Arginine Enhances In Vivo Immune Responses in Young, Adult and Aged Mice1,2,3

ABSTRACT Arginine supplementation enhances in vitro lymphocyte proliferation in healthy adult humans and in rodent models. Studies examining the effect of arginine supplementation on in vivo immune responses are lacking. The purpose of this study was to determine whether arginine supplementation could enhance in vivo immune responses in adult mice and reverse known age-associated alterations in immune function of young and aged mice. Mice (1, 10 and 33 mo old) were fed a 2% arginine or an isonitrogenous diet for 2 wk. Delayed-type hypersensitivity to 2,4-dinitrofluorobenzene–challenged ears and changes in popliteal lymph node weights to injected sheep red blood cells were measured. The mean percentage of increase in ear thickness in challenged vs. unchallenged ears was 27, 35 and 24% with arginine supplementation and 7, 12 and 0% with the isonitrogenous diet in the 1-, 10- and 33-mo-old mice, respectively (P ≤ 0.05 for each age). Across all ages, the mean differences in popliteal lymph node weights were 1.9 ± 0.3 vs. 1.0 ± 0.3 mg with the arginine and isonitrogenous diets, respectively (P ≤ 0.05). Only the 33-mo-old mice fed the isonitrogenous diet did not respond to this immune challenge. These findings suggest that arginine supplementation may enhance in vivo immune responses and/or reverse age-associated changes. J. Nutr. 130: 1827–1830, 2000.

KEY WORDS: • mice • arginine • aged • immune function • delayed-type hypersensitivity

Arginine is a conditionally essential amino acid that is required for optimal growth (Rose 1937). When supplemented at pharmacologic levels, arginine prevents thymic involution and increases mitogen-induced thymocyte proliferation in young-adult rodent models (Barbul et al. 1980a and 1980b). In healthy humans, dietary arginine supplementation enhances mitogen-induced peripheral blood lymphocyte proliferation (Barbul et al. 1981a). Under conditions of stress, arginine supplementation enhances in vivo delayed-type hypersensitivity (DTH) and in vitro mitogen-induced lymphocyte proliferation, and prevents injury-induced atrophy of the thymus (Barbul et al. 1980a, 1981b and 1985, Kennedy et al. 1994, Nirgiotis et al. 1991, Saito et al. 1987). Although arginine supplementation appears to benefit in vitro immune responses in healthy adult populations, there is little, if any knowledge of the effect of arginine supplementation on in vivo immune function or on immune responses across ages. Arginine may be of benefit to immune function in the extremes of life. In the young, the immune system is immature, and in the elderly, immune function is impaired. The age-associated decline in immune function increases susceptibility to infection (Roberts-Thomson et al. 1974, Wick and Grubeck-Loebenstein 1997). Depressed DTH responses measured in elderly nursing home residents are associated with increased mortality (Cohn et al. 1983). This study evaluated DTH and popliteal lymph node weights after an immune challenge in mice of three different ages previously supplemented with a 2% arginine diet. The efficacy of arginine supplementation on reversing the age-associated changes in these immune responses was also evaluated.

MATERIALS AND METHODS

Procedures were approved by the University of Florida Animal Care and Use Committee. On the basis of recommendations from the U.S. National Research Council, National Institute of Aging and others (Makinodan 1995) for aged animal models, hybrid, specific-pathogen–free mice were obtained for this study.

Animals and Diets. Recently weaned 1-mo-old and 10- and 33-mo-old male CB6F1 (BALB/c × C57BL/6) mice (n = 52) were purchased from the National Institute of Aging Colonies at Charles River Laboratories (Stone Ridge, NY). Mice of similar age were housed in groups of two or three in an environmentally controlled room with constant temperature (22°C) and 12-h light:dark cycle. Mice were randomly assigned to either an arginine diet (20 g/kg) or a diet made isonitrogenous to the arginine diet with the addition of alanine. The 1-mo-old mice received an AIN 93 G diet (Reeves et al. 1993) supplemented with arginine or alanine and the 10- and 33-mo-old mice received an AIN 93 M diet supplemented with arginine or alanine. The isonitrogenous AIN 93 G and AIN 93 M diets provided 6.6 and 4.6 g/kg arginine, respectively. All diets were made by Harlan Teklad (Table 1; Madison, WI). After a 7- to 10-d acclimation period, the mice were weighed and given free access to the diets for 2 wk. Free access to water was given throughout the study.

Delayed-type hypersensitivity. On d 9 of the experiment, the mice were anesthetized with methoxyflurane. To sensitize the mice, 50 μL of 5 mL/L 2,4-dinitrofluorobenzene (DNFB) in a 4:1 acetone/olive oil solution was applied to their shaved abdomens. On d 10, the mice were then re-sensitized to DNFB by again applying 50 μL of the above 5 mL/L solution. On d 14, the mice were challenged with DNFB by applying 20 μL of 2 mL/L DNFB in a 4:1 acetone/olive oil solution to the left ear and 20 μL of the 4:1 acetone/olive oil solution to the right ear to serve as an unchallenged control. The mice were

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3 This is Florida Agricultural Experiment Station, Journal Series No. R-07006.
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While the mice were anesthetized on d 9 for the DTH protocol, 1.0 × 10^8 sheep red blood cells (Sigma Chemical, St. Louis, MO) in 50 μL of sterile PBS were injected into the left hind foot pad of the mice and 50 μL of PBS was injected into the right hind foot pad to serve as an unchallenged control. On d 15, the mice were anesthetized and killed by cervical dislocation. The lymph nodes were removed from each leg and weighed.

## RESULTS

### Body weight.
Initial body weights of mice of similar ages were not different between diet groups. After 14 d of diet treatment, the change in weight was not significantly different between age or diet groups (data not shown).

### Delayed-type hypersensitivity.
When diet, age and DTH response were considered in a single statistical model, the DTH response was affected by diet (*P* ≤ 0.05). Across all ages, there was a mean difference of 1.9 ± 0.3 mg in popliteal lymph node weights between the leg challenged with red blood cells and the unchallenged leg in the arginine-fed mice. This was significantly greater than the 1.0 ± 0.3 mg difference in mice fed the isonitrogenous diet (*P* ≤ 0.05). (Fig. 2) When data were analyzed within each age, 

![Graph showing DTH response](https://academic.oup.com/jn/article-abstract/130/7/1827/4686193)

**FIGURE 1** The delayed-type hypersensitivity (DTH) response in 1-, 10- and 33-mo-old mice fed a 2% arginine or isonitrogenous diet. After the mice were sensitized to dinitrofluorobenzene (DNFB), one ear of each mouse was challenged with DNFB. The unchallenged ear served as a control. Ear thickness of both ears was measured. Arginine supplementation significantly increased (*P* ≤ 0.05) the DTH response across ages. Results are expressed as means ± SEM, *n* = 9 (1 mo), 10 (10 mo) or 7 (33 mo). Within an age group: *P* = 0.05 vs. respective unchallenged ears and †*P* = 0.05 vs. isonitrogenous diet.

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**TABLE 1**

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<th>Component</th>
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<th>AIN 93G + arginine</th>
<th>AIN 93M + alanine</th>
<th>AIN 93M + arginine</th>
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<td>200</td>
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<td>140</td>
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<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN 93 vitamin mix</td>
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<td>10</td>
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<tr>
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<tr>
<td>TBHQ2 (antioxidant)</td>
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<td>0.014</td>
<td>0.008</td>
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</tr>
</tbody>
</table>

1 Reeves et al. (1993).

2 tert-Butylhydroquinone.
Within each age group: the proliferative response decreased with advancing age. By 36 mo of age, the response appeared to be maximal. After 8 mo of age, the proliferative response was greatest in mice fed the arginine diet before immune challenge did respond (P < 0.05). Aged mice fed the isonitrogenous diet did not respond to the immune challenge. However, aged mice fed the isonitrogenous diet were also unable to mount a DTH response. The mean percentage of increase in ear thickness in challenged vs. unchallenged ears was 7, 12 and 0% with the isonitrogenous diet and 27, 35 and 24% with the arginine-supplemented diet in the 1-, 10-, and 33-mo-old mice, respectively. This suggests that arginine supplementation increased the DTH response in adult mice and reversed age-associated changes in DTH response in young and aged mice. To our knowledge, this is the first study to report that arginine supplementation enhances the DTH response in normal, healthy mice of any age.

Saito et al. (1987) demonstrated an enhanced DTH response with arginine supplementation in a stressed rodent model. Guinea pigs with burns representing 30% of their total body surface area were fed intragastrically a solution supplemented with arginine at 0, 1, 2 and 4% of total energy intake. Ear thickness in response to DNFB challenge was greatest in the group supplemented with 2% of their total intake as arginine. Additionally, the mortality rates were 56, 29, 22 and 36% in the groups supplemented with 0, 1, 2 and 4% arginine, respectively (Saito et al. 1987). This suggests that arginine supplementation may be beneficial to a point, after which toxicity may occur. We used a 2 g/100 g arginine diet. This amount of arginine would represent ~2% of total energy of the growth and maintenance diets.

Changes in popliteal lymph node weights in response to antigens were also measured in this study. Sheep red blood cells injected into the hind leg footpad enter the lymphatic vessels and become trapped in the popliteal lymph node. T lymphocytes within the node are activated and begin proliferating (Papadimitriou et al. 1983). Lymphocytes from the blood also migrate into the area resulting in visible swelling of the nodes. TH1-type memory T cells preferentially localize into peripheral lymph nodes (Premier et al. 1996). Proliferation and DTH responses (anergy) are associated with increased morbidity and mortality (Cohn et al. 1983, Meakins et al. 1977). Our experiments reported here are consistent with previously published data in that there was no observed DTH response in the 33-mo-old mice fed the isonitrogenous diet. At the other extreme of age, young mice fed the isonitrogenous diet were also unable to mount a DTH response. The mean percentage of increase in ear thickness in challenged vs. unchallenged ears was 7, 12 and 0% with the isonitrogenous diet and 27, 35 and 24% with the arginine-supplemented diet in the 1-, 10-, and 33-mo-old mice, respectively. (Fig. 1). This suggests that arginine supplementation increased the DTH response in adult mice and reversed age-associated changes in DTH response in young and aged mice. To our knowledge, this is the first study to report that arginine supplementation enhances the DTH response in normal, healthy mice of any age.

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DISCUSSION

Young, adult and aged mice supplemented for 2 wk with pharmacologic levels of arginine responded to in vivo immune challenges. Only adult (10-mo-old) mice fed an isonitrogenous diet responded to both immune challenges; however, the DTH response was greater in the adult mice supplemented with arginine. These data suggest that arginine supplementation enhanced in vivo immune responses and/or reversed age-associated changes in immune response.

Early studies by Hori et al. (1973) demonstrated that mitogen-induced murine splenocyte proliferation increased from 2 wk of age until 8 mo of age when functional maturity appeared to be maximal. After 8 mo of age, the proliferative response decreased with advancing age. By 36 mo of age, the proliferative response was 2.5% of maximal (Hori et al. 1973). Aging of adult animals is associated with a progressive decline in immune competence with the major age-related changes attributed to altered T-lymphocyte function. Such changes include thymic involution and decreased mitogen-induced proliferation and DTH responses (Goodwin et al. 1982, Goya et al. 1992, Hori et al. 1973, Waldorf et al. 1968). Changes in the T-cell subsets, determined by cytokine expression, have also been observed with aging. There is a decrease in the T_H1-associated cytokines and a parallel increase in the T_H2-associated cytokine secretion (Hobsb et al. 1991, Kubo and Cinader 1990). A shift in T-cell subsets would account for the decrease in interleukin-2 secretion observed in an aged population.

The DTH response decreases with age (Bender and Tallman 1992, Goodwin et al. 1982, Waldorf et al. 1968), and normal DTH response (anergy) is associated with increased morbidity and mortality (Cohn et al. 1983, Meakins et al. 1977). Our experiments reported here are consistent with previously published data in that there was no observed DTH response in the 33-mo-old mice fed the isonitrogenous diet. At the other extreme of age, young mice fed the isonitrogenous diet were also unable to mount a DTH response. The mean percentage of increase in ear thickness in challenged vs. unchallenged ears was 7, 12 and 0% with the isonitrogenous diet and 27, 35 and 24% with the arginine-supplemented diet in the 1-, 10-, and 33-mo-old mice, respectively. (Fig. 1). This suggests that arginine supplementation increased the DTH response in adult mice and reversed age-associated changes in DTH response in young and aged mice. To our knowledge, this is the first study to report that arginine supplementation enhances the DTH response in normal, healthy mice of any age.

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fed the isonitrogenous diet (P < 0.05) (Fig. 2). These data support the DTH findings in that popliteal lymph node weights and the DTH response across ages were greater in mice fed the arginine diet and that the response to immune challenge in aged mice was evident only after arginine supplementation.

Although T lymphocytes may be the effector cells responsible for directly mediating the observed effects of arginine supplementation (Barbul et al. 1977, Kennedy et al. 1994, Kirk et al. 1992, Reynolds et al. 1988), the mechanism by which arginine may influence immune function is via its ability to increase growth hormone secretion (Merimee et al. 1967). In animal studies, the thymotropic effects of arginine after injury are abolished by hypophysectomy. Additionally, arginine supplementation has no thymotropic effect in hypophysectomized animals given growth hormone replacement therapy (Barbul et al. 1983). In animals and humans, aging is associated with decreases in growth hormone secretion and a reduction in thymus weight and function (Goya et al. 1992, Rudman et al. 1981). Growth hormone treatment increases the proliferative response of thymocytes to the T-cell mitogen concanavalin A by ~57 and 328% in middle-aged and old mice, respectively (Goya et al. 1992). Although arginine may exert some of its trophic effect through the action of growth hormone on the thymus, an intact thymus is not a requirement. Kirk et al. (1992) demonstrated an increase in mitogen-induced splenocyte proliferation and DTH response in athymic nude mice supplemented with arginine. Therefore, growth hormone may stimulate immune function directly in that many immune cells possess receptors for growth hormone and its principal mediator, insulin-like growth factor-I (Eshet et al. 1975, Geffner et al. 1990).

These experiments showed no DTH response in young and aged animals and no increase in popliteal lymph node weight after immune challenge in aged animals fed a control diet. Supplementation of diets with pharmacologic levels of arginine enhanced the DTH response in adult mice and reversed age-associated changes in young and aged mice. Aged mice supplemented with arginine also showed an increase in lymph node weight in response to immune challenge. Because an increase in a specific immune variable does not correlate necessarily with a positive clinical outcome, future studies will examine the effect of arginine supplementation on morbidity and mortality after infectious challenge.

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LITERATURE CITED


