

Detection of Rejection of Human Renal Allografts by Increased RNA Synthesis in Peripheral Lymphocytes

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Current methods of detecting rejection of human renal allografts include clinical and routine laboratory investigation, together with more sophisticated tests. They fail, however, to give positive results until the process has reached a stage at which significant renal damage has already occurred. Among the latter tests ultrasonic measurement of renal blood flow (Samson, 1969), detection of antibody formation (Rowley et al, 1969) and serum complement changes (Carpenter et al, 1969) have yet to prove sufficiently reliable for routine clinical practice.

This study of the functional activity of the peripheral blood lymphocytes in recipients of human renal allografts was undertaken because, in view of the recognised importance of their role in rejection, it seemed possible that detectable changes in function might occur before the rejection process had produced significant renal damage. If such a change in the functional activity could be shown to herald the onset of rejection an appropriate change in the immunosuppressive regime might be made at an early stage in order to prevent further damage occurring.

METHODS

The functional activity of peripheral lymphocytes was assessed by measuring *in vitro* the rate of ribonucleic acid (RNA) synthesis immediately after separation of the cells from a fresh sample of venous blood.

Lymphocytes were extracted from samples of 10-20 ml of fresh defibrinated blood using carbonyl iron powder and methyl cellulose (Coulson & Chalmers, 1967). After sedimentation at 37°C for 30 minutes the supernatant contained a high yield of non-phagocytic mononuclear cells. After further concentration by centrifugation the cells were cultured in T.C. 199 medium (concentration $1.0 - 2.0 \times 10^6$ cells/ml) for thirty minutes. An excess of tritiated uridine was then added and the incubation continued for exactly one hour. After preventing further uptake by freezing, the incorporated

RNA was extracted in trichloroacetic acid and the radioactivity in the insoluble material measured by a 20 minute period of liquid scintillation spectrometry. For the latter the highly efficient method of Davies (1966) was used, the labelled nucleotide being counted in fibre filter discs. The purity of the extract was confirmed by recounting after incubation with ribonuclease. The rate of RNA synthesis was expressed as counts/min/million cells.

PATIENTS

Frequent observations were carried out on the following groups of persons:

- I. Normal volunteers
- II. Patients on haemodialysis
- III. Patients who had received renal allografts both during periods of normal renal function and episodes of rejection.

RESULTS

Group I

The reproducibility of the results is shown in Figure 1. The rate of RNA synthesis in a normal volunteer remained between 45 and 135 cpm/million cells over a period of eighteen months. On three occasions when the count exceeded this range the volunteer was suffering from influenza, atypical lymphocytosis and an upper respiratory tract infection. The average rate of RNA synthesis in all normal volunteers studied was 86 cpm/million cells (S. E. ± 2.5 cpm/ 10^6 cells).

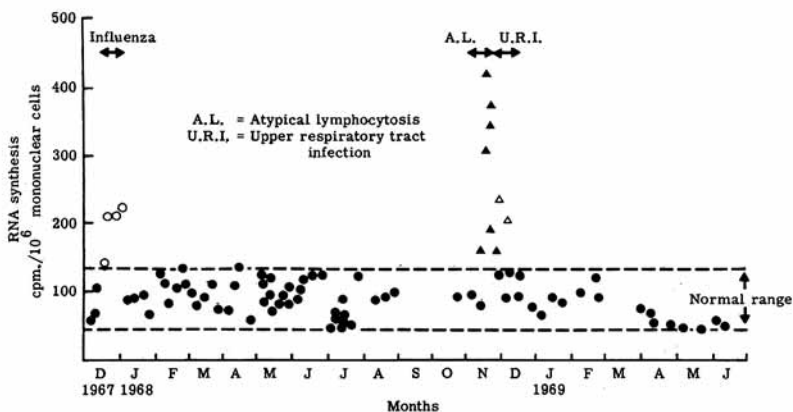


Figure 1. The rate of RNA synthesis in a normal volunteer over a period of eighteen months

Group II

The average rate of RNA synthesis in patients on haemodialysis was found to be 180 cpm/10⁶ cells (S. E. \pm 15.0 cpm/10⁶ cells).

Group III

An example of the rate of RNA synthesis in a patient rejecting a renal allograft is shown in Figure 2. After receiving a cadaver kidney the patient was maintained on prednisone and azathioprine. The kidney failed to function,

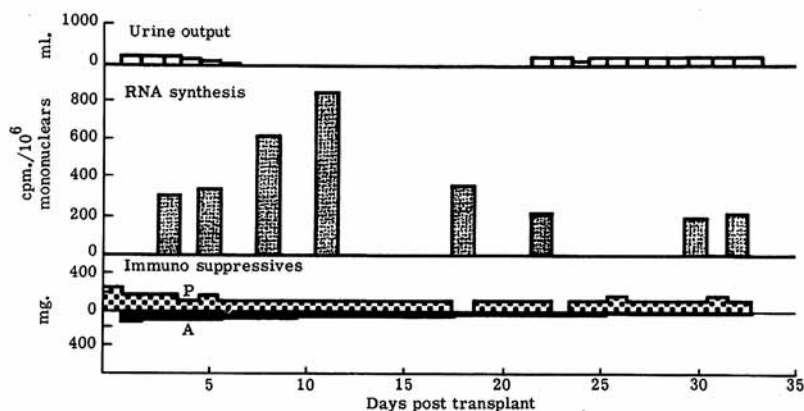


Figure 2. The clinical course and rate of RNA synthesis in a patient with post-transplant oliguria in whom there was clinical evidence suggestive of ischaemia rather than rejection. The oliguria was subsequently shown on biopsy to result from rejection. P = prednisone; A = azathioprine. Urine output in ml/24 hours

the urine volume being reduced to 10-20 ml daily after five days. There being no clinical or routine laboratory evidence of rejection, the oliguria was regarded as being due to damage during the interval of ischaemia between removal from the donor and revascularisation in the recipient. When the kidney had failed to function satisfactorily after sixty days, a biopsy was performed. This showed evidence of severe past rejection. The rate of RNA synthesis showed a rise to 850 cpm/10⁶ cells at the time of maximum oliguria, having risen from a post-transplant level of 300 cpm/10⁶ cells at 3 days. Thereafter the level fell to approximately 200 cpm/10⁶ cells.

A total of seventeen rejection episodes were studied. The clinical diagnosis was made in ignorance of the RNA synthesis data. In Figure 3 a scatter diagram shows the rates of RNA synthesis during periods of six days before and six days after these rejection episodes. The rate of RNA synthesis is above the mean (186 cpm/10⁶ cells) found in pre-transplant patients in all but one of the 44 observations recorded. The mean rate of RNA synthesis

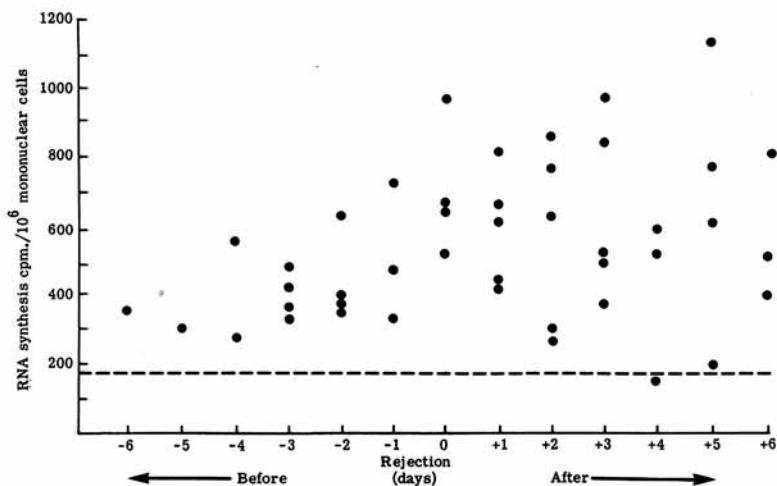


Figure 3. The rate of RNA synthesis during seventeen rejection episodes. The dotted line is the mean value for pre-transplant patients

on the day of maximum rejection was $680 \text{ cpm}/10^6$ cells, a value of more than three times the pre-transplant level. After treatment of rejection the rate of synthesis gradually fell to pre-rejection levels.

During examination of the peripheral blood lymphocytes of these patients undergoing rejection episodes it was noted that periods of increased RNA

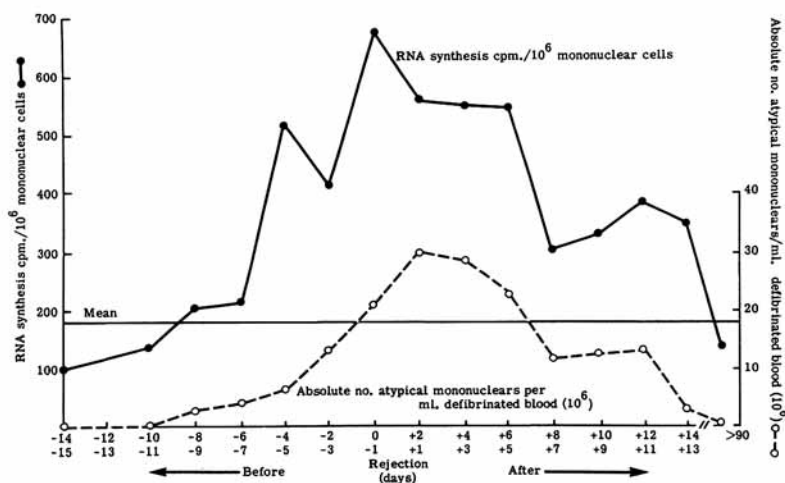


Figure 4. The mean of measurements of the rate of RNA synthesis and the numbers of atypical mononuclear cells during rejection episodes

synthesis coincided with the appearance in increased numbers of large atypical mononuclear cells. Light microscopy, electronmicroscopy and histochemical elucidation of the nature of these cells suggested they were immature cells of the myeloid series rather than of the lymphocytes series. Figure 4 shows the correlation between the increase in RNA synthesis in Group III patients with the increased numbers of these cells. Autoradiographic studies showed that active RNA synthesis was taking place in these cells.

DISCUSSION

The increased rate of RNA synthesis to a mean level of 680 cpm/10⁶ cells on the day of clinical diagnosis of rejection, together with a gradual increase over the pre-transplant mean level of 180 cpm/10⁶ cells during the six days preceding rejection appears to afford the opportunity of employing this three hour test in clinical practice. The recognition of rejection episodes during the oliguric post-transplant phase, which usually follows the use of kidneys from cadavers, is often difficult, methods described hitherto either being impracticable for routine use or unreliable. The coincidental appearance of increased numbers of atypical mononuclear cells in the peripheral blood appears to be the main factor responsible for the increase in the rate of RNA synthesis. That these cells are not specifically sensitised 'effector' or lymphoid cells has been shown by light microscopy, electronmicroscopy and histochemical studies.

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