IgA MESANGIAL DEPOSITS IN C3H/HeJ MICE AFTER ORAL IMMUNISATION

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Summary

In order to develop an experimental IgA nephropathy, C3H/HeJ mice, high producers of IgA, were strongly immunised orally by ferritin and compared to C3H/eB mice. After immunisation, serum IgA and IgG titres increased significantly only in C3H/HeJ mice. Specific antiferritin antibody could be detected in the serum. Mesangial IgA deposits were present in most of C3H/HeJ mice after immunisation and were significantly higher than in C3H/eB mice. No ferritin deposits could be detected in the kidney. No clinical manifestation appeared in these animals.

Introduction

Evidence for the pathogenetic role of IgA immune-complexes in IgA nephropathy has been derived from multiple studies in both humans and experimental animals [1–4]. Clinical and histopathological observations in this disease suggest that the mucosal immune system produces large amounts of specific IgA in response to antigens from food or infectious agents resulting in circulating IgA immune-complexes and renal IgA deposits [5,6]. An immunogenetic abnormality of mucosal immunity can be suspected in these patients [7].

To test this hypothesis experimentally, we orally immunised by ferritin C3H/HeJ mice which are known to be high producers of IgA [8] and we compared them with C3H/eB mice.

We studied serum immunoglobulins, serum antiferritin antibodies and the deposits of immunoglobulins and ferritin in kidney.

Material and methods

Immunisation

Ten female C3H/HeJ and eight C3H/eB mice, 10–12 weeks old, received horse spleen ferritin (Sigma Co) orally: 20mg in 0.2cc by gastric intubation the first
day then 1mg/ml of drinking water for one month. Nine C3H/HeJ mice and 10 C3H/eB mice were not immunised and served as controls.

*Sacrifice and tissue processing*

After 30 days, mice were bled by transection of the right axillary artery under ether anaesthesia. Sera were frozen at -70°C until assayed. Kidneys were frozen at -70°C for immunofluorescence.

*Serum immunoglobulins*

Goat antisera to mouse IgA, IgG and IgM (Cappel Lab) were used in a radial immunodiffusion technique and the results expressed in percentage of the pooled control sera.

*Serum antiferritin-antibody*

The serum level and class of antibodies to ferritin were determined by ELISA technique using peroxidase-conjugated goat antisera to mouse IgA, IgG and IgM (Cappel Lab).

*Renal immunoglobulins and ferritin deposits*

Sections of kidney were stained by the direct method for mouse IgA, IgG and IgM using fluorescein-conjugated antisera (Cappel Lab). In addition, sections of kidney were stained for the presence of ferritin with rabbit antiferritin IgG conjugated to fluorescein [9].

*Haematuria and proteinuria*

At the time of killing, fresh urine from each mouse was tested by Bili-Labstix for protein and blood.

*Results*

*Serum immunoglobulin*

Serum IgA, IgG and IgM were measured in each mouse and the results expressed as the mean ± SD in groups of immunised or non-immunised mice and are summarised in Table I. Serum IgA and IgG were significantly higher in immunised than in non-immunised C3H/HeJ mice but not in C3H/eB mice. IgA were higher in C3H/HeJ mice than in C3H/eB mice before or after immunisation but the difference was not significant. IgG were lower in C3H/HeJ mice than in C3H/eB mice before or after immunisation but the difference was not significant. IgM were higher in immunised than in non-immunised C3H/eB mice but not in C3H/HeJ mice. After immunisation, IgM were significantly lower in C3H/HeJ mice than in C3H/eB mice.
TABLE I. Serum immunoglobulin levels*

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<tr>
<th></th>
<th>IgA</th>
<th>IgG</th>
<th>IgM</th>
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<tbody>
<tr>
<td>Non-immunised</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H/eB</td>
<td>97±20</td>
<td>122±23</td>
<td>112±21</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>104±10</td>
<td>102±14</td>
<td>108±15</td>
</tr>
<tr>
<td>Immunised</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H/eB</td>
<td>108±28</td>
<td>144±54</td>
<td>124±19***</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>138±41**</td>
<td>137±38**</td>
<td>103±16</td>
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</table>

* Means of all mice Ig values in each group ± SD. Results are expressed in percentage of the pooled control sera.

** Different from non-immunised C3H/HeJ (p<0.02)

*** Different from immunised C3H/HeJ (p<0.01)

**Serum antiferritin-antibody**

Serum antiferritin antibody and the class of the antibodies were studied in each mouse. Most of the antibodies were IgG and IgM but the titre was very variable depending on animals. A very small amount of IgA antibody was detectable in both groups of mice without significant difference between strains.

**Renal immunoglobulins and ferritin deposits**

Deposits of IgA, IgG and IgM in kidney of each mouse were studied in immunofluorescence and scored from traces to 4+. Mesangial deposits of IgG and IgM were present before immunisation and did not increase significantly after immunisation. There was no detectable difference between both strains. IgA deposits were detected in the mesangium of few non-immunised mice and they increased significantly after immunisation (Figure 1). IgA deposits were significantly higher in C3H/HeJ mice than in C3H/eB mice (Table II). Deposits of ferritin could not be detected by direct immunofluorescence with antiferritin rabbit IgG.

TABLE II. IgA deposits in the kidney

<table>
<thead>
<tr>
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<th>Intensity of immunofluorescence</th>
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<td></td>
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<td>traces</td>
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<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-immunised</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H/eB</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Immunised</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3H/eB</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>2</td>
<td>2</td>
<td>5</td>
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* Significant difference between C3H/eB and C3H/HeJ (Chi² >5.44; p<0.002)
Figure 1. Glomeruli of mice immunised with ferritin. Mesangial immunofluorescence staining for mouse IgA is higher in C3H/HeJ mice (A) than in C3H/HeB mice (B) x 1000
Proteinuria and haematuria

Proteinuria and haematuria could not be detected in any group at the time of killing.

Discussion

This study demonstrates that C3H/HeJ mice, strongly immunised orally by ferritin present significantly higher serum IgA and IgG than non-immunised animals whereas IgM does not increase. These immunoglobulins are shown to be at least in part specific antiferritin antibody but we cannot exclude a non-specific stimulation by mitogenic effect.

After immunisation, mesangial IgA deposits could be detected in most of C3H/HeJ mice. These IgA deposits were significantly greater in C3H/HeJ mice than in C3H/eB mice. No correlation could be demonstrated between high IgA deposits, high serum IgA or high rate of antiferritin IgA antibody in the same animal. Ferritin could not be demonstrated in the kidney by the technique that we used.

C3H/HeJ mice appear to be a good experimental model to study IgA nephropathy but this study has to be extended to demonstrate that the deposits in the mesangium are ferritin-antiferritin IgA immune-complexes and to produce a glomerulonephritis with clinical manifestations.

References

9 Genin C, Cosio F, Michael AF. Immunology 1984; 51: 225

Open Discussion

BROWNING (Glasgow) This work is similar to that published in 1983 by Emancipator, Gallo and Lamm, who reported the development of IgA nephropathy in Balb/c mice immunised orally with protein antigens. We have tried to reproduce these experiments and, as yet, have been unsuccessful.

GENIN We also have the same results because we used exactly the same protocol with ferritin in the drinking water and it is not sufficient to produce a good immunisation. We need a large amount of antigen at the beginning of the experiment. We demonstrated, for example, that in the C3H/DBA mice we do not get significant IgA deposits. We found specific antibodies from the three classes of immunoglobulins and we found more IgG and IgM than IgA
and Emancipator et al reported only IgA antibodies.

BROWNING I should like to ask if you can explain how the addition of a simple protein antigen to the diet of your immunised mice should lead to the development of a mesangial IgA nephropathy, whilst control mice of the same strain fail to respond to the range of protein antigens present in their daily diet?

GENIN I use a very high quantity of ferritin, 20mg of ferritin initially and then 1mg/ml in drinking water and I think a mouse drinks about 2ml a day. I think it is a very strong immunisation, and the two strains of mice received exactly the same commercial food and they were maintained in sterile boxes to avoid infectious agents. I think the interest of this work is to compare two strains with exactly the same protocol of immunisation.

RITZ (Heidelberg) Could you briefly comment on whether you had any glomerular lesions? You mentioned mesangial IgA deposits, but did you have evidence of glomerulonephritis?

GENIN We had only a light microscopic study: we have to do an electron-microscopic study. We did not find any lesions, it is not really glomerulonephritis, it is IgA deposition. I think the IgA deposits are not sufficient to produce clinical manifestation. We did not find haematuria or proteinuria at the time of sacrifice.

D'AMICO (Milan) Certainly we need a model of immunisation to study IgA nephropathy. Unfortunately in the Emancipator model there is no proteinuria at all. I wonder if you found at least a minor proteinuria, because I heard that you did not find nephritis or renal symptoms.

GENIN I think we have to find what is necessary. I do not know if it is the precipitation of other immune complexes, maybe not IgA immune complexes. Also the dose is not sufficient, you may not have enough immune complexes to develop clinical manifestations.

EGIDO (Spain) I wonder if the lack of correlation between the serum titres of antibodies to ferritin and IgA deposits is due to the small number of animals studied and the timing of the antibody studies. We are working on a model of dextran IgA nephropathy and we have found a close correlation following the injection of dextran between the levels of IgA antibodies and the anti-dextran antibodies in the IgA deposits in the kidney.

GENIN I think it is not specific IgA in the kidney because when you have a 30 per cent increase of IgA and IgG I think it is not only specific antibodies. Maybe you cannot find the antigen and anti-ferritin antibodies in the kidney because it is not only specific IgA. I think we may have a mitogenic effect and we have a non-specific stimulation of all IgA and not only specific IgA.