IMMUNODEFICIENCY IN CHRONIC RENAL INSUFFICIENCY

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with the collaboration of

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Introduction

The existence of immunodeficiency during chronic renal insufficiency was demonstrated in the fifties [1] and the general availability of treatment by repeated haemodialysis and renal transplantation has made the study of this deficiency even more important. Patients survive for very prolonged periods with either weak or no renal function and the factors responsible for this immunodeficiency must be determined. This immune deficiency is fairly deep rooted in most patients, as shown by the prolonged survival of skin allografts and the considerable decrease in retarded hypersensitivity skin reactions. It is also responsible for an acquired susceptibility to infections [2], particularly bacterial germ infections with contingent intracellular parasitism. Furthermore, the evolution of these infections is modified, as demonstrated by the analysis of hepatitis B cases in haemodialysis units [3].

Phagocytosis and bactericidia

By in vitro studies, it has been shown that phagocytic competence was slightly decreased during chronic renal insufficiency, both in polymuclear cells [4] and monocytes [5]: the capacity to reduce tetrazolium nitro-blue is not, in general, modified [6]. The migration of phagocytic cells in the in vivo ‘skin window’ test is only slightly altered, but phagocytosis of Indian ink particles by these cells is weaker in normal subjects [7]. In total, phagocytic activity is decreased to a certain extent in chronic renal insufficiency patients, but this anomaly does not seem to be the principal component of the immunodeficiency, even though it contributes to the aggravation of staphylococcal, Gram positive germ and yeast infections.
Humoral immunity

The synthesis and secretion of antibodies only seems to be moderately affected by chronic renal insufficiency [8–12]. Moreover considerable variations in the capacity to produce antibodies are found in these patients, according to the chemical nature and mode of presentation of the antigen. Antigens, whose nature and presentation determine a thymo-dependent antibody response, are theoretically those which give rise to the production of antibodies most weakened by renal insufficiency. The serum concentrations of immunoglobulins are similar to normal, as is the percentage of B lymphocytes with surface immunoglobulins in the peripheral blood (Table I); but, due to lymphopenia, the absolute number of these cells is decreased. The medullary plasmocytes are normal or slightly decreased in number. The appearance of antibodies after bouts of infection or after vaccinations could be seen in the majority of cases, sometimes with a certain delay or with rather weak titres. Anti-HLA antibodies could be detected in the serum of certain patients after blood transfusions and preliminary results suggest an immunisation frequency slightly weaker than that of normal subjects.

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>CRI (%)</th>
<th>Normals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTLA*</td>
<td>61.3 ± 11.2</td>
<td>66.4 ± 8.1</td>
</tr>
<tr>
<td>E-RFC</td>
<td>58.1 ± 10.8</td>
<td>63.5 ± 9.1</td>
</tr>
<tr>
<td>Slg</td>
<td>20.9 ± 2.9</td>
<td>19.4 ± 3.2</td>
</tr>
</tbody>
</table>

*Percentage of blood lymphocytes with the HTLA phenotype (detected by specific antiserum), the capacity to form E rosettes (E-RFC) with sheep red cells or carriers of surface immunoglobulins (slg). The techniques used have previously been described [25]

Cell mediated immunity

The principal anomalies concern specific cell-mediated immunity.

Lymphocyte populations

Considerable lymphopenia exists in chronic renal insufficiency and, in a previous study [13], we found an average number of small lymphocytes of 996 ± 606 per mm³ of peripheral blood in 30 haemodialysed patients, whereas this number was 2107 ± 643 in normal subjects from the control group. The difference was very significant (p < 0.001). The percentages of T lymphocytes obtained using either a specific antiserum or by the study of E rosette formation were not significantly different from those found in normal subjects (Table I). Taking into account the lymphopenia, the average number of T lymphocytes per mm³ was, however, significantly lower than in normal patients: 611 instead of 1405.
Finally, the study of T lymphocyte sub-populations showed a greater decrease in cells with receptors for the Fc fragment of IgM than those of IgG [14].

**Lymphocyte cultures**

The proliferative response of cultivated total blood lymphocytes stimulated by phytohaemagglutinin is decreased [15].

In contrast, the lymphocytes of these patients, isolated by centrifugation on Ficoll-Hypaque gradient and cultivated in AB serum from normal subjects, have normal proliferative responses [13,16]. Whether this is a stimulation by phyto-mitogens or by allogenic cells, this lymphocytic proliferation determined by tritiated thymidine incorporation is comparable to that obtained with lymphocytes from normal subjects. The proliferative response to antigens (tuberculin, candidin, etc) can be of an amplitude similar to that observed in normal patients, but the frequency of clearly positive responses is lower than that found in the control group. These responses to mitogens, antigens or allogenic cells are decreased when the lymphocytes are no longer cultured in the presence of normal serum, but in the presence of serum from a uraemic patient, whether or not this patient is also the donor of the lymphocytes. Furthermore, the serum from renal insufficiency patients can also inhibit the proliferation of normal lymphocytes stimulated by various agents [16]. This inhibitory activity is found in the serum of the majority of patients, does not disappear completely after haemodialysis and often persists even after the patient’s serum is diluted in normal serum. This is therefore not essentially a deficiency in a substance necessary for cellular growth and proliferation, but an intervention of inhibitory or toxic factors capable of having an effect even after dilution.

**Inhibitory plasmatic factors**

The plasma of renal insufficiency patients contains substances which inhibit lymphocytic proliferation in vitro, which leads to the belief that these plasmatic factors constitute the essential causal element of the noted immunodeficiency. For this reason it was therefore important to identify these factors.

After chromatography of serum from renal insufficiency patients on Sephadex G 25, the inhibitory activity of the response to phytohaemagglutinin or to an allogenic stimulation was found in two principal zones: in the peak of the small molecules (500 daltons) and in the peak of the so-called ‘middle molecules’ (around 1200 daltons). Among the small molecules, urea and creatinine did not reproduce in vitro the inhibitory effect of uraemic serum. Methylguanidine alone, on the other hand, can exercise an in vitro inhibitory activity at concentrations close to those found in the serum of chronic renal insufficiency patients [13,16]. The injection of sublethal doses of methylguanidine into mice did not, however, produce any great prolongation of allograft survival in a strongly incompatible couple. The phenols have an important inhibitory activity in vitro at relatively high concentrations only.

The ‘middle molecules’ are present in much larger quantities in the serum and urine from renal insufficiency patients than in those from normal subjects [17],

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as shown by the comparison of chromatograms. These are peptides which can be
dissociated and whose activity disappears under the effect of proteolytic enzymes.
However, their origin is still uncertain. Do they arise from disturbances in the
metabolism of proteins, which is observed in renal insufficiency as well as in
various states of protein malnutrition? Regardless, these 'middle molecules' have
a very considerable inhibitory influence on cellular proliferation, as studied in
various models. In particular, the response to phytohaemagglutinin, concanavalin
A or pokeweed mitogen, and, to an even greater extent, the allogenic response,
are almost suppressed by even small concentrations of middle molecules [17].
Cellular viability and the capacity to form E rosettes are not, however, affected
by incubation with middle molecules. Other cells, such as platelets, can have some
of their functions modified by these peptides. In vivo, middle molecules also
have a clear inhibitory effect which demonstrates their certain, not negligible role
in immunodeficiency associated with renal insufficiency. In effect, continuous
infusion of middle molecules into rats delays the rejection of a strongly incompati-
ble skin allograft (Table II [18]). The graft versus host reaction was also
weakened when injected allogenic lymphocytes were incubated for 5 hours with
middle molecules (Table III [18]).

**TABLE II. Effect of 'middle molecules' on allograft survival in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of days before skin graft rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Middle molecules'</td>
<td>17.4 ± 1.0</td>
</tr>
<tr>
<td>Saline serum (control group)</td>
<td>12.1 ± 0.3</td>
</tr>
</tbody>
</table>

The difference between the two groups is statistically significant (p < 0.001)

**TABLE III. Effect of 'middle molecules' on the graft versus host reaction in mice**

<table>
<thead>
<tr>
<th>Treatment of lymphocytes</th>
<th>Graft versus host reaction (splenic index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Middle molecules'</td>
<td>1.19</td>
</tr>
<tr>
<td>Saline serum (control group)</td>
<td>1.65</td>
</tr>
</tbody>
</table>

The difference between the two groups is statistically significant (p < 0.005)

Apart from 'middle molecules', which appear to be the element with the pre-
dominant inhibitory activity in serum from chronic renal insufficiency patients,
several other factors can aggravate cell-mediated immune deficiency. In certain
patients, a small inhibitory activity in vitro is found in serum fractions containing
small, non-dialysable proteins, some of which are normally catabolised by kidney
tubular cells. Numerous metabolic modifications induced by renal insufficiency
can also play a part in vitamin B6 deficiency, lowered zinc blood level and disturbances of lipid metabolism. Finally, haemodialysis patients sometimes receive blood transfusions, the effect of which has now been demonstrated on certain immune responses, although the mechanism is still uncertain.

Other in vitro tests

The production of lymphokines, the inhibiting factor of macrophage migration, by lymphocytes from renal insufficiency patients in the presence of antigens seems to be reduced [8].

The intensity of the cytotoxicity exercised by certain T lymphocytes or certain non-T lymphocytes with receptors for the Fc fragment of IgG is significantly weaker in the renal insufficiency patient than in the normal subject [14].

Delayed hypersensitivity

In a previous study [13,19] we confirmed the observations of Kirkpatrick [24], who noted a much smaller percentage of positive delayed hypersensitivity skin reactions in renal insufficiency patients than in normal subjects. By studying delayed reactions to tuberculin, candidin, streptokinase and toxoplasmin, we have been able to show that erythema and induration were weaker in intensity and less frequent for each of these antigens in chronic renal insufficiency patients. At the same time, the percentage of patients with negative reactions to tuberculin and candidin was very high in long-standing renal insufficiency, whereas it was much lower in the initial phase of the disease (Table IV). In most patients these modifications are only partially improved by conventional haemodialysis. In an ongoing study we are searching for a connection between the decrease in delayed hypersensitivity reactions and various parameters. Patients were considered as being anergic when no positive reaction was observed to any of the 7 antigens, each of which produced a clear induration in a high percentage of the normal subjects tested.

<table>
<thead>
<tr>
<th>Groups of subjects</th>
<th>% of subjects with negative reactions to tuberculin and candidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal insufficiency (advanced phase)</td>
<td>58*</td>
</tr>
<tr>
<td>Renal insufficiency (initial phase)</td>
<td>17**</td>
</tr>
<tr>
<td>Normal control subjects</td>
<td>4***</td>
</tr>
</tbody>
</table>

The difference between * and *** is statistically significant (p < 0.001)
The difference between ** and *** is not significant

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Allografts

The prolonged survival of skin allografts was observed by several authors in man or in animals during renal insufficiency [1,20,21]. Rejection is retarded and less acutely developed than in controls. Also, rejections after other organ transplants — kidney, heart — are later in uraemic animals than in normal animals [22,23].

Histology of lymphoid organs

Post-mortem histological examination of the thymus from chronic renal insufficiency patients showed atrophy, various types of cysts and diverse lesions, which varied from patient to patient [24]. No study on thymic biopsies taken from living patients has been reported and it is therefore very difficult to determine which lesions are due to renal insufficiency or to modifications of the thymus in the agonal phase. The activity of thymic epithelial cells could be determined, at least in part, by the level of circulating thymic factors, but recent studies carried out on renal insufficiency have not given concordant results. The spleen often appears to be modified, but the lesions are rather badly recorded; furthermore, a slight decrease in lymphocytic density can be noted in the peri-arteriolar sheath. In the lymph nodes there are predominant lesions, sometimes in the zones favoured by T lymphocytes (deep cortex), but more often in the zones populated by B lymphocytes and plasmocytes (germinative centres, medullary cords) [13].

Conclusion

In summary, chronic renal insufficiency affects most cells, by intervening in the various modes of immune response. This is due principally to the presence in serum and the whole organism of an increased level of several toxic or inhibitory substances, which are retained by insufficient elimination, or defective catabolism in the tubular cells, or produced in excessive quantities due to the metabolic anomalies of renal insufficiency. The same substances are perhaps toxic for other cells — platelets, nerve cells, etc. Although different lymphocytic populations are affected, the decrease in cell-mediated immunity appears to be more important than the disturbances in antibody production. These modifications are important and it is essential to understand with precision their mechanism in the patient in order to prevent intracellular germ infections, to follow the evolution of hepatitis in haemodialysed patients and to determine the immunological balance before renal transplantation.

References

1 Dammin GJ, Couch NP, Murray JE. Ann N Y Acad Sci 1957; 64: 967
2 Montgomery JG, Kalmanson GM, Guze LB. Medicine 1968; 47: 1
4 Brogan TD. Br med J 1967; 3: 596
Open Discussion

BONER (Petah Tikva) We have also been looking at lymphocytes and estimating the T lymphocytes by rosette formation and the activity of the T lymphocytes by graft versus host reaction in the immuno-suppressed rat. There were two interesting findings. First the numbers of T lymphocytes, both relative and absolute, were much lower in patients who had been on dialysis for longer periods of time. Similarly the activity of the T lymphocytes was more affected. Secondly, the day after haemodialysis there was a slight improvement in T cell function and in the number of T cells. These reverted to the former level on the following day. Did you find any difference in the patients who had been on treatment for longer periods of time?

TRAEGER There was no difference in T cell numbers in chronic haemodialysed patients in the long term.

DRUEKE (Paris) Parathyroid hormone has recently been incriminated as interfering with the immune status of haemodialysis patients. Have you personal data on possible interference of parathyroid hormone with lymphocyte function?

TRAEGER We have not studied this.

BRIGGS (Glasgow) I am very interested in your point about the patients on peritoneal dialysis having normal reactivity compared with haemodialysis patients. Have you any evidence that patients who have been on peritoneal dialysis have a poorer transplant survival rate? One other comment I was going to make: we
have been looking at the cell-mediated immune response using a **DNCB skin test** and we have demonstrated, and this was presented two years ago to this association, that although haemodialysis patients as a group have a depressed CMI, as you have pointed out with your studies, there is a spectrum. Some patients have very depressed CMI and have a good transplant survival, whereas in other patients it is either mildly depressed or not depressed at all and these patients have very much higher graft failure rates.