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Participation of Lipid Peroxidation in Rat Pertussis Vaccine Pleurisy. I. Thiobarbituric Acid (TBA) Reactant and Ceruloplasmin

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Female Fischer rats were sensitized to *Bordetella pertussis* vaccine mixed with Freund's complete adjuvant and challenged with pertussis organisms intrapleurally 7, 14 and 21 d later, then compared with non-sensitized rats. The thiobarbituric acid (TBA)-reactive substance and an acute phase reactant, ceruloplasmin, were monitored in the exudate or the serum. The retention volume of exudate in the non-sensitized rats scarcely increased after the intrapleural injection of pertussis organisms. On the other hand, in the sensitized rats, the retention volume of exudate increased rapidly after the challenge, and reached the peak value at 48 or 72 h, whereas the levels of TBA-reactive substance and ceruloplasmin in the exudate reached the maximum values at 24 h; a similar trend was found in the serum of the rats challenged 14 and 21 d later. It was found that the two parameters did not correlate to the retention of exudate and that lipid peroxidation is related to the acute phase response in this inflammatory model.

Keywords——pertussis vaccine pleurisy; thiobarbituric acid reactant; ceruloplasmin; lipid peroxidation

Intrapleural injection of various chemical agents, such as carrageenin,[1,2] dextran,[3] and silver nitrate,[4] has been reported to induce inflammation in the pleural cavity of rats. These models have the advantage of allowing easy determination of various parameters of inflammation.

A number of models have been developed for delayed hypersensitivity reactions; most of them employ the skin as the site of immunity. Yamamoto and his coworkers[5] reported a model of cell-mediated immunity, using the guinea-pig pleural space and tuberculin antigen. Dieppe *et al.*[6] have reported that the intrapleural injection of *Bordetella pertussis* vaccine in sensitized rats produces an inflammatory reaction of delayed hypersensitivity in the pleural cavity of Wistar strain rats. This model is interesting since it appears to be sensitive to drugs which are used in rheumatoid arthritis.[7]

McCord[8] showed that superoxide radicals degrade various high-molecular-weight polymers, such as polysaccharides, and unsaturated fatty acids in the synovial fluid of joints; this may be an important factor in rheumatoid arthritis. Further, Yoshikawa *et al.*[9] showed that an increase of thiobarbituric acid (TBA) reactant level occurs in the synovial fluids of patients with rheumatoid arthritis and in the synovia of rats with adjuvant arthritis. it is known that this substance is a malondialdehyde (MDA) or a nonvolatile precursor of MDA.[10] This TBA-reactive substance (TBA·R) is the most frequently used index of lipid peroxidation in both *in vitro* and *in vivo* experiments.[10]

In this study, we thus examined the lipid peroxidation, as well as the level of ceruloplasmin, in *Bordetella pertussis* vaccine pleurisy.

Experimental

Animals——Five female Fischer rats (SPF), 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 latron, Tokyo. *p*-Phenylenediamine (PPD) was obtained from Tokyo Kasei, Tokyo, and TBA was obtained from BDH Chemicals, Poole, England. The other chemicals were of reagent grade and were used without purification.

Induction of Pertussis Vaccine Pleurisy——The animals were sensitized by subcutaneous injection of 0.2 ml of an emulsion, made of a suspension of *Bordetella pertussis* vaccine and Freund's complete adjuvant (50/50, v/v), into the dorsal surface of both hind paws.

The rats were challenged intrapleurally with 0.2 ml of pertussis vaccine under light ether anesthesia 7, 14 and 21 d after sensitization according to Tarayre *et al.*[11] The animals were killed at 24, 48 and 72 h thereafter by bleeding from the carotid arteries. The blood was centrifuged at $1700 \times g$ for 15 min at 4 °C. The pleural exudate was collected in a plastic tube, and its volume was measured. The supernatant fluids of exudate were obtained by centrifugation at $1200 \times g$ for 10 min at 4 °C.

Determination of Lipid Peroxidation——The levels of TBA·R in the pleural exudate supernatants and in the serum were measured according to the method of Yagi.[12] Lipids and lipid peroxides were precipitated by treating the exudate and serum with phosphotungstic acid, followed by the addition of TBA. The reaction product was then assayed spectrophotometrically (532 nm). The results were expressed as nmol of malondialdehyde formed.

Measurement of Ceruloplasmin——The activities of ceruloplasmin were determined according to the method of Sunderman and Nomoto.[13]

PPD was dissolved in 0.1 M acetate buffer, pH 5.45, to provide a 27.6 mM solution. Next, 2 ml of acetate buffer, and 0.1 ml of test samples were pipetted into each of two test tubes, which were placed in a water bath at 37 °C to reach thermal equilibrium. Warmed PPD solution (1 ml) was added to both tubes. The contents were mixed and the tubes were kept unstoppered in the water bath. After 5 min, 50 μl of 1.5 M sodium azide solution was pipetted into one tube, and the contents were mixed. Exactly 30 min later, 50 μl of sodium azide solution was added to the other tube, and the contents were mixed. The optical density of the reaction mixtures was measured at 530 nm.

Measurement of Protein——The amount of protein was determined by the method of Lowry *et al.*[14]

Statistical Method——The results are presented as mean values ± standard deviation (mean ± S.D.) and the significance of the differences was evaluated by analysis of variance.

Results

Bordetella pertussis vaccine injected into the pleural cavity of sensitized rats was found to bring about a remarkable inflammatory reaction, characterized by the retention of exudate in the pleural space. The results are summarized in Tables I and II.

The changes in the exudate volume were shown to be dependent on the time intervals between the sensitization and the challenge. Thus, for rats sensitized 7 d before challenge, the exudate volume increased to reach the peak value (2.89 ml) 48 h later. A similar trend was observed for rats sensitized 14 d before challenge (3.3 ml at 48 h). For rats sensitized 21 d before challenge, the exudate volume increased slowly but reached a much higher maximal value (4.5 ml) 72 h later.

The changes of ceruloplasmin activity in the exudate were similar for the rats sensitized, 7, 14 and 21 d before challenge; the peak values were observed 24 h after the challenge in every case. In the serum, however, the changes in the ceruloplasmin activity were dependent on the time interval between the sensitization and the challenge. The level of TBA·R, either in the exudate or serum, became maximal 24 h after the challenge, and then decreased slowly in every case. The TBA·R level in the serum of the rats sensitized 7 d before challenge was the only exceptio (no significant peak concentration was observed).

The concentration of protein in the exudate did not exhibit any remarkable change throughout the experiments (data not shown).

The corresponding parameters in the exudate and the serum of the sensitized rats were shown to be correlated. The levels of TBA reactant were shown to have high correlation coefficients (0.990, 0.991 and 0.950). The activity of ceruloplasmin was shown to be correlated

TABLE I. Pleural Responses after Challenge

Day after sensitization	Volume of exudate (ml)			TAB reactant level in exudate Time after challenge (h)			Ceruloplasmin activity in exudate		
	24	48	72	24	48	72	24	48	72
Nonsensitiz.	0.04 ±0.02	0.13 ±0.10	0.01>						
7	2.81 ±0.61	2.89 ±0.20	1.93 ±0.44	4.38 ±0.66	3.61[b] ±0.45	4.44[b] ±0.63	0.85 ±0.18	0.64[a] ±0.15	0.48[a] ±0.16
14	1.93 ±0.38	3.30[a] ±0.54	2.47[b] ±0.56	4.16 ±0.66	3.52[a] ±0.24	3.80 ±0.69	0.53 ±0.15	0.31[b] ±0.10	0.36[b] ±0.06
21	1.95 ±0.35	2.61[b] ±0.62	4.50[a] ±0.72	6.15 ±1.09	4.77[b] ±1.03	5.11 ±1.24	0.73 ±0.12	0.54[b] ±0.20	0.48[a] ±0.10

Each value represents the mean ± S.D. of 5 to 8 rats; TBA reactant levels are shown as nmol MDA/ml. Ceruloplasmin activity is shown as the optical density. The amount of antigen used for sensitization and challenge was 2×10^9 pertussis organisms. *a*) Significant difference from 24 h after challenge at $p < 0.001$, *b*) indicates $p < 0.05$.

TABLE II. Levels of TBA Reactant and Ceruloplasmin in Serum

Day after sensitization	TBA reactant Time after challenge (h)			Ceruloplasmin		
	24	48	72	24	48	72
Nonsensitiz.	2.39±0.36	2.77±0.32	2.74±0.36	0.81±0.31	0.93±0.08	1.06±0.10
7	5.72±0.98	6.54±1.10	6.10±1.38	1.27±0.22	1.30±0.06	1.24±0.24
14	11.80±1.16	7.43±0.73[a]	6.00±0.50[a]	0.92±0.23	1.03±0.17	0.74±0.08[b]
21	17.03±2.11	12.48±4.53[b]	12.50±1.88[a]	1.22±0.10	1.03±0.33	0.84±0.16[a]

Each value represents the mean ± S.D. of 5 to 8 rats; TBA reactant levels are shown as nmol MDA/ml. Ceruloplasmin activity is shown as the optical density. The amount of antigen used for sensitization and challenge was 2×10^9 pertussis organisms. *a*) Significant difference from 24 h after challenge as $p < 0.001$, *b*) indicates $p < 0.05$. The control level of TBA reactant and that of ceruloplasmin were 2.2±0.44 nmol/ml and 0.80±0.19, respectively.

slightly (correlation coefficients 0.773, 0.661 and 0.558).

Discussion

Rowley *et al.*[15] have shown that it is possible to induce active cutaneous hypersensitivity in the rat by using *Bordetella pertussis*. Willoughby[16] and Arrigoni-Martelli *et al.*[17] also studied this reaction in the rat paw. Dieppe *et al.*[7] have shown that it is possible to provoke the reaction within the pleural cavity of the Wistar rat.

The above reaction seemed promising to us since it is sensitive to non-steroidal anti-inflammatory agents, and also to drugs which are used in the treatment of rheumatoid arthritis such as D penicillamine and levamisole.[8] It therefore seemed interesting to study this model in the Fischer rat, which is known to be more sensitive to cellular hypersensitivity phenomena than the Wistar strain. The Fischer strain is much more sensitive to Freund's adjuvant arthritis than Wistar rats.[18]

In the present experiments, we found that the intrapleural injection of *B. pertussis* in the non-sensitized rat was associated with only a slight development of inflammatory reactions; the exudate volume and the level of TBA·R in the serum scarcely increased as compared with those of sensitized rats. In the sensitized rats, both the increase in the exudate volume and that

in the concentration of TBA·R in the serum were found to be greater than those observed in the non-sensitized animals. The TBA·R concentration became maximal 24 h after the challenge. It was also found that the changes in the TBA·R and celuroplasmin levels did not correlate with that of the exudate volume. The reactions were found to be maximal when the challenge was made 21 d after the sensitization.

From the above results, it seems cleat that the Fischer strain is useful as a model for cellular hypersensitivity, and we suggest that the time-course of the TBA reactant level does not reflect the delayed-type reaction but rather relates to the acute phase reactions.

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