Effects of Lycopene and Lutein Supplementation on the Expression of Functionally Associated Surface Molecules on Blood Monocytes from Healthy Male Nonsmokers

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It has been suggested that dietary carotenoids can enhance immune function. Supplementation with β-carotene (15 mg daily) was previously shown to enhance human monocyte function. To examine the effect of other dietary carotenoids, two similar independent studies were done. Healthy adult male nonsmokers were randomly assigned to receive lycopene (study 1), lutein (study 2), or placebo for 26 days, followed by the alternative treatment for another 26 days. The expression of functionally related monocyte surface molecules was quantified by laser flow cytometry before and after each treatment period. There was a significant increase in plasma levels of each carotenoid following dietary supplementation, but the effects on monocyte surface molecule expression were not as striking as those observed after β-carotene supplementation. These findings emphasize that it cannot be assumed that the effect of one carotenoid will be the same as another, even at the same level of intake.

The carotenoids are a group of over 600 naturally occurring colored pigments in plants. Of these, about 24 are common in human foods. These compounds serve two major purposes in plants: as accessory pigments in photosynthesis and in photoprotection. The poylene structure of these carotenoids allows them to absorb light and to quench singlet oxygen and free radicals. There is increasing evidence that dietary components that possess antioxidant properties can help protect the immune system from oxidative damage and thereby enhance cell-mediated immune responses [1, 2]. It is thought that an antioxidant-rich diet can be particularly beneficial in the elderly, possibly restoring immune function to that found in younger persons [3], thereby increasing resistance to infection [4] and tumor formation.

Numerous epidemiologic studies have shown a strong inverse association between the intake of fruit and vegetables and the incidence of cancer [5]. Among the many compounds in these foodstuffs that might possess anticarcinogenic properties, attention has focused on the carotenoids, a group of highly pigmented, fat-soluble antioxidants. One of these compounds, β-carotene, which is present in carrots, broccoli, and other green-leaved vegetables, has had much attention [6] and is effective in preventing cancer in animal models [7]. Other carotenoids, including lycopene (found in high concentrations in tomatoes) and lutein (found in spinach, peas, and watercress) have received less attention but are also associated with a reduced incidence of prostate cancer [8] and lung cancer [9], respectively. However, the precise mechanisms by which these compounds can modulate immune function and thus help to protect against infection and tumor development remain uncertain.

Cell-mediated immune responses are initiated by the stimulation of appropriate T lymphocytes by antigen-presenting cells [10, 11]. A prerequisite for this antigen-presenting cell function is the expression of major histocompatibility complex (MHC) class II molecules (HLA-DR, HLA-DP, and HLA-DQ) [11], which are present on the majority of human monocytes, macrophages, and dendritic cells. Since a person’s degree of immune responsiveness is proportional to both the percentage of MHC class II-positive monocytes and the density of these molecules on the cell surface [12], it is possible that one mechanism by which carotenoids may enhance cell-mediated immune responses is by enhancing the cell surface expression of these molecules. In addition, cell-to-cell adhesion appears to be critical for the initiation of a primary immune response, and the intercellular adhesion molecule (ICAM)-1–leukocyte function–associated antigen (LFA)-1 ligand receptor pair is also capable of costimulating an immune response [13] and of enhancing T cell proliferation and cytokine production.

We previously reported that 4 weeks of supplementation with β-carotene, at a dietary achievable intake of 15 mg/day (equivalent to the average amount present in 150 g of carrots), can elevate the expression of several of these molecules on the surface of blood monocytes in healthy male nonsmokers [14]. In particular, there were significant increases in the percentage of monocytes expressing the MHC class II molecule, HLA-DR,
and the adhesion molecules, ICAM-1 and LFA-3, suggesting that this carotenoid can enhance cell-mediated immune responses within a relatively short time. Given the lack of information on the effect of other dietary carotenoids on immune function, we examined the effects of lycopene, the most common carotenoid in the US diet [15], and lutein on monocyte surface molecule expression in two independent trials. We used the same study design and level of supplementation as in our β-carotene study.

Materials and Methods

Volunteers and study design. Participants were asked not to volunteer for the study if they were providing blood samples for another study or were undergoing any other dietary manipulation. Eligible subjects were healthy male nonsmokers, 18±60 years old, who were not taking regular medication or vitamin supplements, and who were not vegetarian, anemic, or diabetic, and did not consume >280 mL of alcohol per week. All had body mass indexes (BMIs) in kg/m² of <35.

For the lycopene and lutein supplementation studies, respectively, we recruited 23 (age range, 25–58 years [mean, 41]; BMI range, 19.1–31.3 [mean, 25.8]) and 21 (age range, 26–56 years [mean, 39]; BMI range, 20.7–31.6 [mean 25.2]) participants, who were randomly assigned to two groups. Knowledge of the allocated “treatment group” was limited to the study organizer, who took no part in the sample analysis.

After anthropometric measurements were recorded, baseline fasting (12-h) blood samples were taken from all participants. Participants were then immediately commenced on one capsule per day for 26 days (group 1, placebo; group 2, lycopene [study 1] or lutein [study 2]) to supplement their otherwise unchanged diets. Lycopene-rich tomato extract (85% trans-, 15% cis-lycopene) was a gift of LycoRed (Beer-Sheva, Israel); lutein-rich marigold extract (79% trans, 21% cis-lutein) was a gift of Quest International (Cork, Ireland). Lycopene and lutein (500 mg fill weight; 15 mg of carotenoid; common to all leukocytes; Serotec), was used as a positive control.

Statistical analysis. Although the trials were originally designed as full cross-over studies, the carotenoid concentration remained elevated during the placebo period in subjects who had placebo treatment followed by carotenoid supplementation (vol-
unters in group 2 of each study). For this reason, placebo data in the second period of the cross-over studies (from volunteers in group 2) were discarded. Values after treatment (placebo or supplementation) were compared with before-treatment values using Student’s paired t test [19].

Results

Subjects. Volunteers for each of the supplementation studies shared similar descriptive characteristics in terms of age, BMI, and hemoglobin and blood glucose levels (data not shown; details in [20]).

Plasma carotenoid concentration. Compliance was confirmed by the significant elevation in plasma concentrations of lycopene or lutein following 26 days of supplementation (table 1). For comparison, the plasma concentrations of β-carotene recorded in the subjects in our previous study [14] are also shown. Of interest, the increase in plasma concentrations of lutein following supplementation was as striking (although not as high) as that obtained with the same level of β-carotene (about a five-fold increase), while there was only a 63% increase in the mean plasma concentration of lycopene. There were no significant changes in the levels of other carotenoids, ascorbic acid, α-tocopherol, or retinol after either supplementation period (data not shown).

Monocyte surface molecule expression. There was a significant increase in the percentage of monocytes expressing the major MHC class II molecule, HLA-DR, following lycopene supplementation (table 2), but there were no significant changes in the other MHC class II molecules in the adhesion molecules examined. In addition, no significant changes were seen following lutein supplementation (table 2), although due to a technical problem, LFA-3 expression was not evaluated in this study. There were no significant changes in the relative number of MHC class II molecules expressed on blood monocytes following lycopene supplementation, but there was a significant increase in LFA-1 expression (table 3). In contrast, the only significant difference seen in the lutein study was a decrease in the expression of HLA-DQ (table 3).

Discussion

We previously showed that supplementation over 26 days with a dietary achievable level of β-carotene (15 mg/day) can enhance the expression of several functionally associated surface molecules on human blood monocytes [14], suggesting that this carotenoid might enhance cell-mediated immunity by modulating the function of antigen-presenting cells, a crucial factor in the initiation of an immune response to both pathogenic infections and neoplastic cells. To investigate whether this effect of β-carotene was common to other dietary carotenoids, we undertook two further studies. Both studies had the same design and level of supplementation: dietary enrichment with lycopene (study 1) and with lutein (study 2). The only common effect we observed between either of these studies and our previous trial with β-carotene was an increase in the expression of HLA-DR following supplementation with lycopene. In contrast to β-carotene, no changes were observed in the expression of ICAM-1 or LFA-3 in either study; however, there was a significant increase in the expression of LFA-1 following lycopene supplementation, which was not seen after either β-carotene or lutein supplementation.

The less striking effect of either lycopene or lutein supplementation on monocyte surface marker expression than seen after β-carotene supplementation might be related to the lower plasma levels found after supplementation. We previously observed a possible threshold effect of plasma β-carotene concentration on the expression of ICAM-1 and LFA-3 [14], and it is possible that the plasma levels of lycopene or lutein achieved in our studies were not high enough to cause a significant change in the expression of most of the monocyte surface molecules examined. The reason for the difference in plasma levels of these carotenoids following the same level of supplementation is uncertain but could reflect differences in their uptake, metabolism, and excretion or the selective sequestration of different carotenoids to specific sites in the body. It is unlikely that absorption of lycopene from the supplements provided is a problem, since the bioavailability of lycopene from tomato juice and dietary supplements is very similar [21]. However, lycopene is found in higher concentrations in the prostate [22] than in serum, which might contribute to the reduced prostate cancer risk associated with the consumption of tomato-based foods [23]. It is possible that not all beneficial effects bestowed by carotenoids are observed systemically, instead being found only at specific locations in the body.

We know of only one other group that has reported the comparative effects of dietary enrichment with different carotenoids on human immune function, and that group also found con-
Lutein study

Supplement (n = 20)

Placebo (n = 10)

Lutein study

Supplement (n = 20)

Placebo (n = 10)

Lycopene study

Supplement (n = 20)

Placebo (n = 10)

Table 2. Percentage of monocytes expressing each surface molecule.

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<tr>
<td>LFA-1</td>
<td>22.01 (3.00)</td>
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<td>LFA-3</td>
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<td>52.93 (11.79)</td>
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<td>16.57 (2.82)</td>
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<td>HLA-DR</td>
<td>78.32 (4.18)</td>
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<tr>
<td>HLA-DQ</td>
<td>22.01 (3.00)</td>
<td>18.41 (5.13)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>22.54 (5.10)</td>
<td>24.42 (6.79)</td>
</tr>
<tr>
<td>LFA-1</td>
<td>86.81 (3.85)</td>
<td>67.04 (9.53)</td>
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<tr>
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<td>52.92 (7.94)</td>
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NOTE. Data are mean ± (SEM). ND, not determined.

Table 3. Relative median intensity of fluorescence of each monocyte surface molecule.

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Contrasting effects. After a 2-week increase in tomato juice consumption (providing 40 mg of lycopene/day), Watzl et al. [24] observed a significant reduction in lymphocyte proliferation to mitogenic stimulation, which recovered following the subsequent 2-week period of β-carotene enrichment (carrot juice provided 22 mg of β-carotene/day). However, after another 2 weeks of dietary enrichment with lutein (spinach powder provided 11 mg/day of lutein), the proliferative response was again significantly suppressed compared with baseline. These findings emphasize that different carotenoids can affect immune function in different ways. The possibility of an interactive or additive effect of different carotenoids on immune function is also suggested by the results of a study undertaken in a group of premenopausal women. Following dietary depletion of carotenoids, mitogen-stimulated lymphocyte proliferation was reduced compared with baseline. This suppression was not corrected with low-level β-carotene supplementation (0.5 mg/day) but was corrected with a carotenoid complex made from vegetables rich in carotenoids [25]. Therefore, in fruits and vegetables, the influence of the combination of carotenoids they contain on immune function may represent the sum total of different effects and synergistic effects within these combinations [26].

There are several potential mechanisms for an effect of carotenoids on the immune system. It is possible that their ability to quench singlet oxygen (an energized form of oxygen capable of generating free radicals) might lower the free radical burden and protect membrane lipids from peroxidation. Free radicals suppress the expression of the major MHC class II molecule, HLA-DR [27], and we recently showed that dietary supplementation with fish oil containing n-3 polyunsaturated fatty acids (PUFAs), which are more vulnerable to lipid peroxidation than saturated fatty acids, inhibits the expression of HLA-DR and ICAM-1 on human monocytes [18]. However, although there were changes in plasma fatty acid composition associated with the different supplements, there was no evidence that enriching the diets with these carotenoids engenders antioxidant effects that lead to a widespread conservation of plasma PUFAs [20]. Nevertheless, it is possible that the antioxidant properties of carotenoids might still act to modulate immune cell function by modulating the activity of redox-sensitive transcription factors.

It is now appreciated that the transcription factor nuclear factor-κB (NF-κB) is required for maximal transcription of many inflammatory cytokines and adhesion molecules, and it is thought that the generation of reactive oxygen species is a vital link in mediating NF-κB activation by a variety of stimuli [28, 29]. Therefore, it is possible that antioxidant nutrients can influence a variety of inflammatory processes by inhibiting NF-κB activity. If so, this might explain the decrease in surface expression of LFA-1 on monocytes following dietary supplementation with lutein, but the opposite effect following β-carotene supplementation cannot be explained by this mode of action. The divergent effects seen with different carotenoids might be explained by the preferred location of these compounds within the cell. Carotenoids are lipid soluble and thus will be concentrated in the lipid-rich membranes of the cell.
However, their exact location may influence their effectiveness in modulating specific cellular events. To date, little work has been undertaken to explore the distribution of individual carotenoids within mammalian cells.

Changes in eicosanoid production can also influence the expression of cell membrane molecules. Prostaglandin (PG) E2, the major eicosanoid synthesized by monocytes and macrophages, possesses a number of immunosuppressive properties. For instance, it can inhibit the expression of Ia molecules (the murine equivalent of human MHC class II molecules) on stimulated macrophages [30]. It has been suggested that one mechanism by which β-carotene might enhance immune responses is by altering the activation of the arachidonic acid cascade (from which PGE2 is derived), since β-carotene suppresses the generation of arachidonic acid products in vitro from nonlymphoid tissues [31]. The possibility that other carotenoids can modulate the arachidonic acid pathway has not yet been investigated.

In summary, we found that 26-day supplementation with lycopene or lutein, at a dietary achievable level, did not produce as striking an effect on the expression of peripheral blood monocyte surface molecules as we observed following β-carotene supplementation. Thus, if enhancement of these surface molecules is a mechanism by which antioxidant nutrients can enhance immune responsiveness and consequently defense against infection and tumor development, our results suggest that β-carotene is more effective than lycopene or lutein. However, the effects of different levels of intake of these compounds and potential synergistic effects with each other and/or other nutrients present in fruits and vegetables remain to be determined.

Acknowledgments

We thank the volunteers from the Norfolk Fire Service Headquarters, Hethersett, and the Institute of Food Research, Norwich, for taking part in these studies.

References