The Cytotoxic Action of Human Lymphocytes from Graft Recipients on Donor Target Cells in Tissue Culture*

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SUMMARY

Lymphocytes from the recipient of a renal allograft which was undergoing rejection, were shown to be highly cytotoxic to a culture of donor liver cells. This effect was not shown by the lymphocytes of a second recipient who received the other kidney from the same donor.


Lymphocytes that participate in a cell-mediated immune reaction can destroy cells to which they have been sensitized, which can be demonstrated by adding them to cultures of the relevant target cells. This experimental system has wide application, for example in tumour immunology and auto-immune disease. It is of particular value in experiments involving humans, where such manoeuvres as lymphocyte transfer and experimental tumour grafting are not feasible. Destruction of the target cells may be assessed by means such as the release of ³¹Cr (the target cells having been prelabelled with the isotope), the inhibition of colony formation by the target cells, or the easily observed destruction of a target cell monolayer.

We have been using a tissue culture test system in various situations and were recently able to test the lymphocytes of 2 patients who received kidneys from the same donor, against a culture of donor tissue cells.

MATERIALS AND METHODS

Target Cells

At the time of the transplant a piece of fresh liver was removed from the cadaver donor. This was finely minced with scalpel blades, washed in tissue culture medium (TC 199) and then suspended in TC 199 with 20% foetal calf serum. The suspension was poured into Falcon tissue culture flasks, gassed with 5% CO₂ in air, and kept in a 37°C incubator. Continuous monolayers of adherent cells resulted. At the time of the test, excess medium was withdrawn from one of the flasks and replaced by 0.2% trypsin in Hank's balanced salt solution at 37°C for 30 min. The resulting single cell suspension was washed, and then adjusted to a concentration of 50 cells/lambda in TC 199 plus foetal calf serum.

Lymphocytes

Lymphocytes from freshly drawn defibrinated blood were extracted by the method of Coulson and Chalmers and the cell counts adjusted to approximately 10⁷/lambda. The sources of lymphocytes were: (i) recipient 1; (ii) recipient 2; (iii) normal control 1; (iv) normal control 2.

Both recipients had been treated with antilymphocyte globulin, azathioprine and corticosteroids and had low absolute lymphocyte counts. The HL-A groups of recipients, controls, and the donor were determined by the Transplantation Unit of the Natal Institute of Immunology, using the two-stage technique recommended by the National Institutes of Health.

The test was performed 3 weeks after the transplantation of both kidneys from the cadaver donor, one to each previously nephrectomized recipient. At the time, recipient 2 was doing well, his urinary output being about 2.5 litres per day, creatinine clearance 61.2 ml/min, and serum creatinine 1.45 mg/100 ml.

Recipient 1, on the other hand, was showing signs of rejection with a rising serum creatinine (2.5 mg/100 ml), a marked decrease in urinary output, and pyrexia.

We performed a colony inhibition test by combining samples of each lymphocyte suspension with an equal volume of target cell suspension; the resulting ratio of lymphocytes to target cells was 20:1. In another control, equal volumes of medium and target cells were mixed. These various combinations were transferred in 10 lambda aliquots into the wells of Terasaki plates. The plates were sealed in plastic boxes, gassed as before, and incubated for 24 hours at 37°C. The medium was then gently washed out with saline until all loose cells had been removed. The adherent cells were fixed with methanol and stained with May-Grünwald-Giemsa. The wells were examined microscopically and the number of surviving adherent cells counted. Note was also made of lymphocytes which had adhered to the target cells.

RESULTS

If the average number of cells surviving in the 'medium only' wells (50 observations) is taken as 100%, then

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the percentage survival in the other wells was found to be (i) with control 2 lymphocytes—100% (40 observations); (ii) with control 1 lymphocytes—87.5% (30 observations), (iii) with recipient 1 lymphocytes—0% (30 observations); (iv) with recipient 2 lymphocytes—62.5% (30 observations). These results are shown in the accompanying histogram (Fig. 1).

![Histogram of survival of target cells](image)

**Fig. 1. Percentage of target cells surviving after incubation with the various lymphocytes.**

### Lymphocyte Adherence to Target Cells

In all the wells to which lymphocytes were added it was noticed that varying numbers of lymphocytes remained attached to the viable target cells in spite of the washing. We categorized these findings into 4 grades as follows: (+) 1 or 2 lymphocytes per target cell; (+++) 3-5 lymphocytes per target cell; (++++) over 5 lymphocytes on most target cells; and (++++) heavy encrustation of most target cells.

By using this scheme it was found that adherence with lymphocytes of recipient 2 and control 1 averaged +++, while with control 2 the index was ++.

The HL-A groups of the subjects and controls are shown in Table 1.

<table>
<thead>
<tr>
<th>TABLE I. HL-A AND BLOOD GROUPING</th>
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<tbody>
<tr>
<td><strong>Donor</strong></td>
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</tr>
<tr>
<td>Recipient 1</td>
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<tr>
<td>Recipient 2</td>
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<td>Control 1</td>
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<td>Control 2</td>
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**DISCUSSION**

Further exploration of this system is planned, but the results of this preliminary study are clear-cut and correlate closely with clinical criteria of rejection of the allograft in recipient 1. The kidney was, in fact, removed 15 days later and microscopic examination showed accumulations of lymphocytes and plasma cells, with tubular atrophy, increased glomerular cellularity, and arterial intimal thickening. The kidney in recipient 2 was functioning well, and this patient's lymphocytes did not show a similar cytotoxic effect on the donor liver cells in culture.

The results strongly suggest a powerful specific cytotoxic effect of the rejecting recipient's lymphocytes on the culture of donor cells. Such a test system may provide valuable information on immunological responses after transplantation. It may also be of clinical value as a means of assessing the efficacy of immunosuppression, and in the early detection of rejection.

Lundgren et al., assessing lymphocyte cytotoxicity on target cell monolayers, were unable to show an effect when testing lymphocytes from renal allograft recipients, even when the target cells were of donor origin and the lymphocytes taken during episodes of rejection. The colony inhibition technique is possibly more sensitive in that a degree of target cell damage sufficient to prevent colony formation may be unable to produce obvious monolayer destruction.

The significance of lymphocyte adherence to the target cells is not clear, but is a common finding in this type of test. One possibility is that this phenomenon represents exploration of the target cells by the highly motile lymphocytes, as part of the recognition phase of the afferent limb of the immune response. The number of adhering lymphocytes does not appear to be related to the degree of histocompatibility which exists between the lymphocytes and target cells, as is evident from the HL-A types (Table 1).

It might be argued that in this test system where the lymphocytes are mixed before any settling of the target cells has taken place, this non-specific adherence of lymphocytes could mechanically prevent the target cells from attaching themselves to the surface of the culture well. This is not so, because heavy clusters of lymphocytes from the normal controls did not reduce the number of surviving target cells below that seen in the wells to which no lymphocytes had been added.

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**REFERENCES**