Lymphocyte-Epithelium Interaction During Rejection of Nonisogeneic Rat Tracheal Grafts

Bernard P. Lane, MD, Gail S. Habicht, PhD, and Glory S. Jasper, MA

Rat tracheal tissues were maintained in organ culture for 1 to 2 weeks and then implanted heterotopically into syngeneic and outbred hosts. The grafts and surrounding tissues were removed at regular intervals and examined by light and electron microscopy. Allogeneic grafts were consistently rejected, and a constant pattern of immunologic response was observed during rejection. Infiltration by mononuclear cells occurred initially in the subepithelial region. Subsequently, small lymphocytes migrated through the basal lamina and insinuated themselves between the differentiated epithelial cells. Larger cells resembling activated lymphocytes were also present in the intraepithelial infiltrate. Both types of lymphocytes had cellular processes in contact with epithelial cells, but there was no associated death of epithelial cells. Later, the infiltrate in some areas of the epithelium included dividing cells and other large cells exhibiting ultrastructural features suggesting blast transformation. Epithelial cell death was evident during this phase. The sequence of events may reflect a process of recognition and specific cytotoxic action by lymphocytes. (Am J Pathol 86:71–80,1977)

TISSUES AND ORGANS transplanted into nonsyngeneic hosts evoke humoral or cellular immunologic responses which result in rejection of the graft.[1] If the host is not presensitized to donor tissue or its constituents, the rejection reaction consists, at least in part, of infiltration of the graft by mononuclear cells.[2] We have studied cell-mediated rejection of heterotopically implanted tracheal tissue and found that lymphocytes that initially migrate into the epithelium do not cause injury and that there is appearance of activated lymphocytes and lymphoblasts associated with epithelial cell lysis. This intraepithelial process is similar to events which culminate in endothelial cell injury in renal grafts;[3] however, lymphoblast transformation in the epithelium has not been recognized in previous investigations of morphologic features of immunologic rejection of transplanted organs and tissues.

Materials and Methods

Graft Preparation

Sprague-Dawley strain female rats, (Charles River Laboratories, Wilmington, Mass.) served as outbred hosts and donors and Wistar Lewis female rats, obtained from the same source, were selected as an inbred line in which graft acceptance could be studied.

From the Department of Pathology, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, New York.

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Address reprint requests to Dr. Bernard P. Lane, Department of Pathology, Health Sciences Center, State University of New York at Stony Brook, Stony Brook, NY 11794.

All animals received from the supplier were observed for 3 to 5 days for signs of respiratory infections. Healthy animals, selected as sources of tracheas for grafts, were anesthetized by intraperitoneal injection of sodium pentabarbital (4 mg/100 g body weight). Tissues to be grafted were maintained in tissue culture for 1 to 2 weeks prior to implantation. The small fragments thus prepared were free of bacterial contaminants and humoral factors and did not undergo a period of revascularization and hemorrhage as seen in transplanted fresh whole tracheas.[4] The tracheas were excised, trimmed, and sliced transversely into 0.5-mm wide rings [5] which were cultured for 1 to 2 weeks in McCoys 5a medium with 3% calf serum.

Surgical Procedure

Rats of the same strain as the donor were anesthetized with sodium pentobarbital. Small incisions were made along the flanks of each host animal and one cultured fragment placed in each of the subcutaneous pockets formed. Aseptic technique was followed during the implantation of the allografts. To study uniformity of responses by a host to several grafts, one group of 5 rats bearing four grafts each was sacrificed each week for 4 weeks and all of the implanted tissues were removed. Progressive changes in grafts on a single host were assessed by removing a ring together with surrounding tissues each week for 4 weeks from each member of another group of 5 rats. After the results of these experiments were assessed, a third group of 10 rats each received 8 cultured fragments from a single donor and these grafts were excised at 2-day intervals starting with the tenth day after implantation. This use of replicate grafts permitted evaluation of the precise sequence of events in rejection by each recipient.

Histology and Electron Microscopy

Excised tissues were fixed in 3% glutaraldehyde buffered to pH 7.4 with 0.2 M sodium cacodylate and then treated with unbuffered 1% osmium tetroxide. Fixed tissues were dehydrated in alcohols of increasing concentrations, the alcohol replaced with propylene oxide, and the specimen embedded in Epon 812. Thick sections of all grafts were stained with methylene blue–azure II for light microscopic study. Thin sections for electron microscopy were prepared from representative rings.

Results

Gross and Microscopic Appearance

Implants harvested from the subcutaneous sites were embedded in connective tissue and usually adhered to the dermal side of the subcutaneous pocket. The cartilagenous ring and tracheal smooth muscle were lined on the internal surface with ciliated pseudostratified epithelium which extended over the entire surface of the pocket contained within the ring. Only small areas of the epithelium which had been on the external surface of the ring at implantation persisted.

Histopathology of Rejection

Of the 180 grafts implanted into outbred hosts, 143 were recovered. There was considerable variation in the morphology of different grafts studied at the same time interval after implantation, even among those groups which were all implanted on a single host. Nevertheless, a consist-

ent pattern of rejection was determined. During the first few days after implantation, viable grafts possessed normal epithelial architecture. After 10 to 15 days, many grafts were invaded by mononuclear cells. These cells first appeared closely packed in blood vessels and then were concentrated in the epithelium and the immediate subepithelial area (Figure 1). The early lymphocytic infiltration was associated with intercellular edema, but there was no epithelial cell death, and basal cells of the pseudostratified epithelium remained apposed to basal lamina (Figure 2).

The variability among grafts could be ascribed to development of characteristic alterations several days before or after the average time of occurrence. While a majority of grafts studied at each time after implantation exhibited similar histologic changes, 38 of the 143 grafts had features which appeared more than 4 days earlier or later than the average for others in the group. For 6 of 10 host animals, one or two of the eight grafts sequentially excised at 2-day intervals were not infiltrated by significant numbers of mononuclear cells and at least one and as many as three of the grafts on each of the 10 hosts showed diffuse extensive infiltration earlier than was expected. Epithelial cell death did not appear prematurely in any specimens, but in 2 hosts it was not seen in any of the eight grafts. These differences may be related to the site of implantation or to other local host factors, but no systematic variation based upon site could be recognized.

Twenty percent of the Wistar/Lewis grafts were infiltrated by mononuclear cells, but the remaining 80% had well-preserved epithelium and no inflammatory infiltrates throughout the period of sequential sacrifice (Figure 3). In the infiltrated grafts, both type and number of mononuclear cells were similar to these in the grafts removed from outbred hosts at the same time after implantation.

The majority of the infiltrating cells were mononuclear, although an occasional polymorphonuclear cell was observed. In those grafts in which this early infiltrative phase was recognized the invading cells were primarily small lymphocytes. A prominent feature of these cells was the numerous pseudopods at the cell margin. There were also small numbers of a second type of lymphocyte which was elipsoid, measured 9 to 11 μ in greatest dimension, and had more cytoplasm (Figure 2). Pseudopods also extended from the surfaces of these cells, but there was no intracellular evidence of phagocytic activity. Plasma cells were not identified among the infiltrating cells.

Other grafts showed areas of small lymphocyte infiltration and other areas in which lymphoblasts were prominent. Some of these cells were dividing (Figure 4). The bodies of these lymphoid cells were frequently

separated from the epithelial cells by a considerable space, although one or more pseudopods from the lymphocytes frequently came into close association with adjacent epithelial cells. In these areas some of the lymphocytes were apposed to dying epithelial cells. Lymphocytes were also seen in close approximation to other lymphoid cells. Their plasma membranes converged and ran in parallel for about 1 μ. No tight junctions were formed.

Discussion

A number of different mechanisms can underlie immunologic rejection of transplanted tissues. If the host has been exposed to donor antigens prior to placement of the graft, a hyperacute reaction mediated primarily by humoral factors may occur.[6] In hosts not previously sensitized, cell-mediated reactions are characteristic. The infiltrating cells may function largely in recognizing foreign antigen and in developing a humoral response or may be cytolytic.[7]

Reports of histopathologic changes in grafts undergoing rejection include description of infiltration of tissues by mononuclear cells. In renal allografts, most of these cells are intravascular or perivascular and have been considered to produce damage to vascular walls with consequent thrombosis and hemorrhage.[3] Mononuclear cell infiltration of renal tubules is apparently impeded by the thick basal lamina and tubular cell injury is due to ischemia.[8] In cardiac grafts, similar vascular damage with ischemic necrosis of parenchymal cells has been described.[9]

In contrast to the primarily vascular alterations in hearts and kidneys transplanted into nonisogeneic hosts, skin grafts,[10] hepatic grafts,[11] and intestinal grafts [12] exhibit infiltration of epithelium by mononuclear cells with subsequent epithelial cell death. Although cells identified as sensitized lymphocytes or blasts or having ultrastructural features compatible with this designation were described in these reports, mitotic activity or a pattern of infiltration suggesting surveillance followed by cell killing were not discussed. We observed early intravascular localization of mononuclear cells in the tracheal tissues implanted in nonisogeneic hosts, but epithelial cell death did not occur at that time. The tolerance for vascular occlusion may be accounted for by the small size of the graft, permitting exchange of waste and nutrients with surrounding host tissues by diffusion. Subsequently, small lymphocytes were observed migrating through gaps in the basal lamina and entering the widened spaces between epithelial cells. The lymphocytes and a second population of larger cells resembling activated lymphocytes [13] contacted epithelial cells in a manner

which has been interpreted as a manifestation of surveillance function.[14] Extensive epithelial cell death was not observed to be associated with these early contacts by lymphocytes. In some grafts, mitotically active lymphoblasts were found within the epithelium. When the tissues exhibited this sort of infiltrate, epithelial cell death was common, and a close association of lymphocytes with some of the dying or dead cells was apparent. Some of the lymphocytes exhibited cell-to-cell interactions seen both in sensitized cell populations [15] and in phytohemagglutinin-stimulated cultured cells.[16]

Some of the cells in perivascular infiltrates in renal grafts undergoing rejection have been noted to divide,[3] and it has been suggested [17] that this mitotic activity is provoked by donor lymphocytes in a manner analogous to *in vitro* mixed lymphocyte culture.[18] Similarly, the observed loss of immunogenicity with retained antigenicity of some tissues cultured before implantation has been interpreted as resulting from death of lymphocytes during the period of culture.[19]

We have examined many tracheal cultures histologically in the course of other studies [5] and after a week in culture have not encountered lymphocytes in any of them. All of the lymphocytes seen in the transplanted cultures are therefore host derived, and the mitotic activity cannot be analogous to mixed lymphocyte culture as suggested for renal allografts.

It appears, rather, that the changes in lymphocyte populations and the mitotic activity which we observed is a response to epithelial cells. Without labeled cell populations,[20] the relationship between the different mononuclear populations cannot be established, but it is clear that the early infiltrating cells are not cytotoxic while those of the mixed population present later, if not themselves cytotoxic, seem to be at least indirectly involved in epithelial cell lysis. These observations are consistent with differentiation of the lymphocytes into two populations—recognition or amplification cells and killer cells.[21]

The process we describe may serve as an *in vivo* model in which the processes of cellular recognition and cell lysis by lymphocytes, shown to occur *in vitro*,[22] can be studied. Another use could be as a sensitive method for recognizing neoantigens induced by carcinogen treatment.[23] Tracheal cultures have been used for carcinogenesis studies[24] and are now being developed into a large scale bioassay for chemical carcinogens.[5] Early recognition of small numbers of cultured cells transformed by the carcinogens might be achieved by screening for immune responses after transplantation into inbred hosts.

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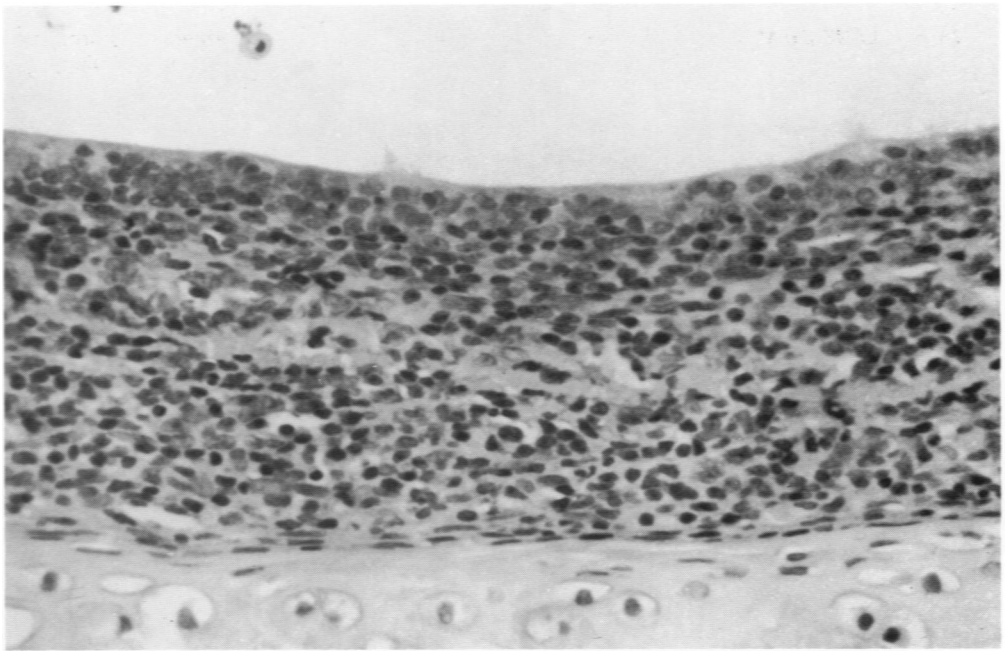
[*Illustrations follow*]



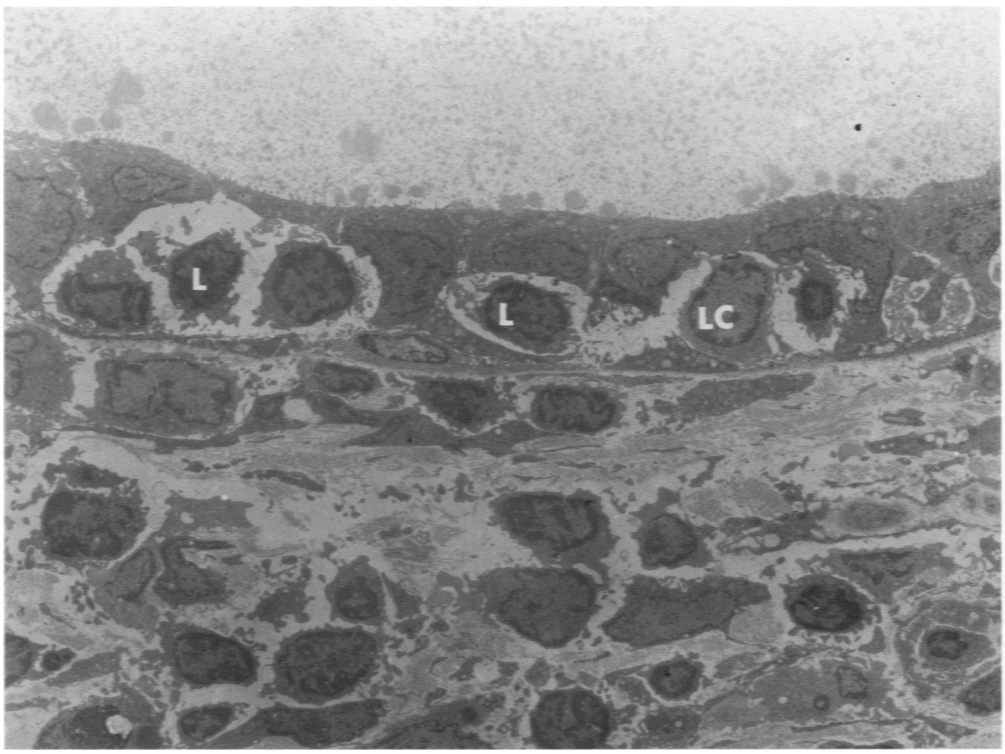


Figure 1—The mucosa of a tracheal culture implanted 10 days previously in a subcutaneous site on a nonisogeneic recipient is infiltrated by host lymphocytes (× 530). **Figure 2**—The cells infiltrating the edematous epithelium are predominantly small lymphocytes (*L*), but occasional larger cells (*LC*) with convoluted nuclei and abundant cytoplasm are present. Processes of the lymphocytes contact the uninjured epithelial cells. (× 1950)

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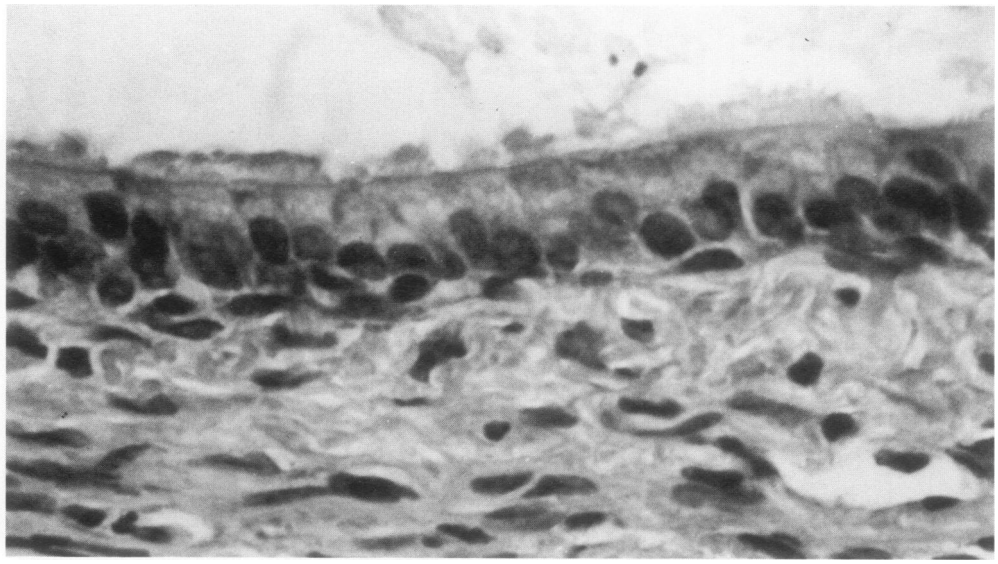

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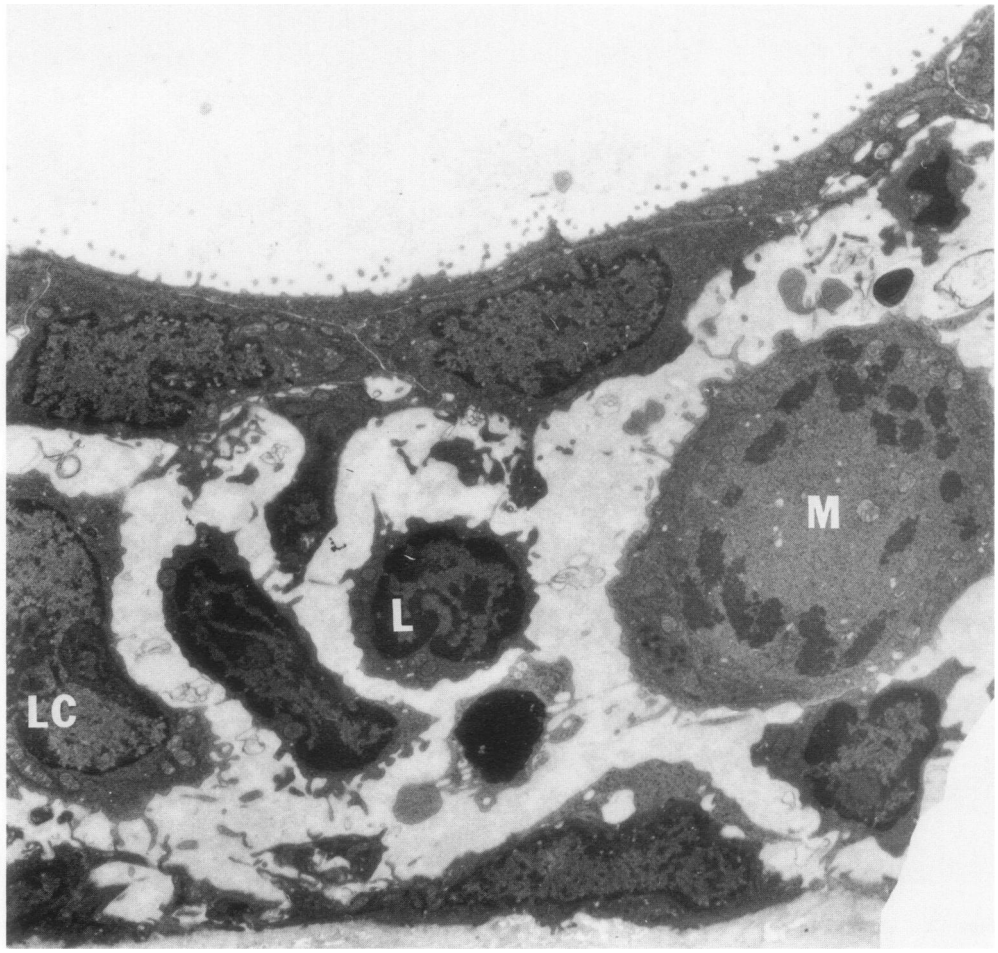

Figure 3—The mucosa of a tracheal culture implanted on an isogeneic host is not infiltrated by lymphocytes. The cell-differentiated epithelium is intact and is not edematous. (× 900) **Figure 4**—By 14 days, a mixed infiltrate is present in the epithelium of some of the tracheas implanted on nonisogeneic recipients. In addition to small lymphocytes (*L*), there are larger lymphocytes (*LC*) and lymphoblasts in mitosis (*M*). (× 6000)