Participation of Lipid Peroxidation in Rat Pertussis Vaccine Pleurisy. III. Thiobarbituric Acid (TBA) Reactant and Lysosomal Enzyme

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Activity of lysosomal enzymes, such as N-acetyl-β-D-glucosaminidase (NAG), was assayed in exudate on a rat model of Bordetella pertussis vaccine pleurisy. Thiobarbituric acid (TBA)-reactive substance (TBA-R) and superoxide dismutase (SOD) activity were then monitored in the exudate on the acute phase response in this inflammatory model.

Retention of the exudate in the pleural space increased rapidly after the challenge, and the exudate volume at 24 h reached about three times the volume at 6 h.

The activity of NAG showed high levels at 6 h after the challenge, and then increased slightly at 24 h. This activity correlated with the retention of exudate and TBA-R levels.

The activity of SOD at 6 h was shown to be higher than that at 24 h after the challenge, thus showing negative correlations with TBA-R levels and exudate volume. The levels of TBA-R rapidly increased and reached maximum values at 24 h. It was concluded that the above three parameters correlate to the acute phase response in this inflammatory model.

Keywords
pertussis vaccine pleurisy; N-acetyl-β-D-glucosaminidase; thiobarbituric acid-reactive substance; superoxide dismutase; lipid peroxidation

Introduction
After the discovery and characterization of lysosomes by de Duve and his coworkers,11 evidence has been accumulated on the role of these subcellular particles in mediating connective tissue injury, such as carrageenin-induced pleurisy and adjuvant-induced arthritis.

The leakage of lysosomal contents, especially from leukocytes, is considered generally to be a key step in bringing about various inflammatory reactions. The mechanism of the anti-inflammatory action of drugs has been explained by this concept, at least in part. On the other hand, an increase in thiobarbituric acid (TBA)-reactive substance (TBA-R) has been known to occur during intoxication of ethanol and carbon tetrachloride, after exposure to ionizing irradiation, and also as a result of aging and acute or chronic inflammation, such as carrageenin paw edema and arthritis.

Biomembranes and subcellular organelles are the major sites of lipid peroxidation damage. Of importance to cellular damage is the lability of lysosomal membranes to rupture, followed by the concurrent release of an array of hydrolytic enzymes, which initiate intracellular digestion and catabolism. Tappel and his coworkers30 showed that isolated lysosomes underwent the peroxidation of lipids less rapidly than mitochondrial and microsomal fractions, and showed that lysosomes peroxidized at one-third the rate of mitochondria and one-tenth the rate of the microsomal fractions. Yet, release of lysosomal enzymes occurred relatively rapidly, and initiates further damage to the structural and functional parts of the cell.

In this study, in order to prove the relationship between the inflammatory process and lipid peroxidation, and also to manifest the change of the superoxide dismutase (SOD) activity in the inflammatory process, we examined the level of TBA-R, the activity of SOD, the activity of lysosomal enzymes such as N-acetyl-β-D-glucosaminidase (NAG) and the retention of exudate in the pleural space on the acute phase response in a rat inflammatory model of Bordetella pertussis vaccine pleurisy.

Experimental
Animals Five female Fischer rats (SF), 11 weeks old and weighing 160—180 g were used. The animals were obtained from Charles River Japan, Kanagawa.

Materials Bordetella pertussis vaccine was obtained from Chiba Serum Institute, Chiba. Freund’s complete adjuvant was obtained from Iatron, Tokyo. Phenyl-β-N-acetyl-glucosaminide, 4-amino antipyrine and potassium ferrocyanide were obtained from Nakarai Chemicals, Kyoto. TBA was obtained from BDH Chemicals, Poole, England. The other chemicals were of a reagent grade and were used without purification.

Induction of Pertussis Vaccine Pleurisy Female Fischer rats were used, and pertussis vaccine pleurisy was induced as described in the preceding paper.31 Animals were sacrificed at 6 and 24 h after induction by bleeding from the carotid artery. The other procedures were the same as described in the previous report.30

Determination of Lipid Peroxidation The levels of TBA-R in the rat exudate supernatants were measured according to the method of Yagi.40 Lipids and lipids containing peroxides were precipitated by treating the exudate with phosphotungstic acid, followed by the addition of TBA. The reaction product was then assayed spectrophotometrically (532 nm). The results were expressed as μmol of malondialdehyde formed.

Measurements of NAG Activity The activities of NAG in the exudate were assayed according to the modified method of Walker and his coworkers.51 Phenyl-β-N-acetyl-glucosaminide was dissolved in 0.05 M citrate buffer pH 4.3, to provide a 5 mm solution. A test sample (0.1 ml) and the substrate solution (1.5 ml) was mixed and incubated at 37°C for 30 min. The reaction was stopped by the addition of 1 M borate buffer (pH 8.8, 0.5 ml). The liberated phenol was measured by reading the optical density at 510 nm with a spectrophotometer. The enzyme activity was expressed as μg of liberated phenol during 30 min.

Measurement of SOD-like Activity The activity of O2− scavengers was assayed according to the nitrite method described by Oyanagi.60 To a tube containing 0.2 ml of buffer solution (final concentrations of 13 mm KH2PO4 and 7 mm Na2B4O7) which contained 0.5 mm ethylene diamine tetraacetic acid, disodium salt (EDTA-2Na) (pH 8.2), the following were added and mixed: 0.5 mm hypoxanthe (0.2 ml), 10 mm hydrogen peroxide plus 8.85 mm hydroxylamine O-sulfonic acid solution (0.1 ml), 0.1 ml of the sample, and 0.2 ml of water. The reaction was started by adding 0.2 ml of 1 M/L/mn xanidine oxido (XOD). This mixture (1.0 ml) was incubated for 30 min at 37°C. To this solution was added 2.0 ml of a coloring reagent which contained 30 μm N-naphthylethylendiamine, 3 μm sulfanilic acid and 25% acetic acid. The optical absorption was measured at 550 nm.

Measurements of Protein The amount of protein was determined by the method of Lowry et al.71

Statistical Method The results are expressed as mean values ± standard deviation (mean ± S.D.) and the probability for significance of the differences was determined by analysis of variance.
TABLE I. Levels of TBA Reactant and Enzyme Activities in the Exudate of Rat Pertussis Vaccine Pleurisy

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Volume (ml)</th>
<th>TBA-R</th>
<th>Concentration in exudate</th>
<th>Protein</th>
<th>Amount of per g protein in exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NAG</td>
<td>SOD</td>
<td>TBA-R</td>
</tr>
<tr>
<td>6</td>
<td>0.70 ± 0.24</td>
<td>1.50 ± 0.35</td>
<td>1.38 ± 0.15</td>
<td>6.62 ± 1.57</td>
<td>42.4 ± 4.2</td>
</tr>
<tr>
<td>24</td>
<td>2.05 ± 0.35</td>
<td>2.19 ± 0.39</td>
<td>1.68 ± 0.77</td>
<td>5.50 ± 1.57</td>
<td>41.0 ± 6.0</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 5 rats; TBA-R, TBA reactants are shown as nmol of malondialdehyde (MDA)/ml or nmol/g protein. NAG activities are shown as μg/ml or μg/g protein. SOD activities are shown as U/ml or U/g protein. Protein amounts are shown as mg. TBA-R were measured spectrophotometrically according to the method of Yagi. 

Results

Bordetella pertussis vaccine injected into the pleural cavity of sensitized rats was found to bring about a remarkable inflammatory reaction, which is characterized by retention of exudate and a transient increase of TBA-R in the exudate. The results are summarized in Table I.

The exudate retention volume increased promptly. Six hours after the challenge, 0.70 ± 0.24 ml (S.D.) of the exudate was collected from the pleural cavity, and at 24 h the exudate level reached about three times as high as at 6 h. The levels of TBA-R in the exudate increased transiently, and reached to about 55.5 nmol/g protein at 24 h.

The activity of SOD in the exudate achieved a maximum value at 6 h, and then decreased significantly. This activity changed in an opposite manner to the variation of the level of TBA-R, and shows a negative correlation with the exudate volume (−0.656), the level of TBA-R (−0.714) and NAG activity (−0.716).

The activity of NAG in the exudate reached its high level at 6 h (1.38 μg/ml), and then increased slightly at 24 h. The variation of this activity correlated with the retention of exudate (0.638) and the level of TBA-R (0.800).

The concentration of protein in the exudate exhibited no remarkable change throughout the experiments.

Discussion

Increase of lipid peroxidation in the inflammatory site, such as synovial fluids of rheumatoid arthritis and synovia of rats with adjuvant arthritis, were reported by Yoshikawa and his coworkers.8) These increases implied a significant correlation with the activities of lysosomal enzymes.

We used a pertussis vaccine in the present experiments, and studied the level of TBA-R and the activities of SOD and NAG in the acute phase response of pertussis vaccine pleurisy in rats. The levels of TBA-R in the exudate increased transiently and reached maximum values at 24 h. By contrast, the activity of SOD showed high levels of activity at 6 h and decreased slightly, then reached minimum values at 24 h. From the above facts we conclude that the increase of lipid peroxidation may be caused by diminution of SOD activity or the generation of a superoxide by leukocytes which migrated into the pleural space.9) Yoshikawa et al. used a TBA reactant to examine the damage induced by lipid peroxidation and the protection by SOD (from bovine liver: Orgotein) in rat adjuvant arthritis. It was reported that the TBA reactant was reduced significantly by the injection of SOD.10) There are few papers, however, which describe the variation in the activity of SOD at inflammatory sites.

The activity of a lysosomal enzyme such as NAG in the exudate exhibited a high value at 6 h and increased slightly at 24 h; this correlated with the level of TBA-R. The results suggest that the increase of lipid peroxidation caused the release of a lysosomal enzyme; this agrees with the results reported by Yoshikawa and his coworkers in the case of adjuvant arthritis.

From the above results, we suggest that the changes in TBA-R level and SOD activity reflect the acute phase reactions; the increase of NAG activity may be caused secondarily by the increase of lipid peroxidation. So, we conclude that the determination of the level of lipid peroxides and activity of SOD are useful in investigating the inflammatory process.

References