Nutrition and the Aging Immune Response

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Dysregulation of immune function with advancing age has been well documented [1–3]. Age-related dysregulation of the immune system contributes to the increased incidence of infectious, inflammatory, and neoplastic diseases observed in the elderly as well as to their extended post-illness recovery periods. Some of the diseases more frequently seen in the aged reflect the deterioration of immune function in this population. These include but are not limited to: upper respiratory infections; tuberculosis; shingles; autoimmunity, and neoplastic diseases. Furthermore, studies suggest that elderly individuals with low delayed-type hypersensitivity skin test (DTH) responses, an \textit{in vivo} measure of cellular immunity, are less self-sufficient and have a higher incidence of morbidity, postoperative complications, and mortality compared to those who have normal DTH responses [4–6].

Immune responses are categorized as innate or acquired according to the nature of the immune cells that respond to the insult. The innate or the nonspecific response is provided by phagocytic cells – macrophages and neutrophils – and natural killer (NK) cells as the first line of defense against the many common pathogens. The innate response involves immune surveillance and killing mechanisms, which do not require a previous encounter of these immune cells with the pathogen. On the other hand, acquired immunity necessitates a previous encounter with the invading pathogen for the
Table 1. Age-associated changes in immune function

<table>
<thead>
<tr>
<th>Macrophages</th>
<th>T cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑PGE₂ production</td>
<td>Thymic involution</td>
<td>↓Antibody production</td>
</tr>
<tr>
<td>↑NO production</td>
<td>↑Memory/Naive T cell ratio</td>
<td>↑Auto-antibody production</td>
</tr>
<tr>
<td></td>
<td>↓Delayed-type hypersensitivity response</td>
<td>↓Natural killer cell activity</td>
</tr>
<tr>
<td></td>
<td>↓In vitro T cell proliferation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓IL-2 production</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓Response to IL-2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Altered Th₁ vs. Th₂ cytokine profile</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓Cytotoxic T cell activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓Mobilization of intracellular calcium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓Early activation signals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Altered expression of cell surface molecules</td>
<td></td>
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<td></td>
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</tbody>
</table>

priming of a very specific immune response. Cells of the immune system that participate in acquired immunity include B cells, T cells, and macrophages. Upon encounter with an antigen, B cells develop into plasma cells that secrete their B cell receptors as antibodies. T cells, however, have a receptor that is not secreted but when bound to an antigen peptide in the context of a self-major histocompatibility molecule on an antigen-presenting cell initiates an intracellular signal that among other things results in T cell activation and the production and secretion of various cytokines and growth factors. These cytokines and growth factors provide regulatory signals for other cells of the immune system as well as T cells to react to the pathogen and thus result in enhanced host response. Therefore, T cells play a central role in adaptive immunity as well as providing secreted factors that regulate immune cells of both the adaptive and the innate immune responses.

The decline in immune response with age (Table 1) has been shown to coincide with a decrease in in vitro mitogen-activated T cell proliferation and the production of and responsiveness to interleukin 2 (IL-2), a major T cell growth factor that regulates clonal expansion of T cells during the immune response [7]. Many factors have been described as contributing to the age-related decline in T cell function. These include changes in the ability of T cells to receive and respond to activation signals and phenotypic changes within the T cell population [8–11]. One of the hallmarks of age-related changes that take place in the T cell compartment of the immune system is a gradual shift toward greater proportions of antigen-experienced memory
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T cells with fewer T cells of the virgin or naive phenotype. This phenotypic change contributes to reduced ability of the elderly to mount an effective immune response to new antigens. Age-related changes in the cytokine profile of effector T cells have also been shown to occur. The activation of a naive T cell upon initial encounter with an antigen on the surface of an antigen-presenting cell results in the production of great amounts of the IL-2 as well as the expression of high affinity IL-2 receptors (IL-2R). This drives clonal expansion of responding T cells and differentiation into either interferon-γ (IFN-γ) and IL-2 producing T helper 1 (Th1) or T helper 2 (Th2) cells that produce IL-4, IL-5, and IL-10. Reports indicate an age-related decrease in the production of Th1 cytokines with increased Th2 cytokines [12]. Furthermore, increased production of T cell suppressive factor prostaglandin E2 (PGE₂) by macrophages in the aged has been reported to contribute to the dysregulation of T cell function in these subjects [13, 14]. No clear consensus exists on the age-related changes in natural killer (NK) activity. Reviews on NK activity and aging summarize reports on decreases, no change, and increases in NK activity in the aged [15–17]. These differences in results can conceivably be explained by differences in sample sizes, criteria used for the inclusion of ‘healthy elderly’, age ranges used, as well as the ever present possibility of contamination by other cell types [18].

Some decline in B cell function has also been observed, particularly the loss of high-affinity cell surface receptors for antigen and for cytokines [19]. Proliferation of B cells is fairly well maintained; however, old animals show impaired responses to foreign antigens that activate CD5⁻ B cells. On the other hand, the response of CD5⁺ B cells to autoantigens remains intact [20]. Thus, while the ability to respond to foreign antigens declines with age, the autoantibody response increases, which may be a contributing factor to the age-related increase in autoimmune diseases [21–23].

The role nutrients play in immunological aging has been given increased attention over the past two decades. Malnutrition is a significant problem amongst the elderly due to a multitude of factors such as limited income, limited food preferences, and overall health status resulting from chronic disease. These problems are even greater in the developing world where poverty is widespread and access to food may be severely limited. Intakes below the recommended dietary allowance (RDA) by the elderly have been reported for zinc and vitamins E, C, and B₆ [24]. Infections are more common among the elderly, particularly when they are malnourished [25]. Linn and Jensen [26] studied the prevalence of malnutrition in young (<65 years) and old (>65 years) outpatients of an ambulatory care unit, excluding those with infection, autoimmune diseases, and other major medical conditions. Malnutrition was more prevalent in the older than in the younger group with malnourished in both age groups showing decreased immune measures. Additionally, Chandra and Puri [27] showed that nutritional supplementation of 30 malnourished elderly (70–84 years) men improved their antibody
response to influenza vaccine. Consequently, micronutrient and macronutrient supplementation in the frail and healthy elderly has been considered to improve immune function and health [28–30].

Several micronutrients have been shown to play a regulatory role in the immune response. Deficiency of some of these nutrients is associated with impairments in T cell-mediated function, similar to those observed in the elderly. In a landmark study by Chandra [31], the immunomodulating and clinical effects of a 1-year multivitamin/mineral supplementation at RDA levels; except for vitamin E and β-carotene (as provitamin A), which were given at levels four times greater than the RDA, were examined in 96 men and woman over the age of 65 years. This was a randomized, double-blind, placebo-controlled trial with subjects that had no known chronic or serious illnesses or were taking medications known to alter immunological or nutritional status. One year after supplementation had begun, significant reductions in deficiencies of vitamin A, β-carotene, vitamin B₆, vitamin C, iron, and zinc were seen among those taking the supplement with no change among those taking placebo. There was a significant increase in total T cells as well as CD₃⁺IL-2Rα⁺CD₄⁺ T cell subsets and NK cells in the supplemented group. Lymphocyte function was also improved with increased T cell proliferation in response to phytohemagglutinin A (PHA), IL-2 production, IL-2R release, NK activity, and B cell response to influenza vaccination. Lesourd [32] studied the effects of protein-calorie undernutrition on a group of healthy young adults (20–50 years), healthy elderly (78.7 ± 7 years), and healthy elderly (79.4 ± 7.5 years) with low nutritional status as indicated by serum albumin levels of between 30 and 35 g/l. Several indices of immune response were measured. The authors noted that, while certain measures of immune response (percent CD₃⁺ cells, T cell mitogenic response, IL-2 production, and B cell response to influenza vaccine) were lower in both elderly groups compared to the young, other measures (percent CD₄⁺ cells and DTH responses) were lower only in the undernourished elderly group compared to the young. Moreover, in almost all measures of immune function, the age-related differences were more pronounced in the undernourished group. In the same study, the magnitude of decrease in nutritional status, as determined by serum albumin levels, was an important factor in determining the immune response in elderly. It is important to note that the low serum albumin levels in these studies reflected low nutritional status rather than the presence of disease. Hence it was demonstrated that undernutrition contributes to the decline in immune response with aging. This is also supported by the observation that undernourished elderly who were supplemented with 500 kcal/day ready-to-use complete nutritional supplement, had a better immune response compared to undernourished elderly not receiving supplements. These observations, however, do not provide information on how much of the effect is due to protein-calorie undernutrition and how much is due to micronutrient deficiencies present under these conditions.
It is recognized that the immune response in the aged responds to certain types of fatty acids. Changes in the dietary composition of fatty acids modulate membrane phospholipid fatty acid composition [33], resulting in modification of eicosanoids, the oxygenated products of arachidonic acid (AA) [34, 35]. Eicosanoids generated from AA are the most abundant and frequently the most active lipid mediators. Polyunsaturated fatty acids (PUFAs) of the n-3 series can interfere with AA metabolism at the cyclooxygenase (COX) and lipoxygenase levels. Several AA metabolites, including prostaglandin (PG), leukotriene, and hydroxyeicosatetraenoic acid, are produced by immune cells in response to various activators. Generally, cellular and humoral immune responses are negatively regulated by COX products. For example, PGE2 inhibits lymphocyte proliferation [36], production of IL-2 [37], the generation of cytotoxic cells [38], and NK activity [39]. Because PGE2 production has been shown to increase with age [14] and contribute to the age-related decrease in T cell proliferation and IL-2 production [13], the effect of n-3 PUFA supplementation on inflammatory cytokine production and T cell-mediated function of healthy young and elderly subjects was evaluated [40]. Supplementation with n-3 PUFA for 3 months resulted in a greater increase in plasma levels of eicosapentaenoic and docosahexaenoic acid in old compared to young. In addition, n-3 PUFA supplementation decreased AA in old but not in young subjects. The production of IL-1β, TNF-α, and IL-6 was reduced in n-3 PUFA-supplemented subjects, with a greater effect in old compared to young subjects. The production of IL-2 was lower in old subjects compared to young, which was further decreased with n-3 PUFA supplementation in the old.

Black currant seed oil (BCSO) is rich in both α- and γ-linolenic acids and has been shown to modulate eicosanoid production and membrane lipid composition. A 2-month randomized, double-blind, placebo-controlled trial studying the immunologic effects of BCSO in 40 healthy elderly subjects (>65 years old) was recently conducted [41]. Supplementation with BCSO resulted in an increased DTH response and reduced PGE2 production compared to that of baseline or placebo control. However, there were no significant effects on T cell proliferation or IL-2 production in the BCSO-supplemented group. Conjugated linoleic acid (CLA) has been shown to have immunomodulating properties. Hayek et al. [42] investigated the effect of 1% dietary CLA for 8 weeks on a series of immune measures in young (4 months) and old (22 months) mice. Supplementation with CLA increased all CLA isomers in hepatic neutral lipids and phospholipids. Young mice fed CLA had increased splenocyte proliferation in response to concanavalin A (Con A) and PHA compared to that of control fed mice. Old CLA-fed mice had a greater proliferative response to Con A and IL-2 production compared to old control mice. There was no effect of CLA on NK activity, production of PGE2, or DTH response in young or old mice.
The effects of mineral and vitamin supplementation on the immune response of the elderly have been examined in several studies [24], although interpretation of results has been hampered by study design flaws, such as too few subjects, inadequate characterization of health status of subjects, lack of placebo-treated control groups, and the use of immune measures that do not change with age. More recently however, studies have been conducted which strongly suggest that the immunological status of the elderly can be improved by supplementation with single or multiple vitamins and minerals (Table 2).

Selenium (Se) has been shown to be an essential nutrient for the maintenance of the immune response. Recent animal studies have indicated that Se supplementation may improve the immune response in the aged. Old mice supplemented with 2 ppm Se for 8 weeks had higher mitogenic response to PHA and cytolytic T cell activity against malignant cells [43]. This effect of Se was not due to increases in IL-1, IL-2, or IFN-γ production but was related to the ability of Se to enhance the expression of IL-2Rα and/or β subunits on activated T cells. A double-blind, placebo-controlled trial of Se supplementation (100 µg/day) in institutionalized elderly (78 years old) for 6 months resulted in significantly greater lymphocyte proliferation in response to pokeweed mitogen, a B cell mitogen [44]. However, there was no effect of Se supplementation on T cell response. No significant correlations between plasma Se levels and lymphoproliferative responses were observed in this study, although the greatest increase in B cell response occurred in subjects who had the lowest plasma Se at baseline. The authors concluded that Se supplementation enhances immune response in the elderly. However, the data presented do not fully support this conclusion. The greatest age-related immunological defects are observed in T cells and this study did not report an effect of supplemental Se on T cell response to mitogens.

Talbott et al. [45] found that in a group of healthy subjects over 65 years of age, a 2-month supplementation with 50 mg/day of vitamin B₆ as pyridoxine hydrochloride increased lymphocyte proliferation in response to T and B cell mitogens. In addition, percentages of helper but not cytotoxic T cells increased. The effect of B₆ supplementation was greatest in subjects with the lowest initial plasma pyridoxal phosphate levels, indicating that higher than RDA levels of B₆ may be needed to achieve optimal immune function in the elderly. Another study, which examined the effects of vitamin B₆ supplementation on the immune response in a group of elderly men, involved a depletion period of 20 days or less followed by a 3-week repletion period (3.00, 15.00, 22.50, and 33.75 µg B₆/kg body weight), and a final 4-day supplementation period of 50 mg/day [46]. Vitamin B₆ deficiency resulted in decreased percentage of lymphocytes, reduced T and B cell proliferative responses, and less IL-2 production. These measures correlated with decreased plasma pyridoxal phosphate levels. Vitamin B₆ repletion in part restored these immune measures to values similar as those of baseline levels. However, over half of the subjects did not reach baseline values for these immune measures upon repletion. The
Table 2. Effects of nutrient supplementation on immune function in the aged

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Results</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multivitamin + minerals</td>
<td>↑Immune response</td>
<td>Small number of subjects</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>↓Antibiotic use</td>
<td>Methods not well defined</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↓Number of sick days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivitamin</td>
<td>No effect on infection</td>
<td>Short duration</td>
<td>64</td>
</tr>
<tr>
<td>Mixture of vitamins A, E, and C</td>
<td>↑In <em>in vitro</em> immune measures</td>
<td>Short duration</td>
<td>65</td>
</tr>
<tr>
<td>Selenium</td>
<td>No effect on T cells</td>
<td>Confounding factors in yeast</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑In B cells response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>↑In deficient subjects</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>↓In replete subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Mixed results</td>
<td>Design problems</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inappropriate measurements</td>
<td></td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>↑In subjects with low B&lt;sub&gt;6&lt;/sub&gt; status at baseline</td>
<td></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>↓In depleted subjects, reversed by repletion</td>
<td></td>
<td>46, 66</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>No effect on T cells</td>
<td></td>
<td>52, 67</td>
</tr>
<tr>
<td></td>
<td>↑NK cell activity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Supplements</th>
<th>Results</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>No effect on infection</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>↑T cell proliferation and IL-2 production ↓ PGE2 production</td>
<td>↑Immune function in frail elderly</td>
<td>47, 68</td>
</tr>
<tr>
<td>Fish oil</td>
<td>↓Production of IL-1β, TNF-α, IL-6, and IL-2</td>
<td></td>
<td>40, 69</td>
</tr>
<tr>
<td>Conjugated linoleic acid</td>
<td>↑T cell proliferation ↑IL-2 production No effect on NK activity, PGE2 production or DTH response</td>
<td></td>
<td>42</td>
</tr>
<tr>
<td>Black currant seed oil</td>
<td>↑DTH response ↓PGE2 production No effect on T cell proliferation or IL-2 production</td>
<td></td>
<td>41</td>
</tr>
</tbody>
</table>
authors suggested that the elderly might have a higher requirement for vitamin B₆ to maintain normal immune response. The mechanism by which vitamin B₆ could be operating in maintaining immune responsiveness may involve its role as a coenzyme for the synthesis of DNA and RNA. Nucleic acid synthesis is crucial for lymphocyte maintenance and antigen-induced proliferation.

Vitamin E, the most important fat-soluble antioxidant, is best known for its role in protecting phospholipids of cell membranes from peroxidative damage. We reported that when old mice were fed 500 ppm vitamin E, macrophage production of PGF₂ was lowered [47]. In addition, measures of cell-mediated immune function, such as DTH response, in vitro T cell proliferation, and IL-2 production in response to the T cell mitogen Con A were increased compared to those fed a control diet containing 30 ppm vitamin E. The effects of aging and vitamin E supplementation on the immune function have also been reported for human subjects. A dietary supplement of 800 mg/day vitamin E for 30 days to men and woman over 60 years of age, increased DTH, the mitogenic response to Con A, the production of IL-2, and decreased PGF₂ production [48]. In a subsequent randomized double-blind, placebo-controlled trial, Meydani et al. [49] supplemented healthy elderly (>65 years old) subjects with 60, 200, or 800 mg/day vitamin E or placebo for 235 days. All three doses of vitamin E used, enhanced DTH responses with those receiving 200 mg/day showing the highest percent increase. In addition, antibody titer against the T cell-dependent hepatitis B vaccine was significantly increased in subjects receiving supplemental vitamin E of 200 or 800 mg/day over those receiving the placebo control (Table 3). Those consuming 200 mg/day also had a significant increase in antibody response to tetanus toxoid vaccine.

Previous studies from our laboratory show that vitamin E exerts its effect in part by reducing macrophage PGF₂ production [13, 47]. However, unpublished data from our laboratory also suggest a direct effect of vitamin E on T cells that is independent of its effect on reducing the production of PGF₂ by macrophages. This direct effect of vitamin E was found to be mediated through increased proliferation and IL-2 production of naive T cells [50]. Thus, supplementary vitamin E may enhance T cell function in the elderly by two mechanisms: both indirectly via reduced PGF₂ production by macrophages, and directly by enhancing the function of naive T cells.

The immunostimulatory effects of vitamin E on T cell function in the aged has not been observed by β-carotene, another antioxidant. Santos et al. [51] showed that neither short-term, high-dose (90 mg/day for 3 weeks) or long-term, moderate-dose (50 mg/every 2 days for 12 years) supplementation with β-carotene had a significant effect on in vitro or in vivo indices of T cell-mediated function. However, long-term supplementation with β-carotene did increase NK activity in the elderly [52].

Food consumption and dietary intake data indicate that the intake of zinc by the elderly is marginally adequate [53]. Several investigators have evaluated the effect of supplemental zinc on the immune response of older subjects, but
Table 3. Effect of vitamin E supplementation on delayed-type hypersensitivity skin response and antibody titer to hepatitis B in elderly subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>DTH response induration index, mm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibody titer to hepatitis B IU/ml&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% with detectable hepatitis B titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>end of study</td>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Placebo</td>
<td>22</td>
<td>24</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>60 mg E&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24</td>
<td>29</td>
<td>0.03</td>
</tr>
<tr>
<td>200 mg E</td>
<td>17</td>
<td>27</td>
<td>0.006</td>
</tr>
<tr>
<td>800 mg E</td>
<td>17</td>
<td>28</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data represent mean values for DTH response and geometric mean for antibody titer to hepatitis B.

<sup>b</sup> Compared to baseline using Wilcoxon signed rank test followed by Bonferroni correction for multiple comparisons.

<sup>c</sup> Not significant (p > 0.05).

<sup>d</sup> Vitamin E as dl-α-tocopherol (mg/day).

From Meydani et al. [49].

with conflicting results [54]. A study of zinc-deficient elderly (65–78 years) that were supplemented with 60 mg zinc/day for 4.5 months found a significant improvement in the number of positive antigens in the DTH response [55]. A recent double-blind, randomized, placebo-controlled trial showed that dietary supplementation with zinc sulfate (25 mg/day) for 3 months among 118 elderly subjects resulted in improved lymphocyte proliferation [56]. Also, zinc supplementation resulted in greater numbers of CD4<sup>+</sup>/DR<sup>+</sup> T cells and CD3<sup>+</sup>/CD16<sup>+</sup>/CD56<sup>+</sup> cytotoxic T cells compared to those who received placebo. Other studies have reported no effect of supplemental zinc on certain immune measures in elderly subjects [57, 58].

Glutathione (GSH) is the most abundant intracellular nonprotein thiol. This ubiquitous tripeptide is involved in numerous cellular functions, including DNA and protein synthesis and protection of cells from harmful effects of radiation, oxygen intermediates, and free radicals [59]. It has been suggested that GSH status is inversely related to aging and may be a predictor of morbidity and mortality [60]. A limited number of studies have investigated the effect of GSH on immune response in the aged. We conducted a study to investigate whether the decline in immune response with age can be reversed by dietary GSH supplementation [61]. Young, middle-aged, and old mice were fed diets containing different amounts of GSH (0, 0.1, 0.5, and 1.0%) for 4 weeks. There was an age-related decrease in GSH levels, and both in vivo (DTH response) and in vitro (T cell proliferation) measures of immune response. Dietary GSH supplementation reversed the age-associated decline.
in GSH levels while significantly improving the immune response. To further extend these findings to humans, an in vitro GSH supplementation was conducted [62]. At concentrations between 2 and 10 mmol/l, GSH enhanced the mitogenic response of peripheral blood mononuclear cells from both young and old subjects. Protein-bound polysaccharides (PSP), found in abundance in certain mushrooms, are a class of compounds that have been associated with enhanced immune response and reduced tumor burden [63]. There are, however, few well-controlled published studies, particularly in healthy subjects, to support the PSP-mediated immunoenhancing claims that have been made. Therefore, we examined the effect of dietary supplementation with mushroom extract containing PSP on immune function in young (5 months) and old (23 months) mice. Supplementation with PSP for 1 month had no effect on mitogenic response to Con A, PHA, or lipopolysaccharide. In addition, the production of IL-1, IL-2, IL-4, and PGE$_2$ was not significantly different between PSP and control fed mice. On the other hand, old mice fed PSP had a significantly greater DTH response compared to old control fed mice whereas there was no effect of PSP on the DTH response in young mice. These results indicate that PSP-containing mushroom extract may have a modest immunoenhancing effect in aged but not in young mice.

References

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**Discussion**

*Dr. Bunout:* With the multi-test CMI (measures delayed type hypersensitivity response), you can have a boosting effect when you do repeated measurements. Have you seen that boosting effect, and if so how do you deal with it when you interpret the results?

*Dr. Meydani:* We haven’t seen any boosting effect with multi-test CMI. In our studies there is a slight increase in the placebo group, but the effects we see with some of the oils or with vitamin E are greater. We deal with this in the usual way, by comparing the change in the treatment group with the change in the placebo group, and if it is significantly different then we accept that it a real change. The multi-test CMI has the advantage over traditional techniques in that it does not have a substantial boosting effect.

*Dr. Weindruch:* You showed in vitro evidence to suggest that vitamin E causes expansion or increased proliferative capacity of the naïve T cells. Have you observed any evidence or suggestion in vivo that that aspect of immunologic aging might be sensitive to treatment?

*Dr. Meydani:* If you take animals that have not been challenged and look at the percentage of naïve and memory T cells in the blood, there is no effect of vitamin E compared with untreated naïve animals. However, what is important in the way the immune system works is that the cells are responsive to pathogens. What we haven’t done is to look at animals that have been challenged, to see whether the situation is similar to what happens in vitro. In vitro, if we look at zero time without any stimulation, there is no effect of vitamin E on the percentage of memory cells or naïve cells; it is when we stimulate them and look at the ability of each particular cell to go through cell division that we see the effect.

*Dr. Lesourd:* Could you comment on the discrepancy between what is going on in the lymphocytes and what is going on in the monocytes? It seems that the
increase in activation of monocytes is probably the main mechanism involved. Is that a compensatory mechanism for the decline in T-cell function, or does it mean there is permanent activation of the monocytes, or are we dealing with something else?

**Dr. Meydani:** There are very different regulatory aspects for each of these populations of cells. Macrophages seem to be hyperactive in their production of mediators, and some of their functions, for example phagocytosis, do not appear to change much with aging. Why there is a discrepancy between cell types with aging, I don’t know. If aging caused similar changes in all the different systems it would have made our life much easier. I don’t know if we are dealing with a compensatory mechanism, as the increase that we see in the mediator seems to be more of in T cell suppressive factor. If it was the helper factors that were being increased then perhaps that would have been compensatory.

**Dr. Lesourd:** What I am suggesting is that there is compensation for the fact that the T cell is not able to respond, so the macrophage becomes more responsive and releases more suppressive factor, because that is one of the side effects of macrophage function.

**Dr. Meydani:** But we don’t have any evidence that it is hyperactive in so far as its ability to phagocytose bacteria or to kill viruses is concerned. It is not hyperactive in that way, so it is not compensating for the lack of T-cell function. It does produce more of some of the suppressive factors for T cells but its own function is not exaggerated with aging.

**Dr. Taylor:** How reproducible is the delayed-type hypersensitivity test for vitamin E? And secondly, what were the vitamin E levels in the placebo? Were they very low?

**Dr. Meydani:** In animals there is a lot of evidence that reproducibility is good. In humans, doing this type of clinical trial carefully is not an easy thing, so there are not very many similar studies with vitamin E or other similar types of nutrient. However, we were very pleased to see that our results were comparable with those of the Netherlands group [1], who supplemented with vitamin E at a different level from us (50 and 100 units a day as opposed to 60, 200, and 800). When you combine the two studies there is a nice relationship between dose and response, so the method seems reproducible in these two studies at least. However, such studies are not easy to do, and a lot of quality control has to be applied before you can get good reproducibility.

As to your second question, the placebo group was getting an adequate level of vitamin E, based on current recommendations. They were certainly not vitamin E-deficient subjects. In fact it is very difficult to induce vitamin E deficiency in humans.

**Dr. Rosenberg:** Can you use the DTH test to predict who is going to respond to vitamin E? For example, when you did your intervention study could you decide not to give everyone vitamin E, but to screen the population and give vitamin E only to the ones with a reduced delayed type response? Wouldn’t that give a greater public health response to your intervention?

**Dr. Meydani:** That’s an excellent question. We will be able to answer that in the larger study that we are doing now. The studies we have done so far have not been large enough to test that hypothesis but in our current study, which has 640 participants, we expect to see a large variation in DTH response, and we are doing DTH tests in all of them.

**Reference**