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Effect of Antibodies to Red Cell Superoxide Dismutase on Its Activity in the Blood of Patients with Rheumatoid Arthritis

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A relationship is revealed between the level of antibodies to superoxide dismutase and the activity, course, and form of a disease. The production of antibodies to red cell superoxide dismutase increases as the pathological process progresses. By reducing the resistance of erythrocyte membranes to reactive oxygen species, antibodies to red cell superoxide dismutase promote the development of anemia in patients with rheumatoid arthritis. The results indicate that inhibition of the active center of this enzyme by specific immunoglobulins is one of the causes of reduced activity of red cell superoxide dismutase or the lack of an appreciable increase in its activity during hyperproduction of reactive oxygen species.

Key Words: *superoxide dismutase; glutathione reductase; antibodies; rheumatoid arthritis*

The increase of free-radical oxidation in rheumatic diseases is associated with a reduction of superoxide dismutase (SOD) activity [5,7,9,10]. This reduction may be caused not only by depletion of the antioxidant system during hyperproduction of reactive oxygen species, but also by inhibition of the enzyme by the respective antibodies. The antigenic nature of the enzyme is confirmed by antibody production to plasma SOD and to C-terminal peptide of human liver manganese SOD during immunization of laboratory animals [12].

We have been unable to find any reports about antibody production to antioxidant enzymes in rheumatic diseases.

The aim of this research was to detect the production of antibodies to SOD in patients with rheumatoid arthritis (RA) and to elucidate the effects of specific antibodies and circulating immune complexes (CIC) on enzyme activity.

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MATERIALS AND METHODS

Sera from 104 patients with RA of different degrees of activity, forms, and course were tested in enzyme immunoassay. Commercial preparations of erythrocytic SOD (activity 3000 U) in a concentration of 100 μg/ml were used as antigen. The antigen was diluted with 0.05 M carbonate-bicarbonate buffer, pH 9.6. Sera from 30 normal subjects were tested for control.

Effects of antibodies to SOD were studied using the soluble and immobilized forms of the enzyme. The enzymatic activity was measured as described elsewhere [8]. The enzyme was immobilized by "mechanical" incorporation in the spatial lattice of the gel, followed by additional covalent fixation after a previously described method [11]. Sera of RA patients with high antibody titers selected beforehand

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TABLE 1. SOD Activity and Level of Antibodies to the Enzyme in Patients with RA of Different Degrees of Activity ($M \pm m$)

Group	Number of examinees	SOD activity		Antibodies to red cell SOD
		erythrocytic	plasma	
Healthy subjects	30	40.0±2.6	5.4±0.19	0.06±0.004
Patients				
1st degree of activity	16	45.5±4.46	6.0±0.27	0.102±0.011
2nd degree of activity	77	47.6±2.34	11.4±0.48	0.123±0.006
3rd degree of activity	11	47.8±5.79	11.4±0.66	0.229±0.051

(extinction >0.2) were the source of specific immunoglobulins. "Pure" antibodies to red cell SOD were obtained using a special antigenic immunoadsorbent [2]. After dialysis against buffered normal saline followed by concentration through a semipermeable Sartorius membrane system until the initial volume of the serum was attained, the antibodies were used to study their interaction with the enzyme. Normal saline and sera of normal subjects were used in control tests.

In parallel with this, SOD activity [3] and the level of CIC following their precipitation with 7% polyethylene glycol [4] were measured in the plasma of RA patients. SOD activity and protein concentration in dissolved CIC were then measured [13].

RESULTS

Increased levels of antibodies to SOD (0.131±0.007) were detected in the sera of 75 (72.1%) patients (0.06±0.004 in the control, $p<0.001$). A reliable relationship was revealed between the level of antibodies and the activity and course of the disease ($p<0.05$, Tables 1 and 2). The results indicate increased production of antibodies to red cell SOD during activation of the pathological process. For example, the level of specific immunoglobulins differs appreciably from the control even in disease of the first degree of activity ($p<0.001$). A fulminant course was associated with a higher level of antibodies in comparison with slow progression of the disease ($p<0.05$). This permits us to consider antibodies to SOD as a criterion of RA severity. We detected an inverse correlation between antibodies to erythrocytic SOD and the red cell count and hemoglobin level in the blood of RA patients ($r=-0.20$, $p=0.07$). Hence, antibodies to red cell SOD promote the development of anemia in RA patients by reducing erythrocyte membrane resistance to reactive oxygen species. This may account for the increase of plasma SOD activity (Tables 1 and 2) due to the release of erythrocytic SOD during cell membrane destruction.

Incorporation of the enzyme in the rigid structure of the polyacrylamide gel permitted us to rule out any kind of conformational changes in the structure of the molecule during the interaction with antibodies when enzymatic activity remained intact (Table 3).

Measurements of the enzymatic activity of immobilized SOD after preliminary interaction with purified antibodies to erythrocytic SOD revealed a 99% reduction of enzyme activity in comparison with the control. Such a time course appears to be due to antibody blocking of the active center of the enzyme, which simultaneously functions as an antigenic determinant.

Measurement of the activity of soluble SOD following interaction with specific antibodies showed a 65% decrease. These data indicate a higher activity of soluble SOD after its reaction with specific antibodies in comparison with its immobilized forms (Table 3). This might be due to the appearance of SOD activity during the formation of the antigen(SOD)-antibody complex, an activity which, according to some reports [1,6], is intrinsic to any immune complex. Hence we naturally tried to assess possible SOD activity in the plasma CIC of RA patients. At a protein concentration of 880 μg/ml it was 2.4 per ml. Thus,

TABLE 2. SOD Activity and Level of Antibodies to the Enzyme in Patients with Different Patterns of RA ($M \pm m$)

Group	Number of examinees	SOD activity		Antibodies to red cell SOD
		erythrocytic	plasma	
Healthy subjects	30	40.0±2.6	5.4±0.19	0.06±0.004
Patients				
with slow progress of disease	70	38.7±2.77	6.9±0.29	0.116±0.06
with rapid progress of disease	34	51.5±2.4	12.3±0.41	0.149±0.0175

TABLE 3. Comparison of Activities of Immobilized and Soluble SOD after Interaction with Antibodies ($M \pm m$)

SOD	Enzyme activity in 1 ml		
	antibodies to SOD	donor	normal saline
Granulated	0.34±0.002	33.4±0.19	37.3±0.06
Soluble	13.5±0.09	32.5±0.104	37.5±0.08

when measuring plasma SOD activity in rheumatic patients, one should not disregard possible SOD activity of CIC.

Hence, inhibition of the active center of the enzyme by specific immunoglobulins is one reason that erythrocytic SOD activity decreases or does not appreciably increase during hyperproduction of reactive oxygen species.

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