

# Aged Garlic Extract Modifies Human Immunity<sup>1–3</sup>

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## Abstract

Garlic contains numerous compounds that have the potential to influence immunity. Immune cells, especially innate immune cells, are responsible for the inflammation necessary to kill pathogens. Two innate lymphocytes,  $\gamma\delta$ -T and natural killer (NK) cells, appear to be susceptible to diet modification. The purpose of this review was to summarize the influence of aged garlic extract (AGE) on the immune system. The author's laboratory is interested in AGE's effects on cell proliferation and activation and inflammation and to learn whether those changes might affect the occurrence and severity of colds and flu. Healthy human participants ( $n = 120$ ), between 21 and 50 y of age, were recruited for a randomized, double-blind, placebo-controlled parallel-intervention study to consume 2.56 g AGE/d or placebo supplements for 90 d during the cold and flu season. Peripheral blood mononuclear cells were isolated before and after consumption, and  $\gamma\delta$ -T and NK cell function was assessed by flow cytometry. The effect on cold and flu symptoms was determined by using daily diary records of self-reported illnesses. After 45 d of AGE consumption,  $\gamma\delta$ -T and NK cells proliferated better and were more activated than cells from the placebo group. After 90 d, although the number of illnesses was not significantly different, the AGE group showed reduced cold and flu severity, with a reduction in the number of symptoms, the number of days participants functioned suboptimally, and the number of work/school days missed. These results suggest that AGE supplementation may enhance immune cell function and may be partly responsible for the reduced severity of colds and flu reported. The results also suggest that the immune system functions well with AGE supplementation, perhaps with less accompanying inflammation. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT01390116. *J Nutr* 2016;146(Suppl):433S–6S.

**Keywords:** aged garlic extract, human immunity, NK cell,  $\gamma\delta$ -T cell, colds, flu

Garlic has been widely known for centuries to influence health and to provide benefits to almost all physiologic systems. Aged garlic extract (AGE)<sup>4</sup> is manufactured from organically grown garlic cloves (*Allium sativum* L.) that are sliced and soaked in an aqueous ethanol solution and extracted and aged up to 20 mo. Numerous compounds have been detected in AGE that have the potential to affect immunity, including the lectin family, which is known to interact with pathogen recognition receptors on immune cell surfaces (1, 2). Fructo-oligosaccharide and N $\alpha$ -fructosyl arginine are aged garlic compounds that have structures resembling pathogen-associated molecular patterns, with the potential to interact with immune cells (3). Chandrashekar and Venkatesh (4) showed that the fructans from AGE were a well-tolerated adjuvant to IgG production when mice were treated with ovalbumin as the antigen.

The antioxidant properties of AGE have also been studied for their impact on human health. Tetrahydro- $\beta$ -carboline (5), N $\alpha$ -(1-deoxy-D-fructos-1-yl)-L-arginine (6), and S-allyl-L-cysteine (SAC) (7, 8) are compounds in AGE that were shown to have antioxidant and protective effects against neurodegeneration, cardiotoxicity, and diabetic complications.

The immune system is complex and redundant. In healthy persons, the immune system lives in surveillance mode, with cells migrating through the blood and lymph systems to detect

pathogens in the body (Figure 1). When a potential pathogen is discovered, the immune system can often eliminate it without symptoms, although pathogens have unique ways to evade the body's surveillance. When the amount of pathogen becomes a serious threat, the immune system goes into response mode. This response includes the proliferation of immune cells and the subsequent activation of those cells, characterized by increased expression of cell surface receptors and the synthesis and secretion of cytokines. Once the immune system has eliminated the pathogen, it then returns to surveillance mode by an active process of resolving inflammation.

The resolution of inflammation is a balance of positive and negative feedback from the immune cells and the tissue microenvironment. Neutrophils switch from synthesizing leukotrienes and prostaglandins to lipoxins and resolvins, among others (9, 10). Fibroblasts and other stromal cells remove their survival signals, resulting in apoptotic death of immune cells that are no longer needed (11). T-regulatory cells are involved in the transition from inflammation to resolution of inflammation (12), and there is an active coordinated process of apoptosis and clearance of cells by macrophages (13).

Inflammation is the product of an immune cell response, which is necessary and valuable for pathogen elimination and

wound healing. In healthy humans, inflammation usually resolves itself. However, when the body is unable to resolve inflammation and revert to a surveillance mode, an unhealthy state of chronic inflammation develops. Chronic inflammation results from the prolonged activation of immunity or an inability to resolve the immune response and is associated with many disease states such as metabolic syndrome, high blood pressure, atherosclerosis, diabetes, and arthritis (14, 15). Tissue damage caused by an overactive immune system creates a cycle whereby the immune system attempts to repair the damage, which, in turn, creates more inflammation and potentially more damage (Figure 1).

Can diet, or more specifically AGE, improve immune surveillance, improve the immune response, and/or help inflammation subside? Surveillance is the state in which the immune system removes the pathogen without causing symptoms related to illness. Improving the response means improving the ability to proliferate and the ability to activate (express receptors on the surface and secrete cytokines). Do the antioxidant properties of AGE help protect against the free radicals that damage the host and that set up a vicious cycle of tissue damage, inflammation, and more tissue damage? Is there a health outcome associated with functional immune cell changes?

Recent studies have shown the effectiveness of AGE on the immune response against implanted tumors in mice. Ebrahimpour et al. (16) and Fallah-Rostami et al. (17) investigated immune responses to implanted fibrosarcoma cells in mice. Naltrexone, an opioid antagonist, was used with or without AGE. AGE plus naltrexone increased survival times, tumor growth inhibition, and the immune response, mainly cytotoxicity, as well as the CD4<sup>+</sup>-to-CD8<sup>+</sup> ratio and IFN- $\gamma$  production (16). AGE alone also increased survival time, reduced tumor growth, and increased

the production of IFN- $\gamma$  (17). Larypoor et al. (18) examined the immunostimulatory aspects of AGE against the immunosuppression by aflatoxin B1. They concluded that AGE alters the cytokine pattern toward T helper (Th) 1, whereas aflatoxin B1 alters the cytokines into a Th2 pattern. The Th1 pattern is beneficial to immunity in general and to antitumor immunity specifically.

In our study completed in 2011 (19), we used healthy adults to examine the effects of AGE on 2 specific innate-like lymphocytes, the  $\gamma\delta$ -T cell and the NK cell, as our primary outcome. The effect of AGE on NK cells has been studied in patients with cancer (20). No studies, to our knowledge, have examined the effect of AGE on  $\gamma\delta$ -T cells, and this cell has been shown by our previous studies to be modified by diet (21, 22). Although not many  $\gamma\delta$ -T cells are found in the blood, they are numerous in the intestine and epithelial linings of the lung and genitourinary tract. For our randomized, double-blind, placebo-controlled, parallel-intervention study, we recruited 120 healthy individuals, 60 for each group, to determine the effect of consuming 2.56 g AGE daily. Capsules were consumed for a total of 90 d, and the blood draw was taken at the beginning and at 45 d to determine if immune changes had occurred. The study continued for another 45 d to capture the full cold and flu season. Participants' average age was 26 y and 60% were female. Overall compliance determined by capsule count was good, with an average of 85% taking their capsules as directed for the entire study. Compliance was also shown by increased SAC concentrations in the serum and increased reduced glutathione (GSH) concentrations in the cytosol of peripheral blood mononuclear cells (PBMCs), as seen in Table 1.

In a population of cells from participants who consumed AGE for 45 d, the  $\gamma\delta$ -T and NK cells were able to proliferate better in ex vivo cultures than cells from those who consumed the placebo (19). The  $\gamma\delta$ -T cells nearly doubled their ability to proliferate, whereas the NK cells almost tripled their proliferation numbers when compared with preconsumption values. The

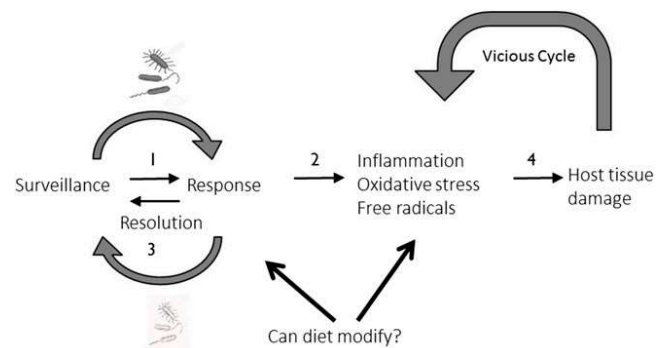
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<sup>4</sup> Abbreviations used: AGE, aged garlic extract; GSH, reduced glutathione; NKG2D, natural killer group 2 member D; PBMC, peripheral blood mononuclear cell; SAC, S-allyl-L-cysteine; Th, T helper.



**FIGURE 1** Potential mechanisms by which diet may influence immunity, inflammation, and/or oxidative stress. Surveillance by the immune system allows for the destruction of potential pathogens, without any accompanying signs of illness. When a potential pathogen overwhelms the surveillance system, cells of the immune system respond (arrow 1), resulting in immune cell proliferation and activation and symptoms such as fever, congestion, etc. (arrow 2). Once the threat has been removed, the immune system returns to surveillance mode (arrow 3). The resolution of the response and return to surveillance is important because if the immune system does not return to normal, chronic inflammation persists. This inflammation is destructive to host tissues and organs and may lead to disease (arrow 4). Diet may play a role in aiding the surveillance processes by improving the initial response to potential pathogens and by helping to resolve the inflammation caused by the response. In addition, diet may play a role by supplying antioxidants that protect host tissues and organs during an inflammatory response.

expression of CD314 [natural killer group 2 member D (NKG2D)], a marker of activity, on the surface of NK cells was significantly greater after AGE supplementation, suggesting the potential for more cytolytic activity by NK cells. This cytolytic activity is responsible for killing infected and tumor cells but may also play a role in inducing the apoptosis of responding immune cells, as the need for them is decreased when the pathogen has been eliminated. In addition,  $\gamma\delta$ -T cells have been shown to kill macrophages during the resolution of bacterial infections (23, 24). Thus, there may be a role for AGE to help resolve inflammation by improving conditions for immune cell death by apoptosis.

Participants ( $n = 120$ ) were instructed to keep a daily diary of symptoms related to illness as a secondary study outcome. After 90 d of AGE/placebo supplementation, the diaries were collected and the entries were analyzed. With 55% of the placebo group and 45% of the AGE group reporting illnesses, no significant difference in the incidence of colds and flu was seen between the groups (Figure 2A). Participants who consumed AGE appeared to have a reduction in the severity of their illness, as noted by a reduction in the number of symptoms reported (Figure 2B), and a reduction in the number of days they functioned suboptimally and/or missed work or school due to illness (Figure 2C). These results suggest that, although not helping to prevent an illness, the addition of AGE to the diet could be useful in reducing illness severity.

In determining compliance, we found improved GSH concentrations in PBMC cytosol and improved thiol status in the serum (Table 1). These 2 antioxidants serve to protect host tissues in times of oxidative stress. Immunity requires GSH for 2 reasons. First, because the immune system produces free radicals to kill pathogens, GSH serves to protect the host immune cells with its antioxidant activity. Second, lymphocytes are dependent on adequate GSH concentrations; T cell proliferation and NK cell cytotoxic activity are functions that are impaired under low GSH conditions (25, 26). It is unclear why the individuals in the placebo group had lower GSH concentrations after the study than they did before the study began.

The change in cytokine concentrations detected in the cell culture supernatant of PBMCs stimulated with phytohemagglutinin for 24 h was also noteworthy. Participants who had consumed AGE for 45 d had reduced concentrations of both TNF- $\alpha$  and IFN- $\gamma$ , although the values did not achieve significance (19).

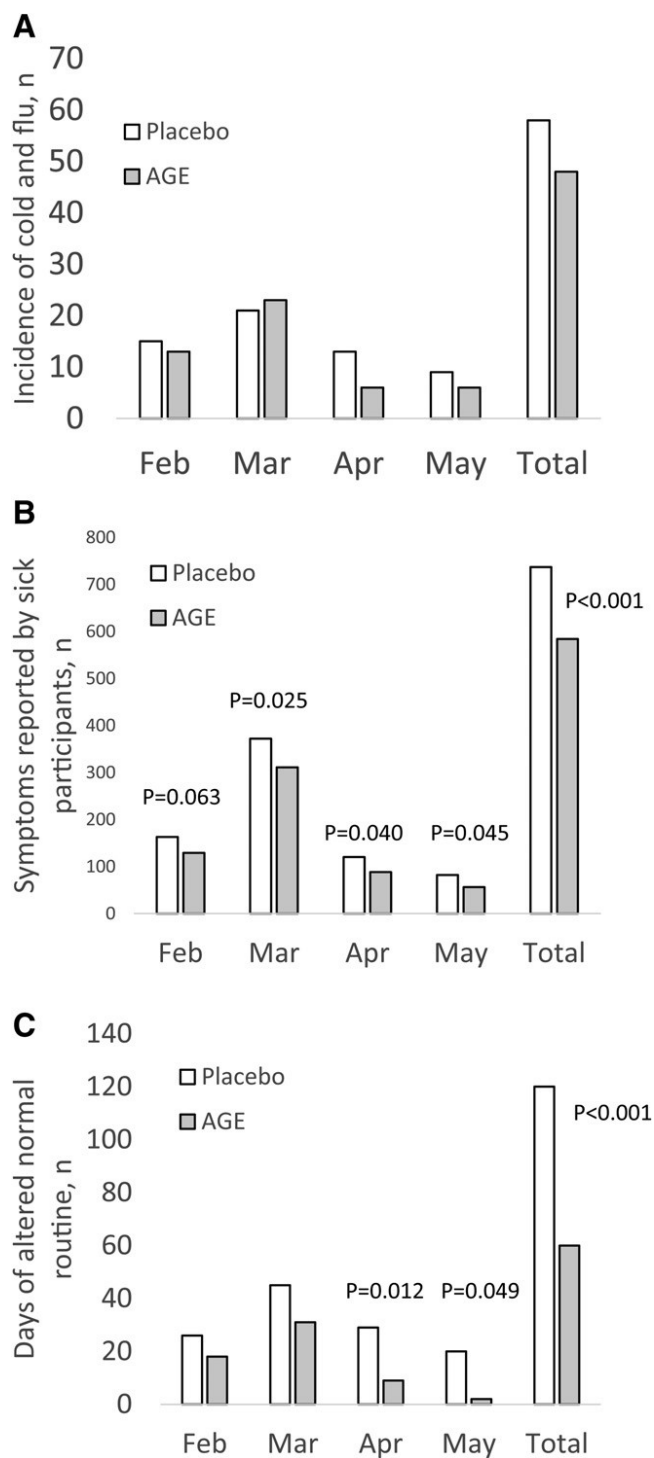
Taking these results into consideration, we concluded that supplementing the diet with AGE resulted in enhanced immune cell function, and perhaps under less inflammatory conditions. This led us to hypothesize that AGE supplementation might reduce inflammation in a chronically inflamed population.

It is well known that obesity is associated with chronic inflammation in both adults (27) and children (28). Immune function is reduced in obese and overweight people, and yet they are in a chronic state of inflammation. Adipose tissue actively secretes adipokines, cytokines, and chemokines, and the more adipose

**TABLE 1** Changes in serum SAC and PBMC cytosol GSH concentrations in adults who received 2.56 g AGE/d or placebo for 45 d<sup>1</sup>

	AGE ( $n = 56$ )	Placebo ( $n = 56$ )	<i>P</i>
SAC, ng/mL	228.0 $\pm$ 15.0	109.7 $\pm$ 18.8	<0.001
GSH, nmol/L	144.1 $\pm$ 0.1	-55.1 $\pm$ 0.1	0.036

<sup>1</sup> Values are means  $\pm$  SEMs. Baseline values were subtracted from the values at 45 d. The negative value in the placebo group indicates that GSH concentrations were lower at the end of the study. AGE, aged garlic extract; GSH, reduced glutathione; PBMC, peripheral blood mononuclear cell; SAC, S-allyl-L-cysteine.



**FIGURE 2** Incidence of self-reported cold or flu (A), number of symptoms reported by participants who became ill (B), and number of days that participants reported a decrease in their normal routines due to illness during consumption of an AGE supplement (2.56 g/d;  $n = 56$ ) or a placebo ( $n = 56$ ) for 90 d. *P* values are shown when the groups differed significantly (*z*-test of proportions). AGE, aged garlic extract.

tissue one has, the more inflammatory mediators will be secreted (29). In the peripheral blood of overweight and obese individuals, increased inflammation has been represented by a higher neutrophil-to-lymphocyte ratio, elevated high-sensitivity C-reactive protein, and increased chemokines (30).

In addition, Lautenbach et al. (31) found a reduction in NK cells in the livers of obese individuals. It is worth noting that after AGE consumption, when immune cells were stimulated ex

vivo we found both enhanced NK proliferation and increased expression of the surface receptor NKG2D on those cells (19). NKG2D, the cytolytic receptor found on NK cells and several other tumoricidal immune cells (32, 33), is thought to be important in resolving inflammation through apoptosis of the immune cells that are no longer required once a response has ended.

The literature is replete with research that endorses the concept of inappropriate immunity leading to inflammation, whether as chronic inflammatory activity or an inability to resolve an immune response. Theories to define what might be responsible for creating this problem have included the following: alterations in adipokine synthesis and secretion, oxidative stress, adipose tissue hypoxia, endoplasmic reticulum stress, and FA-induced inflammation (29). It is likely that a combination of these theories may eventually result in a plausible explanation. What is important, however, is that many, if not all, of our disease risks are associated with chronic inflammation. If a dietary supplement derived from garlic could help to alleviate the consequences of this type of inflammation, this could only lead to improved health outcomes in the general population.

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