Human T-cell function in experimental ascorbic acid deficiency and spontaneous scurvy

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ABSTRACT Studies in animal models suggest that ascorbic acid deficiency impairs T-cell-mediated immunity. We studied five normal volunteers hospitalized on a metabolic unit and consuming a strictly controlled diet deficient in ascorbic acid 1) after a 5-wk control period of ascorbic acid supplementation (75 mg/day) and 2) after a 9-wk period of no supplementation. Three of the subjects were restudied after a 5-wk period of ascorbic acid supplementation after the deficient period. At the end of both control periods ascorbic acid levels in plasma ranged from 0.9 to 1.3 mg/dl and in leukocytes from 19 to 30 μg/10⁶ cells. At the end of the deficient period levels of ascorbic acid in plasma ranged from 0.09 to 0.15 mg/dl and in leukocytes from 6.2 to 10 μg/10⁶ cells, levels at or below those frequently found in frank scurvy. None of the T-cell parameters were ascorbic acid deficiency within one year after the control periods tested including mitogen responsiveness to phytohemagglutinin and percentage of T-cells bearing receptors for IgM (helper cells) and IgG (suppressor cells) was different in the deficient period compared to the control periods. One patient with spontaneous scurvy (plasma ascorbic acid 0.07 mg/dl, leukocyte ascorbic acid 4.9 μg/10⁶ cells) was studied at the time of admission and after vigorous ascorbic acid repletion. All T-cell parameters after repletion were unchanged from admission. We conclude that in man ascorbic acid deficiency, even at the scurbitic level, does not alter T-cell numbers or impair in vitro T-cell function. Am J Clin Nutr 1982;36:127–130.

KEY WORDS T-lymphocytes, cellular immunity, delayed hypersensitivity, suppressor cells, helper cells

Introduction
Even the most affluent Western societies include substantial population groups subclinically deficient in ascorbic acid (1–4), although progression to frank scurvy is unusual. Studies in animal models suggest that this subclinical deficiency results in certain metabolic abnormalities including impaired drug metabolism (5) and altered cholesterol oxidation (6). In addition, normal leukocytes are rich in ascorbate, and this vitamin appears to be essential for some kinds of leukocyte function. Specifically, ascorbate-deficient animal models suffer from altered T-cell-mediated immunity as judged by prolonged skin allograft survival (7), suppressed reactivity to tuberculin (8), and impaired cell-mediated cytotoxicity (9). Antibody-mediated immunity, however, appears unaffected by ascorbate deficiency (10).

In human volunteers, ingestion of 1 to 3 g/day of ascorbate has been shown to double or triple lymphocyte transformation in response to mitogens (11). However, little or no information is available regarding the effects of ascorbate deficiency on leukocyte number or function. Crandon et al. (12) noted a slight reduction in total white blood cell count in a human subject made experimentally scurbitic, but did not evaluate leukocyte function. Because impaired T-cell function in populations subclinically deficient in ascorbate could predispose to both infectious and neoplastic diseases, we undertook a study of this nutritional state in human subjects under carefully controlled conditions. In accordance with the results of animal studies, we

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Received September 18, 1981.
Accepted for publication December 8, 1981.

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focused this investigation on T-lymphocyte function.

Methods

All studies were carried out in accordance with the principles of the Declaration of Helsinki and were approved by the Human Studies Subcommittee of the Minneapolis Veterans Administration Medical Center and the Committee on the Use of Human Subjects in Research of the University of Minnesota. Informed consent was obtained from all subjects before the study. Five paid male volunteers were recruited at random and admitted to the Special Diagnostic and Treatment Unit of the Minneapolis VA Medical Center. All subjects were without disease by previously published criteria (13). Their ages were 31, 50, 55, 56, and 61 yr. Throughout their hospital stays, subjects were fed a controlled diet providing 3 to 4 mg ascorbic acid daily. The diet was composed of normal foods excluding foods containing significant amounts of vitamin C. In order to increase the palatability of the diet, the menu was varied each day of the week and repeated on a weekly basis. The diet was supplemented with ascorbic acid as follows: control period 1 (days 1 to 33), three 25-mg capsules per day; depletion period (days 34 to 96), no supplement; repletion period (days 97 to 102), four 250-mg capsules per day; and control period 2 (days 103 to 138), three 25-mg capsules per day. Ascorbic acid levels in plasma and white blood cells were determined by standard techniques (14, 15) biweekly throughout the entire study. Two of the five subjects left the study after the repletion period.

In the last 3 days of both control periods and the deficient period, 20 to 30 ml heparinized blood were drawn from each volunteer. For each sample T-lymphocytes were purified as described previously (16). Briefly, peripheral blood was subjected to Ficoll-Hypaque and lymphocytes harvested from the interface. The peripheral blood lymphocytes were then rosetted with sheep erythrocytes and subjected to another Ficoll-Hypaque. Rosetted cells were then harvested and the erythrocytes lyzed by brief exposure to sterile distilled water. This procedure routinely resulted in 95 to 98% T-cell purity. A dose response curve of 3H-thymidine incorporation in response to phytohemagglutinin was determined using a 4-day in vitro incubation period (17). In addition, the purified T-cells were examined for the presence of Fc receptors for IgG (T4) or IgM (T8) (18).

Results

Plasma levels of ascorbic acid are depicted graphically in another publication (19). At the end of the first control period these levels ranged from 0.87 to 1.20 mg/dl and at the end of the second control period they ranged from 1.08 to 1.34 mg/dl. Leukocyte ascorbic acid levels at the end of these two control periods ranged from 19.4 to 29.0 µg/10⁶ cells and from 22.2 to 29.5 µg/10⁶ cells, respectively. At the end of the deficient period, plasma ascorbic acid levels ranged from 0.09 to 0.15 mg/dl having been below 0.20 mg/dl for at least 3 wk in all subjects. Leukocyte ascorbic acid levels at the end of the deficient period ranged from 6.2 to 10.0 µg/10⁶ cells. Levels below which signs of frank scurvy appear are 0.2 mg/dl in plasma and 10 µg/10⁶ cells in leukocytes (20). None of these normal control subjects developed signs or symptoms of scurvy.

As shown in Figure 1, percentages of T-cells with the membrane markers T₄ and T₈ were indistinguishable in all three periods. Moreover, mitogen response of purified T-cells to phytohemagglutinin (Fig. 2) during the deficient period was not significantly different from that in either control period. Total absolute lymphocyte and T-cell numbers were also not altered by ascorbate deficiency. In control period 1, deficient period, and control period 2, mean total lymphocyte counts (cells/mm³) were 3805, 3840, and 3960, respectively, while T-cell numbers were 2960, 2915, and 2890, respectively.

Finally, we fortuitously had the opportunity to study a patient admitted to the Minneapolis VA Medical Center with clinical signs of scurvy including gingivitis and follicular hemorrhages. On admission this patient,
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FIG. 2. Incorporation of $^3$H-thymidine by T-cells in response to four doses of phytohemagglutinin (20, 15, 10, and 5 µg/dl). Again subjects were studied after a 5-wk control period ($C_t$), after 9-wk of ascorbic acid deficiency ($Def$), and after a subsequent 5-wk control period ($C_r$).

who was an alcoholic with other nutritional problems as well, had a plasma ascorbate level of 0.07 mg/dl and a leukocyte level of 4.92 µg/10$^9$ cells. One week after supplementation with ascorbic acid, 250 mg four times each day, his plasma ascorbate level had risen to 2.06 mg/dl and his leukocyte level to 59.6 µg/10$^9$ cell. Parameters of in vitro T-lymphocyte function on admission (Table 1) were virtually identical to those after ascorbate repletion, and both were within the normal range of our laboratory.

Discussion

The present study demonstrates that subclinical ascorbic acid deficiency has no effect on in vitro T-cell function in man. Several features of the study design permit this conclusion to be drawn with considerable certainty. First, the entire study was carried out on a metabolic ward with subjects eating only food of controlled composition prepared under the supervision of a dietitian. Second, each subject served as his own control since each was studied during an initial period of ascorbate supplementation followed by a period of ascorbate deficiency. In addition, three subjects were studied in a postdeficiency period of ascorbate supplementation to rule out any hidden effect of prolonged adherence to the study protocol. Third, both plasma and leukocyte ascorbate levels were monitored to assure that the anticipated degree of deficiency or supplementation had actually been achieved.

Finally, we used two sensitive techniques known to reliably reflect certain critical aspects of human T-cell function. Mitogen responsiveness of T-cells to phytohemagglutinin is an established, validated indicator of T-cell involvement in in vivo hypersensitivity (21). T-cell membrane Fc receptors, however, reflect the presence of at least two functional T-cell subsets including helper ($T_h$) and suppressor ($T_s$) activity (18). That neither of these two measurements were changed during the deficiency period suggests that T-cell function was fully intact despite plasma and leukocyte levels of ascorbate at the scorbutic level.

It also seems unlikely that further depletion of ascorbate would impair T-cell function in man since our one subject with frank scurvy had normal mitogen responsiveness and Fc receptor levels that were unaffected by depletion with ascorbate. To be absolutely certain on this point, one would have to study a larger number of subjects with clinical scurvy. However, a positive result in such a study would be difficult to interpret because of the almost inevitable presence of additional nutritional deficiencies.

The authors are grateful to Dr. George Sarosi for referring the patient with scurvy. Members of the nursing and dietetic staff of the Special Diagnostic and Treatment Unit of the Minneapolis Veterans Administration Medical Center provided invaluable assistance in caring for the subjects. Nancy Haas provided skillful technical assistance.
References