Cellular Rejection*

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Introduction and Background

Delayed or cellular hypersensitivity is the primary immunologic mechanism in the rejection of organ homografts. Mounting evidence implicates the small lymphocyte as the cell responsible for implementing this reaction.

The immunologic role of the lymphocyte in the graft-versus-host reaction was first recognized by Simonsen (1957). He showed that if newborn or heavily irradiated mice are injected with foreign adult lymphoid cells, the animals will waste and die. He further demonstrated that the cells causing the graft-versus-host reaction are present in the peripheral blood of the mouse. Other investigators have since demonstrated that this cell is the small lymphocyte.

Strober and Gowans (1965) have demonstrated that there is interaction between small lymphocytes and a foreign organ graft. In this study, lymphocytes were first perfused through isolated allogenic kidneys and then injected back into the animal from which the lymphocytes were derived. It could then be demonstrated that these animals reacted to a skin graft from the kidney donor with a secondary response, indicating prior sensitization. This study seemed to indicate that the small lymphocyte is involved in the afferent (sensitization) limb of the homograft response.

There is also experimental evidence to suggest that the small lymphocyte is intimately involved in the efferent (destruction) limb of the homograft response. The earliest indication of this was histologic interpretation of the events surrounding the homograft reaction. In serial biopsies taken daily following renal transplantation in dogs, the events can be examined unmodified by immunosuppressive agents. In biopsies from such dogs, very little histologic alteration can be seen for the first 48 hours, but by the third day there are well-established pyronine-positive mononuclear cells in the perivascular interstitial areas. In the ensuing days this infiltration spreads and tubular cell degeneration occurs, followed by infarction. The glomeruli remain remarkably normal during this entire process of primary graft rejection. In the immunosuppressed animal this perivascular infiltration of immunocytes does not occur, and organ function is prolonged.

Experimental Data

Interested in this observed phenomenon, my colleagues and I designed several experiments to study the relationships of the lymphocyte to the efferent arc of transplant immunity and the effect of lymphocyte depletion upon the temporal and histologic events of the homograft response.

Because of the marked radiosensitivity of the lymphocyte, it seemed possible that lymphocyte depletion could be produced in the experimental animal by the use of radiation. Hume and Egdahl (Hume et al., 1960) had previously reported that lethal whole-body radiation in the dog abrogated the immune response, whereas sublethal doses of radiation did not alter the immune response.

Our first experiments (Wolf et al., 1966) utilized small pellets of Yttrium^{90}, a powerful beta emitter having a half-life of 64 hours. The pellets were encased in Silastic tubing and implanted into the abdominal aortas of dogs. Thus, all the blood flowing through the aorta was irradiated. These animals all showed a prompt and profound lymphopenia (Fig. 1), which persisted for two to three weeks following the cessation of radiation and dropped to levels of 10% of control values.

Serial biopsies of mesenteric lymph nodes in these animals showed progressive lymphocyte depletion from germinal follicles. Bizarre cells were seen in the circulating blood of these animals during the radiation period; the cells showed clumping of the cytoplasm and nuclear vacuolization.

A group of these animals received renal homografts in addition to blood irradiation (Fig. 2). The untreated control kidneys had a functional survival of 5.2 days, while the irradiated animals had a mean functional survival of 16 days, the longest functional survival being 34 days. From this study, it appeared that a relation-

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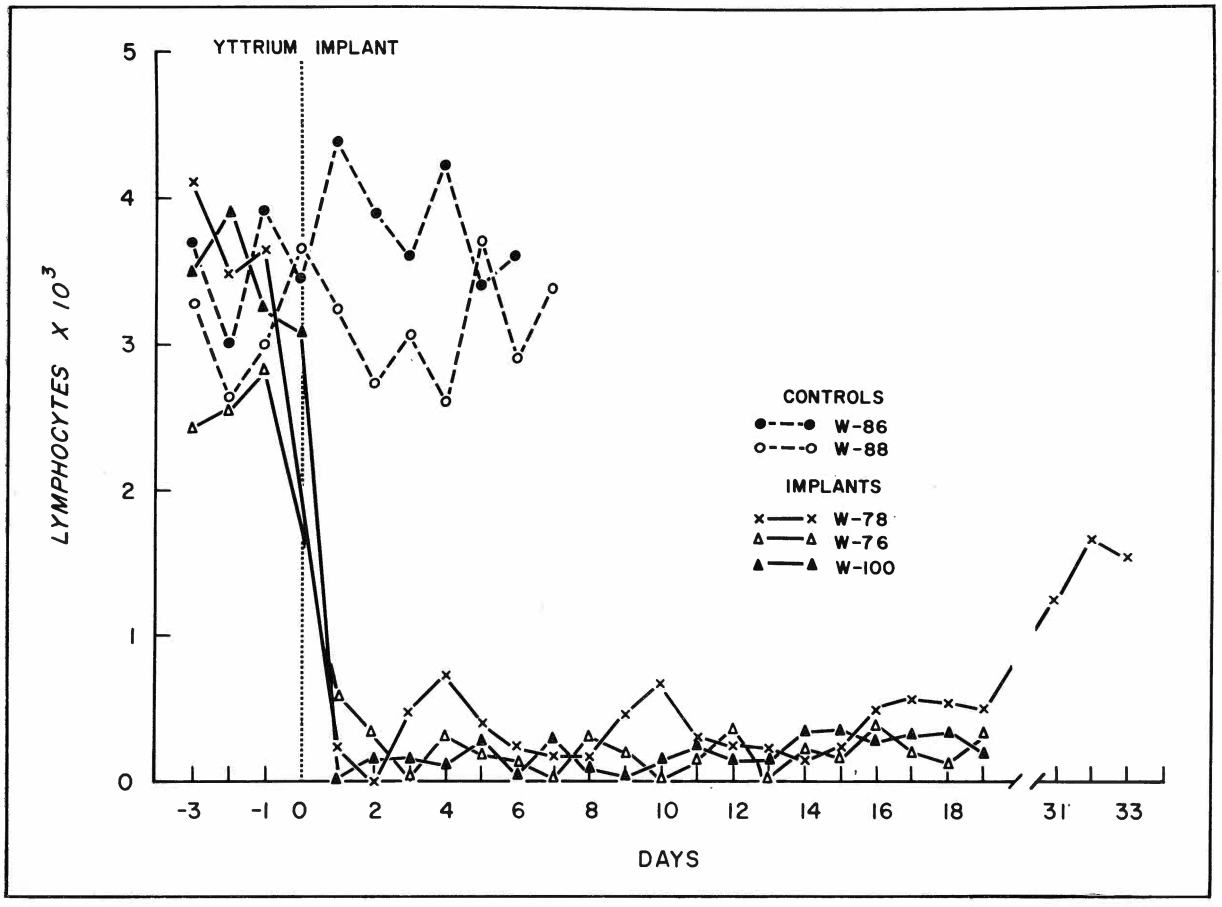

Fig. 1—Dogs receiving Y^{90} implants show a marked and prolonged lymphocytopenia.

ship existed between total lymphocyte mass in the dog and its ability to produce homograft destruction.

To examine further the effect of the lymphocyte on the efferent arc, we designed an experiment in which we tried to keep the afferent arc constant (Wolf, McGavic and Hume, 1969). To do this, we simultaneously transplanted two kidneys from the same donor into the same recipient. One kidney was placed in the pelvis and the other in the neck. One of the two kidneys received local graft irradiation in a dose of 150 roentgens on days one, three, five and seven post transplantation. Thus, the animal had one kidney to which he was normally sensitized and which had received no form of immunosup-

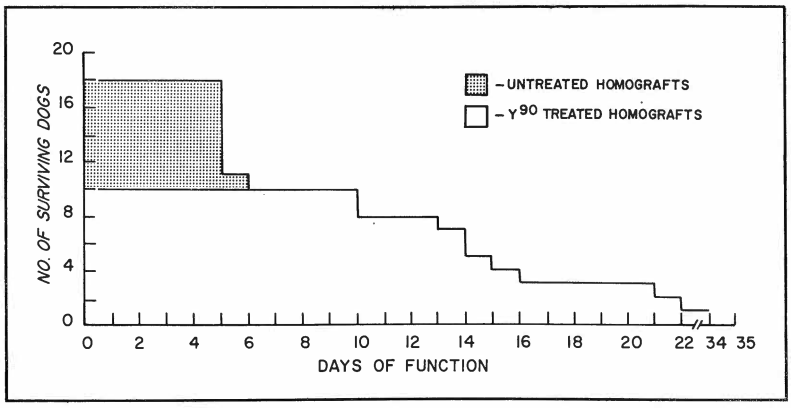

Fig. 2.—Dogs receiving Y^{90} radiation concurrent with renal transplantation have prolonged homograph survival.

pression, while the other kidney received only local graft irradiation. Serial biopsies were then taken simultaneously in both kidneys in several dogs. In each biopsy the unirradiated kidney showed more marked cellular infiltration on any given day than the irradiated kidney. The nonirradiated kidneys rejected in a mean time of 5.7 days, while the irradiated kidneys had a mean survival time of 12.1 days, with 24 days the longest period for a surviving kidney.

It would appear from this experiment that the animal was normally sensitized—as indicated by rejection in the normal time of the untreated kidney—but that the local irradiation served as an immunosuppressant, probably by destroying sensitized lymphocytes in the kidney. This experiment, then, gives further evidence that the lymphocyte is involved in the primary immune response.

To further manipulate the cellular environment in an attempt to study the effect of the lymphocyte on the efferent arc of transplant immunity, we designed some tissue culture experiments (Wolf, Fawley and Hume, 1969). In these studies, renal cells were cultured from transplanted kidneys removed from patients who had rejected either chronically or acutely. These cells were grown in tissue culture at 37C, using Eagle's Minimum Essential Medium to which was added 20% serum and penicillin, streptomycin, and L-glutamine. After these monolayer cultures had been established for a period of five to seven days, lymphocytes—from the patient, from the kidney donor, or from an indifferent donor—were added to the cultures. Figure 3 shows one such renal cell monolayer culture to which indifferent lymphocytes have been added. The lymphocytes did not appear to have any effect on the cultures, and the culture continued to grow in a normal fashion. However, as seen in Figure 4, when the patient's own lymphocytes were added to the culture, even in the absence of complement or autologous serum, there was destruction of most of the kidney monolayer within 12 to 14 hours. Nine human kidneys which had been previously transplanted for periods of 1 to 18 months have thus far been studied, as have been six non-transplanted kidneys. In seven of the nine transplanted kidneys which had histologically established rejection patterns, the recipients' lymphocytes were markedly cytotoxic to the kidney cells, showing destruction of most of the mo-

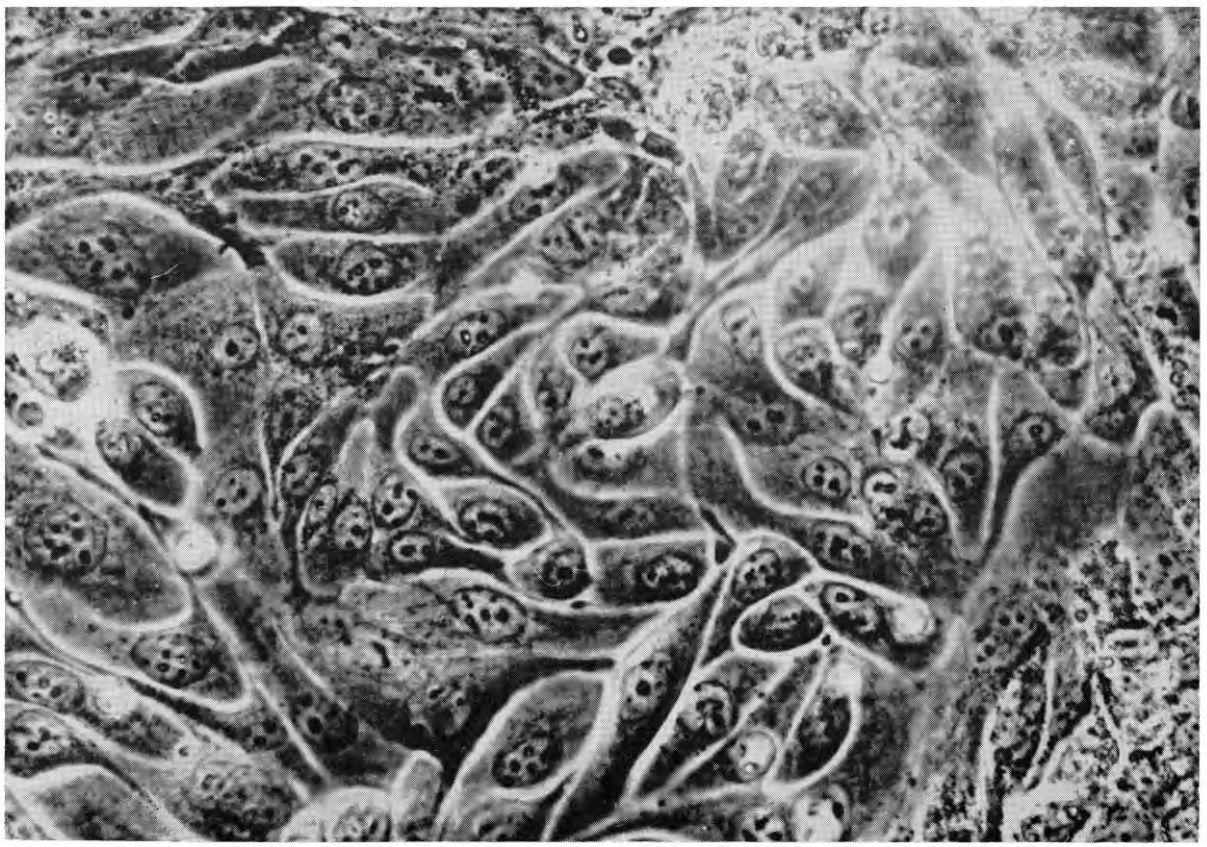

Fig. 3—Renal cell monolayer culture reacted with indifferent lymphocytes and showing no evidence of renal cell destruction (× 380).

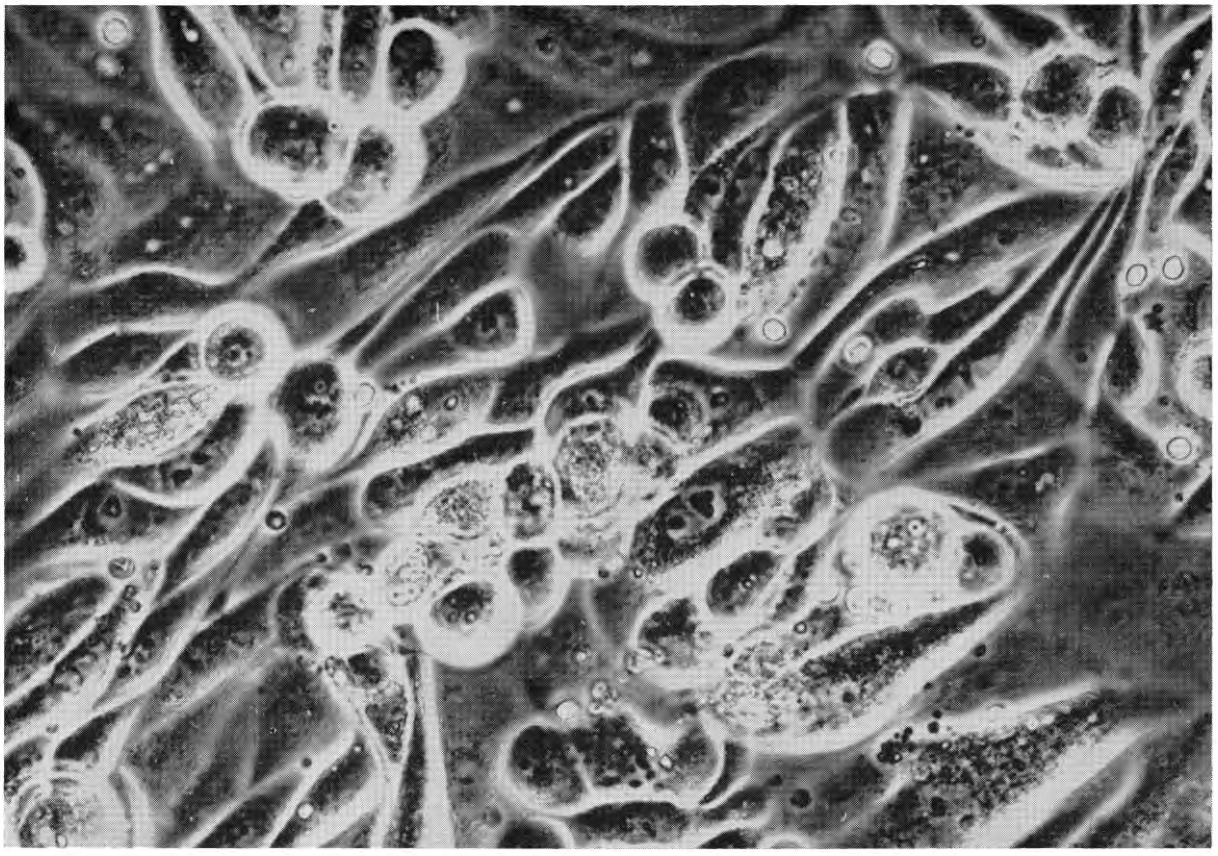

Fig. 4—Renal cell culture reacted with specifically sensitized lymphocytes and showing marked renal cell destruction (× 380).

nolayer within 12 to 14 hours, even in the absence of complement source and regardless of the source of the serum. Indifferent lymphocytes, donor lymphocytes, or lymphocytes from other transplant recipients did not have this same effect.

Conclusion

From these studies we have concluded that: 1) sensitized lymphocytes in close physical contact to the target cell can produce destruction of the target cell; 2) deficiency of lymphoid mass within the organ homograft recipient can produce attenuation of the homograft reaction; and 3) the lymphocyte target cell reaction in vitro is individual-specific, but not complement-dependent, is rapid, and proceeds in the absence of autologous serum source.

Mounting experimental data suggest that the homograft reaction is intimately related to the small lymphocyte. It is evident that both the afferent and efferent arcs of transplant immunity are dependent upon the intactness of the lymphoid system. The lymphocyte has the ability to recognize the foreign antigen, is involved in the activity of cell-bound antibodies, and may be involved in the inductive processes leading to antibody formation.

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