ORIGINAL RESEARCH

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Immune Status and the Development of *Listeria monocytogenes* Infection in Aged and Young Guinea Pigs

Abstract

Purpose: Consuming even low numbers of the foodborne pathogen, *Listeria monocytogenes*, places the elderly at risk for severe illness. The impact of immunomodulation on the development of listerial infection within a young and aged population after low dose challenge with *L. monocytogenes* was investigated.

Methods: Animals received daily supplementation of vitamin E for a period of 21 days to promote immunomodulation, and were then orally challenged with 100 CFU of *L. monocytogenes*. Levels of CD8⁺, CD4⁺ and CD3⁺ T cells were used as markers to determine the influence of daily supplementation with vitamin E on immune response; the spleen and liver were harvested for microbiological analysis.

Results: Higher numbers of animals became infected in control groups than in vitamin E-treated group. During the post-challenge period, vitamin E-treated aged animals showed faster CD8⁺ T cell proliferation than control aged animals.

Conclusion: Daily supplementation with vitamin E was more beneficial in young compared with aged animals in mitigating listerial infection. Results suggest exposure to even low numbers of *L. monocytogenes* can result in infection in both healthy young and aged populations.

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Listeriosis is a serious infection that is associated with high mortality rate and the greatest number of hospitalizations in the United States compared with other foodborne pathogens [1]. The causative agent, *Listeria monocytogenes*, is able to grow under a wide range of conditions and is ubiquitous in the environment [2]; therefore, it has a high potential to contaminate food products. *L. monocytogenes* is a facultative intracellular bacterium that has the ability to evade and escape the hosts' immune system, resulting in the systemic disease listeriosis. Listeriosis occurs more frequently in immunocompromised people, such as cancer patients, pregnant women and the elderly. Survey data suggests that people consume *L. monocytogenes*-contaminated food on a daily basis [3]. In European countries, the incidence of listeriosis among persons ≥ 60 years of age is increasing [4]. The authors suggested that the increase may be associated with the consumption of food contaminated with low-levels of *Listeria*. The severity of *L. monocytogenes* infection is underscored by the recent outbreak of listeriosis in the United States that was linked to consumption of contaminated cantaloupe in which 147 persons became infected, 33 died, and one miscarriage [5]. This Center for Disease Control report indicated that most ill persons were 60 years or older. Consuming as few as 100 CFU *L. monocytogenes*/g may result in illness. Indeed, an outbreak involving frankfurters resulted in 101 cases of listeriosis and 21 fatalities although the level of contamination was extremely low; <0.3 CFU/g [6].

Research directed at lowering the incidence and the severity of listeriosis is important and desperately needed. Vitamin E is an important antioxidant in human plasma and tissue, and is necessary to maintain the function of the hosts' immune system [7]; however, the recommended daily allowance (RDA) of vitamin E is not sufficient to maintain elevated immune function. Orthomolecular doses of vitamin E have been studied with respect to immunological and clinical implications and have been shown to promote both the cellular and humoral immune responses in humans and animals. In particular, supplementation of the diet with vitamin E was demonstrated to stimulate the immune system of the elderly [8-12] at least in part by mitigation of viral and bacterial infections [8]. Research suggests that vitamin E has a positive effect on proliferation and stimulation of cytotoxic T cells [8, 13-15]. Others have reported that supplementation with 750 mg led to an increase in CD4:CD8ratio. Conversely, some studies show no immunomodulatory effect following vitamin E supplementation [16-18]. Experimental condition, subjects condition and supplement administration may explain these discrepancies.

L. monocytogenes mainly induces a cell-mediated immune response, since the majority of *L. monocytogenes* remain intracellular during infection. In the cell-mediated immune response, T lymphocytes play a major role. In the present study, orthomolecular levels of dietary vitamin E were evaluated as an immunoenhancer in aged and young animals, based on the implication among vitamin E supplementation, T cell response and listerial infection.

The elderly comprises 7% of the global human population and this number is expected to increase rapidly during the next five decades [19]. Immunosenescence is well-known to correlate with aging; thus, the elderly are at greater risk of developing listeriosis because of immunosenescence in combination with declining health status and a litany of other factors. Aged and young guinea pigs were used in this study to compare the influence of vitamin E supplementation on listerial infection in an aged and young population. The aged guinea pig model has been used previously [12, 20].

Guinea pigs were used as the animal model since listerial infection via the oral route in mice and rats is not reproducible, and does not become systemic. It can be induced in guinea pigs because *L. monocytogenes* can cross the intestinal barrier and disseminate throughout the body. The internalization of *L. monocytogenes* in humans is specific, relying on the interaction between human E-cadherin and Internal A (InlA) of *L. monocytogenes* [21, 22].

In this study, a diet containing an orthomolecular level of vitamin E was provided to guinea pigs and followed by intragastric gavage with low numbers of *L. monocytogenes*. Levels of CD3⁺, CD4⁺, and CD8⁺ T cells were monitored during the pre-challenge and post-challenge period as markers for determining influence of vitamin E supplementation on the immune system. Levels of CD3⁺ T cell populations were monitored since CD3⁺ proteins are associated with nearly all subsets of T cells [23]. CD4⁺ T cells are reported to be involved in response to listerial infection during primary infection in activation of dendritic cell, antigen-specific antibody and memory CD8⁺ T cells [24-28]. CD8⁺ T cell populations were monitored since they are involved in clearance of intracellular pathogens through adaptive immunity and were reported to increase in animals receiving orthomolecular levels of vitamin E [12, 29]. Plasma vitamin E concentrations were also monitored throughout the study. Incidence and latency of infection were evaluated post-challenge. The study was specifically designed to concurrently gauge the response of young and aged animals to exposure to *L. monocytogenes* and orthomolecular dietary vitamin E supplementation.

Materials and Methods

Bacteria

A cocktail of three *L. monocytogenes* serotype 4b strains, J1-110 (food epidemic), N1-225 (human epidemic), MMS97-1 (raw beef), were used. Growth conditions and cocktail preparation were according to Pang *et al.* [12].

Animals

Outbred female Dunkin-Hartley guinea pigs weighing *ca.* 250-300 g and retired breeders weighing *ca.* 1000 g were designated as young and aged animal models, respectively. Animals were purchased from Charles River Lab. Inc. (Wilmington, MA). All animals were housed under standard conditions (50% humidity and 12 h-dark-12 h-light cycle) at the Rutgers animal facility, and each animal was housed in individual cages. Fresh potable water and food was available *ad lib*. Upon arrival at the animal facility, all animals were weighted and randomly assigned to one of four groups: vitamin E young, control young, vitamin E aged and control aged. A total of 75 and 77 animals were included in analysis for duplicate experiments. All animal experiments were conducted in accordance with federal guidelines and were approved by the Rutgers University animal care and facilities committee.

Dietary vitamin E supplementation

Supplemental vitamin E was incorporated into the diet. Purina guinea pig chow was supplemented with 5,000 IU vitamin E [DL- α -tocopherol acetate] /kg feed (Research Diets Inc., New Brunswick, NJ). This level was used to achieve *ca.* 200 IU vitamin E per day. Animals from the control groups received only Purina guinea pig chow. Animals were maintained on the respective diets for the duration of the study.

Blood and plasma samples collection

Blood and plasma samples were collected according to Pang *et al.* [12]. Blood samples were used immediately for indirect immunostaining and plasma samples were frozen immediately at -80 °C until required for further analysis.

Indirect immunostaining

A 100 μ L whole blood sample was incubated with 100 μ L of IQ lyse (IQ Products, The Netherlands) for 10 min at room temperature. Cells were washed with PBS containing fetal bovine serum (FBS, 2% v/v). Monoclonal mouse anti-guinea pig CD8⁺, CD4⁺, and CD3⁺ antibodies conjugated to FITC, PE

and APC (AbD Serotec Inc., Raleigh, NC), respectively, were added to blood sample preparations in series. Samples were incubated for 10 min at room temperature in the dark, and cells were washed between each incubation. Working concentrations of antibodies were used according to the manufacturer's recommendations. Coulter Flow Count (100 μ L) (Beckman Coulter Inc., Hialeah, FL) and PBS containing 2 % FBS (900 μ L) were added to cells immediately prior to flow cytometry analysis. Samples incubated with a single or no antibody preparation served as negative controls. Samples were analyzed in a Coulter Cytomic FC500 Flow Cytometer (Becton-Dickinson, North Carolina, USA).

HPLC analysis of α -tocopherol concentration in the plasma

HPLC analysis of α -tocopherol concentration in the plasma was according to Pang *et al.* [12]. Plasma samples stored at -80 °C from both control and vitamin E-treated guinea pigs were analyzed. Samples were processed for extraction and analyzed using a DIONEX Ultimate 3000 HPLC (California, USA) under the following conditions: mobile phase, methanol; flow rate, 0.85 mL/min; UV detector, 295 nm and running time, 18 min.

Intragastric Listeria monocytogenes challenge

Food was withheld for 12 h prior to intragastric challenge in order to prevent regurgitation during challenge. Each animal received *ca.* 1.5×10^2 CFU of the *L. monocytogenes* serotype 4b cocktail using a 38 cm nasogastric feeding tube (Jorgensen Laboratories Inc., Loveland, CO) fitted onto a 3 mL syringe.

Organ collection and microbiology

Liver and spleen were collected aseptically *in toto*. Organ samples were weighed and placed into sterile plastic tubes to which a volume equal to the weight of the sample of cold PBS containing 0.001 % Triton-X was added. The samples were then homogenized using a tissue homogenizer (Polytrons, Kinematica, Switzerland). A 100 μ L volume was plated onto duplicate Rapid L'mono plates (Bio-Rad Laboratories, Inc., Richmond, CA) and the plates incubated at 37 °C for 24-48 h. *L. monocytogenes* colonies were counted and numbers expressed as mean log CFU/g of organ. Remaining organ samples were stored at 4 °C.

Enrichment of samples

All organ samples were also subjected to enrichment procedures. An amount of 2 X BHI broth (1 ml for spleen samples,

10 ml for liver samples) were added to the organ samples and samples incubated at 37 °C for 24-48 h. A (100 µL) aliquot was plated onto Rapid L'mono plate and PALCAM agar plates (EMD Chemicals Inc. Gibbstwon, NJ, USA), in duplicate and the plates were incubated at 37 °C for 24-72 h to detect the presence of *L. monocytogenes*.

Statistical analysis

Microbiological data was analyzed using a Z-test to compare the significant differences on the total numbers of animals infected between groups. Animals were considered infected based on isolation of *L. monocytogenes* from the spleen or liver. All T cell population data was analyzed using Microsoft Excel and SAS. The significant differences between the time point means were determined by SAS Duncan's multiple range test, glm (general linear model) procedure. Data were presented as means $\pm$ STDEV. The level of statistical relevance for all comparisons was set at $P < 0.05$.

Results

Data representing pre-challenge period were labeled wk0, wk1, wk2, wk3 and data representing post-challenge period were labeled D0, D3, D6, D9, D12, D16, D20, where Wk3 was identical to D0. Wk0 and wk3/D0 were considered as the

baseline levels for the pre-challenge and post-challenge period, respectively, for all statistical comparisons.

Levels of plasma α -tocopherol concentration were greater in animals receiving the vitamin E diet

Concentration of plasma α -tocopherol was analyzed by HPLC and converted according to the concentration of α -tocopherol acetate (internal standard). The mean plasma α -tocopherol concentration at wk0 in aged animals was greater (114.88 nM) compared with the young animals (50.54 nM) (Fig. 1). Levels of plasma vitamin E remained unchanged in the young control group ($P > 0.05$); however, in the aged control group, plasma vitamin E concentration declined nearly 50% by wk1 and continued to decrease significantly ($P < 0.05$) at wk2 and wk3 compared with wk0 (Fig. 1A). In the vitamin E young group, the plasma vitamin E level was 2- and 3-fold greater at wk1 and wk2 ($P < 0.05$), respectively, compared with wk0, and remained 1.8-fold greater at wk3 however this difference was not statistically significant (Fig. 1B). In vitamin E aged animals, the concentration of plasma α -tocopherol remained fairly constant from wk0 to wk3 ($P > 0.05$) (Fig. 1B).

During the post-challenge period, in aged animals receiving vitamin E, the starting plasma vitamin E concentration was 93.2 nM (wk3/D0) and then peaked at D6 (413.4 nM) and

TABLE 1. Infection of spleen and liver samples following oral challenge with 100 CFU *L. monocytogenes*.

Day post-challenge	Organs	Young		Aged	
		Vitamin E	Control	Vitamin E	Control
Day 3	Liver [‡]	0/6	3/6	2/6	2/6
	Spleen [‡]	0/6	2/6	0/6	3/6
Day 6	Liver	0/6	0/6	2/6	3/6
	Spleen	0/6	0/6	1/6	0/6
Day 9	Liver	0/6	0/6	1/6	0/6
	Spleen	0/6	0/6	0/6	0/6
Day 12-20	Liver	0/20	0/19	0/20	0/21
	Spleen	0/20	0/19	0/20	0/21
Total no. of animals infected		0/38	3/37*	5/38	6/39
Percentage of animals infected		0%	8%	13%	15%

[‡]Liver and spleen samples were obtained from two independent experiments before oral gavage with *L. monocytogenes* and on day 3, 6, 9, 12, 16 and 20 post-challenge. All organ samples were negative for *L. monocytogenes* by direct plating. Total number of animals infected was based on the presence of *L. monocytogenes* in the liver or spleen following subculture.

*Significant difference in total numbers of young vitamin E and control animals infected, $P < 0.05$.

D20 (272.03 nM) (Fig. 1B); however, plasma vitamin E concentration remained constant until D16 ($P>0.05$) and then peaked at D20 ($P<0.05$) in the control aged animals (Fig. 1A). A similar response profile occurred in the vitamin E young animals, although the initial peak occurred at D3 (Fig. 1B). In control young animals, plasma vitamin E concentration increased ($P<0.05$) at D3 and D12 (Fig. 1A).

*Listerial infection following oral challenge with ca. 100 CFU *L. monocytogenes**

All animals were intragastrically challenged with ca. 100 CFU *L. monocytogenes* and infection was determined based on microbiological culture of liver and spleen samples. Diarrhea was sporadically observed but few animals became infected and no

FIGURE 1. Plasma α -tocopherol concentration of young (A) and aged (B) guinea pigs during pre-challenge period (wk0-wk3) and post-challenge period (D3-D20). Blood samples were obtained from two independent experiments. Plasma vitamin E concentration was expressed as mean $\pm$ STDEV (nM). Significant differences were analyzed by GLM Duncan's multiple range test; comparisons were not between groups.

* Statistical comparisons of pre-challenge period (wk0-wk3) were within group and based on wk0, $P<0.05$.

† Statistical comparisons of post-challenge period (D3-D20) were within group and based on wk3, $P<0.05$.

FIGURE 2. Changes in the levels of total (CD3⁺) T lymphocytes of young (A) and aged (B) guinea pigs during pre-challenge period (wk0-wk3) and post-challenge period (D3-D20). Data of CD3⁺ T cell were presented in the absolute number of cells per micro liter of plasma (cells/ μ L). Blood samples were obtained from two independent experiments. Each curve represents the mean $\pm$ STDEV of two independent experiments. Significant differences were analyzed by GLM Duncan's multiple range test; comparisons were not between groups.

* Statistical comparisons of pre-challenge period (wk0-wk3) were within group and based on wk0, $P < 0.05$.

† Statistical comparisons of post-challenge period (D3-D20) were within group and based on wk3, $P < 0.05$.

deaths occurred. The results of microbiological analysis of liver and spleen samples showed that all animals were negative for listerial infection after direct plating (data not shown). All organ samples were processed for microbiological enrichment (Table 1). When comparing the percentage of total animals infected, vitamin E-treated young group had a significant lower percentage of infected animals compared to other groups ($P < 0.05$). None of the young guinea pigs receiving vitamin E developed infection, in contrast to untreated young guinea pigs in which 8% of animals became infected. Infections were not evident in young animals (control and vitamin E groups) beyond day 3 post-challenge. For aged animals, 13 % of vitamin E-treated and 15 % of control animals became infected. Those animals that were infected, developed infection by day 3, 6 or 9 post-challenge. No animals developed infection at day 12 or beyond.

Change in T cell proliferation associated with dietary vitamin E supplementation

Levels of CD3⁺ T cells of young and aged control animals increased significantly by wk3 ($P < 0.05$) compared with wk0 (Fig. 2A); there were no significant changes in CD3⁺ T cells during the pre-challenge period in vitamin E-treated young and aged guinea pigs (Fig. 2B). Levels of CD3⁺ T cells fluctuated over the post-challenge period for all groups (Fig. 2A and B). On day 3 post-challenge, only the vitamin E-treated aged group showed a significant (28.0 %, $P < 0.05$) increase in CD3⁺ T cell levels; levels tended to decline in the other groups. In the control young group, there was a significant decrease ($P < 0.05$) in numbers of CD3⁺ T cell on D6, D12, D16 and D20 compared to D0 (Fig. 2A). In the vitamin E-treated young animals the CD3⁺ T cell population fluctuated compared with D0, but not significantly. In control aged animals, a significant decline in numbers of CD3⁺ T cells occurred at D9, D12, D16 and D20 compared to D0 ($P < 0.05$) (Fig. 2A). In the vitamin E aged animals, CD3⁺ T cells peaked at D3 and D9 ($P < 0.05$) and then significantly decreased by D12 ($P < 0.05$) (Fig. 2B). The absolute numbers of CD3⁺ T cells remained near or below the base line level in both control groups (Fig. 2A); however, in both vitamin E-treated groups elevated numbers of CD3⁺ T cells were observed periodically during the post-challenge period (Fig. 2B).

During the pre-challenge period levels of CD4⁺ T cells increased significantly by wk2 in all groups except for the control young group. During the post-challenge period, fluctuations in levels of CD4⁺ T cells occurred similarly in young and aged animals (Fig. 3). CD4⁺ T cell levels declined sharply ($P < 0.05$) on D3 and D12 compared to D0 in control and vitamin

E young animals (Fig. 3A and B). CD4⁺ T cell levels decreased significantly on D3 and D16 in control and vitamin E aged animals.

During the pre-challenge period, levels of CD8⁺ T cells remained nearly constant in all groups ($P > 0.05$) (Fig. 4). During the post-challenge period the percent CD8⁺ T cells significantly increased compared to D0: vitamin E aged - D3, D9, D12, D16; control aged - D9, D16; control young - D9, D20; and vitamin E young - D12 (Fig. 4).

Discussion

In this study, aged (retired breeders, *ca.* 2 years of age) and young (*ca.* 6 months of age) guinea pigs served as surrogates for aged and young adult human population, respectively. The incidence of listerial infection steadily increases from age 40 to 64 with a more dramatic increase at age 65 onwards, indicating that age or age-related reasons are predisposing factors for developing listeriosis [3]. This guinea pig model represents aged adults in the 'at risk' group. Immunomodulation through dietary vitamin E supplementation and the development of listerial infection was investigated. The duration of the vitamin E supplementation period prior to challenge for this study was selected based on a previous study reporting a significant increase in the plasma α -tocopherol level achieved by day 21 in aged guinea pigs supplemented for 35 days [12]. The challenge dose, *ca.* 100 CFU, was a level that based on statistical probabilities would be expected to result in only a limited number of infections; however, this level of *L. monocytogenes* can result in listeriosis. The 10² CFU dose used in this study was selected based on studies using guinea pigs demonstrating 100%, *ca.* 90%, 25% and 13 to 50% of animals becoming infected when challenged orally with 10⁸, 10⁶, 10⁴ and 10² CFU *L. monocytogenes*, respectively [12, 30]. Changes in T cell levels were used as a marker to gauge the immunomodulatory effect of vitamin E supplementation and immune response to *L. monocytogenes* infection.

All guinea pigs in the treated groups received daily *ca.* 200 IU vitamin E, which is approximately 8-fold greater than the RDA for adult humans. The RDA for a 70 kg human is 22.5 IU vitamin E/ day, and the upper limit (UL) is 1500 IU vitamin E/ day. Many animal and clinical studies have shown that there are no important adverse effects of high dose vitamin E supplementation; although a few clinical cases have reported that vitamin E supplementation has adverse results [31-34]. In this study, no adverse effects were observed. The vitamin E diet was feed throughout the experiment to eliminate any potential bias (rejection of feed, subsequent weight loss, etc.) that may result from change in diet. The initial 21 day period of vitamin

FIGURE 3. Changes in the levels of helper (CD4⁺) T lymphocytes of young (A) and aged (B) guinea pigs during pre-challenge period (wk0-wk3) and post-challenge period (D3-D20). Percent CD4⁺ T cells were calculated by dividing the number of CD4⁺ T cell by number of CD3⁺ (total) T lymphocytes and multiplying by 100. Blood samples were obtained from two independent experiments. Each curve represents the mean $\pm$ STDEV of two independent experiments. Significant differences were analyzed by GLM Duncan's multiple range test; comparisons were not between groups.

* Statistical comparisons of pre-challenge period (wk0-wk3) were within group and based on wk0, $P < 0.05$.

† Statistical comparisons of post-challenge period (D3-D20) were within group and based on wk3, $P < 0.05$.

FIGURE 4. Changes in the levels of cytotoxic (CD8+) T lymphocytes young (A) and aged (B) guinea pigs during -challenge period (wk0-wk3) and post-challenge period (D3-D20). Percent CD8+ T cells were calculated by dividing the number of CD8+ T cell by number of CD3+ (total) T lymphocytes and multiplying by 100. Blood samples were obtained from two independent experiments. Each curve represents the mean $\pm$ STDEV of two independent experiments. Significant differences were analyzed by GLM Duncan's multiple range test; comparisons were not between groups.

* Statistical comparisons of pre-challenge period (wk0-wk3) were within group and based on wk0, $P < 0.05$.

† Statistical comparisons of post-challenge period (D3-D20) were within group and based on wk3, $P < 0.05$.

E supplementation had a stimulatory effect on plasma α -tocopherol concentration in both young and aged animals. The change in plasma α -tocopherol levels indicates the absorption of vitamin E from the intestine [35]. Research demonstrated that plasma α -tocopherol concentrations are limited and levels can only increase 2- to 3-fold, regardless of the supplementation regime [36-39], because of the rapid turnover rate of α -tocopherol in the plasma. The same trend was observed in the vitamin E-treated young animals in the present study. In addition, Hollander and Dadufalza [40] concluded that the total amount of vitamin E absorbed increases with aging due to the high demand for antioxidants in the elderly. The study suggests that there is higher total absorption of vitamin E in aged rats than in young rats. In the present study, higher basal levels (wk0) of plasma vitamin E were observed in the aged animals compared to young animals, likely a result of high demand for antioxidants associated with stress occurring during transportation from the vendor to the research facility.

During the post-challenge period levels of plasma α -tocopherol were significantly increased at D3, D16, D20 and D6, D20 for vitamin E young and aged animals, respectively. Indeed, plasma vitamin E concentrations were an average of 3.2-fold greater days 3 and 6 post-challenge in vitamin E-treated guinea pigs, although levels dramatically dropped by day 9. The initial significant increase may have been due to the acute demand for antioxidants during an immune response to combat the listerial challenge. Macrophage activation and various immune responses produce free radicals, and the host requires antioxidants immediately to eliminate those radicals. Vitamin E was probably mobilized to neutralize the production of reactive oxygen species during immune responses [41]. The mechanism of the mobilization is not yet known. The body likely responds with the release of tocopherol from the liver under conditions of stress. The liver plays an important function in regulation of vitamin E disposition, metabolism, and excretion through α -TTP [42]. Pincemail *et al.* [43] demonstrated that the plasma tocopherol levels increased significantly in subjects during intensive exercise, which also caused the production of oxidative radicals. The mobilization of tocopherol could help to prevent lipoperoxidation phenomena occurring in skeletal muscle during exercise. The rapid turnover rate of tocopherol in plasma may result in the marked decline of plasma tocopherol concentration. The fluctuation in plasma α -tocopherol levels may demonstrate the animals attempt to address physiological requirements and achieve homeostasis.

Under conditions of the present study, short-term (21 days) daily dietary supplementation with vitamin E did not provide a significant net positive immunoenhancing effect in

young or aged guinea pigs based on limited change in CD3⁺ (Fig. 2B), CD4⁺ (Fig. 3B), and CD8⁺ (Fig. 4B) T cell populations in the pre-challenge period ($P>0.05$). The functional capacity of T cells may have been enhanced through dietary vitamin E supplementation based on fewer infections in animals receiving the orthomolecular levels of vitamin E. Studies conducted by Adolfsson *et al.* [44] using T cells from vitamin E supplemented animals showed that vitamin E supplementation increases cell-dividing capacity, IL-2-production capacity, and high affinity IL-2R of the naïve T cells in aged mice.

During the post-challenge period, the results suggest that dietary vitamin E supplementation promoted CD8⁺ and CD3⁺ T cell response in aged guinea pigs. The promotion is not evident in the young animals, which may be due to the fact that young guinea pigs would have a healthy robust immune system capable of clearance of *L. monocytogenes* without induction of a robust and urgent T cell production. Vitamin E resulted in early expansion of the CD8⁺ T cell population and promoted CD3⁺ T cell response in the vitamin E aged group. CD8⁺ and CD3⁺ T cells increased significantly by D3 in the vitamin E aged animals. A previous study has shown that notable changes in the CD8⁺ T cell response requires 4-5 days, and peaks at 7-9 days post-infection [45]. In the control aged animals, the CD8⁺ T cell response follows the normal expansion trend. The expansion in the vitamin E young group of the CD8⁺ T cell population on D12 suggests the production of memory CD8⁺ T cells after infection; however, the expansion on D9 in control group indicated the normal expansion of cytotoxic T cells for pathogen elimination. Levels of CD8⁺ T cells, for all four groups, remained elevated above wk3/D0 throughout the entire post-challenge period suggesting cytotoxic T cells were induced upon listerial infection. Vitamin E supplementation had a limited effect on CD4⁺ T cell populations after listerial infection, since similar trends were observed in both control and vitamin E-treated animals. In contrast to CD8⁺ T cells, levels of CD4⁺ T cells remained below wk3/D0 levels throughout the entire post-challenge period, this may indicate the utilization of helper (CD4⁺) T cells for memory T cells, antigen-specific antibody production [46]. In the present study, listerial infection in general stimulated CD3⁺, CD4⁺ and CD8⁺ T cell response during the post-challenge period. No vitamin E-treated young animals became infected post-challenge, consistent with the maintenance of a relatively stable CD3⁺ T cell population.

In this study, dramatic and long-term significant change in CD3⁺, CD4⁺ and CD8⁺ T cell proliferation in the pre-challenge period was not observed. This is in contrast to studies done by other groups [9,12,47]; however, higher levels of vita-

min E or longer treatment periods were used in the previously published studies. In our study, an insufficiently high level of vitamin E in the diet and limited period of vitamin E supplementation prior to *L. monocytogenes* challenge may have attributed to the failure of enhanced CD8⁺ T cells, helper (CD4⁺) T cells and total (CD3⁺) T cell proliferation in young and aged guinea pigs. Dietary vitamin E supplementation near or at orthomolecular levels does not always result in desired effects over an extended supplementation period [16-18].

Epidemiological reports and dose-response studies on animals showed that the infectious dose for *L. monocytogenes* can be low. In the present study, regardless of treatment, the greatest numbers of animals were positive for listerial infection on day 3 post-challenge. This trend was also observed by other researchers [48]. After day 12 post-challenge, none of the guinea pigs were positive for *L. monocytogenes*; demonstrating that the latency of listerial infection was not seen under the conditions evaluated. Infection status suggests that dietary vitamin E supplementation was more beneficial in young guinea pigs than in aged guinea pigs.

In conclusion, the study design permitted the concurrent evaluation of young and aged animals following orthomolecular dietary supplementation of vitamin E and challenge with *L. monocytogenes*. To the best of our knowledge, this has not been reported previously. Short-term daily dietary vitamin E supplementation resulted in significantly lower numbers of infected young animals but no significant change in number of infected aged animals. The effect of vitamin E supplementation was not overtly beneficial in promoting T cell proliferation in young or aged guinea pigs based on the three weeks of vitamin E supplementation. Young and aged animals became infected following challenge with low levels of *L. monocytogenes* (ca. 100 CFU) and latency of infection was not apparent. Dietary vitamin E supplementation promoted CD8⁺ T cell proliferation especially in infected aged animals. It is likely that the robust nature of the immune system of young animals was capable of combating exposure to the low number of *L. monocytogenes* encountered without having to mount a substantial immune response. These results may suggest that short-term daily dietary vitamin E supplementation may achieve a sufficiently robust enough immunomodulatory response to mitigate or protect against listerial infection in aged animals. The immunomodulatory effect of vitamin E supplementation was more evident in young animals.

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