SUPEROXIDE DISMUTASE ACTIVITY OF T LYMPHOCYTES AND NON-T LYMPHOCYTES

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1. Introduction

Superoxide dismutase (SOD, EC 1.15.1.1), which catalyzes the dismutation of superoxide anion, $O_2^{\cdot -}$, to less toxic hydrogen peroxide and molecular oxygen, is an ubiquitous enzyme in oxygen-metabolizing cells and serves to protect them from the potentially harmful effects of this reactive free radical [1]. Clinically, SOD activity has been extensively determined in peripheral blood cells in various conditions [2–7]. It was reported that peripheral lymphocytes of human blood have higher SOD activity than do granulocytes [8–10].

The purpose of this study is to extend this observation and to differentiate between the SOD activities of T and non-T lymphocytes.

2. Materials and methods

Peripheral blood lymphocytes were isolated from 50 ml heparinized venous blood of 6 healthy adult donors by centrifugation over a Conray-Ficoll gradient. For separation of T and non-T lymphocytes, a method based on the spontaneous rosette formation of human T lymphocytes with sheep erythrocytes (SRBC) was used [11]. A mononuclear cell suspension, 1 vol. (5×10^6/ml) and 1 vol. 2% SRBC in fetal calf serum were mixed. Mononuclear cells forming SRBC rosettes were then separated from cells not forming rosettes by centrifugation over a Conray-Ficoll gradient as noted above. Unrosetted cells remaining at the interface and rosetted cells sedimenting to the bottom of the gradient were then removed separately; the SRBC in the pellet fraction were disrupted by Tris-buffered ammonium chloride and the remaining cells were used as T lymphocytes. The interface cells were re-rosetted and centrifuged over a second Conray-Ficoll layer in order to remove residual rosetting T lymphocytes and were employed as non-T lymphocytes. Cytochemical identification of monocytes in the cell preparation was by the method in [12]. After three washings with phosphate-buffered saline, the cell pellet was suspended in 0.1% Triton X-100 and disrupted by sonication for 5 min at 200 W (Insonator Model 200 M, Kubota). Sonicated materials were centrifuged at 25 000 × g for 30 min in the cold, and the clear supernatant was assayed for SOD activity and protein content, the latter being determined by the Lowry method [13] using bovine serum albumin as the standard. SOD activity was measured by inhibition of superoxide-mediated reduction of ferricytochrome c using carbonate buffer, at pH 10.0 [1,14]. Calculation of activity units was done by logit paper [15]. The amount of specimen required to inhibit the rate of ferricytochrome c reduction by 50% was defined as 1 unit of activity [1], which was expressed either as units/mg protein or 10^7 cells. Cyanide sensitivity was assessed with 1.0 mM potassium cyanide [14]. Granulocytes were obtained from the remaining pellet after sedimentation of erythrocytes by 3% dextran–saline solution. Contaminating erythrocytes were similarly removed. Each granulocyte preparation was >95% pure. The cell pellet was treated in the same way as the lymphocyte pellet. The presence of Triton X-100 did not interfere with the determination of enzyme activity.

Table 1
Contamination by other types of cells in T and non-T lymphocyte preparations

Preparation	Contaminating cells (%)		
	Granulocytes	T in non-T Non-T in T	Monocytes
T lymphocytes	3.0 ± 3.9[a] (0 – 8.1)[b]	1.5 ± 1.3 (0 – 2.9)	2.7 ± 2.5 (0 – 5.0)
Non-T lymphocytes	5.2 ± 4.2. (0 – 12.9)	0.8 ± 0.6 (0 – 2.0)	21.3 ± 10.3 (10.0 – 30.0)

[a] Mean ± 1 SD
[b] Range

3. Results

3.1. *Contamination of T and non-T lymphocyte preparation by other types of cells*

As shown in table 1, T lymphocyte preparations were relatively pure, free from other types of cells. On the other hand, ~10–30% of the non-T lymphocyte preparation was monocytes and 0–12.9% granulocytes. The remaining cells were B lymphocytes as identified by their surface immunoglobulin.

3.2. *SOD activity of each cell preparation*

Table 2 shows the SOD activities of the three cell preparations. T lymphocytes had about 1.8-times the activity of non-T lymphocytes as expressed either in terms of units/mg protein or units/10^7 cells. The difference among these three cell populations was significant ($p < 0.005$). When the granulocyte activity is taken as 1, T lymphocytes have 3.3- and 4.2-times and non-T lymphocytes 1.9- and 2.4-times higher activity, respectively. The protein content of T and non-T lymphocytes was almost the same. A cyanide-sensitive fraction, i.e., cytosol SOD, of granulocytes was higher than that of non-T lymphocytes ($p < 0.05$) and lower than that of T lymphocytes ($p < 0.05$).

4. Discussion

As described in [8] and as confirmed above, lymphocytes of peripheral blood have a higher SOD activity than granulocytes. When the lymphocyte population was divided into T and non-T lymphocytes, the former, with ~95% purity in the present study, showed 1.8-times the activity of the latter. On the other hand, the non-T lymphocyte population was a heterogeneous one, so it could not be determined how much each cell population contributed to the total activity, although it is probable that most of the activity was that of B lymphocytes. Without the contaminating granulocytes, the activity of which was comparatively low, the total non-T lymphocyte activity would have been slightly higher than the present value. Unfortunately, the way in which monocytes, accounting for 10–30% of the

Table 2
Superoxide dismutase activity of T and non-T lymphocytes and granulocytes, with ratio of activity in parentheses

Cell	units/mg protein	units/10^7 cells	Cyanide-sensitive fraction (%)
T lymphocytes	52.3 ± 3.3[a] (3.3)	27.6 ± 3.0 (4.2)	81.4 ± 4.4
Non-T lymphocytes	29.7 ± 4.6 (1.9)	15.6 ± 5.4 (2.4)	66.8 ± 10.7
Granulocytes	15.9 ± 3.7 (1.0)	6.5 ± 2.0 (1.0)	76.0 ± 5.4

[a] Mean ± 1 SD

total cell number, affected this value could not be evaluated. No correlation, however, was found between contaminating monocyte number and non-T lymphocyte SOD activity. Further studies of monocyte SOD are in progress.

Granulocyte SOD may well play an important role, since the oxygen metabolites, the first of which is superoxide, are intimately related to the intracellular killing mechanism of granulocytes [16]. However, the reason that lymphocytes are provided with a higher activity remains to be elucidated. The role of lymphocyte SOD may be related both to lymphocyte longevity and to such immune functions as the production of immunoglobulins and lymphokines. It is known that T lymphocytes are generally more long-lived than B lymphocytes and that the half-life of circulating polymorphonuclear leukocytes is 6–7 h [17]. Taking this fact into consideration, it is interesting to note that the life span of these 3 cell populations parallels the amount of SOD in each type of cell. According to [18], immunoglobulin production is most active at pO_2 of 33 mmHg, which falls in the pO_2 range of venous blood but ⩽33% of the level in normal arterial blood. This observation appears to indicate that plasma cells, into which B lymphocytes differentiate, must handle surplus oxygen in order to maintain an efficient immunoglobulin production at its production sites. Although nothing is known as to how environmental oxygen regulates the lymphokine production of T lymphocytes, an analogous situation may be expected to be present. Further studies are needed to clarify the relationship between the functions of a cell and its SOD activity.

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