INFECTION AND IMMUNITY, Apr. 1981, p. 394-397
0019-9567/81/040394-04$02.00/0

Vol. 32, No. 1

NOTES

Role of Adenosine Deaminase in Activation of Macrophages

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The intracellular adenosine deaminase activity of induced macrophages was twice that of resident cells. Coformycin, a specific inhibitor of adenosine deaminase, inhibited the activation of macrophages, as measured by superoxide generation.

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4.; ADA) is an enzyme in the purine salvage pathway which is primarily responsible for the intracellular disposition of transported adenosine. Patients who inherently lack this enzyme have defects in both humoral and cellular immunity that are manifested as severe combined immunodeficiency disease (5). Numerous functional studies have shown that ADA is of critical importance for the maturation and function of lymphocytes, especially those of T lineage. Lymphocytes rendered artificially ADA deficient by the addition of a specific inhibitor of ADA fail to respond to mitogens and lose their capacity to reject allografts (1, 2, 3). Also, although the addition of an ADA inhibitor results in the failure of morphological maturation of human monocytes, little is known about the role of ADA in the functional activation of monocytes or macrophages (4).

Our study focuses on a correlation between the function of peritoneal macrophages and ADA activity in guinea pigs.

Guinea pigs were injected intraperitoneally with 20 ml of liquid paraffin. After 4 days, peritoneal exudate cells were collected. Resident and induced macrophages were purified as described previously (13).

ADA activity was assayed by the modified method of Kalcker (9). Briefly, macrophages were suspended at 4°C in 0.15 M sodium phosphate buffer (pH 7.2) and sonicated. The suspension was centrifuged at 1,000 × *g* for 10 min, and the supernatant was immediately used for the ADA assay. The ADA assay reaction was carried out in a cuvette (1-cm path length) which contained 0.2 ml of 1.0 mM adenosine (Sigma Chemical Co., St. Louis, Mo.) in 1.6 ml of 0.15 M sodium phosphate buffer (pH 7.2) equilibrated at 30°C. The reaction was started by the addition of 0.2 ml of cell extract and was followed by recording the decrease in optical density at 265 nm with a double-beam spectrophotometer (Hitachi 556; Hitachi Ltd., Tokyo).

Protein was determined by the method of Lowry et al. (7).

The superoxide (O_2^-) assay was performed by the method of Nakagawara (8). Briefly, 1 ml of a reaction mixture consisting of 2 mM glucose, 1 mM $CaCl_2$, 80 μM ferricytochrome *c*, 5 μg of cytochalasin E, and 2 × 10^5 macrophages was incubated at 37°C for 10 min in a microcell for spectrophotometric analysis. A 50-μg amount of wheat germ agglutinin or 50 μg of antibody equivalent immune complexes was then added, and the reduction of cytochrome *c* was measured continuously with a double-beam spectrophotometer at 550 to 540 nm. The amount of reduced cytochrome *c* was calculated using a molar absorption coefficient of 19,000.

The immune complexes used as stimulants for O_2^- generation were prepared by mixing egg albumin and affinity-purified egg albumin-specific 7S antibodies at a molar ratio of 1:2. The specific antibodies were purified from the sera of hyperimmunized guinea pigs by the method of Wofsy and Burr (12).

Fc receptor was assayed as previously reported (13). Briefly, 3 × 10^6 macrophages were incubated with I^{125}-labeled antigen-antibody (Ag-Ab) complexes (7 μg of antibody equivalent; 136,000 cpm) for 2 h at 4°C with continuous rotation. The cells were then washed two times with buffer, and the cell-bound radioactivity was counted in a gamma scintillation counter. The I^{125}-labeled Ag-Ab complexes were prepared by mixing egg albumin and I^{125}-labeled anti-egg albumin antibodies at a molar ratio of 2:1. The chloramine T method was used for the I^{125} labeling of the antibodies.

As shown in Table 1, macrophages induced in vivo by liquid paraffin injection contained two-

fold as much total measurable ADA activity as the resident macrophages. However, the specific activity of the enzyme remained at about the same level for the two groups because of a 90% increase in intracellular protein content after induction (0.458 mg versus 0.887 mg per 10^7 resident versus induced macrophages).

The effect of coformycin (CFM), a potent inhibitor of ADA (10), on macrophages induced by liquid paraffin injection was examined. Guinea pigs were injected with paraffin on day 0. On the same day and 3 days thereafter CFM (250 μg per kg of body weight) was injected intraperitoneally. Peritoneal exudate cells were collected on day 4 and used as CFM-treated macrophages. Control macrophages were obtained in the same way except that 1 ml of phosphate-buffered saline was used instead of CFM; the cells thus obtained from control animals were used as control macrophages. The total number of exudate cells obtained from control or CFM-treated guinea pigs was about the same. However, the peritoneal exudate cells obtained from CFM-treated guinea pigs contained 20 to 30% polymorphonuclear cells, whereas over 95% of the exudate cells from the control group consisted of macrophages, as confirmed by Giemsa and esterase stainings (Fig. 1).

TABLE 1. *ADA activities and O_2^- generation of resident and induced macrophages*[a]

Macrophage[b]	ADA activity[c]		O_2^- generation (nmol/min per 2 × 10^5 cells)
	Unit	nmol/min per mg of protein	
Resident (5)	5.2 ± 0.71	94.7 ± 22.1	1.6 ± 0.20
Induced (5)	10.3 ± 3.70 ($P < 0.025$)	105 ± 32.0 (NS)	3.0 ± 0.36 ($P < 0.001$)

[a] Results are given as the mean ± standard deviation.
[b] Numbers in parentheses represent the number of individual experiments.
[c] One unit of ADA activity is defined as the amount of enzyme in 10^7 cells that produces a decrease in optical density of 0.01 per min. The P values represent the comparison between the values of resident and induced macrophages. NS, Not significant.

ADA activities, amount of O_2^- generation, and Fc receptor-mediated binding capacities of I^{125}-labeled Ag-Ab complexes (Fig. 2) were compared in CFM-treated macrophages and the control group. For removing polymorphonuclear cells from the exudate cells, cells were separated by centrifugation on Ficoll-Hypaque solution and incubated on a glass dish for 24 h. Thereafter, the adherent cells were collected and used as purified macrophages. Viability of the cells thus obtained was over 95%, and there was no contamination of polymorphonuclear cells. As shown in Fig. 2a, ADA activity was almost completely inhibited by CFM treatment. O_2^- generation, assayed by using the combination of wheat germ agglutinin and cytochalasin E or Ag-Ab complexes as a stimulant, was also inhibited to 25% of the control macrophages (Fig. 2b). This difference in the level of O_2^- generation between CFM-treated and nontreated macrophages was not due to the difference in the amount of immune complexes bound, as both CFM-treated and nontreated control cells bound comparable amounts of I^{125}-labeled Ag-Ab complexes (Fig. 2c). Also, the effect of CFM on O_2^- generation was examined as follows: CFM (500 μg per kg of guinea pig weight) was administered intraperitoneally only once at day 3 after liquid paraffin injection. The peritoneal macrophages, collected on day 4, had no detectable ADA activity; however, O_2^- generation remained at a normal level as compared with that of control cells (data not

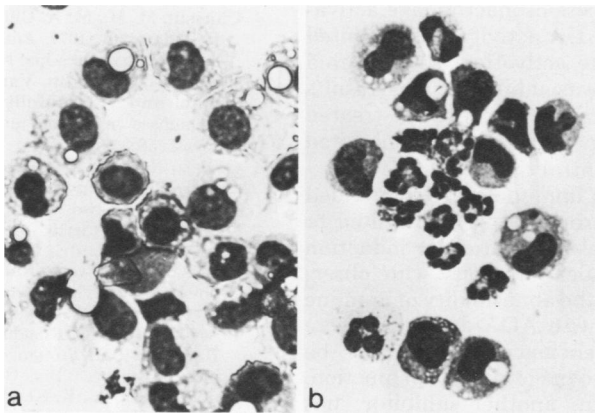

FIG. 1. *Differences in populations of guinea pig peritoneal exudate cells (Giemsa staining of cytocentrifuge preparations, ×400). (a) Control (liquid paraffin injected only); (b) liquid paraffin plus CFM injected.*

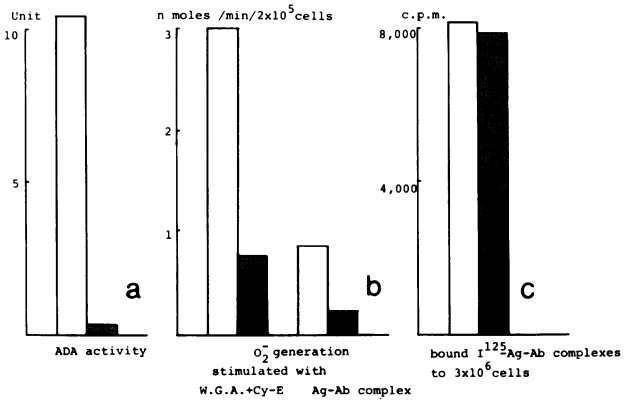

FIG. 2. *Effect of CFM on the activation of guinea pig macrophages. CFM (250 μg per kg body weight) was administered intraperitoneally on days 0, 1, 2, and 3 after liquid paraffin injection, and peritoneal exudate cells were collected on day 4. The combination of wheat germ agglutinin and cytochalasin E (W.G.A. + Cy-E) or 50 μg of antibody equivalent immune complexes was used as a stimulant for O_2^- generation. See Table 1, footnote c, for explanation of ADA activity units. Open columns, controls; shaded columns, CFM-treated macrophages.*

shown).

ADA activity in lymphocytes, erythrocytes, and fibroblasts is either absent or markedly reduced among 30 to 50% of patients with severe combined immunodeficiency disease. This disease is characterized by lymphopenia, thymic aplasia or hypoplasia, and defective proliferative response of lymphocytes to mitogens, combined with defective antibody synthesis (5). The patients usually die at an early age due to infection.

Little is known about monocyte function in patients with ADA-deficient severe combined immunodeficiency disease. To address this problem, we assayed O_2^- generation as a marker of functional activity in peritoneal macrophages obtained from guinea pigs rendered artificially ADA deficient by CFM. All our data shown here suggest that ADA might play an important role in the induction process of macrophage activation because, when ADA activity was inhibited by CFM, macrophage activation, as measured by O_2^- generation, was inhibited. Our results show that O_2^- generation of the CFM-treated peritoneal exudate macrophages was inhibited to 25% that of the control cells.

Recent progress in immunology has revealed that the role of macrophages is not limited to phagocytosis but is also essential for induction of humoral and cellular immunity. Our observations indicate that the abnormality of immune function in patients with ADA-deficient severe combined immunodeficiency disease may be due, at least in part, to macrophage dysfunction.

2′-Deoxycoformycin, another inhibitor, has now been clinically investigated for the treatment of acute lymphoblastic leukemia patients, whose blast cells usually have high ADA activity (11). However, our observations here indicate that caution should be exercised in the clinical use of 2′-deoxycoformycin or CFM until their full effect on the immune system has been adequately investigated.

We thank H. Umezawa and T. Takeuchi for their gift of coformycin and K. Onoue and H. Okano for reviewing the manuscript.

This study was supported in part by a Grant-in-Aid for cancer research from the Ministry of Health and Welfare, Japan.

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