUTOLOGOUS AND HOMOLOGOUS TRANSPLANTS

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Alfred C. Standen
John R. Esterly
Bjarne Pearson

SEPTEMBER 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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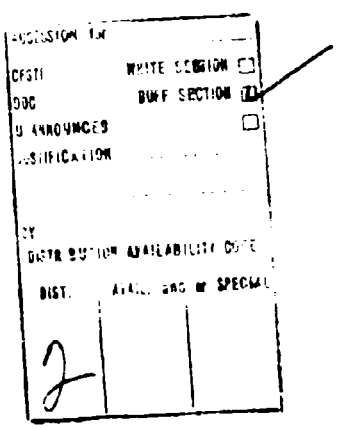

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Pathology Division
MEDICAL SCIENCES LABORATORY

Project 1T013001A91A

September 1968

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

A progressive increase in the number and confluency of reticular cells was observed in the lymphoid portion of the appendix of rabbits following transplantation of homologous thymus beneath the kidney capsule. These changes were most striking at 96 hours following transplantation. Similar, but less severe, changes were also found in the sacculus rotundus but were not seen in other intestinal lymphoid tissue, peripheral lymph nodes, spleen, or recipient thymus. Significant reactive changes were not seen following homologous muscle transplants or autografts of either thymic or muscle tissue. These studies indicate that the appendiceal reticular reaction may be nonspecific and in part represent a transplantation reaction. The results of histochemical studies suggest that these reactive cells have neither macrophage-like nor active protein synthetic functions.

I. INTRODUCTION

Only recently has the significance of the mammalian appendix been appreciated as a central lymphoid organ necessary for the development of complete immunologic competence.[1] Because appendectomy in the neonatal rabbit results in a markedly depressed response to soluble antigen, the appendix has been considered a mammalian equivalent of the bursa of Fabricius.[2]

Histologic response to antigens has not been found in the thymus, possibly because of its "blood barrier." However, reactive changes in the rabbit appendix, which is histologically similar to the thymus, have been noted in a series of transplantation studies carried out in this laboratory. This reaction is evanescent and nonspecific and is characterized by a striking increase in the number and aggregation of large, pale-staining reticular cells. Similar, but less marked, changes were found in the appendix of the rabbit receiving an intravenous injection of 7S human gamma globulin. The duration and cell counts of this latter reaction were described in a preliminary report.[3] In the present experiment, autologous and homologous transplants of thymus or muscle were performed to evaluate the factors in the appendiceal reaction. Additional lymphoid tissues were examined to further determine the extent and distribution of the reaction. Histochemical techniques were used to determine β-galactosidase and β-glucuronidase changes in the reactive cells.

II. MATERIALS AND METHODS

Adult New Zealand rabbits raised in our laboratory were anesthetized with Fluothane,* and sections of the thymus or biceps brachii muscle measuring 0.5 cm^3 from the homologous donor were transplanted under the kidney capsule of the recipient. Autologous thymus and muscle transplantations were performed in the same manner. In addition, thymus and muscle homograft studies were repeated in rabbits obtained from a commercial source to insure genetic heterogeneity. Likewise, the changes following homologous thymic and muscle transplants were evaluated in an inbred strain of Fischer 344 rats. At the time of transplantation, sections of donor tissue of the organs studied were retained and served as normal controls. Rabbits subjected to sham operations and rabbits injected intravenously with 7S human gamma globulin were utilized as additional controls. Three to 28 animals were used in each experiment, and two histologic sections of each appendix were evaluated. Donor tissue was transplanted to only one recipient in each experiment.

* Ayerst Laboratories, New York, New York.

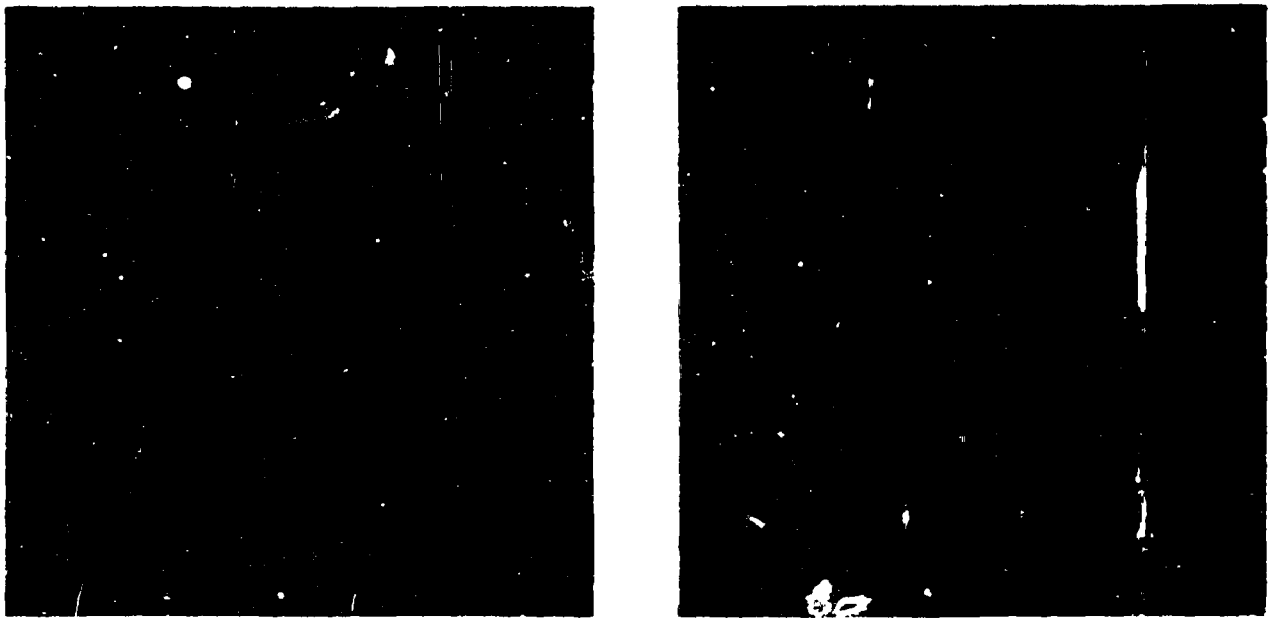
Following transplantation, animals were sacrificed at intervals from 4 hours to 7 days. The appendix, sacculus rotundus, jejunum, spleen, thymus, and mesenteric and popliteal lymph nodes were removed. A portion of each tissue was fixed in alcoholic formalin (10 ml formaldehyde in 90 ml of 80% alcohol), and routinely prepared paraffin sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), and methyl green - pyronin Y. Sections for enzymatic histochemistry were prepared from portions of fresh tissue frozen in test tubes immersed in a Dry Ice and acetone bath and cut in a cryostat at -15 C. Reactions for β-galactosidase and β-glucuronidase were demonstrated with halogen substituted indolyl substrates according to our procedures previously described.[4,5]

Reaction severity was determined and graded from examination of the H&E sections. Where the reaction was noted, reticular cell counts were made in unit areas of 0.0784 mm^2; the areas were selected at random. The results were analyzed and expressed in terms of the Student "t" test.

III. RESULTS

The rabbit appendix is composed of (i) closely packed lymphoid cells arranged in a luminal portion, or dome, separated by intestinal villi, and (ii) a more continuous basilar portion irregularly divided by connective tissue septa containing blood vessels and lymphatics. The luminal surface of the dome is covered with modified intestinal epithelium referred to as the cap epithelium, composed of one to several layers of cuboidal cells without nuclear polarization. The normal rabbit appendix illustrated in Figure 1 depicts these structures and the close association of the epithelium and lymphoid cells.

Normal histologic differences occur in the cap epithelium of the dome and villi.[6] The villous epithelium, like other intestinal mucosa, contains goblet cells. The cap epithelium is different in that it is devoid of goblet cells. The lymphocyte is the predominant cell in the dome and the base (Fig. 2 and 3). Reticular cells have an abundant, pale-staining eosinophilic cytoplasm and are especially prominent near corticomedullary junctions. Their morphology and location are similar to that of the reticular cells in the adult rabbit thymus. The structural relationship between endodermal and mesodermal tissues in the appendix is also analogous to that in the thymus and unlike that of peripheral lymph nodes.



A B

Figure 1. Normal Rabbit Appendix. A: Transmural section. The lumen is at top, and the serosal surface at the bottom. H&E (105X). B: Diagram indicating dome (D) and base (B). The lymphoid cells in the base are separated by connective tissue septa containing blood vessels and lymphatics. The villous epithelium is columnar, but that covering the dome is modified. The arrows mark the junction between the two types of epithelium.

Figure 2. Dome Portion of the Normal Appendix Showing Lymphocytes and Occasional Large Pale-Staining Reticular Cells. H&E (165X).

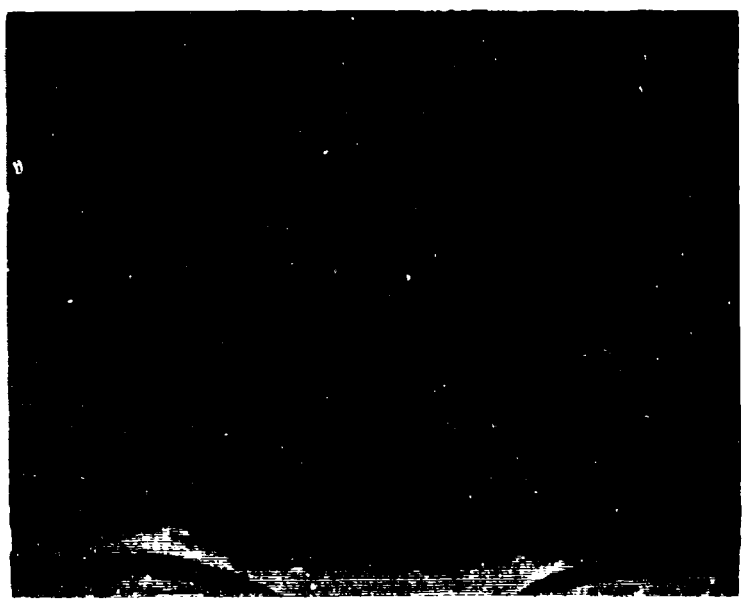


Figure 3. Lymphoid Elements in the Base of the Normal Appendix Segregated by Connective Tissue Septa. Note pale-staining medullary area of the centrally located follicle. H&E (165X).

The changes observed in the appendixes of rabbits that received homologous thymus transplants consisted of an increase in the number and confluency of reticular cells. Reactive changes were first apparent at 4 hours and were most prominent at 96 hours (Fig. 4). Reticular cell aggregates were present in both the dome and base. They appeared to be located near the corticomedullary junction, but, in the base, nodules of reticular cells were found adjacent to the connective tissue septa. By 7 days these reactive changes were no longer apparent. Seven-day specimens could not be distinguished from the appendixes of donor control animals.

The reticular cell counts of the reactions observed in the rabbit appendix in the experimental and control studies included in this report are listed in Table 1. The changes in the appendixes from the donor controls and sham-operated animals were not significant. However, animals given intravenous 7S human gamma globulin showed significant changes at 24, 48, and 96 hours,[3] but in no case were the reactions as marked or consistent as in the animals that received homologous thymic transplants.

The appendixes of rabbits that received homologous muscle transplants showed equivocal reactions, and the p value of the reticular cell counts was not significant. Minimal reactions were also seen after autologous grafts both of muscle and thymic tissue. Autologous thymic transplanted animals showed significantly lower values than the controls. Thymic transplants in the inbred rats produced no changes in the ileocecal lymph node or other intestinal lymphoid tissues.

The most striking reactions were found in the sections of animals that received homologous transplants of tissue from New Zealand rabbits obtained from a commercial source ("foreign" rabbits). The cell counts in these appendixes for both thymic and muscle transplants were significantly different ($p < 0.001$) from those in locally bred rabbits receiving similar homologous transplants as well as from donor control animals.

The findings in other organs in the recipient animals with the strongest appendiceal reactions were similar but less severe in the sacculus rotundus (Fig. 5). An occasional reactive focus was identified in the mesenteric lymph nodes. No changes were seen in the intestinal reticular cells in the lamina propria of the jejunum, Peyer's patches, spleen, peripheral lymphoid tissue, or thymus. At 96 hours the homologous donor tissue showed signs of graft rejection. In many cases, the autologous graft tissue also showed signs of rejection. In these cases of rejection, in addition to congestion and hemorrhage, necrosis was observed.

A

B

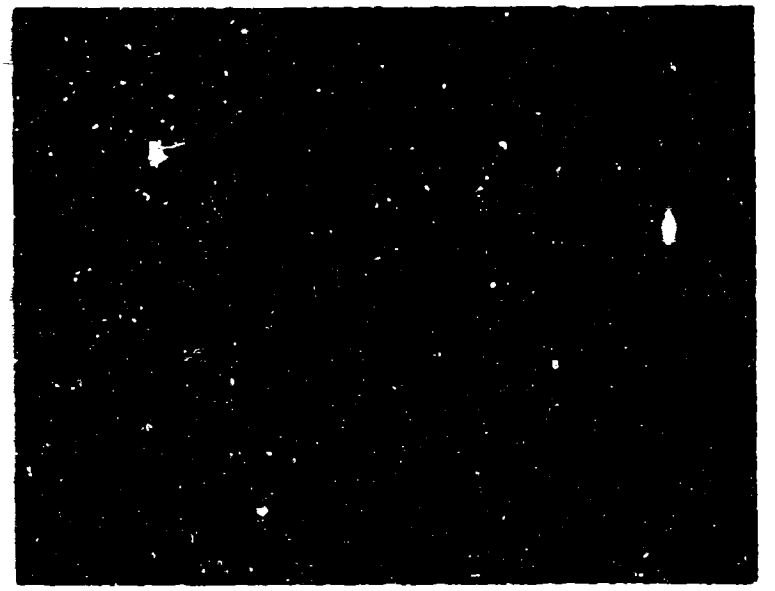


Figure 4. Appendix Section from Rabbit that had Received an Homologous Thymic Transplant 96 Hours Previously. A: Confluent groups of pale-staining reticular cells present along the septa and corticomedullary junction of the appendix. H&E (35X). B: Higher magnification of A showing the homogeneous appearance of the reactive reticular cells. H&E (165X).

TABLE 1. RETICULAR CELL COUNTS IN RECIPIENT APPENDIX

Group	Observations[a]	Mean[b]	Probability (p)
Homologous thymus	42	25.0	<0.001
Donor control	56	13.8	-
7S gamma globulin	12	21.4	<0.001
Sham-operated	12	13.3	0.7 to 0.5
Homologous muscle	22	14.5	0.7 to 0.5
Autologous thymus	12	9.9	0.01 to 0.001
Autologous muscle	16	12.8	0.5 to 0.3
"Foreign" thymus	6	43.1	<0.001
"Foreign" muscle	6	25.9	<0.001

a. Two observations per animal.
b. Unit area = 0.0784 mm^2.

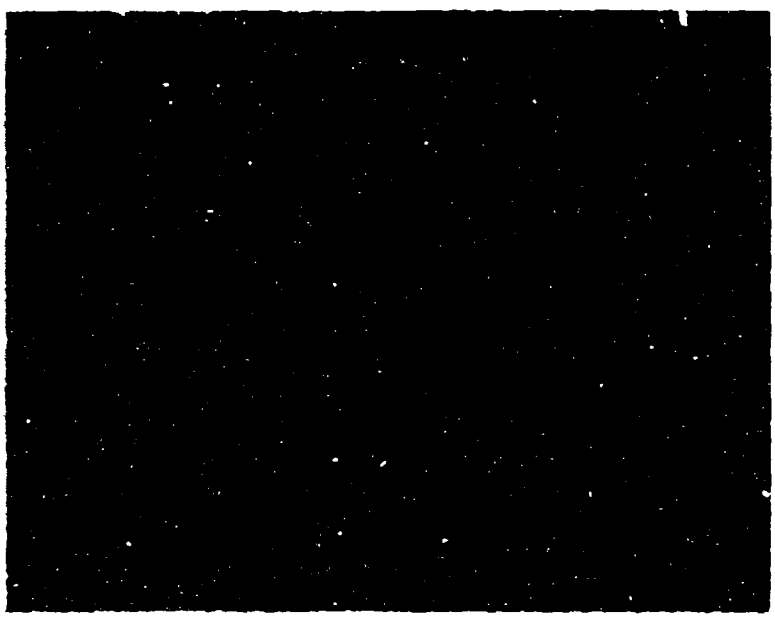


Figure 5. Similar but Less Severe Reactive Changes in the Sacculus Rotundus. H&E (165X).

Reticular cells in the normal appendix were strongly PAS-positive. In sections showing the transplantation reaction, reticular cells displayed less intense Schiff reactivity. In general, PAS reactivity was inversely related to the number of reticular cell aggregates present. Plasma cells were not observed in the normal appendix or in the experimental specimens as demonstrated with methyl green - pyronin Y staining. However, methyl green staining of the reticular cell nuclei was usually more intense than that of the nuclei in the control slides.

Occasional galactosidase- and glucuronidase-positive cells were demonstrated in the sections of normal rabbit appendix. No significant changes in reactivity for these enzymes were detected in the experimental animals.

IV. DISCUSSION

The appendix, sacculus rotundus, mesenteric lymph nodes, and Peyer's patches make up the intestinal lymphoid tissue of the rabbit. The embryological development of this system precedes that of peripheral lymph nodes, and several of the components, such as the thymus, form in approximation to endoderm.[7] Because of its bursa-like function in the ontogeny of circulating antibody, it was of interest to examine the appendix for histologic reactions in animals undergoing transplants. The results show that a nonspecific reticular cell reaction is a consistent finding that varies somewhat in degree. This reaction is most marked in the appendix, but it also occurs in the sacculus rotundus and is found on occasion in the mesenteric lymph nodes. The reticular cell location in the corticomedullary zone is similar to that which has been described previously in antigenic reactions.[8] The presence of reticular cell aggregates along connective tissue septa suggests relationship of the lymphatic nodules to the vascular supply. However, no thrombosis was seen.

The distribution of the reaction within the intestinal lymphoid tissues probably reflects the route of lymphatic drainage. Because the appendix is not in the path of lymphoid drainage from the retroperitoneally located kidney, it is difficult to explain the reaction in the appendix. The absence of changes in the Peyer's patches is most likely due to their parallel but independent drainage.

The origin of the reactive reticular cells is uncertain. The frequent mitoses seen at early time intervals suggest local proliferation; however, other origins cannot be excluded. The relationship of the reticular cells to the "hemocytoblasts" described in imprints of lymph nodes draining skin

transplants[9] is also uncertain, but it is clear that these reactions can be distinguished from the plasma cell proliferation that follows stimulation with soluble antigen.[10-12]

Schiff-reactive elements in the lymphoid system usually reflect phagocytosis of PAS-positive material, and the explanation for positive-staining histiocytes in the appendix is their bacterial content.[13] The present finding of diminished Schiff reactivity in the numerous reticular cells of the animals subjected to transplants also suggests proliferation with dilution of the reactive material.

Because the morphology of these reticular cells does not resemble that of plasma cells, it is understandable that pyroninophilia was not a prominent finding. The increased methyl green staining indicates nucleic acid content of recently divided cells.

Galactosidase and glucuronidase activity has been associated with activation of the reticular endothelial cells.[14-16] The absence of such changes in these cells suggests that they may not have an active macrophage-like function.

The qualitatively similar, but less striking, reactions found after intravenous gamma globulin and autografts demonstrate that this reticular reaction is nonspecific. However, the severe and statistically significant reactions seen in animals that received homologous tissue suggest that transplantation is an important stimulus. The differences between the reactions to thymus and muscle tissues indicate that thymic tissue is a more potent antigen.

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13. ABSTRACT

A progressive increase in the number and confluency of reticular cells was observed in the lymphoid portion of the appendix of rabbits following transplantation of homologous thymus beneath the kidney capsule. These changes were most striking at 96 hours following transplantation. Similar, but less severe, changes were also found in the sacculus rotundus but were not seen in other intestinal lymphoid tissue, peripheral lymph nodes, splee. or recipient thymus. Significant reactive changes were not seen following homologous muscle transplants or autografts of either thymic or muscle tissue. These studies indicate that the appendiceal reticular reaction may be nonspecific and in part represent a transplantation reaction. The results of histochemical studies suggest that these reactive cells have neither macrophage-like nor active protein synthetic functions.

14. Key Words

*Reticulum cells
*Rabbits
*Transplants
Homologous
Lymphoid
Appendix
Thymus

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