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Symposium on
'Micronutrients and the immune response'

Vitamin E and the immune response

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Undernourishment has been suggested to be the most frequent cause of secondary immunodeficiency in man (Chandra *et al.* 1978) and a practical consequence of this is that nutritional deficiency leads to a significantly increased susceptibility to infection. The association of immune deficiency and protein–energy malnutrition is well described and reviewed (Dowd & Heatley, 1984) and there is now increasing awareness as well as published evidence, that individual nutrients may have a major influence on immune function. The number of individual micronutrients reported to have an influence on immune function is ever increasing and includes the elements, zinc, copper, iron, selenium and magnesium, as well as the vitamins, folic acid, B_{12}, C, B_6, A, β-carotene and E. Several of these individual micronutrients will be considered in the present symposium and this communication will examine the evidence in relation to vitamin E.

Since single nutrient deficiency is uncommon in human populations, the majority of studies reported in relation to vitamin E and indeed other micronutrients, have been performed in experimental animals. One is then immediately faced with the unanswered problem of extrapolating results in animals to humans and since many of the studies in animals have looked at the extremes of nutritional state, either gross depletion or supplementation at levels greatly in excess of recommended intakes, extrapolation becomes even more difficult. Added to these difficulties, the complexity of the immune system, involving processes such as mucosal and cell-mediated immunity, complement function, phagocytosis and intracellular killing, further complicates interpretation of results.

Very few studies have been performed on the effect of vitamin E on immune function in man and in earlier studies the numbers of subjects studied have been small and, therefore, it is, as yet, not possible to draw firm conclusions. Also in earlier studies matched control groups were usually not included, making the interpretation of the findings even more difficult. In a retrospective study of 100 apparently healthy subjects over the age of 60 years, a relationship was found between serum vitamin E levels and the incidence of infections, subjects with the highest vitamin E levels (greater than 13·5 mg/l) having the lowest number of infections (Chavance *et al.* 1985). Baehner *et al.* (1977) supplemented three subjects with 1600 mg vitamin E/d and reported improved

neutrophil phagocytosis but decreased bactericidal activity, together with decreased oxidative damage to neutrophils. The decreased bactericidal activity may be explained by the effect of vitamin E on the respiratory burst pathway of neutrophils, since other studies in humans have demonstrated decreased hydrogen peroxide levels in neutrophils following vitamin E supplements (Butterick *et al.* 1983). In a further uncontrolled trial, vitamin E supplementation with 300 mg/day for 3 weeks was reported to decrease bactericidal activity and mitogen-induced lymphocyte proliferation. However, there was no effect on in vivo delayed hypersensitivity response to phytohaemagglutinin (PHA) (Prasad, 1980). In a study describing the effect of vitamin E supplements on T-lymphocytes and their sub-sets, Haberal *et al.* (1988) reported that in burns patients intramuscular vitamin E increased the percentage of T-cells in peripheral blood and that this increase was due to a specific increase in the T-helper-cell sub-set.

More recent well-controlled studies have confirmed that vitamin E supplementation in older subjects does influence immune function. In a recently reported study subjects over the age of 60 were randomized to receive either placebo or 800 mg vitamin E/d for 3 weeks and various aspects of immune status were assessed (Meydani *et al.* 1990). There was a significant improvement in delayed-type hypersensitivity, lymphocyte proliferation response to concanavalin-A (con-A), but not PHA, and interleukin-2 production in reponse to both con-A and PHA, together with decreased prostaglandin E_2 production and plasma lipid peroxide levels. Serum immunoglobulin levels were not altered. Purkins *et al.* (1990), in a study of elderly institutionalized patients, randomly allocated to receive either placebo or vitamin E (100 mg/d for 28 d), showed that in the supplemented patients a significant increase occurred in T-cell numbers and a significant alteration in T-cell sub-sets. There was a 50% increase in T-cell numbers, due almost exclusively to an increase in the T-helper-cell sub-set, the T-cytotoxic sub-set remaining unchanged. This alteration in T-cells was not accompanied by improved mitogenic stimulation to PHA. These same authors, in an earlier randomized controlled study using an antioxidant cocktail (vitamin C 100 mg, vitamin A 2·4 mg, vitamin E 50 mg) for 28 d, demonstrated a similar effect on T-cell numbers and sub-sets and also significantly improved mitogenic response to PHA (Penn *et al.* 1989).

Further clinical trials are required, of longer duration, to establish both the time scale and dosage of vitamin E required maximally to influence immune function. In at-risk malnourished hospital populations isolated vitamin E depletion is unlikely and, therefore, synergism with other nutrients may also have to be considered.

The effect of vitamin E on resistance to infection has been studied in a wide variety of animal species (Table 1). Large doses of vitamin E, much greater than those usually recommended for animals have been used in these situations but favourable responses have generally been reported.

Peripheral blood lymphocytes play a central role in immune response mechanisms and, therefore, it is not surprising that the effect of vitamin E on lymphocyte proliferation in response to mitogenic stimulation has been studied in several animal species, using a variety of mitogens. Both the T-cell mitogens, con-A and phytohaemagglutinin and the B-cell mitogen lipopolysaccharide, have been studied. There are many differences in experimental conditions used by various groups, such as different species of animal, different strains of the same species, age of the animals, supplemental dose of vitamin E used, duration of feeding, laboratory procedures and others, which makes comparison of results difficult (Table 2). However, despite some exceptions, the overall

Table 1. *The effect of vitamin E on the response to infections in experimental animals*

(The range of vitamin E supplement used was 180–360 mg/kg diet)

Species	Infective agent	Response seen
Chicks	*Escherichia coli*	Increased humoral immunity Decreased mortality
Mice	*Diplococcus pneumoniae*	Improved survival Improved phagocytosis Decreased mortality
Guinea-pig	*Mycobacterium tuberculosis*	Increased delayed-type hypersensitivity
Swine	*E. Coli*	Increased antibody titres
Turkey	*E. Coli*	Increased humoral immunity
Cattle	Bovine herpes virus	Increased antibody titre

Table 2. *The effect of vitamin E supplementation on mitogen-stimulated lymphocyte proliferation*

Species	Lymphocyte proliferation	Vitamin E (mg/kg diet)	Reference
Rat	Improved	200	Gabriel *et al.* (1984)
Rat	Improved	150	Eskew *et al.* (1985)
Rat	Improved	50	Bendich *et al.* (1986)
Mice	Improved	50	Corwin & Shloss (1980)
Mice	Improved	500	Meydani *et al.* (1990)
Rat	No effect	500	Ip & White (1987)
Mice	No effect	?*	Lim *et al.* (1981)
Rat	No effect	100	Purkins *et al.* (1990)

* The amount of vitamin E used is unclear but stated to be twenty times that of the control diet.

conclusion is that vitamin E depletion depresses mitogenic proliferation and vitamin E supplementation restores or enhances the response to normal or above, though the dietary intake of vitamin E used is usually greater than the recommended dietary requirement for the rat of 30 mg/kg diet.

Three separate strains of rat were studied by Gabriel *et al.* (1984): Sprague-Dawley, Wistar-Kyoto and a spontaneously-hypertensive Wistar strain. The latter strain was more sensitive to the development of vitamin E deficiency, though all strains showed decreased body-weight, myopathy, testicular degeneration and splenomegaly. After 17 weeks receiving a deficient diet all three strains had depressed lymphocyte proliferation to the T-cell mitogens con-A and phytohaemagglutinin and the B-cell mitogen lipopolysaccharide from *Escherichia coli*. Supplementation with vitamin E at 200 mg/kg resulted in significant increase in lymphocyte proliferation to all three mitogens in the Wistar-Kyoto and spontaneously hypertensive rats. However, in the Sprague-Dawley strain the improvement in proliferation to con-A did not reach significance. Further work by the same group suggested that in rats (spontaneously hypertensive strain), supplementation with 50 mg vitamin E/kg diet produced optimal mitogenic response with no further improvement at 200 or 1000 mg/kg (Bendich *et al.* 1986). The effect of vitamin E

deficiency and vitamin E supplementation has been confirmed by other workers in both rats (Eskew *et al.* 1985) and mice (Corwin & Shloss, 1980; Meydani *et al.* 1990). Bendich *et al.* (1986) suggested that the vitamin E requirement of rats should be higher if it was based on lymphocyte proliferation than on other more traditional indicators of deficiency, such as peroxidative haemolysis of erythrocytes or indicators of muscle degeneration. However, further studies will be required before such recommendations can be made, particularly since some studies have shown that pharmacological doses of the vitamin may have a deleterious effect on some aspects of immune function (Yasunaga *et al.* 1982; Lim *et al.* 1981).

Phagocytosis and ingestion by polymorphonuclear leucocytes and neutrophil chemotaxis have been studied in relation to vitamin E status. In children with chronic cholestasis, neutrophil chemotaxis is depressed and returns to normal following prolonged vitamin E therapy. The authors suggest that vitamin E deficiency may impair neutrophil function in such patients and, thus, increase the risk of infection (Sokol *et al.* 1984). Supplementation of healthy young men with 300 mg vitamin E/d depressed the bactericidal activity of leucocytes (Prasad, 1980). It has also been stated that prolonged ingestion of high dose, 1600 mg/d, of α-tocopherol can lead to selective inhibition of polymorphonuclear leucocyte phagocytosis and indicated that the vitamin may need to be administered in moderation (Boxer, 1986). Premature babies have a decreased ability for chemotaxis, phagocytosis and bactericidal activity compared with adult controls and maturation of this aspect of the immune system has been shown to occur more rapidly in babies given vitamin E (Chirico *et al.* 1983).

A patient with glutathione peroxidase (*EC* 1.11.1.9) deficiency has been described, whose granulocytes showed depressed ability to kill bacteria, despite normal phagocytosis and increased hydrogen peroxide production. A 3-month course of vitamin E (400 mg/d) resulted in reversal of these abnormalities (Boxer *et al.* 1979). Polymorphonuclear leucocytes from normal adults when incubated in vitro in the presence and absence of vitamin E showed decreased superoxide production with high doses of the vitamin (100 mg/l) but not with lower levels (Engle *et al.* 1988). The authors suggest that this may help to explain, at least in part, the observations of others (Johnson *et al.* 1985), that low-birth-weight infants maintained at pharmacological levels of vitamin E are at increased risk of developing sepsis and necrotizing enterocolitis.

In both rabbits and rats vitamin E depletion has also been demonstrated to influence neutrophil function. Using Sprague-Dawley rats Harris *et al.* (1980) showed that neutrophil chemotaxis and ingestion were depressed in leucocytes from vitamin E-deficient rats and these abnormalities could be increased by administration of vitamin E. In rabbits the return of neutrophils to the circulation after chemotactic challenge was promoted by vitamin E, the suggested mechanism being reduced adherence to endothelial tissues (Lafuse *et al.* 1983).

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