Circulating immune complex in murine autoimmune hepatitis

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High level of circulating immune complexes (CIC) in the serum has been reported in different forms of hepatitis particularly in complicated cases of viral hepatitis due to hepatitis B virus (HBV) infection. In this study CIC level in experimental autoimmune hepatitis were assessed by detection of polyethylene glycol (PEG) index. The sera of mice with established autoimmune hepatitis (EAH) confirmed by histopathological study showed higher PEG index (C57BL/6 mice: 34.56 ± 6.28 and C3H mice: 31.95 ± 28.99). The control healthy mice showed lower PEG index (C57BL/6 mice: 19.48 ± 6.85 and C3H mice: 21.27 ± 6.1). The high level of PEG index in EAH was found statistically significant. The role of CIC in the development of autoimmune hepatitis is discussed.

On exposure to most antigens an individual responds by synthesizing specific antibodies that subsequently may interact with the inciting agents and unite non-covalently with them to form immune complexes. These immune complexes precipitate in different tissues causing pathological changes as it is evident in certain cases of glomerulonephritis. Immunoglobulins are important for the removal of foreign antigens that have forced the primary immunological barriers and entered into the circulation. Soluble circulating IgG immune complexes (IgG-ICs) are physiologically eliminated by the liver but under certain circumstances when liver tissue is not in proper healthy condition there is a rise of extra hepatic deposition.

Earlier works on hepatitis in animal model showed an increase in circulating immune complexes (CIC) level in hepatitis. A virus infection, in which anti-HAV antibody associated with the complex was found to be IgM class. CIC's in patients with HBV infection was detected by Almeida. Chronic hepatitis B infection may be associated with glomerulonephritis in which HBsAg, IgG and complement component are deposited in glomeruli. Immune complex associated features example serum sickness like syndrome and extra hepatic manifestations like arthritis, nephritis or polyarteritis nodosa. This might be one of the cause of high level of CICs in HbsAg positive patients.

In animal model CICS were characterized, it was found to be composed of IgG, C3 and anti-HAV of IgM class with HAV capsid polypeptide and viral RNA. In chronic autoimmune hepatitis usually presence of autoantibodies in the serum help in the diagnosis of the disease but their role in formation of CIC and hence in pathogenesis has not been evaluated. In the present study an attempt has been made to find alteration in the level of circulating immune complex in experimentally developed autoimmune hepatitis whether the same has any role in the pathogenesis of this type of hepatitis.

Six weeks old male and female C57BL/6 and C3H strain mice were purchased from CDRI, Lucknow, maintained and bred in the departmental animal house, IMS, BHU. Adequate food, water and temperature was maintained.

Animals—Total of 120 mice of inbred strain (C57BL/6 and C3H), 6-7 weeks old male mice and weighing approximately 25 g were selected for the experiment.

Preparation of syngeneic liver antigen (S-100)—Animals were sacrificed, Liver dissected out, flushed with cold PBS (phosphate buffer saline). Homogenized in potter homogenizer then centrifuged in 3000 rpm for 7 min (in cold 4°C). The supernatant was ultracentrifuged at 20,000 rpm for 1 hr. The final supernatant is labeled as S-100, protein concentration of S-100 estimated to be 2.5 mg/ml.

Dose of antigen—The selected animals were inoculated with 200 μl S-100, 100μl PBS, 200μl of CFA, thrice weekly for four weeks. Each animals who had received a complete dosage of 12 inoculation of antigen were sacrificed.

Serum collection—Blood collected from ocular vessel rupture. Serum separated and stored at -20°C for immunological study.
Table 1—Strains of mice for induction of experimental autoimmune hepatitis

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Strains</th>
<th>Number</th>
<th>PBS</th>
<th>S-100</th>
<th>CFA</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>C57BL/6</td>
<td>40</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>II</td>
<td>C3H</td>
<td>40</td>
<td>100</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>III*</td>
<td>C57BL/6</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV*</td>
<td>C3H</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Control animals

Table 2—Presence of circulating immune complexes in experimental serum

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Strains</th>
<th>Number</th>
<th>Range</th>
<th>Mean±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>C57BL/6</td>
<td>40</td>
<td>20-50</td>
<td>34.56±28.28</td>
<td>&lt;0.005</td>
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<tr>
<td>II</td>
<td>C3H</td>
<td>40</td>
<td>20-50</td>
<td>31.75±28.99</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>III*</td>
<td>C57BL/6</td>
<td>20</td>
<td>0-25</td>
<td>19.48±6.85</td>
<td></td>
</tr>
<tr>
<td>IV*</td>
<td>C3H</td>
<td>20</td>
<td>0-25</td>
<td>21.27±6.18</td>
<td></td>
</tr>
</tbody>
</table>

*Control animals

Detection and estimation of circulating immune complexes—Detection of PEG (polyethylene glycol) index

Chemicals required: Polyethylene glycol (6000) Ranbaxy - 4.16% in borate buffer
Borate buffer: 0.1 M Boric acid—6.184 g, 25 mM disodium tetraborate—9.536 g and 75 mM sodium chloride—4.84 g. were dissolved in 1 l. distilled water pH was adjusted 8.4.
Method—Polyethylene glycol precipitation method of Creighton with slight modification was used for this purpose.
Serum diluted in ratio 1:3 with 0.1 M borate buffer (pH 8.4)
0.22 ml of diluted serum was mixed with 2 ml of 4.16% PEG (6000) in borate buffer.
(Final serum dilution was 1:30 and final PEG concentration was 3.75%).
0.22 ml of diluted serum was mixed with 2 ml of borate buffer (pH 8.4) as control.
Both were incubated at 4°C overnight.
The absorbance was measured in spectrophotometer at 450 nm to assess the turbidity due to precipitation of circulating immune complexes in PEG containing tube was matched with the absorbance of control tube containing borate buffer.
PEG Index was calculated as follows:

Absorbance with PE at 450 × 1000
Absorbance with borate buffer at 450 nm

A total of 80 cases of experimentally induced autoimmune hepatitis diagnosed on the basis of histopathological and immunological alteration in C57BL/6 and C3H murine model, along with 40 control cases i.e. normal individuals were included in our present study. Level of circulating immune complexes as PEG index calculated from PEG precipitate (using 4.16% PEG in borate buffer saline) in different study groups are shown in Table 2.

A total of 80 cases of experimentally induced autoimmune hepatitis diagnosed on the basis of histopathological and immunological alteration in C57BL/6 and C3H murine model along with 40 control cases were included in the present study. Level of circulating immune complexes expressed as PEG Index calculated from PEG precipitate in different study groups are shown in Table 2. Highest value was observed in group-I (mean±SD = 34.56±6.28) followed by group-I (mean±SD = 31.75±28.99). Both the values are statically significant when compared with control. Wands reported an undetectable level of CICs in control groups during their study on CIC in hepatitis in humans. The presence of non specific immune complexes or cryoglobulins precipitate.

CIC value in experimentally induced autoimmune hepatitis is appreciable high in our study with statistically significant P value < 0.005, <0.01 Autoimmune hepatitis is associated with circulating immune complexes but its frequency of positive result appears to be lower than viral hepatitis. It requires further research into the mechanisms of CICs presence in serum of experimentally induced hepatitis and do they have any special role in hepatocellular damage? The high levels of CICs in serum may be...
due to an increased formation as well as due to decreased clearance of RE cells. RE cells of liver, which are claimed to be the clearing machinery of immune complex are known to be depressed in hepatitis. In experimental model, have shown that both liver endothelial cells and Kupffer cells are involved in hepatic handling of soluble IgG immune complexes but elevation of CICs in autoimmune hepatitis may be a secondary response to the liver damage and it do not have any direct role in liver pathogenesis.

Reference