PAPERS AND ORIGINALS

Further Assessment of Rosette Inhibition Test in Clinical Organ Transplantation

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Summary

The rosette inhibition test was used in the clinical management of organ allografts to estimate the amount of immunosuppressive drugs necessary to prevent rejection. In patients surviving more than three months renal function appeared to be better than in a similar group of patients managed without the test. It is suggested that this was due to a reduction in the number of clinical or subclinical rejection episodes. On the other hand, the test indicates that in many cases the level of immunosuppression should be much higher, and if this advice is followed the patients become increasingly exposed to the risk of infection. In other words, those patients with good renal function remained well, whereas those who might otherwise have rejected their kidney and survived had in fact died of sepsis.

Introduction

In clinical transplantation, whether of kidneys or other organs, the most difficult problem in management is the assessment of the immune status—especially that of the cellular system.

In-vitro tests of lymphocyte transformation (Tennenbaum *et al.*, 1968) and leucocyte migration (Bendixen and Søborg, 1969; Smith *et al.*, 1969) are useful and accurate tests of lymphocyte activity but do not give results quickly enough to be of much clinical use. Estimates of RNA synthesis by lymphocytes

(Parker, 1969; Parker and Mowbray, 1971; Posen *et al.*, 1971) will give a clinically useful result, but if antilymphocyte globulin (A.L.G.) is used or if the patient has a viral infection the test is invalidated.

Zaalberg (1964) first described the "cluster" or "rosette" formation of sheep red blood cells around immunized mouse lymphocytes. Bach and Antoine (1968) noted that a small proportion of "background" or "spontaneous" rosettes was formed in this system. They first proposed a sensitive in-vitro test based on the inhibition of this spontaneous rosette formation. The test was modified by Munro *et al.* (1971) and has been used continuously in the renal transplant programme at King's College Hospital and Guy's Hospital over the past two years. The object of this paper is to present some of the lessons learnt during this time.

What is a Rosette?

The actual configuration of a rosette is now a well-recognized entity. It consists of a central mononuclear cell surrounded by four or more adherent heterogeneic red blood cells. In man it has been shown by Lay *et al.* (1971) that spontaneous rosettes are formed when using sheep red blood cells and, to a less extent, pig red blood cells. They are not formed with horse, ox, rabbit, guinea-pig, mouse, or human ABO blood group red cells. In mice and rats Bach and his colleagues (Bach and Antoine, 1968; Bach *et al.*, 1969; Bach *et al.*, 1970) and others (Biozzi *et al.*, 1967; Brain and Gordon, 1970, 1971; Duffus and Allan, 1971) have shown that sheep red blood cells will form numerous rosettes, whereas in dogs only human red cells were found to produce rosettes (Kelly *et al.*, 1972). We still have been unable to find a red cell that will produce rosettes in pigs.

The exact identity of the central cell is still the subject of much debate, which is reviewed by Biozzi (1972), Bach and Dardenne (1972), and McConnell (1972). Bach and Dormont (1971) suggested that it is probably a T-Lymphocyte. Lay *et al.*, (1971) suggested that it is in most instances a thymic-dependent cell, as they were able to show many more rosettes when using thymocytes than with Burkitt lymphoma cells. Duffus and Allan (1971), Reyes and Bach (1971), Storb *et al.* (1969), and Gudat *et al.* (1970) have shown that the rosette-forming cells are

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no rosettes could be found. During the ensuing 12 hours rosette-forming cells began to appear in the peripheral blood. By 24 hours an appreciable number of cells could be found and an estimate of the level of immune activity obtained. Within two or three days of terminating a course of A.L.G. treatment there usually appeared to be an increase in the number of rosette-forming cells, but two to three weeks later this settled down to a more normal level—that is, the level seen on routine immunosuppression with azathioprine and prednisone (Fig. 3).

ROUTINE IMMUNOSUPPRESSION

The results of 36 consecutive first cadaver renal transplants carried out in our unit are shown in Table I and Fig. 4. Sixteen patients survived varying periods from 3 to 26 months with

TABLE I—Results of 36 Consecutive First Cadaver Renal Transplants

Case No.	Date of Transplant	Outcome	Plasma Creatinine (mg/100 ml)
1	23/1/70	Rejection at 3 months	
2	23/1/70	Alive and well	1.2
3	26/1/70	Rejection at 3 months	
4	23/2/70	Alive and well	1.2
5	13/3/70	Alive and well	1.5
6	1/4/70	Died of sepsis at 2 months	
7	13/4/70	Rejection at 3 months	
8	13/4/70	Alive and well	1.7
9	11/5/70	Alive and well	1.6
10	12/5/70	Alive and well	1.2
11	19/5/70	Rejection at 1 year	
12	19/5/70	Rejection at 1 year	
13	16/5/70	Died of sepsis at 1½ months	
14	27/6/70	Died of sepsis at 9 months	
15	27/6/70	Alive and well	1.2
16	26/8/70	Alive and well	0.9
17	18/11/70	Rejection at 1 month	
18	28/11/70	Rejection at 2 months	
19	30/1/71	Died of sepsis at 2 months	
20	25/3/71	Infected arterial patch at 2 months	
21	27/3/71	Alive and well	1.5
22	17/6/71	Died of sepsis at 3 months	
23	22/6/71	Alive and well	1.0
24	27/6/71	Alive and well	1.5
25	30/6/71	Died of sepsis at 2 months	
26	8/7/71	Alive and well	1.4
27	26/7/71	Rejection at 3 months	
28	5/8/71	Died of sepsis at 1 month	
29	24/10/71	Rejection at 1 month	
30	24/10/71	Alive and well	0.9
31	24/10/71	Alive and well	2.2
32	28/10/71	Rejection at 1 month	
33	11/12/71	Died of sepsis at 1 month	
34	24/12/71	Alive and well	0.9
35	1/1/72	Alive and well	0.9
36	10/1/72	Rejection at 3 weeks

FIG. 4—Outcome of 36 cadaver renal transplants.

good renal function and only nine clinical rejection episodes. Overall the standard of renal function was better than in a similar group of patients managed on our previous regimen. We believe that this is because by monitoring the activity of the rosette-forming cells we can inhibit them before they have a chance to manifest themselves by some degree of renal impairment. On the other hand, 20 kidneys were removed for either overwhelming rejection or infection. Ten patients in this group died, eight of sepsis as a result of over-immunosuppression—that is, the rosette test suggested that more immuno-

suppression was necessary, but as a result the patient succumbed to infection. It is of note that 11 patients rejected their kidneys despite increases in immunosuppression. In case 36 rejection was after a week-end at home when the patient failed to take his drugs. In case 18 the patient rejected the kidney despite enormous doses of A.L.G. and steroids. In the other nine it was felt that the level of immunosuppression could not be increased despite the rosette test suggesting that it should be. These kidneys failed and were removed. We have not completed our investigations yet, but there seems to be a correlation between the tissue-matching and the amount of immunosuppression used—that is, the greater the number of mis-matches the more immunosuppression was needed. This is not entirely true, because in Case 2 (Fig. 1) the patient had a three mis-match kidney and yet at two years had good renal function on routine doses of azathioprine and prednisone. The immunological and clinical values in case 24 (Fig. 5) were notable in that both clinically and according to the rosette test the patient had a severe rejection episode on the fourth day. Despite enormous doses of prednisone the rosette test

FIG. 5—Case 24. Clinical and immunological values eight months after receiving renal allograft.

suggested that he was still unsuppressed. A.L.G. was subsequently used and we were able to establish a satisfactory level of immunosuppression as judged by the test, although he remained anuric for a further 10 days. The patient survived, and although his renal function is not good it is slowly improving and he has no proteinuria. It is interesting to note that he had a two mis-match kidney, the two antigens being

TABLE II—Use of Radiotherapy in Clinical Rejection Episodes

Year	No. of Transplants	No. of Radiotherapy Treatments
1967	6	11
1968	13	16
1969	20	19
1970	33	26
1971	30	6

on the second sublocus. Oliver *et al.* (1972) suggested that the results for patients with two mis-matches on the second sublocus are very bad indeed.

Further evidence that the test is reducing the number of clinical rejection episodes seen in our unit is contained in Table II. It is the routine in our unit to give radiotherapy to the graft for three days in moderate and severe clinical rejection episodes. Since using the test routinely from about the middle of 1970 we have reduced the amount of radiotherapy used.

USE OF A.L.G.

As stated above, the number of rosettes during the actual administration of A.L.G. is severely reduced, but when this occurs we suggest that the right dose of that particular A.L.G. is being given to the patient. In Case 34 (Fig. 3) it is seen that the number of rosettes per 1,000 lymphocytes in the control sample was small during administration but that the number increased after stopping the treatment. Indeed the patient had a mild rejection episode with an increase in her proteinuria and a slight change in her creatinine clearance on the 14th day. This was corrected subsequently by increasing the steroids. It is of note that during the administration of A.L.G. the steroid level was reduced.

Of greater interest are the clinical and immunological values shown in Fig. 6. This was the case of a second kidney in a girl with

FIG. 6—Clinical and immunological values 18 months after receiving renal allograft.

numerous cytotoxic antibodies. She had a negative cross-match at the time of transplantation. Routine A.L.G. was given and we adjusted the dose on the rosette test until we achieved immunosuppression. We were able to reduce her prednisone dosage during this treatment but had to increase it greatly immediately afterwards. The dosage was then reduced steadily over the next six months, when, unbeknown to the unit, she stopped taking the prednisone altogether. In view of the fact that her renal function had not changed we did not reinstitute the prednisone. She continued on azathioprine 150 mg/day and had good renal function at 18 months. The rosette test suggested that she was still suppressed.

HUMORAL ANTIBODY PRODUCTION

The immunological values of a lung transplant patient from King's College Hospital (Hugh-Jones *et al.*, 1971) are shown in Fig. 2. Four rejection episodes were diagnosed clinically and confirmed by the rosette test. Each episode was treated with a variety of immunosuppressive agents, and although the patient died his lungs at necropsy showed minimal changes suggestive of rejection (Cullum *et al.*, 1972). The interesting thing about the rejection episodes was that a lung-binding antibody was detected 24–48 hours before the rosette test suggested that rejection would occur, and this persisted for two to three days after adequate immunosuppression and clinical improvement was achieved.

Conclusion

Although the immunological basis for the rosette inhibition test is still obscure, it does seem to be a useful test in the clinical management of organ transplants. It allows the clinician to reduce the immunosuppression safely in patients where the immune system appears to be inhibited. On the other hand, it also encourages the clinician to give more immunosuppression than is safe for the survival of a patient. This increase in the level of immunosuppression in some patients seems to reflect the degree of tissue-matching. It should be pointed out that this test does not indicate when any degree of tolerance or enhancement is present, and in some cases we may be over-immunosuppressing patients, especially the long-term ones. As all these patients were on minimum immunosuppression and had good renal function we felt that we would be jeopardizing their graft by reducing the drugs still further with little added benefit to the patient.

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References

- Ada, G. L., and Byrt, P. (1969). *Nature*, 222, 1291.
- Bach, J. F., and Antoine, B. (1968). *Nature*, 217, 658.
- Bach, J. F., and Dardenne, J. F. (1972). *Transplantation Proceedings*. In press.
- Bach, J. F., and Dormont, J. (1971). *Transplantation*, 11, 96.
- Bach, J. F., Dardenne, M., and Fournier, C. (1969). *Nature*, 222, 998.
- Bach, J. F., Muller, J. Y., and Dardenne, M. (1970). *Nature*, 227, 1251.
- Bendixen, G., and Sjøborg, M. (1969). *Danish Medical Bulletin*, 16, 1.
- Biozzi, G. (1972). *Transplantation Proceedings*. In press.
- Biozzi, G., Stiffel, C., and Mouton, D. (1967). In *Immunity, Cancer and Chemotherapy*, ed. E. Mihich, p. 103. New York, Academic Press.
- Brain, P., and Gordon, A. J. (1970). *South African Medical Journal*, 44, 784.
- Brain, P., and Gordon, A. J. (1971). *Journal of Clinical and Experimental Immunology*, 8, 441.
- Coombs, R. R. A., Gurner, B. W., Wilson, A. B., Holm, G., and Lindgren, B. (1970). *International Archives of Allergy and Applied Immunology*, 39, 658.
- Cullum, P. *et al.* (1972). *British Medical Journal*, 2, 71.
- Duffus, W. P. H., and Allan, D. (1971). *Immunology*, 20, 345.
- Greaves, M. F., and Hoog, J. (1970). In *Proceedings of the 3rd Sigrid Juselius Symposium on Cell Interactions in Immune Responses*, ed. O. Makela and A. Cross. New York, Academic Press.
- Gudat, F. G., Harris, T. N., Harris, S., and Hummeler, K. (1970). *Journal of Experimental Medicine*, 132, 448.
- Hugh-Jones, P. *et al.* (1971). *British Medical Journal*, 3, 391.
- Kelly, G. E., Mears, D. G., and Sheil, A. G. R. (1972). *Transplantation*. In press.
- Laskov, R. (1968). *Nature*, 219, 973.
- Lay, W. H., Mendes, N. F., Bianco, C., and Nussenzweig, V. (1971). *Nature*, 230, 531.
- McConnell, I. (1972). *Transplantation Proceedings*. In press.
- McConnell, I., Munro, A., Gurner, B. W., and Coombs, R. R. A. (1969). *International Archives of Allergy and Applied Immunology*, 35, 209.
- Munro, A. *et al.* (1971). *British Medical Journal*, 3, 271.
- Oliver, T., Sachs, J. A., and Festenstein, M. (1972). In preparation.
- Parker, J. R. (1969). Ph.D. thesis, London.
- Parker, J. R., and Mowbray, J. F. (1971). *Transplantation*, 11, 201.
- Posen, G., Harris, J., Page, D., and Stewart, T. (1971). *Proceedings of the European Dialysis and Transplant Association*, 8, 270.
- Reyes, F., and Bach, J. F. (1971). *Cellular Immunology*, 2, 182.
- Smith, M. G. M. *et al.* (1969). *British Medical Journal*, 4, 275.
- Storb, U., Bauer, W., Storb, R., Flidner, T. M., and Weiser, R. S. (1969). *Journal of Immunology*, 102, 1474.
- Tennenbaum, J. I., St. Pierre, R. L., and Cerilli, G. J. (1968). *Transplantation*, 6, 986.
- Wiggall, H., and Makeld, O. (1970). *Journal of Experimental Medicine*, 132, 110.
- Zaalberg, O. B. (1964). *Nature*, 202, 1231.