ABNORMALITIES OF IMMUNE REGULATION IN PATIENTS WITH IgA MESANGIAL GLOMERULONEPHRITIS (BERGER'S DISEASE)

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Summary

Regulation of the immune response was studied in 22 patients with IgA nephropathy. A significant increase in the IgA production by Pokeweed-stimulated peripheral mononuclear cells maintained in culture for seven days was observed. These patients had significantly less IgA suppressor cell activity, as assessed by the Concanavalin A-generated suppressor cell assay, than the normal controls. The fact that most of the patients studied had increased activity of helper T cells on IgA synthesis, together with an augmentation in the percentage of OKT4+ cells, suggest that the abnormalities in helper T cell function might be the primary defect in this nephropathy. The existence of similar alterations in some of the healthy relatives of the patients further supports a genetic basis for susceptibility to this disease.

Introduction

IgA nephropathy is characterised by the presence of IgA, and sometimes C3 and/or other immunoglobulins, in the mesangium. This, together with high concentrations of serum IgA frequently observed in these patients, has focused attention on the role of IgA [1]. We recently published evidence on the existence of high serum concentrations of true polymeric IgA in a large majority of patients [2], as well as its presence in the mesangium [3], a finding recently confirmed by Bene et al [4]. However, hitherto few studies have been published on the immune regulation of IgA in such patients. The existence of an increased number of IgA-bearing lymphocytes had been reported [5], but this has not been confirmed using anti-IgA F(ab')2 fragments [6]. Furthermore the activity of IgA-specific suppressor T cells was lower in eight patients with IgA nephropathy than in patients with other nephropathies and control subjects [7].

In order to investigate the regulation of the immune response in IgA nephropathy we studied T-lymphocyte subsets using monoclonal antibodies and the
suppressor and helper activity of peripheral blood mononuclear cells (PBM) in vitro.

Material and methods

We have studied 22 patients with biopsy proven IgA nephropathy, and 17 healthy first-degree relatives of five of the patients. None had symptoms or signs of systemic disease. Medical students and staff members matched for age and sex were used as controls. Neither patients nor controls had ingested any drug in the 48 hours before examination.

PBM suspensions were obtained from fresh heparinised blood of patients and controls by Hypaque-Ficoll gradient centrifugation (Pharmacia Fine Chemicals, Uppsala, Sweden) following the method of Böyum [8]. The interphase cells were collected and washed three times with Hank’s solution and once with RPMI-1640 medium. The last wash was found to be free of immunoglobulins as measured by radioimmunoassay [2]. The cells at a concentration of 2 x 10^6 cells/ml in RPMI-1640 medium were incubated in the presence or absence of 10μl/ml of Pokeweed mitogen (PWM) at 37°C under conditions previously described [9]. After seven days of culture the amount of IgA, IgG and IgM in the supernatant was measured.

T-cell subsets were identified in the mononuclear cell population, obtained as described above, by using specific monoclonal antibodies, termed OKT3+, OKT4+, OKT8+ (Ortho Pharmaceuticals, Raritan NJ, USA), produced by mouse hybridomas [10]. Functional in vitro assays have demonstrated that OKT4+ and OKT8+ cells represent the helper/inducer and cytotoxic/suppressor T-lymphocyte subpopulations, respectively [11]. The immunofluorescence test was performed as described by the manufacturers with modifications described elsewhere [12]. Results were expressed as the percentage of each T cell subset with respect to the total number of mononuclear cells counted.

Suppressor cells were activated by incubation with 25μg/ml of Con A (Grade IV, Sigma Chemical Co, St Louis, Mo, USA), by adding Con A and PWM at the beginning of the culture. The percentage of suppression of PWM-induced synthesis of immunoglobulin was calculated [12,13].

Helper T cell function was examined in an in vitro assay of immunoglobulin production by the use of a helper T cell dependent activator (PWM) in cultures containing allogeneic mixtures of B cells from patients or controls with T cells from either controls or patients with IgA nephropathy [12].

Results

PBM IgA production after PWM-stimulation was significantly greater in patients (mean 590ng/ml) than in controls (mean 240ng/ml) (p<0.0025). No differences were seen in IgM and IgG synthesis.

The percentages of OKT3+, OKT4+ and OKT8+ cells and OKT4+/OKT8+ cell ratio in patients and controls are shown in Table I. There was a significant increase in the percentage of OKT4+ cells in patients compared with the control
TABLE I. Mean percentage values of peripheral blood T-lymphocyte subsets in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>OKT3⁺ cells %</th>
<th>OKT4⁺ cells %</th>
<th>OKT8⁺ cells %</th>
<th>OKT4⁺/OKT8⁺ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11</td>
<td>53.32 ± 1.72</td>
<td>33.37 ± 1.18</td>
<td>22.83 ± 0.92</td>
<td>1.47 ± 0.06</td>
</tr>
<tr>
<td>Patients</td>
<td>22</td>
<td>58.99 ± 3.11</td>
<td>45.46 ± 1.47</td>
<td>26.23 ± 2.28</td>
<td>1.79 ± 0.05</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>NS</td>
<td>&lt;0.00025</td>
<td>NS</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Values are the means ± SEM

group. Consequently, an increase in the OKT4⁺/OKT8⁺ cell ratio was also observed. Three patients examined on two different occasions with an interval of four to six months showed persistently elevated percentages of OKT4⁺ helper cells.

In patients with IgA nephropathy the generation of IgA suppressor cells at 25μg/ml of Concanavalin A was significantly decreased compared with the control group (Figure 1). No significant differences were seen in the percentages of suppression of IgG and IgM synthesis in most of the patients (data not shown).

Figure 1. Suppression of Pokeweed-induced IgA synthesis by peripheral blood mononuclear cells exposed to 25μg/ml of Concanavalin A for seven days of culture in controls and in patients with IgA nephropathy (p<0.05). Each symbol represents a subject. No significant differences were seen in the percentages of suppression of IgG and IgM synthesis in most of the patients.
Figure 2. Percentages of OKT8+ (suppressor/cytotoxic) cell subset measured by monoclonal antibodies after stimulation with Pokeweed mitogen (PWM) alone (basal) or PWM plus Concanavalin A (50μg/ml) after seven days of culture of peripheral blood mononuclear cells. Each symbol represents a subject. p values are calculated in relation to basal values (PWM-stimulation alone).

To examine changes in the suppressor T cell subset (OKT8+ cells) during the generation of Concanavalin A induced suppressor cells the patients were phenotyped with specific monoclonal antibodies at the beginning and end of the culture (seventh day). At the end of the culture period (PWM plus Con A treated cells) the percentage of OKT8+ cells in the control group increased (p<0.0025) in relation to the basal percentages of OKT8+ cells (with PWM alone) (Figure 2). By contrast, in the patient group there was no significant changes in the generation of OKT8+ cells at the dose of Con A employed. These data are in agreement with the functional studies shown in Figure 1.

T cells obtained from nine patients were significantly more efficient than T cells from nine controls in providing specific IgA helper activity for normal allogeneic B cells (520ng/ml versus 210ng/ml). Similar results were obtained when T cells from patients and controls were co-cultured with allogeneic B cells from patients.

In 17 healthy first-degree relatives of five patients with IgA nephropathy some of the above experiments were also performed. Eight out of 17 relatives synthesised significantly more IgA after seven days of culture than the control group (mean 240ng/ml plus 1SD). Seven out of 17 relatives had significantly higher percentages of OKT4+ cells (greater than 2SD of mean from controls).
However only two presented a significant increase in the OKT4⁺/OKT8⁺ cell ratio in relation to the control group. Only two relatives had a decrease of IgA-specific suppressor T cell activity.

Discussion

The present study shows that patients with IgA nephropathy have a significant increase in IgA secretion by Pokeweed stimulated mononuclear cells maintained in culture for seven days. This phenomenon seems specific for IgA since no changes were observed in the production of IgG and IgM. Furthermore these patients had significantly less IgA-suppressor cell activity, as assessed by the Concanavalin A-generated suppressor cell assay, than the normal controls. These data are in agreement with previous findings reported by Sakai et al [7]. The apparent contradiction between a normal basal suppressor population, as assessed by monoclonal antibodies and the functional studies, might presumably be explained by the few clones of suppressor T cells involved in IgA regulation. However the generation of cells bearing OKT8⁺ antigens was significantly lower in patients than in controls confirming the existence of an alteration in the immunoregulatory role of suppressor cells as shown in the functional studies.

The enhanced IgA production by in vitro mitogen-stimulated peripheral blood mononuclear cells from patients with IgA nephropathy could be due to the effect of an inadequate suppressor system as well as an overactive helper cell population. The fact that we have found abnormalities in both systems makes it difficult to assign a preponderant role to either mechanism. However, some data such as the increased activity of helper T cells on IgA synthesis, the constantly high percentage of OKT4⁺ cells, the normal percentage of OKT8⁺ cells in the majority of patients, as well as the findings in the relatives, suggest that the involvement of helper cells may be the primary phenomenon and the IgA-specific abnormalities in suppressor cells its consequence. It is possible that IgA immune complexes could serve to modulate suppressor cell activity, just as IgG complexes do [14].

From our studies we cannot completely exclude the existence of a B-cell abnormality as a primary defect, and the T-cell abnormalities a secondary or subsequent phenomenon. In fact, it has recently been observed that the number of T cells with surface membrane receptors for IgA (Tα cells) are increased in mice with IgA secreting myelomas, a phenomenon closely linked to the high serum concentrations of IgA [15]. Furthermore Tα cells in vitro seem to have an IgA-specific helper activity [16].

The existence in the relatives of an in vitro increased production of IgA after polyclonal stimulation together with some abnormalities in the immune regulation of IgA and in the T cell subsets, that are in some way similar to those found in patients with IgA nephropathy, suggest a genetic basis for the susceptibility to this disease. This might explain the existence of familial IgA nephropathies and the association between certain antigens of the HLA system with this disease, although this last point remains controversial [1]. The reason why only a small number of genetically conditioned subjects develop the nephropathy is unknown. In summary, the immunoregulatory abnormalities described in this paper could be the first step in understanding the pathogenesis of the disease.
Acknowledgments

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References


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Open Discussion

RITZ (Heidelberg) My compliments for this beautiful presentation. I may have missed one point. Did you investigate the sub-classes of IgA that were synthesised in vitro. You are certainly aware of the controversies between Conley et al and André et al relating to whether or not it is IgA2 or IgA1.

EGIDO No, we have not examined the sub-classes of IgA in the supernatant cultures. We think that to obtain information about the polymeric nature of IgA it is better to study the ability for binding the secretory component rather than to establish the predominance of IgA1 or IgA2 sub-classes.