Antioxidants, Cytokines, and Influenza Infection in Aged Mice and Elderly Humans

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The age-associated dysregulation of the immune response contributes to higher incidences of infectious diseases in the aged. Of note, there is dysregulation of cytokines, including a change in T helper (Th) 1/Th2 cytokine balance and an increase in production of proinflammatory cytokines. Synthesis of many cytokines is influenced by changes in the cellular oxidant/antioxidant balance. Because vitamin E supplementation reduces oxidative stress and improves the immune response in the aged, a series of experiments was conducted to determine the effect of supplementation with vitamin E and other antioxidants on resistance to influenza infection in aged mice and the role of cytokines in vitamin E–induced increase in resistance to influenza infection. The results of these studies plus findings by other investigators on the effects of age and antioxidants on production of cytokines in human and animal models are reviewed.

Despite the development of antibiotics and vaccines, infectious diseases are a continuing threat to humans, especially the elderly. The age-associated decline of immune function contributes to the increased susceptibility of the aged to infection. The influenza virus is one of the most infectious human agents, and influenza epidemics occur every year in the United States and worldwide. The incidence of influenza-related deaths in the United States ranges from about 10,000 in years with low influenza activity to more than 40,000 during severe influenza epidemics with an average about 20,000 per year [1].

Influenza infection induces a cascade of nonspecific and specific immune functions such as phagocytosis, natural killer (NK) and cytotoxic T lymphocyte (CTL) activities, and production of antibodies and various cytokines. While a number of cytokines have immunoregulatory and antiviral properties that may be important in the control of influenza infection, others are more likely to contribute to the symptoms and pathology associated with the infection. Many aspects of immune function, including production of cytokines, are dysregulated in the elderly, and cytokines are important in the development of effector activity of various immune cells against many organisms. Therefore, age-associated changes in cytokine profiles will affect immune response and resistance against pathogens.

Antioxidants, such as vitamins E and C, β-carotene, and glutathione, enhance some parameters of immune function when added to isolated immune cells in vitro or given as supplements to humans or animals in vivo. One mechanism for the immunostimulatory effects of antioxidants is their effect on production of immunoregulatory molecules such as cytokines. This review focuses on the effects of age and antioxidant supplementation on resistance to influenza infection as mediated through changes in cytokine production.

Cytokines and Influenza Infection

Following influenza infection, a wide spectrum of cytokines is produced by bronchoalveolar lavage, mediastinal lymph node, and spleen cells. Hennet et al. [2] reported an early increase of interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor, and interferon (IFN)-γ in cell-free bronchoalveolar lavage fluid of mice during influenza infection. Levels of these cytokines peaked between 36 h and 3 days after infection, with the exception of IL-6, which remained elevated throughout the infection. IL-2, IL-3, and IL-4 were undetectable in the samples. These results suggest that mononuclear phagocytes, possibly alveolar macrophages, play a prominent role in the initiation of an immune response during the early stages of influenza infection. Sarawar and Doherty [3] reported that production of IL-2, IL-10, and IFN-γ by mediastinal lymph node cells from mice infected with influenza virus were prominent throughout the response, but there was minimal evidence for IL-4, IL-5, and TNF-α secretion. Infection of human monocytes, rat alveolar macrophages, and murine PUS-1.8 macrophages with influenza virus result in production of TNF-α, IFN, and prostaglandin E₂ (PGE₂) [4]. In vitro studies using human lung epithelial cells [5] and human peripheral blood
mononuclear cells (PBMC) [6] showed increased IFN-α production and antiviral MxA gene expression following influenza virus infection.

It is difficult to determine the individual contributions of the various cytokines produced following influenza infection in infectivity and pathogenesis, because the cytokines are pleiotropic and redundant in their functions and exert synergistic or antagonistic effects on each other. However, in general, it is believed that among the cytokines produced after influenza infection, IFN-α/β, IFN-γ, and IL-2 have protective roles against influenza infection, while IL-1, TNF-α, and IL-6 seem to be involved in the inflammatory phase of the infection.

IFN-α/β induces an antiviral state in cells by stimulating the transcription of several genes coding for proteins, such as 2',5'-oligoadenylate synthetase (2-5 OAS), double-stranded RNA-dependent protein kinase, and the Mx proteins [7]. 2',5'-oligoadenylate, converted from ATP by the action of 2-5 OAS, binds to and activates a latent cellular endoribonuclease that degrades single-stranded viral and cellular RNAs [7]. Protein kinase P1/eIF2 (a serine-threonine kinase also known as p68 kinase), before and after induced by IFN, blocks viral protein synthesis and thus limits virus spread [8]. Murine Mx1 protein, which has a distinct nuclear targeting signal, can specifically inhibit the replication of influenza virus at a transcriptional level, and human MxA protein inhibits the replication of influenza virus at posttranscriptional and translational levels [9]. Tough et al. [10] suggested that release of IFN-α/β during viral infection could play a role in the stimulation of memory cells as well as act as an adjuvant augmenting both the intensity of primary response to viruses and the survival of early memory cells.

IFN-γ produced by T helper (Th) 1 and NK cells exerts not only direct antiviral effects but also regulates several aspects of immune response including stimulation of macrophages and NK cell activity, up-regulation of expression of major histocompatibility complex molecules, and control of immunoglobulin class switching [11]. IL-2, another Th1 cytokine produced by activated T cells, does not have direct antiviral activity. However, IL-2 is an essential component of the immune response as it stimulates the rapid proliferation of T cells, which orchestrate many immunologic events. The cytokines secreted by Th cells affect not only local inflammation but also the overall antibody production and the nature of the antibody available to fight off infectious agents. For example, IL-4 stimulates B cells to make IgG1 and IgE, whereas IFN-γ tends to induce B cells to make IgG2a in mice [12]. In fact, neutralization of IFN-γ led to a significant reduction in virus-specific titers of IgG2a and IgG3 in serum and in the magnitude of the cellular infiltrate in lung tissue following influenza infection in BALB/c mice [13].

Symptoms of influenza infection are similar to those observed with dysregulation of cytokines [14]. Conn et al. [15] noted that a decrease in food and water intake, body temperature, and general locomotor activity is associated with elevated levels of IL-1, IL-6, and TNF-α in the lungs of mice infected with influenza virus. The IL-1 family consists of IL-1α, IL-1β, and IL-1 receptor antagonist (Ra). IL-1α and IL-1β can induce fever, sleep, anorexia, and hypotension. IL-1Ra provides some protection against the disease-provoking effects of IL-1 by inhibiting IL-1 activity by blocking the binding of IL-1 to its cell surface receptors [16].

TNF-α appears to play a dual role in influenza infection; it is considered both beneficial and harmful due to its cytotoxic and proinflammatory effects. TNF is produced by different immune cells, including monocytes, NK, B, T, and neutrophils [17]. In vitro treatment with TNF-α results in decreased production of infectious virus, inhibition of viral protein synthesis, and a reduction in cytopathic effect of virus [18]. Systemic administration of TNF-α produces fever and anorexia via hypothalamic centers that regulate body temperature and appetite. Other functions of TNF-α include induction of other cytokines (e.g., IL-1 and IL-6), induction of apoptosis in mature T cells, and increased phagocytic activity [17].

IL-6, produced by both lymphoid and nonlymphoid cells, is a multifunctional cytokine that regulates immune and acute-phase responses and hemopoiesis. IL-6 is involved in T cell activation, growth, and differentiation and in B cell differentiation. Dysregulated expression of IL-6 has been implicated in the pathogenesis of a variety of diseases, especially autoimmune disorders, and several chronic proliferative diseases [19].

Changes in Immune Functions with Aging

Aging is a complex process that affects a wide variety of body functions including those of the immune system [20]. Decreased delayed type hypersensitivity response, lymphocyte proliferation, and CTL activity and an increase in memory T cells and antibody response plus altered cytokine patterns are among the changes observed with aging [21] (table 1).

Production of IL-2, a key factor in cell-mediated responses, declines with age in both mice and humans. IL-2 production by splenocytes is significantly lower in old C57BL mice infected with influenza virus [35]. PBMC from persons >62 years old produce significantly less IL-2 after influenza vaccination than PBMC from younger subjects [36]. Because the generation of high-affinity IL-2 receptors after stimulation is also lower in T cells from older persons, IL-2 supplementation by itself can only partially restore T cell responses in culture [21, 37]. In some studies, IFN-α production was significantly lower in the elderly than in the young. Abb et al. [38] demonstrated that production of virus-induced IFN-α is significantly decreased in mononuclear cell cultures of older subjects compared with those of younger persons. Sindermann et al. [24] found that the elderly have significantly decreased levels of IFN-α in whole blood cultures stimulated with Newcastle disease virus.

Reports on age-related changes in the production of IFN-γ are inconsistent. IFN-γ production is higher in phytohemag-
peripheral blood mononuclear cells. TNF-α is lower in the production of IL-1β in elderly subjects. However, there was no significant difference in the production of IL-1β, TNF-α, and PHA-stimulated IL-6 between the two age groups. Daynes et al. [30] also reported higher levels of IL-6 in serum and in cultured splenocytes of aged mice and higher levels of IL-6 in plasma and in PBMC cultures from donors 65–83 years old compared with younger control groups. However, Peterson et al. [31] found similar levels of TNF-α and IL-6 in the serum of healthy elderly (mean age, 80 years; range, 75–92) and younger controls (mean age, 30; range, 22–35). Beharka et al. [32] found no significant difference in serum or ex vivo PBMC production of IL-6 between young and old healthy subjects or in ex vivo IL-6 production by mouse splenocytes. No age-related differences in IL-6 production were observed when PBMC from 30 young and 270 elderly subjects were stimulated in vitro with trivalent influenza vaccine before and after influenza immunization [44]. The discrepancies in age-related IL-6 production appear to be due to differences in the health status of the subjects studied. Also, higher IL-6 production reflects the presence of age-associated diseases rather than of the aging process itself.

### Antioxidants and Cytokines

Antioxidants, such as vitamins E and C, β-carotene, and glutathione, enhance immune function when administered supplementally to animals or humans in vivo or to culture systems in vitro [45]. One mechanism for immunostimulatory effects of these antioxidants is the ability to modulate the production of cytokines that regulate immune function (table 2).

Several studies describe the effects of vitamin C on IFN production. Siegel [49] reported higher circulating IFN levels in mice supplemented with vitamin C in drinking water and subsequently infected with Rauscher leukemia virus. Vitamin C also enhanced the IFN levels produced by human embryo lung fibroblasts stimulated with Newcastle disease virus and polyinosinic-polycytidylic acid (poly(rI)-poly(rC)) [51] by mouse L cells and mouse embryonic fibroblasts stimulated with poly(rI)-poly(rC) [50].

Glutathione plays an important role in maintaining intracellular redox balance and in cellular defense against oxidative stress. In vitro supplementation of glutathione to PBMC from both young and old subjects significantly increased the production of IL-2 [48]. IL-2 production was enhanced by 140% in cells of young subjects and by 372% in cells of older subjects. The effect of glutathione on proinflammatory cytokines has also been reported. In vitro incubation of alveolar macrophages with glutathione significantly reduced the secretion of lipopolysaccharide (LPS)-induced TNF-α, IL-6, and IL-8 [57]. Significant decreases in monocytes, TNF-α, IL-6, and IL-8 production with increases in whole blood glutathione levels were reported in cirrhosis patients treated for 9 days with oxazolidine-4-carboxylic acid (OTZ) [56]. OTZ is metabolized intracellularly by 5-oxo-L-prolinase to a compound that spontaneously decarboxylates to L-cysteine and therefore increases intracellular cysteine and promotes glutathione synthesis.

Among antioxidants, vitamin E has been the most extensively studied with regard to immunostimulatory effects. Dietary vitamin E supplementation increases IL-2 production in both

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Effects of aging</th>
<th>Observed in</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>Lower production</td>
<td>Human PBMC</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Lower mRNA</td>
<td>Human PBMC</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>Lower receptor</td>
<td>Human PBMC</td>
<td>[22]</td>
</tr>
<tr>
<td>IFN-α</td>
<td>Lower production</td>
<td>Human whole blood culture</td>
<td>[24]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Higher production</td>
<td>Splenic T cells of mice</td>
<td>[25, 26]</td>
</tr>
<tr>
<td></td>
<td>Higher mRNA</td>
<td>Mouse splenocyte</td>
<td>[27]</td>
</tr>
<tr>
<td>IL-1</td>
<td>Lower production</td>
<td>Splenic T cell and peritoneal macrophage coculture of mice</td>
<td>[33]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Higher levels</td>
<td>Human plasma</td>
<td>[29]</td>
</tr>
<tr>
<td></td>
<td>Higher production</td>
<td>Human PBMC</td>
<td>[30]</td>
</tr>
<tr>
<td></td>
<td>Similar levels</td>
<td>Human serum</td>
<td>[31, 32]</td>
</tr>
<tr>
<td>IL-1</td>
<td>Higher production</td>
<td>Human PBMC</td>
<td>[34]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Higher production</td>
<td>Human PBMC</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>Similar levels</td>
<td>Human serum</td>
<td>[31]</td>
</tr>
</tbody>
</table>

**NOTE.** IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; PBMC, peripheral blood mononuclear cells.
animals and humans. Meydani and colleagues [46, 47] showed that IL-2 production was significantly higher in elderly subjects (>60 years old) given 800 mg/day of vitamin E for 30 days, while IL-2 production was two-fold higher in aged mice fed the vitamin E–supplemented diet for 6 weeks compared with mice fed the control diet (adequate in vitamin E). Wang et al. [53] showed that infection of LP-BM5 in mice causes murine AIDS and is associated with lower IL-2 and IFN-γ and higher IL-6 and TNF-α production by splenocytes than seen in uninfected mice. Supplementation of LP-BM5–infected mice with vitamin E increased production of IL-2 and IFN-γ and decreased release of IL-6 and TNF-α by splenocytes. Vitamin E supplementation (800 mg/day for 60 days) also prevented exercise-induced elevation in IL-1 and significantly decreased the production of IL-6 [54]. Short-term high-dose intramuscular injection of vitamin E (600 mg/day for 3 days) to pigs resulted in lower peak plasma levels of IL-6 following LPS injection [55]. In rats, short-term high-dose enteral supplementation of vitamin E (100 mg/kg/day for 5 days) resulted in significantly lower production of LPS-stimulated TNF-α by whole blood and peritoneal macrophages [59].

How do these antioxidants regulate the production of cytokines? There are two possible mechanisms—first, through their effect on transcription factors that are regulated by redox status, and second, by influencing PGE2 synthesis, which plays a key role in Th1 response and regulation of proinflammatory cytokines. At least two transcriptional factors, nuclear factor (NF)-κB and activator protein (AP)-1, are regulated by redox status. Reduction/oxidation can either up- or down-regulate DNA binding and transactivation activities (or both) in transcriptional activator–dependent and cell type–dependent manners [60]. Many cytokines (e.g., IL-2, IFN, IL-1, IL-6, and TNF-α) contain NF-κB and AP-1 binding sites in the promoter and enhancer regions of the genes encoding them. Vitamin E or its derivatives inhibit NF-κB activation in mice [61], human Jurkat T cells [62], and rat Kupffer’s cells [63]. However, differential regulation of cytokines in different cells, all with NF-κB and AP-1 binding sites, by vitamin E and other antioxidants indicate that regulation of these cytokines is cell type-specific.

PGE2 has a direct inhibitory effect on the early stage of T cell activation, resulting in decreased IL-2 production and decreased IL-2 receptor expression [64]. PGE2 can modulate Th1 and Th2 cytokine secretion through its effect on IL-12, a heterodimeric cytokine that plays a central role in increasing Th1 responses by promoting the differentiation of naïve T cells into a population of Th1 cells capable of producing large amounts of IFN-γ following activation and by serving as a costimulus required for maximum secretion of IFN-γ by differentiated Th1 cells responding to specific antigen [65]. PGE2 inhibits LPS-induced IL-12 production in human whole blood culture [66] and inhibits IL-12 receptor expression and IL-12 responsiveness in human PBMC [67]. In addition, PGE2 can regulate IL-6 and TNF-α production. Thivierge and Rola-Pleszczynski [68] showed that PGE2 can increase production of IL-6 by macrophages. However, PGE2 can suppress TNF-α expression in macrophages [17]. Previously, we showed that vitamin E supplementation inhibits cyclooxygenase activity and decreases PGE2 production in old mice [69].

### Table 2. Effects of antioxidants on cytokine production.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Effects of antioxidants</th>
<th>Observed in</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>IL-2</td>
<td>Higher production vitamin E</td>
<td>Human PBMC</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouse splenocytes</td>
<td>[47]</td>
</tr>
<tr>
<td>IFN</td>
<td>Higher production vitamin C</td>
<td>Human PBMC</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>Higher production vitamin C (in vitro)</td>
<td>Serum of mouse challenged with leukemia virus</td>
<td>[49]</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Higher production vitamin E</td>
<td>Splenocytes of mouse challenged with influenza</td>
<td>[52]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Lower production vitamin E</td>
<td>Human PBMC</td>
<td>[54]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plasma of LPS-challenged pig</td>
<td>[55]</td>
</tr>
<tr>
<td>IL-1</td>
<td>Lower production vitamin E (in vitro)</td>
<td>Human PBMC</td>
<td>[58]</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Lower production vitamin E</td>
<td>Whole blood culture of rat</td>
<td>[59]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Splenocytes of mouse infected with LP-BM5</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monocytes of cirrhosis patients</td>
<td>[56]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human alveolar macrophages</td>
<td>[57]</td>
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<tr>
<td></td>
<td></td>
<td>Human alveolar macrophages</td>
<td>[57]</td>
</tr>
</tbody>
</table>

**NOTE.** GSH, glutathione; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; TNF, tumor necrosis factor.

**Antioxidants and Influenza Infection**

Influenza infection can change redox status of cells and activate transcriptional factors. Furthermore, influenza infection results in decreased total concentrations of antioxidants, glutathione, and vitamins C and E from the lung, especially in the early stages of the infection, an occurrence attributed to in-
In addition to reactive oxygen species (ROS) generated by phagocytes following viral infection, influenza virus itself seems to be involved in the generation of ROS directly. Pahl and Baueerle [71] reported that expression of influenza virus hemagglutinin (HA) activates NF-κB DNA binding and transactivation in HeLa and 293 cells. Activation was inhibited in the presence of the antioxidant di-thiothreitol, suggesting that HA increases the production of ROS within the cell, which may act as a second messenger to activate the NF-κB. AP-1 is also activated in T cells following exposure to influenza virus [72]. Increased oxidative stress observed with influenza infection can cause direct tissue damage, changes in the infectivity of influenza virus, or indirect pathogenesis by influencing cytokine profile.

Since old mice have lower antioxidant status and higher ROS production than young mice, we investigated the effect of vitamin E supplementation on influenza virus titer in young and old mice. We [73] showed that old mice fed a diet high in vitamin E (500 ppm) had significantly lower lung virus titer than old mice fed a diet containing adequate levels of vitamin E (30 ppm) following influenza infection (figure 1). A highly significant inverse correlation was observed between virus titer and vitamin E levels on day 5. The effect of vitamin E on lowering influenza virus titer could not be explained by a change in CTL activity, as there was no difference in CTL activity in aged mice fed adequate or high levels of vitamin E. NK activity was significantly higher in old mice fed the vitamin E-supplemented diet than in old mice fed the diet containing adequate vitamin E, but the magnitude of decrease in virus titer (25-fold) could not be explained solely by the increased NK activity (3-fold) [73]. Therefore, to delineate further the mechanisms of vitamin E on lowering influenza virus titer, we studied the effects of supplementation with vitamin E and other antioxidants on cytokine production and resistance to influenza infection in young and old mice.

To determine whether the effect of vitamin E on influenza virus titer is mediated through its antioxidant function, we compared the effect of vitamin E supplementation with that of other antioxidants such as glutathione, melatonin, and strawberry extract [74]. Vitamin E supplementation was effective in lowering virus titer and preventing weight loss and also decreased food intake following influenza infection of aged mice (table 3). This beneficial effect was not observed with the other dietary antioxidants tested.

The effect of vitamin E on preventing weight loss following influenza infection appeared to be due to a reduction in pulmonary IL-6 and TNF-α levels. The vitamin E-supplemented group had significantly lower pulmonary IL-6 and TNF-α levels following infection than the control group (about 50% of the control), and there was a significant positive correlation between weight loss and pulmonary IL-6 and TNF-α levels [74]. To determine further the mechanism of the vitamin E effect, Th1 and Th2 cytokines were measured in young and old mice infected with influenza virus and supplemented with adequate (30 ppm) or high (500 ppm) levels of vitamin E for 8 weeks [52]. Old mice fed the vitamin E-supplemented diet had lower virus titers than those fed the control diet on days 2 (P < .1), 5 (P < .05), and 7 (P < .1). Old mice had impaired Th1 response following influenza infection as evidenced by their significantly lower production of IL-2 and IFN-γ following influenza infection compared with young mice. Vitamin E supplementation increased production of Th1 cytokines, IL-2, and IFN-γ in old mice (by ~100%). The higher IFN-γ production was associated with lower virus titer [74]. In conclusion, the protective effect

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Weight loss/5 days after infection</th>
<th>Food intake/5 days after infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.78 ± 1.39</td>
<td>6.28 ± 3.04</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1.84 ± 0.91</td>
<td>16.86 ± 2.60</td>
</tr>
<tr>
<td>Vitamin E + glutathione</td>
<td>5.06 ± 1.88</td>
<td>7.92 ± 1.55</td>
</tr>
<tr>
<td>Glutathione</td>
<td>4.98 ± 1.00</td>
<td>9.24 ± 3.14</td>
</tr>
<tr>
<td>Melatonin</td>
<td>5.76 ± 0.99</td>
<td>7.52 ± 2.39</td>
</tr>
<tr>
<td>Strawberry</td>
<td>5.40 ± 1.89</td>
<td>7.80 ± 4.12</td>
</tr>
</tbody>
</table>

NOTE: Values are mean ± SEM, n = 5. Mice (18 months old) were fed the appropriate diets for 6 months before infection with influenza virus.

Significantly different from control group by Fisher’s least significant test (P < .05)
of vitamin E on lowering influenza virus titer is mainly due to the enhancement of Th1 response. In addition, a moderate increase by vitamin E in NK activity might also contribute to its beneficial effects [73].

Conclusion

The important role of cytokines in the regulation of immune and inflammatory responses associated with infectious diseases is recognized with the availability of purified cytokines, antibodies against them, and the use of transgenic mouse models. In addition, there has been significant progress in the understanding of the signal transduction pathways involved in regulation of cytokine production [75]. It is clear that the oxidant/antioxidant balance plays an important role in regulation of cytokines, and there is evidence that influenza infection increases ROS production and reduces tissue antioxidant levels in the host. Vitamin E supplementation significantly decreases influenza virus titer following influenza infection in young and old mice. There is also evidence for the beneficial effect of antioxidants in other viral infections. However, our understanding of the mechanisms (cytokine- and noncytokine-mediated) through which the antioxidants exert their effects on reducing viral infectivity is limited. Further studies are needed to delineate the mechanisms involved in the protective effect of antioxidants such as vitamin E against influenza infection. Whether changes in the production of a specific cytokine contribute to the beneficial effects of antioxidants against infectious agents can be determined by the use of transgenic animal models that lack a specific cytokine production or by blockage of a specific cytokine in vivo by a monoclonal antibody against it. The beneficial effect of vitamin E on reducing the pulmonary influenza virus titer in old mice should be tested in humans and for other viral infections. Clinical intervention trials are needed to investigate the effect of vitamin E supplementation on infectious diseases in humans to determine the efficacy of vitamin E supplementation in reducing the burden of influenza infection in the elderly.

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