Preliminary Communications

Changes in Leucocyte Migration after Renal Transplantation

British Medical Journal, 1969, 4, 275-278

Summary: The leucocyte migration test, an in-vitro measure of cellular immunity, has been used to follow the changes in cell-mediated hypersensitivity to kidney and histocompatibility antigens in three patients after renal transplantation.

Inhibition of leucocyte migration, indicating strong sensitization to the antigens used, occurred in each patient, starting five to seven days after transplantation. Satisfactory renal function had not been established in any of the patients at this time. In one case inhibition of leucocyte migration persisted almost continuously until the 24th day and was associated with poor renal function proved histologically to be due to rejection. Treatment with increased dosage of prednisone was associated with a rapid reversion to normal of the migration index and improvement in renal function. Later, inhibition of migration occurred again, and shortly afterwards the graft ceased to function. In the other two cases the migration index became normal without alteration in immunosuppressive therapy and a satisfactory diuresis followed. It is suggested that this simple test should prove useful in the specific diagnosis of rejection and in control of immunosuppressive therapy.

INTRODUCTION

Rejection of a solid graft is probably mediated by sensitized lymphocytes (Russell and Monaco, 1964), and if these could be detected in vitro the diagnosis of rejection might be made with more certainty and treatment instituted more rapidly. The leucocyte migration test has been shown, in man, to be a specific measure of delayed hypersensitivity (Søborg and Bendixen, 1967). In a preliminary report we described the modification of this test for use in the diagnosis of rejection following liver transplantation, and showed that changes in leucocyte migration in the presence of fetal liver homogenate preceded definite changes in conventional liver function tests (Eddleston, *et al.*, 1969). We here describe the technique and use of this test following renal transplantation in three patients. Fetal kidney homogenate and an extract of donor leucocytes were used as antigens.

DETAILS OF TECHNIQUE

Measurements of leucocyte migration were made with a modification of the method of Søborg and Bendixen (1967). Twenty millilitres of peripheral venous blood was taken and added to 200 units of heparin and 2 ml. of 6% dextran (average M.W. 110,000) in a sterile glass bottle. The red cells were allowed to sediment for 45 minutes at 37° C. and the leucocyte-rich supernatant was then transferred to a sterile siliconized centrifuge tube. The leucocytes were separated by centrifugation at 150 *g* for five minutes, and washed in Hanks's balanced salt solution. After further centrifugation at 350 *g* for five minutes the cell button was resuspended in 370 to 750 μ l. of 199 medium. Six to 12 capillary tubes of 1 mm. internal diameter were then filled with the cell suspension and one end was sealed by heating. After centrifugation at 350 *g* for 10 minutes the tubes were cut just below the cell/fluid interface, and the portions containing the leucocytes were mounted

in small tissue-culture chambers. Two capillary tubes were placed in each chamber so that duplicate readings of the migration area could be obtained. A control chamber was filled with 199 medium and 10% horse serum, and test chambers were filled with the same solution to which a measured quantity of antigen had been added. The chambers were then sealed and incubated at 37° C. for 20 hours. The areas of migration were measured by means of a projection microscope and a planimeter. The migration index was calculated by dividing the mean area of leucocyte migration in the test chamber by the mean area of migration in the control chamber. A value less than one indicated inhibition and a value greater than one stimulation of migration.

PREPARATION OF ANTIGENS

The kidney antigen was prepared by homogenizing fetal kidney in an equal volume of 0.9% saline solution. After centrifugation at 1,500 *g* for five minutes the supernatant was transferred in 5-ml. aliquots to sterile containers, which were stored at -20° C. The protein content of the homogenate was measured by Lowry's method and leucocyte migration was measured by use of both high and low concentrations of antigen (400 and 200 μ g./ml.). The donor leucocyte antigen was prepared from heparinized peripheral venous blood. The red cells were sedimented in the presence of dextran, the leucocytes being obtained by centrifugation of the leucocyte-rich supernatant. The leucocytes were resuspended in sufficient 199 medium to produce a concentration of 10^7 cells per ml. The cells were then disrupted by rapid freezing and thawing and the suspension was stored at -20° C. Leucocyte migration was measured at two concentrations of this antigen (0.2 ml. and 0.1 ml. per 1.5 ml. of 199 medium).

PRESENTATION OF RESULTS

Søborg (1968) has shown that, at any one concentration of antigen, stimulation of migration of the leucocytes is indicative of weak sensitization whereas inhibition of migration is associated with strong sensitization to that antigen. Thus with increasing sensitization an initial episode of stimulation is followed by a progressive inhibition of migration (Fig. 1). During the transition from stimulation to inhibition values of the migration index can be obtained which fall within the normal range, though there is increased sensitization to the antigen (position C, Fig. 1). At any one level of sensitization to an antigen, however, inhibition of migration is found when a high antigen concentration is used in the test chamber and stimulation with a lower concentration (Søborg, 1968). Values for the migration index which fall within the normal range but which indicate increased sensitization can therefore be identified by showing stimulation of migration when tested against a

FIG. 1.—Diagrammatic presentation of the changes in migration index observed with increasing sensitization.

lower antigen concentration (position C, Fig. 1). Points on the descending part of the stimulation curve with the same values for the migration index as points on the ascending limb of the curve can also be distinguished by measuring against two antigen concentrations. During the ascending part of the curve, when there is little sensitization, the migration index with a low concentration of antigen is less than that with a high concentration (position A, Fig. 1). On the descending part of the curve, when sensitization is increasing, the migration index with the high concentration of antigen is less than that with the low concentration (position B, Fig. 1).

By measuring the migration index with a low as well as a high concentration of antigen it is possible to place any particular value for the migration index on the curve of increasing sensitization. This has enabled us to produce a more informative presentation of the results, and the scale of values on the ordinate of Figs. 2 and 5 represents increasing sensitization; values for the migration index which represent inhibition being found above those indicating stimulation. Because inhibition is the more important phenomenon the scale during this phase is larger than the one used for the phase of stimulation.

RESULTS

A summary of the operative and preoperative details is given in the Table. Two of the patients were operated on at Addenbrooke's Hospital, Cambridge, and one at Guy's Hospital, London. Daily blood samples were sent to King's College Hospital, and the migration indices were measured without knowledge either of the patient's clinical state or of the results of laboratory determinations of renal function. The immunosuppressive regimen consisted of prednisone, azathioprine, and antilymphocyte globulin. The dosage for these drugs was decided on the clinical state of the patient and on the results of haematological and renal function tests only. Each operation was technically successful, but the postoperative course of one patient differed greatly from the other two.

Clinical and Operative Data Relating to the Three Patients						
Case No.	Age and Sex	Preoperative Diagnosis	Trans-plant	Ischaemia Time (Minutes)		Tissue Match*
				Warm	Total	
1	33 M	Chronic glomerulonephritis	Cadaver	91	451	2 major incompatibilities
2	16 F	Chronic glomerulonephritis	Cadaver	64	306	1 major, 1 minor incompatibility
3	16 M	Chronic glomerulonephritis and pyelonephritis	Cadaver	48	178	1 major incompatibility

* According to Batchelor (1968) and Joysey (1969).

CASE 1

This patient, a 33-year-old bricklayer, had had nocturia with episodes of loin pain and dysuria for 17 years. Renal biopsy in December 1968 showed chronic glomerulonephritis with evidence of superadded infection. He was started on peritoneal dialysis in January 1969 and on maintenance haemodialysis in February. Bilateral nephrectomy was carried out in late February and a cadaver kidney transplant on 24 April.

After the operation he was anuric for 14 days. The first measurement of the migration index was made on the fifth postoperative day, when there was already inhibition of migration (Fig. 2). Throughout the remainder of the anuric phase the migration index was abnormal, with prolonged periods of inhibition. After initial high dosage the immunosuppressive therapy with prednisone and azathioprine remained at a constant level during this time.

DIURETIC PHASE

A diuresis first occurred on the 14th day, and the urine volume slowly increased to 1,340 ml./24 hours; the urinary urea rose to 11.4 g./24 hours by the 22nd day (Fig. 2). For most of this period there was inhibition of leucocyte migration. Although there was a sudden return of the migration index to normal on the 20th day, this was at once followed by a further inhibitory episode. During

this increase in the urine volume and urinary urea the dosage of prednisone had been reduced and that of azathioprine increased.

FIG. 2.—Case 1. Changes in migration index, renal function, and immunosuppression.

After the 22nd day renal function deteriorated, and a biopsy taken on the 26th day showed histological evidence of rejection in that there was diffuse infiltration of small and large mononuclear cells in the interstitium with denser foci perivascularly. There was some glomerular capillary collapse and amorphous eosinophilic material in the tubular lumen (Fig. 3). The prednisone was increased to 200 mg./day; after this the migration index very rapidly became normal. The 24-hour urine volume and urinary urea rose to a maximum on the 33rd and 34th days, but as the prednisone was decreased—despite the addition of intravenous antilymphocyte globulin therapy—the migration index became abnormal again and the urinary urea and urine volume diminished. The histological appearances of a second biopsy, taken on the 40th day, were worse.

FIG. 3.—Case 1. Renal biopsy taken on 26th day after transplantation, showing histological evidence of rejection, with diffuse infiltration of small and large mononuclear cells in the interstitium and denser foci perivascularly. (H. and E. $\times 350$.)

The patient became anuric on the 43rd day, and on the following day the transplanted kidney was removed. Macroscopically the removed kidney was large, with multiple discrete petechial haemorrhages. Microscopically the outer cortical glomeruli were largely necrotic and there were adhesions in Bowman's space—the capillaries were blocked with eosinophilic material, probably platelets, and with fibrin. The tubular cells were atrophic and there was very substantial interstitial mononuclear cell infiltration, with many

pyroninophilic cells. There was intimal thickening of vessel walls with subendothelial mononuclear cell infiltration. Some of the veins were thrombosed (Fig. 4).

FIG. 4.—Case 1. Kidney at transplant nephrectomy, showing substantial interstitial mononuclear cell infiltration and intimal thickening of vessel walls. (H. and E. $\times 88$.)

Within 72 hours of the transplant nephrectomy the migration index became normal. Unfortunately the patient died of pulmonary oedema secondary to left ventricular failure one week later.

CASE 2

This patient was a 16-year-old girl who had developed a nephrotic syndrome in August 1967 shortly after leaving school. A renal biopsy in December 1968 showed evidence of chronic glomerulonephritis. She was started on peritoneal dialysis in January 1969 and on maintenance haemodialysis one month later. Bilateral nephrectomy was carried out in February and she received a cadaver kidney transplant on 3 May.

FIG. 5.—Case 2. Changes in migration index, renal function, and immunosuppression.

She was anuric for the first 11 postoperative days. The migration index was determined in the presence of donor leucocyte extract as well as homogenized fetal kidney, and overall the changes in both indices were very similar (Fig. 5). The first abnormality occurred on the sixth postoperative day, and after a period of inhibition of migration both indices returned to normal by the 13th day, though there was no significant change in treatment at this time.

DIURETIC PHASE

A diuresis first occurred on the 11th day, but the 24-hour urine volume did not exceed 2 litres until the 14th day—just after the migration indices returned to normal. Throughout the rest of the study the urine volume remained between 2 and 4 litres/24 hours.

A second episode of inhibition began on the 16th day and continued until the 26th day. Though the 24-hour urine volume did not decrease there was a significant fall in the urinary urea shortly after this time—from a maximum of 27 g. down to 12 g./24 hours on the 35th day. Apart from one further abnormal reading on the 34th day, the migration index became normal and the urinary urea rose again. Throughout the diuretic phase the dosage of prednisone was slowly decreased to 40 mg./24 hours and that of azathioprine slowly increased to 125 mg./24 hours.

Three months after transplantation the patient was at home and was very well.

CASE 3

This patient was a 16-year-old boy. He developed nocturia in 1958, and in January 1968 a renal biopsy showed proliferative glomerulonephritis with evidence of chronic pyelonephritis. He was started on peritoneal dialysis in March 1968 and later in the year underwent bilateral nephrectomy. Maintenance haemodialysis was begun in March 1969 and a cadaver kidney was transplanted on 2 May. Initially, as in the previous patients, daily blood samples were tested, but after he left hospital on the 14th day the migration indices were measured only twice a week. Both homogenized fetal kidney and an extract of donor leucocytes were used as antigens, and during the time that daily blood samples were being examined it was apparent that changes in the migration index with use of the donor leucocyte extract occurred before changes in that when using the fetal kidney homogenate. After this, when the indices were measured less frequently, the values appeared to correspond.

The patient remained oliguric for 11 days postoperatively (Fig. 6). During this period there was inhibition of migration from the 6th until the 10th day with donor leucocyte antigen and from the 8th to the 11th day with fetal kidney antigen. Azathioprine dosage was maintained at 150 mg. daily after the first day, but the prednisone was gradually reduced over this phase.

FIG. 6.—Case 3. Changes in migration index, renal function, and immunosuppression.

DIURETIC PHASE

The 24-hour urine volume exceeded 500 ml. for the first time on the 12th day but a substantial output did not occur until the 18th day. The urinary urea rose in parallel with the urine volume. A second peak of inhibition of migration occurred on the 14th day, but the migration index had returned almost to normal by the 18th day. On the 22nd day another inhibitory reading was obtained,

but by the 26th day the index was practically normal again. The width of the second and third peaks of inhibition shown in Fig. 6 may be exaggerated because the index was measured only twice a week during this period. These inhibitory episodes were not accompanied by a significant fall in 24-hour urine volume or urinary urea.

The final reading on the 50th day was normal. This patient returned to work as a trainee accountant and was well at the time of writing.

DISCUSSION

The diagnosis of rejection after transplantation of the kidney, with the use of conventional tests of renal function, can be difficult. Alterations in the tests may be due to a variety of causes, including renal artery stenosis, renal infarction or infection, and ureteric obstruction. They are therefore not specific to rejection. Furthermore, a kidney may be extensively damaged by rejection before a significant alteration in renal function can be detected. Indeed, biopsy evidence of active rejection has been reported with normal renal function tests (Ogden *et al.*, 1967). By the time rejection episodes cause abnormalities in such tests, histological evidence is always found on biopsy (Kincaid-Smith, 1967). Recently, a retrospective analysis of eight simple tests of renal function in relation to rejection episodes has shown that the earliest and most reliable changes occurred in the 24-hour urinary urea and urinary osmolality, though alterations in the 24-hour urine volume and the peripheral blood platelet count were also significant (Chisholm *et al.*, 1969). Serial determinations of 24-hour urinary urea and urine volume were readily available in the present cases and were therefore used for the correlation with the migration index.

Most experimental immunological work suggests that the initial rejection of a renal homograft is primarily cell-mediated. The first set rejection of canine renal homografts is associated with a dense infiltration of lymphocytes and plasma cells. Subsequently there is capillary endothelial disruption with oedema and exudation of plasma, leading to a fall in renal blood flow and tubular necrosis (Kountz *et al.*, 1963). Similarly, acute first set rejection of human renal grafts shows a dense cellular infiltrate (Porter *et al.*, 1965). But human grafts rejected after some months usually show damage to arteries and arterioles, often with little or no cellular infiltrate. This has led some workers to suggest that circulating antibodies are important in chronic rejection (Porter, 1965). Serum levels of antibody, however, do not correlate with rejection episodes, and it is possible that these changes are also primarily cell-mediated.

Extensive experimental work by Søborg (1968) has shown that the leucocyte migration test detects specific changes in cellular immunity rather than in circulating antibodies. In the present study the changes in leucocyte migration in the presence of fetal kidney homogenate may have indicated the development of sensitivity either to organ-specific kidney antigens or to histocompatibility antigens, which are present in the kidney as in other tissues. Rejection is initiated by histocompatibility antigens present in the graft but not in the host. In one patient the similarity between the changes in leucocyte migration with the use of donor leucocytes and pooled fetal kidney homogenate might suggest that the response to the kidney antigen was dependent on its content of histocompatibility antigens. In the other patient, however, the changes in the two migration indices did not occur at the same time, at least during the initial inhibitory episode, suggesting that an immune response to organ-specific antigens had also occurred. Experimental studies are in progress to clarify this point.

Serial renal biopsies in a number of patients would be necessary to show conclusively that changes in the migration index reflect the activity of the rejection process. In Case 1, however, renal tissue was obtained on three occasions when there was inhibition of leucocyte migration. Both of the renal biopsies and the sections of the graft after removal showed the histological changes of progressive rejection. In Case 1 changes in

the urine volume and the urinary urea occurred at much the same time as the alterations in migration index, but in Case 2 the second episode of inhibition preceded the fall in urinary urea by several days. This also occurred in Case 3, though the fall in urinary urea was not so pronounced. The explanation for this difference may be that the graft in Case 1 had been extensively damaged in the anuric phase, so that subsequent small changes in renal blood flow due to rejection were immediately reflected in a fall in urinary urea and volume.

In each patient significant abnormality of the migration index developed towards the end of the first week, suggesting that rejection may be contributing to the early anuria. Similar changes at this time have been observed in our patients after hepatic transplantation, in whom they may precede or accompany a cholestatic jaundice, considered histologically to be due to rejection (Eddleston *et al.*, 1969; Williams *et al.*, 1969).

The effect of immunosuppressive therapy on the migration index is uncertain, for in Cases 2 and 3 the index returned to normal without alteration in the drug therapy—in Case 1, however, a large increase in the dosage of prednisone was followed by a return of the index to normal, with concomitant improvement in renal function. Moreover, as the prednisone was decreased, despite the administration of antilymphocyte globulin, the index became abnormal again and renal function deteriorated.

It remains to be seen whether or not this simple test will help in the diagnosis of chronic rejection. If this process is even partly cell-mediated there is every reason to suppose that it will. Whether more exact titration of immunosuppressive therapy, guided by alterations in the migration index, will improve renal function in the early stages and later on prevent insidious organ destruction remains to be determined.

We thank Dr. Basil Herbertson for the renal histology. Mrs. Angela Birbeck prepared the drawings, and the Wellcome Trust Foundation gave generous support. One of us (J. A. D.) was supported by the University of Valencia.

M. G. M. SMITH, M.A., M.B., M.R.C.P.,

A. L. W. F. EDDLESTON, M.A., B.M., M.R.C.P.,
Research Fellows.

J. A. DOMINGUEZ, M.D.,

Visiting Research Fellow, Liver Unit, King's College
Hospital, London S.E.5.

D. B. EVANS, M.B., M.R.C.P.,

Consultant Physician, Addenbrooke's Hospital, Cam-
bridge.

M. BEWICK, F.R.C.S.,

Lecturer in Surgery, Guy's Hospital Medical School,
London S.E.1.

ROGER WILLIAMS, M.D., F.R.C.P.,

Consultant Physician and Director, Medical Research
Council Group on Metabolism and Haemo-
dynamics of Liver Disease, King's College Hospi-
tal, London S.E.5.

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