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NOTES

Effect of Adrenocorticosteroid on Glucose Metabolism in BCG-Sensitized Alveolar Macrophages

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The increases in oxidation of glucose-*1*-^{14}C and glucose-*6*-^{14}C in BCG-sensitized alveolar macrophages are inhibited by administration of methylprednisolone sodium succinate. Both resting and phagocytizing cells are affected.

Recent evidence suggests that cellular immunity and delayed hypersensitivity may be mediated by common mechanisms (7, 8). Both states are associated with an increase in hexose monophosphate shunt activity (HMPS) in macrophages (1, 5). The HMPS activity of macrophages is increased in the presence of purified macrophage-inhibiting factor (MIF) and this increment correlates with enhanced cellular immunity (7). We recently observed that adrenocorticosteroid prevents the formation of MIF in lymphocytes of rabbits sensitized with BCG (W. B. Casey and C. E. McCall, Immunology, *in press*). Since adrenocorticosteroid profoundly suppresses both delayed hypersensitivity and cellular immunity, we have studied the effects of adrenocorticosteroid on glucose oxidation in pulmonary alveolar macrophages which have been collected from rabbits sensitized with BCG.

Three groups of New Zealand White rabbits weighing 1.5 to 2.0 kg were used: (i) nonsensitized, untreated animals; (ii) sensitized rabbits, not treated with adrenocorticosteroid; and (iii) sensitized rabbits, treated with adrenocorticosteroid, beginning on the day of sensitization. The adrenocorticosteroid used was methylprednisolone sodium succinate (supplied as Solu-Medrol by the Upjohn Co., Kalamazoo, Mich.), which was administered in daily intramuscular doses of 3.5 mg/kg. This dose effectively blocks the adaptation to delayed hypersensitivity (Casey and McCall, *in press*). Rabbits were sensitized by injecting 100 μg of heat-killed BCG in Bayol F into an ear vein. This preparation resulted in the formation of extensive granulomata in the lung. Cells were then collected after at least 14 days by the method of Myrvik et al. (6) and suspended in Earle's medium 199, and the total and differential counts were performed in a white cell-counting chamber by phase microscopy. Cell viability was determined by staining with 1% Trypan Blue dye.

Glucose oxidation via the Krebs cycle and the HMPS was estimated by methods previously described (2), employing glucose differentially labeled at the C-1 or C-6 position. Studies were performed before and after phagocytosis of latex particles (Difco) 0.8 μm in diameter. Reactions were initiated by addition of 0.15 ml of latex particles to a cell suspension containing 5×10^6 macrophages and allowed to proceed for 1 hr at 37 C. Cell suspensions were adjusted to a constant number of viable macrophages to give a comparable number of phagocytes. Lymphocytes were not removed.

The evolution of $^{14}CO_2$ from the metabolism of glucose-*6*-^{14}C and glucose-*1*-^{14}C in resting and phagocytizing cells is compared in three experimental groups in Table 1. Results are expressed as the mean in counts per minute per 5×10^6 macrophages. Glucose-*6*-^{14}C and glucose-*1*-^{14}C oxidation were significantly increased in both the nonphagocytizing and the phagocytizing cells collected from BCG-immunized, untreated rabbits when compared with nonimmunized controls ($P < 0.01$).

When adrenocorticosteroid was administered to BCG-immunized rabbits, these increases in glucose oxidation were not observed, and the values were not statistically different from those of control cells. When results from resting cells are compared with those obtained during phago-

TABLE 1. *Effect of adrenocorticosteroid on $^{14}CO_2$ production from glucose-6-^{14}C and glucose-1-^{14}C in resting and phagocytizing rabbit alveolar macrophages*[a]

Condition	Glucose-6-^{14}C[a]		Glucose-1-^{14}C[a]	
	Resting	Phagocytizing	Resting	Phagocytizing
Control (3)[b]	163 ± 4	233 ± 6	357 ± 29	682 ± 121
BCG, −steroid (4)	321 ± 34	374 ± 24	1518 ± 173	2400 ± 242
BCG, +steroid (4)	196 ± 25	245 ± 44	528 ± 104	566 ± 22

[a] Values are expressed in counts per minute per 5×10^6 alveolar macrophages ± 1 standard error of the mean.
[b] Values in parentheses indicate number of rabbits studied.

TABLE 2. *Differential cell counts of rabbit alveolar exudates*

Condition	Macrophages[a]	Lymphocytes[a]	Neutrophils[a]
Control (3)[b]	88 ± 5	7 ± 2	5 ± 4
BCG, −steroid (4)	69 ± 2	22 ± 3	9 ± 2
BCG, +steroid (4)	66 ± 5	23 ± 4	11 ± 4

[a] Values represent mean per cent ± standard deviation.
[b] Values in parentheses indicate number of rabbits studied.

cytosis, glucose-1-^{14}C oxidation increased in the control group and in the BCG-immunized, nontreated group after phagocytosis ($P < 0.05$). No such increment was observed after phagocytosis by cells from the BCG-immunized, adrenocorticosteroid-treated group. A slight increase in the glucose-6-^{14}C oxidation occurred in each of the three groups after phagocytosis, but the variations were too great to be statistically significant.

Alveolar exudates from control rabbits had fewer lymphocytes than BCG-immunized rabbits, whereas the per cent of macrophages, lymphocytes, and neutrophils was similar in BCG-immunized rabbits either receiving or not receiving adrenocorticosteroid (Table 2). Since cell populations in treated or untreated BCG-immunized rabbits were not significantly different, results cannot be explained by variations in cell populations. No difference in cell viability was present among the three experimental groups.

The mechanism by which adrenocorticosteroid suppresses glucose oxidation is unknown. We have found no effect of these drugs on the activities of hexokinase pyruvate kinase, glucose-6-phosphate dehydrogenase, or 6-phosphogluconate dehydrogenase (*unpublished data*). Mandell reported that adrenocorticosteroid added in vitro to neutrophils inhibits reduced nicotinamide adenine dinucleotide oxidase activity, which could then account for the decrease in HMPS and decreased bactericidal activity (3). Munck observed that, in thymus cell suspension, cortisol produces a block at the level of glucose transport (4). The reduction in the oxidation of glucose-6-^{14}C and glucose-1-^{14}C observed in this study, both in resting and in phagocytizing cells, would support inhibition in the transport of glucose. It is possible that the reduction in increment in HMPS activity observed in phagocytizing macrophages treated with adrenocorticosteroid is caused by inhibition of an enzyme responsible for the respiratory burst associated with the HMPS. For example, adrenocorticosteroid might reduce the availability of nicotinamide adenine dinucleotide phosphate, which is rate limiting for the HMPS, by stabilizing lysosomes which contain reduced nicotinamide dinucleotide phosphate oxidase.

Our data do not establish that the physiological consequences of reduced delayed hypersensitivity and cellular immunity caused by adrenocorticosteroid therapy are the result of alterations in glucose metabolism, but the role of the HMPS in adaptation of macrophages both to delayed hypersensitivity and to cellular immunity should be explored further.

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