Reactive Lymphocyte Blastogenesis (RLB) in the Immunologic Assessment of Human Renal Allograft Recipients

GERALD POSEN, JULES HARRIS,
DENNIS PAGÉ, THOMAS STEWART
Department of Medicine, University of Ottawa, Ottawa, Canada

We have previously reported our experience with an immunologic assay for renal allograft rejection (Pagé et al., 1971a, Pagé et al., 1971b, Harris et al., 1971). The assay * detects the presence of increased numbers of circulating DNA-synthesizing mononuclear cells (RLB) in the peripheral blood by means of their $^3$H-thymidine uptake (Tennentbaum, 1968). This paper further extends our observations with this test.

**METHODS AND MATERIALS**

Our patient population consisted of 7 males and 3 females, all of whom experienced at least one rejection crisis. Their age range was 17 - 52 years - median age 26.5 years. All had a pregraft diagnosis of chronic glomerulonephritis and all had received cadaver kidneys. Two additional patients, a male age 28 years and a female age 19 years, both of whom had received a cadaver kidney for a pregraft diagnosis of chronic glomerulonephritis and neither of whom had to this time suffered a rejection reaction, were also evaluated. The control population consisted of 42 healthy volunteers, 22 males and 20 females – age range 11 - 50 years, median 29 years. None of these had a history of allergy or viral or bacterial infection at the time of study.

Technical details have been previously published (Pagé et al., 1971b). Briefly, about 30 ml of heparinised blood is drawn and after dextran sedimentation cultures were set up containing $10^6$ lymphocytes in 13 x 100 mm tubes with 2 ml Eagle’s spinner modified media and 1 ml autologous plasma. Two $\mu$Ci $^3$H-thymidine – specific activity 6.7 $\mu$Ci/mM – were immediately added and the cultures incubated in 5% CO$_2$ in air for 2 hours at 37°C. They were processed for liquid scintillation counting at the end of that time. Phytohemagglutinin (PHA) studies were performed concurrently using $10^5$ and

* See also Parker, J. and Mowbray, J. (1971) Transplantation 11, 201 (Editor's note)
10^6 responding cells. These cultures were incubated for 5 days and after a terminal 3-hour incubation with 2μCi ^3H-thymidine harvested for counting. In the micromethod or 'fingertip' method for RLB (Junge-Dent Test), 0.05 ml unheparinized fingertip blood was placed at once in 2 ml of Eagle's medium and incubated for 3 hours with 2μCi ^3H-thymidine (Dent, 1970).

RESULTS

RLB results

Figure 1 shows the results of 91 studies performed in our 10 patients during a total of 15 rejection crises. These are compared to 38 values obtained in our control group. The vertical bars give ranges; the horizontal lines are drawn through median values. In the patients studied counts ranged between 1466 - 31,354 cpm - median 5,679 cpm. In the normal subjects the median count was 696 cpm - range 78 - 1289 cpm. In addition to identifying an immunologic cause for renal impairment, monitoring of RLB counts will give up to one week's warning of rejection. Counts will rise up to one week before clinical evidence of rejection develops.

Figure 2 illustrates results obtained with the micromethod of Junge-Dent. Thirty-four studies were conducted in 7 patients during 8 rejection crises. Results ranged between 137 - 1706 cpm, median 377 cpm. This compared to a median of 75 cpm, range 31 - 196 cpm established with 27 values obtained in the control group. No relationship was found between
Figure 2. Fingertip micromethod study of reactive lymphocyte blastogenesis. Thirty-four studies were performed in 7 patients during 8 rejection crises. The vertical bars give ranges; the horizontal lines are drawn through median values.

Figure 3. The phenomenon of 'PHA escape' observed in 4 allograft recipients. The vertical bars give ranges; the horizontal lines are drawn through median values.

Peripheral blood absolute lymphocyte count and the counts registered with the fingertip micromethod. We have previously shown that the micromethod will provide essentially the same information as the test requiring $10^6$ lymphocytes (Page et al, 1971b).

**PHA results**

Figure 3 illustrates the phenomenon of 'PHA escape' - recovery of normal
responsiveness to PHA at the time of rejection crisis despite a dose of immunosuppressive medication which previously suppressed lymphocyte reactivity. PHA escape has not been noted to occur before the development of gross clinical evidence of rejection. Figure 3 shows the PHA response in control subjects and in 4 patients before and at the time of graft rejection. The 42 control subjects had a response of 20, 342 - 112, 510 cpm, median 44, 376 cpm. Eighteen observations made in the 4 patients during the 2 - 3 month interval before rejection yielded a range of 577 - 13, 109 cpm, median 1524 cpm. Eleven studies of PHA response at the actual time of rejection - before any increase in immunosuppressive medication - give a rebound to a median of 27, 985 cpm - range 15, 474 - 90, 697 cpm.

Figure 4. Illustrating normal PHA reactivity in patients with stable renal function receiving apparently adequate doses of immunosuppressive medication. The vertical bars give ranges; the horizontal lines are drawn through median values

In some cases apparently adequate immunosuppressive medication fails to suppress PHA responsiveness despite its effectiveness in preserving stable renal function. This, in addition to 'PHA escape,' throws seriously into question the usefulness of the PHA response as a measure to assist with the titration of patients with immunosuppressive drugs. Figure 4 details 34 observations of PHA responsiveness for both $10^5$ and $10^6$ lymphocytes made in 4 allograft recipients over a 3-month period. During that time they had each received a minimum of 25 mgm prednisone and 150 mgm azathioprine and enjoyed stable renal function as assessed by serum creatinine. Their lymphocyte responsiveness did not differ significantly from the control subjects who had not been receiving immunosuppressive drugs; median response $10^6$ lymphocytes 76, 701 cpm (range 16, 297 - 159, 503 cpm) cf. normal
Figure 5. A portion of the clinical course of patient RP showing PHA responses within the normal range despite immunosuppressive medication. No rejection crisis has been experienced. RLB counts remain normal.

Figure 6. A portion of the clinical course of patient YL. Normal RLB counts in the presence of a rising serum creatinine provided evidence to search for a non-immunologic cause of renal impairment.

median 44,376 cpm (range 20,342 - 112,510 cpm); median response $10^5$ lymphocytes 80,491 cpm (range 25,082 - 206,622 cpm) cf. normal median 65,358 (range 27,516 - 165,641 cpm).

Monitoring patients following transplantation
Serial monitoring of the PHA response of lymphocytes is unsatisfactory for judging the effectiveness of immunosuppressive treatment. This contention
is further supported by information schematically presented in Figure 5 which represents the portion of the clinical course of a patient who has not as yet undergone rejection crisis since allograft placement. It depicts the course of patient RP from November 1970 to May 1971. Prednisone and azathioprine have been kept constant at 20 mgm and 250 mgm respectively. Renal function has remained normal. The PHA response of both $10^5$ and $10^6$ lymphocytes has remained generally within normal limits without any adjustment in immunosuppressive therapy. This index of the lymphocyte’s functional integrity cannot be used to modify the dose of immunosuppressive drugs given. Determinations of RLB in this case have given values within the normal range and this test serves as a valuable guide for changes in treatment.

Figure 6 shows a portion of the clinical course of patient YL. PHA responsiveness has remained within the normal range. Elevation in the serum creatinine despite normal RLB counts was discovered to be due to renal artery stenosis. In this case monitoring of RLB counts allowed a distinction to be made between an immunological and a non-immunological cause for renal impairment. This avoided an unnecessary increase in immunosuppressive medication.

The usefulness of RLB values in the differential diagnosis of the oliguria which frequently follows cadaver transplants is illustrated in Figure 7. This figure illustrates a portion of the clinical course of patient DC. She received a cadaver transplant on March 21, 1971. She was oliguric following the operation and required repeated haemodialysis. One week following the operation she developed leukopenia necessitating a decrease in azathioprine dose. At the same time her RLB values increased. She was afebrile, had no kidney tenderness and there was no change in urine output. On the basis of the increasing RLB she was treated as a rejection crisis with increasing

![Figure 7: A portion of the clinical course of patient DC illustrating the usefulness of serial RLB determinations in the differential diagnosis of post-transplant oliguria (see text)](image-url)

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doses of prednisone. A renal biopsy 4 days after the start of the increased RLB demonstrated histological evidence of rejection. The RLB decreased and her kidney function returned soon after. Subsequently she has done well with stable RLB values and stable renal function.

SUMMARY

In conclusion we feel that determination of reactive lymphocyte blastogenesis (RLB) is a simple, rapid test that may prove useful in early diagnosis of rejection crises before clinical evidence of deteriorating renal function and in determining the efficacy of the immunosuppressive therapy. We also conclude that the lymphocyte response to PHA is an unsatisfactory measurement for these purposes. The clinical utility of RLB studies is now well established. Additional experience with its use especially in transplant situations complicated by haemolysis, infection or rapidly recovering leukopenia is required.

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REFERENCES


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tation, 6, 986
OPEN DISCUSSION

FESTENSTEIN (London): I would like to ask the speaker one or two technical questions. First, you said that the response to an antigenic stimulus was being measured. Do you mean that the kidney is the antigen, or do you put some antigen into the cultures as well?

POSEN: No, the kidney is the antigen. We are measuring the response of the lymphocytes in the draining lymph nodes of the kidney. These lymphocytes undergo blast changes in response to stimulation by the antigen in the kidney, are released into the circulation and then attack the renal graft.

FESTENSTEIN: The other question was about the category of ‘normals’ in the comparison of blastogenesis. Are these normals transplanted patients who are not rejecting, or are they a normal population? If these are a normal population, have you got figures for transplanted patients not undergoing a rejection episode?

POSEN: Yes, these were normal persons. We do have figures for transplanted patients who were not rejecting but I do not have them with me. In the non-rejecting phase the transplanted recipients have RLB counts in the normal range which rise shortly before the appearance of clinical signs of rejection.

FESTENSTEIN: One more question. The figures you showed were the range of the series; can you make a confident prediction in the individual case?

POSEN: That is a very good question. At present the series is still small and it was only this last case that really convinced me. We need a lot more experience with the test but I think it has great potential. It is a little complicated to set up but it is not too bad. We have been doing the test with increasing frequency and are now doing it daily on a group of patients. The first time that we used these results clinically was in this last girl when she was oliguric. We were worried about it so we did her with immunosuppressive therapy and then did the biopsy; to our delight the biopsy confirmed our suspicions.

J DORMONT (Paris): I want to ask you something which has already been discussed. Were any of your controls patients with hepatitis on prednisone therapy? We performed this same test and had the feeling that it was influenced by many factors which were not decidedly related to rejection, including prednisone therapy and viral diseases.
POSEN: It is very important to know that the test is invalid during treatment with ALG which we have not used. We have not done any studies on patients with other renal diseases treated with prednisone or azathioprine. The patients of course have acted as their own controls in that the results of the RLB test were normal when they were not rejecting and were receiving azathioprine and prednisone. None of these cases have shown either false negatives or false positives. In microangiopathic haemolytic anaemia and in autoimmune haemolytic anaemia you may get false positives although we have not as yet seen any, nor have we seen false positives in patients with infections.

DORMONT: When you have rejection and a positive result with your test, you either start or increase the dose of prednisone. What happens to the test – does it become negative, and if so have you seen a subsequent increase in level?

POSEN: The only time we found a second increase was when we decreased the prednisone too quickly and the patient's renal function deteriorated again. This correlated very nicely with the RLB count and we presented this data in Ottawa in January. We increased the steroids and the RLB count fell again; the patient's renal function improved and has remained good since then.

J S CAMERON (London): You mentioned that stimulation with PHA was no good. Have you tried to improve this aspect by using other antigens such as streptokinase or candidin, or even kidney tissue itself?

POSEN: No we have not, but we are in the process of studying that now, particularly with kidney tissue. But the problem of stimulation with PHA or any antigen is that there is a three to five day culture period. The RLB test takes us only five hours.