Lysozyme in chronic liver disease: a biochemical and histological study

IH MANIFOLD, FM BISHOP, P CLOKE, DR TRIGER, JCE UNDERWOOD

From the University Departments of Medicine and Histopathology, The Royal Hallamshire Hospital, Sheffield

SUMMARY  Serum lysozyme activities and semiquantitative analysis of tissue lysozyme distribution were studied in patients with primary biliary cirrhosis (PBC), chronic hepatitis (CH), miscellaneous liver diseases, and normal subjects. Serum lysozyme was significantly raised in PBC and CH. Portal venous blood has similar lysozyme activities to peripheral venous blood in a group of various liver diseases. Lysozyme-containing intralobular cells were decreased in all liver diseases studied but portal tract lysozyme was increased only in PBC and CH. Thus the increase in serum lysozyme in PBC and CH appears to originate from the portal inflammatory infiltrate, seen in these diseases.

There have been several studies of serum lysozyme as a possible marker of chronic inflammatory activity. Raised activity has been reported in sarcoidosis, correlating with extent and activity of the disease, and in tuberculosis. Many investigations of serum lysozyme have been carried out in inflammatory bowel disease but results are conflicting. The rationale for these studies is that lysozyme is found in granulocytes, monocytes, and macrophages and is more abundant in cells involved in active inflammation than in normal tissue.

Lysozyme is also found in Kupffer cells. There have been relatively few studies of serum lysozyme in hepatic disease. Raised activities have been reported in acute and chronic hepatitis, but results in cirrhosis have been conflicting. We have therefore studied serum activities and intrahepatic tissue distribution of lysozyme in various liver diseases and controls. Simultaneous portal and peripheral venous samples were also obtained from a small group of patients with various liver diseases to assess the possible contribution to serum activity of gastrointestinal tract and splenic lysozyme.

Material and methods

SERUM LYSOZYME

Serum lysozyme was measured turbidimetrically by the rate of lysis of Micrococcus lysodeikticus (Worthington Diagnostics). The absorbance change from 30 to 60s after enzyme addition was measured on a Unicam SP1800 spectrometer. Sera were measured in batches, each batch separately standardised with egg albumen lysozyme and incorporating quality control. Specimens were stored at −20°C and assayed within one month. Patients with raised serum creatinine were excluded from the study. Serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (AP) were measured by autoanalyser and differential white cell counts performed.

DEMONSTRATION OF TISSUE LYSOZYME

A modified indirect immunoperoxidase technique was used on paraffin sections (4μm) of formalin-fixed liver biopsies. Sections were initially treated with rabbit antihuman lysozyme, washed, and exposed to peroxidase-conjugated goat antirabbit IgG (Dako Immunoglobulins Ltd). Subsequent treatment with diaminobenzidine (BDH) and hydrogen peroxide formed the brown final reaction product. Appropriate controls were included; non-immune sera and diaminobenzidine alone were used to assess non-specific binding and endogenous peroxidase (non-immune goat serum, Sera Lab Ltd, non-immune rabbit serum, Gibco Bio-Cult Diagnostics Ltd). Sections were stained in batches with controls known to be positive for lysozyme. Haematoxylin was used as a counterstain.

Coded sections were analysed by the same observer throughout. The mean number of lysozyme positive intralobular cells per microscope field was determined. The amount of lysozyme in portal tracts was assessed semiquantitatively on a five point scale.
The scores from the first five tracts analysed for each biopsy, were added (the number of portal tracts per biopsy varied widely) thus deriving a single independent variable per biopsy.

SUBJECTS

Serum lysozyme
All cases of liver disease were biopsy proven. Thirty-two patients with primary biliary cirrhosis (PBC), 14 with chronic active and chronic persistent hepatitis (CH), and 15 with miscellaneous liver diseases (hepatoma, hepatic fibrosis, extrahepatic cholestasis, alcoholic liver disease, granuloma, schistosomiasis and halothane hepatitis) were studied. Forty-seven control subjects were drawn from hospital staff, out-patients with minor complaints and the local population. Hepatic or other inflammatory disease was excluded as far as possible from the control group. Simultaneous portal and peripheral blood samples were obtained either at laparotomy for portacaval shunting or during the course of percutaneous transhepatic cholangiography when portal vessels were incidentally entered. This group included the following diseases: PBC, CH, extrahepatic obstruction, cholangiocarcinoma, acute hepatitis and cryptogenic cirrhosis.

Tissue lysozyme
The following cases were studied: 20 with PBC, 20 with CH, 40 with miscellaneous liver diseases including alcoholic liver disease, primary and secondary malignancy, granuloma, extrahepatic cholestasis, acute hepatitis, haemosiderosis and cryptogenic cirrhosis. A control group of 20 biopsies showing normal histology on conventional staining was also studied.

STATISTICAL METHODS
Comparisons of serum lysozyme between groups and numbers of lobular lysozyme positive cells between groups were made using Student's t test with Welch's approximation for inequality of variance where appropriate.18 Correlation coefficients were calculated to test for significant linear relations between serum lysozyme activities and creatinine, ALT, AST, AP and peripheral blood leucocyte counts. Portal and peripheral lysozyme activities within the same patient were compared using the paired t test and, because of small numbers, the non-parametric, Wilcoxon paired rank summation test and the sign test, were also used. Since the data on portal tract lysozyme was semiquantitative, it was analysed using a contingency table in which the members of each group were classified into a high score or low score category. Significance was assessed by means of the $\chi^2$ test. A small number of cases had to be excluded because too few (or no) portal tracts were analysable.

Fig. 1 Serum lysozyme activities in normal and disease groups.

Fig. 2 Simultaneous portal venous and peripheral venous blood lysozyme activities in a group of miscellaneous liver disease patients.
Lysozyme in chronic liver disease: a biochemical and histological study

Results

Serum lysozyme activities were significantly raised in PBC (p < 0.001) and CH (p < 0.01) but there was no significant difference between these two groups. The miscellaneous liver disease group was not significantly different from normals but the variation in the group was very large (Fig. 1). Serum lysozyme correlated with peripheral blood monocyte counts in PBC (r = 0.44, p < 0.05, n = 23 patients) and with peripheral blood granulocytes in normals (r = 0.43, p < 0.05, n = 23 subjects). Serum creatinine concentrations were not significantly different between patients and controls and did not correlate with serum lysozyme activities. Serum AST, ALT, and AP activities did not correlate with serum lysozyme activities in patients. Portal venous lysozyme did not differ significantly from peripheral venous activity by any of the tests used (Fig. 2).

Lysozyme was seen in biopsies in intralobular sinusoidal-lining cells and in some portal tract cells. There were significantly fewer lysozyme-positive

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Portal tract lysozyme. Normal and disease group patients classified into high or low portal tract lysozyme scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Score</td>
</tr>
<tr>
<td></td>
<td>Low (0–2)</td>
</tr>
<tr>
<td>PBC</td>
<td>3</td>
</tr>
<tr>
<td>CH</td>
<td>5</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>23</td>
</tr>
<tr>
<td>Normal</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 2 Portal tract lysozyme significance levels

<table>
<thead>
<tr>
<th></th>
<th>PBC</th>
<th>CH</th>
<th>Miscellaneous</th>
<th>Normals</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>&lt; 0.001</td>
<td>&lt; 0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normals</td>
<td>&lt; 0.005</td>
<td>&lt; 0.05</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS = not significant.
in normal, although this observation would not be amenable to quantification.

d) raised serum lysozyme activities are seen in viral hepatitis, a condition associated initially with Kupffer cell proliferation rather than depletion.

Lysozyme has been found in granulocytes and it has been suggested that the serum activities in normal individuals largely reflect the rate of physiological granulocyte turnover. It has also been suggested that any severe infection will increase lysozyme release because of leucocyte destruction. Our findings of a correlation between serum lysozyme and peripheral granulocyte counts in normal individuals is in accord with a granulocyte origin of serum lysozyme in this group. However the cellular inflammatory infiltrate in the liver in PBC and CH is almost completely composed of mononuclear cells such that granulocyte lysozyme would seem to be of little importance in the excessive production of the enzyme in these diseases. The pathological significance of the correlation between peripheral blood monocyte counts and serum lysozyme in PBC is offset by the fact that the mean monocyte count was lower in PBC than in controls (0.26 x 10^3/μl vs 0.46 x 10^3/μl), whereas the mean serum lysozyme was higher in PBC than in controls. This suggests that lysozyme secreted by circulating monocytes is not the cause of the observed difference in serum activities. Also the similarity between mean creatinine concentrations in normals and patients and the lack of correlation with serum lysozyme suggests that the raised lysozyme activities are not due to any impairment of renal excretion in liver disease.

IH Manifold was in receipt of a research grant from the Sheffield Special Trustees Fund while this work was being carried out.

References

Lysozyme in chronic liver disease: a biochemical and histological study


Requests for reprints to: Dr IH Manifold, Western Park Hospital, Whitham Road, Sheffield S10 2SJ, England.