Studies of Cellular Immune Responses in Patients with Minimal Change Nephropathy

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Summary

Patients with a 'minimal change' nephropathy (MCN) were tested for cell-mediated immunity to renal antigens. By a lymphocytotoxicity test it was shown that lymphocytes from patients with MCN showed greater toxicity to renal cells than those from normal subjects. Using the leukocyte migration test (LMT) 21/29 patients reacted to a homogenate of human neonatal kidney but only 5/9 patients reacted to a glomerular basement membrane antigen. A serum factor which blocked LMT was present in all patients. In sequential studies it was observed that during a relapse steroid, and sometimes cyclophosphamide, therapy temporarily abolished the reaction to renal antigens.

Introduction

There have been several previous reports of cell-mediated immunity to kidney-derived antigens in patients with glomerulonephritis (Benedixen, 1968, Rocklin et al, 1970; Macanovic et al, 1972; Mahieu et al, 1972; Mallick et al, 1972). However the only report of such reactions in patients suffering from a steroid-responsive 'minimal change' nephrotic syndrome was that of Mallick et al (1972), although the possibility that T-lymphocytes are involved in the pathogenesis of this disease has been discussed by Shalhoub (1974), and Lagrue et al (1975) have described a lymphokine causing increased vascular permeability produced by lymphocytes during relapse of this condition.

Although the nephrotic syndrome associated with minimal light microscopic change is usually responsive to steroids, with or without cyclophosphamide, evidence for the participation of immunological mechanisms in the disease
process is sparse. Deposition of immunoglobulin or complement is not constantly found in the glomeruli as judged by immunofluorescent techniques. Changes in the complement system and immunoglobulin which have been related to relapse are also not constantly found (Ngu et al, 1970).

The aim of the present work was to study the reaction of these patients to kidney-derived antigens by two tests which are generally accepted as in vitro correlates of delayed hypersensitivity reactions; the leukocyte migration inhibition assay and the direct lymphocyte-mediated cytotoxicity test.

METHODS

The patients studied suffered from a relapsing nephrotic syndrome responsive to steroids with or without cyclophosphamide. There was no biopsy evidence of glomerular damage by light microscopy or deposition of immunoglobulin or complement in the glomeruli. A total of 29 patients were tested on at least two separate occasions using the leukocyte migration test and 12 of these were also tested in the direct lymphocyte-mediated cytotoxicity test.

The leukocyte migration test

The leukocyte migration test was performed as described by Mallick et al (1972). Peripheral blood leukocytes were washed several times and allowed to migrate out of capillary tubes into tissue culture medium which did or did not contain antigen. The area of migration was measured and the results were expressed as a migration index, which is the ratio of the area of migration with antigen to the area without antigen. If there is an antigen to which the leukocytes have been sensitised in the medium the migration is inhibited.

Two antigen preparations were used in the migration assay. One was the supernatant from a homogenate of human neonatal kidneys. The other antigen was a soluble glomerular basement membrane preparation formed by collagenase treatment of basement membranes from glomeruli of an adult human kidney.

Direct lymphocyte-mediated cytotoxicity

Kidney epithelial cells were obtained by trypsinisation of human neonatal kidneys and placed in the wells of a Microtest II tissue culture plate. Peripheral blood lymphocytes were prepared by the method of Coulson and Chalmers (1967) and added to the kidney target cells in different ratios of lymphocytes to target cells. After three days incubation the number of cells in the wells to which lymphocytes had been added was compared with that of the control wells with no lymphocytes.
RESULTS

Leukocyte migration test

Using the leukocyte migration test we have previously made an extensive study of normal subjects. This has established normal values of migration indices for these antigens of 0.95 (SD ± 0.075). We have taken the normal range to be two standard deviations either side of the mean. Therefore inhibition of migration producing a migration index of less than 0.8 is considered to be abnormal.

Of the 29 patients with a ‘minimal change’ nephropathy 21 showed inhibition of migration in the presence of the kidney homogenate. All of these patients were in clinical remission at the time of testing.

TABLE I. Number of Patients with a Reaction to Each of the Two Antigen Preparations

<table>
<thead>
<tr>
<th>Kidney homogenate</th>
<th>Glomerular basement membrane</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Ten patients were tested with both the kidney homogenate and the glomerular basement membrane preparation. Table I shows the reaction of these patients to the different antigens. The variation in the reaction suggests that patients do not necessarily react to only basement membrane antigens, and that different antigens are involved in the reaction of different patients.

Lymphocyte-mediated cytotoxicity

Twelve patients with ‘minimal change’ renal disease and 10 normal control subjects were tested. The minimum lymphocyte to target cell ratio which gave a significant

TABLE II. Distribution of Minimum Ratio of Lymphocytes to Renal Epithelial Cells Required to Induce a Significant (p < 0.01) Kill in Patients and Normal Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Minimum ratio for significant kill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100:1</td>
</tr>
<tr>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td>‘Minimal change’ nephropathy</td>
<td>1</td>
</tr>
</tbody>
</table>
target cell kill ($p < 0.01$) was calculated for each subject. Table II shows these results. When the two groups are compared using a $X^2$ test there is a greater cytotoxicity against kidney epithelial cells of lymphocytes from the group of patients with 'minimal change' renal disease than from normal subjects.

In order to test the specificity of lymphocytotoxicity, lymphocytes from 7 of the patients with 'minimal change' disease were compared with those from a group of 10 normal subjects for their capacity to kill a bladder cancer cell population. Significant differences were not found, suggesting that the renal epithelial cell killing by the 'minimal change' patients was specific.

**Alteration of leukocyte migration by autologous serum**

The leukocyte migration test was normally performed in a medium containing 20% foetal calf serum. At each time of testing it was also performed in 20% heat-inactivated autologous serum taken on the day of testing. In all cases when inhibition of migration was observed in calf serum it was reduced or abolished by autologous serum. This blocking of the inhibition was specific as the serum

![Graph](image)

**Figure 1.** Migration Indices obtained in the presence of calf serum, autologous serum and fractions of autologous serum (AS).
did not affect the leukocyte migration inhibition produced by an unrelated antigen, tuberculin PPD, in tuberculin-positive individuals.

A sample of serum which blocked the migration inhibition was fractionated on a Sephadex G-200 column and each fraction was tested for blocking activity in the leukocyte migration test. Figure 1 shows the migration indices obtained using three concentrations of kidney homogenate in the presence of calf serum, autologous serum and each of the major fractions of this serum. Fraction I is the 19S fraction, Fraction II which contains most of the blocking activity is the 7S fraction and Fraction III is the 4.5S fraction.

Leukocyte migration inhibition in relapse and remission

The leukocyte migration test was performed regularly on each patient at four-monthly intervals for up to two years while patients were in remission. During this time seven relapses were monitored. All were in patients who had previously reacted to the kidney homogenate. Figure 2 shows the migration indices obtained during one such relapse. As in all other patients the migration inhibition was

![Diagram](image)

*Figure 2. Patient F.F. showing migration indices and steroid therapy before, during and after a relapse.*
abolished during steroid therapy. However the reaction to kidney antigens returned as the steroid dose was reduced.

Cyclophosphamide therapy which was given in all seven patients showed no effect on the migration inhibition in three of them but abolished it in the other four. In three of these four the inhibition of migration returned after cyclophosphamide had been withdrawn. The results for the remaining patient are shown in Figure 3. This patient had suffered repeated relapses of his nephrotic syndrome over a period of 14 years. Whenever steroids were reduced below a critical level of about 10 mg/day proteinuria returned. However, after a prolonged course of cyclophosphamide the leukocyte migration test became negative and has remained so, and no further periods of relapse have occurred since the withdrawal of cyclophosphamide therapy.

**DISCUSSION**

The present study indicates that cell-mediated immunity to kidney-derived antigens is present in the majority of patients with a relapsing steroid-responsive ‘minimal change’ nephrotic syndrome and is detected by both leukocyte migration inhibition to renal antigens and by lymphocytotoxicity to a renal cell culture. The homogenate of perinatal kidneys used in the leukocyte migration test provided a variety of renal antigens, some of which may have been foetal antigens.
The glomerular basement membrane preparation was also probably composed of several antigens, all represented in the glomerular basement membrane. The differing response of patients to these antigen preparations indicates that patients can become sensitised to more than one antigen. The high percentage of patients with a reaction to kidney homogenate, which is predominantly of tubular origin, suggests that there may be a foetal antigenic determinant, represented strongly but not exclusively in the tubules, to which these patients respond.

The pathogenesis of 'minimal change' renal disease is unknown. The finding of cell-mediated immunity to renal antigens, and of a serum factor which appears to block specifically such cell-mediated immunity, as measured by the leukocyte migration test, may be of relevance. We have been unable to show quantitative changes in leukocyte migration inhibition or in the level of blocking activity correlating with relapse or remission, and the sporadic nature of relapse makes it impossible to obtain repeated tests in the period immediately preceding the onset of proteinuria. Further, present tests of cell-mediated immunity are not easy to quantitate. Nevertheless, the increasing evidence that T-lymphocytes may be involved in 'minimal change' nephropathy is of interest in view of the response of the condition to steroids and cyclophosphamide therapy.

Acknowledgment

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References

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Open Discussion

PIERIDES (Newcastle upon Tyne) May I ask whether these changes are found only in the minimal change nephrotic syndrome, or do you find them in the nephrotic syndrome from other causes of glomerulonephritis?
EYRES Yes, we have in fact tested a total of 60 other patients with assorted glomerulonephritis and quite a large percentage also react. There is a difference between different histological groups; we find a majority of people with mesangiocapillary disease who react, a relatively small percentage, about 20%, of patients with membranous glomerulonephritis react and the proliferatives are in between. About half of them will react to these antigens.

PIERIDES May I ask, in that case, how much importance you would place on your findings, then? Is it a mysterious factor you have been looking for, or just a non-specific effect of the nephrotic syndrome?

EYRES Obviously our results do not present any evidence that it is the causative effect of minimal change glomerulonephritis, although the response to steroids and the apparent lack of evidence of other immunologically connected events in this particular disease point to it being of some importance. I think in the other patients it might probably be almost a completely separate thing.

KERR (Newcastle) I am delighted at your findings of immunological abnormalities in the one renal disease that responds to immunosuppressive drugs. Last year you probably remember Robson and his colleagues in the New England Journal of Medicine described persistently elevated IgM levels between relapses. Have you looked for this and have you found it in these patients?

EYRES No, we have not really looked for it specifically, so I cannot really say whether they have got it. He is suggesting that it is also a T cell mediated effect; obviously of a completely different nature from ours. I am afraid I cannot say anything about our patients in that respect.