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Research Article

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Prevention of Autoantibody Formation and Prolonged Survival in New Zealand Black/New Zealand White F₁ Mice Fed Dehydroisoandrosterone

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Abstract

Dehydroisoandrosterone, administered orally to New Zealand Black/New Zealand White F₁ hybrid mice, prevented the formation of antibodies to double-stranded DNA and prolonged survival in this murine model of lupus erythematosus.

Introduction

New Zealand Black/New Zealand White F₁ hybrid (B/W)¹ mice serve as an excellent animal model for the study of systemic lupus erythematosus (SLE) (1, 2). Spontaneously, these mice develop an autoimmune disorder that is characterized by the formation of anti-DNA and antinuclear antibodies (ANA). In these mice, there are circulating immune complexes; and there are immunoglobulin deposits in the renal glomeruli and along dermal-epidermal junctions (3). The maximal degree of severity of the immune complex glomerulonephritis (GN) is attained at 9 mo of age and death most often occurs before 12 mo of age.

Hurd et al. (4) demonstrated a beneficial effect of an essential fatty acid (EFA)-deficient diet on the time of onset and on the severity of lupus GN in these mice. In a preliminary study designed for a purpose totally independent of the study of murine lupus, we found fatty acid esterification of steroids, including dehydroisoandrosterone (DHA), in incubation mixtures that contained kidney tissue (5, 6). To test the hypothesis that these two experimental findings may be interrelated by way of a common mechanism, we conducted the present investigation to evaluate the effect of DHA, administered orally, in this murine lupus model.

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1. *Abbreviations used in this paper:* ANA, antinuclear antibodies; B/W, New Zealand Black/New Zealand White F₁ hybrid mice; DHA, dehydroisoandrosterone; DHT, 5 α -dihydrotestosterone; dsDNA, double-stranded DNA; EFA, essential fatty acid; GN, glomerulonephritis; PG, prostaglandin; T, testosterone.

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Methods

Female B/W mice (2 mo of age) were purchased from Jackson Laboratories, Bar Harbor, ME. The mice were maintained in our animal resources center with free access to food and water. The animals were weighed at monthly intervals. Commencing at 2 mo of age, the nontreated mice were fed standard laboratory chow, and the treated animals were fed a diet that consisted of standard laboratory chow which was impregnated with DHA (0.45%, wt/wt) (ICN Nutritional Biochemicals, Cleveland, OH). This amount of DHA was computed to provide ~450 mg/kg body wt per 2 d. Blood (~200 μ l) was obtained from the retrobulbar venous plexus of the mice each month. Plasma was separated and stored at -70°C until assayed for anti-double stranded DNA (anti-dsDNA) autoantibodies. Radiolabeled dsDNA was purchased from Electro Nucleonics, Inc., Columbia, MD. Antibodies to [³H]dsDNA were evaluated by use of a cellulose ester filter radioimmunoassay that has been described in detail (7).

Results

The cumulative mortality, as a function of age, for the nontreated and DHA-treated female B/W mice is presented in Fig. 1. The nontreated mice began to succumb to lupus at 6.5 mo of age, and, by 12 mo, only 4 of 24 (17%) survived. By contrast, the DHA-treated mice began to die at 9.7 mo of age and 16 of 25 (64%) were alive at 12 mo of age.

The weight gain for each group of mice is presented in Fig. 2. There was no significant difference in the mean weight of the mice of the two groups at any time in the study. The results of the anti-dsDNA autoantibody assay of plasma from both groups of animals are presented in Fig. 3. An abrupt and striking increase in anti-dsDNA autoantibodies commenced between 4 and 5 mo of age in the mice that were not fed DHA. In contrast, the anti-dsDNA autoantibodies in the plasma of the mice treated with DHA did not increase appreciably throughout the course of the study.

To determine the effect of DHA-treatment on the development of immune complex GN, DHA-treated and nontreated B/W mice were sacrificed at 6 mo ($n = 3$) of age. Immunoglobulin deposition in renal glomeruli was evaluated by direct immunofluorescence microscopy of cryostat sections that were stained with fluorescein-conjugated anti-mouse IgG. Three investigators, who had no knowledge of the identity of the specimens, evaluated independently the immunofluorescence in these sections, and the brightness of immunofluorescence was assigned a value of 0 to 3. The immunofluorescence staining in glomeruli of DHA-treated mice was scored as 1, whereas that in nontreated animals at 6 mo of age was much more intense (grade 3). The lack of deposition of immuno-

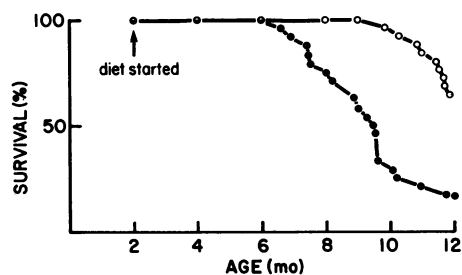


Figure 1. Comparison of the survival rates for the DHA-treated (○) and nontreated mice (●). The nontreated mice began to succumb earlier and the mortality rate was greater.

globulin in renal glomeruli of DHA-treated B/W mice is consistent with the finding that DHA treatment is associated with prevention of autoantibody formation in and the prolonged survival of these animals.

Discussion

The findings of a number of investigators are indicative of a fundamental role for EFAs or metabolites thereof in murine lupus erythematosus in B/W mice. Zurier et al. (8, 9) found that prostaglandin (PG) E_1 treatment of B/W mice caused a decrease in the severity of lupus. These investigators administered PGE_1 (200 μ g) once or twice daily from 6 to 52 wk of age. The treated mice did not develop anemia or GN, and survived longer than nontreated mice (8). Subsequently, they demonstrated that PGE_1 -treatment prevented the glomerular deposition of immunoglobulins and complement, and prevented the development of proliferative GN (9). These findings were confirmed in a study conducted by Hurd et al. (4), who also found that PGE_1 -treatment was beneficial in delaying the time of onset and severity of murine lupus in B/W mice. Hurd and co-workers extended their investigations by examining the effects of diets in which there was a high concentration of linoleic acid, thus rich in EFA, and diets that were deficient in EFA on murine lupus. Linoleic acid is a precursor of both dihomo- γ -linolenic and arachidonic acids that can be converted to prostaglandins by way of the reaction catalyzed by the cyclooxygenase pathway or to leukotrienes by way of the lipoxygenase pathway. Surprisingly, the results of their study were the antithesis of what they had predicted. Namely, whereas both groups of investigators found that PGE_1 -treatment was salutary in delaying the onset of murine lupus (4, 8, 9), inexplicably, Hurd and associates found that the feeding of an EFA-deficient diet was extraordinarily beneficial with respect

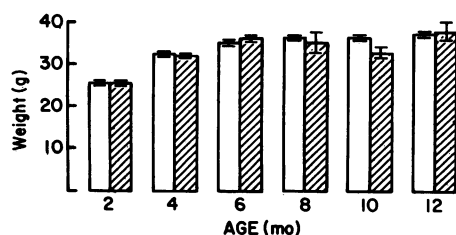


Figure 2. Weights (mean \pm SEM) of the DHA-treated mice (open bar) and the nontreated mice (hatched bar) as a function of age. There was no significant difference between groups.

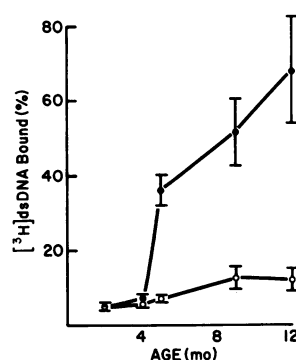


Figure 3. Comparison of anti-dsDNA autoantibodies (mean \pm SEM) in plasma of DHA-treated (○) and nontreated (●) mice as a function of duration of treatment. Percent [3 H]dsDNA bound is reflective directly of the amount of anti-dsDNA autoantibody in plasma.

to prolongation of survival in mice destined to develop lupus. The EFA-deficient animals survived longer, GN was less severe, and ANA and anti-dsDNA levels were appreciably lower than were those in animals fed a normal or else an EFA-rich diet.

We have demonstrated the formation of fatty acid esters of DHA in kidney tissue of B/W mice (6). Based on this observation, we considered the possibility that the esterification of DHA might provide a "sump" for EFAs, and arachidonic acid in particular, and thus produce a beneficial effect similar to that demonstrated in B/W mice that are fed an EFA-deficient diet. Whereas this proposition led us to conduct the present study, we have no data in support of the proposition that the results of this study are attributable to a fatty acid "sump" effect.

Androgens are known to be beneficial in delaying the time of onset and severity of murine lupus erythematosus. Prepubertal castration of male B/W mice causes an increased incidence of lupus, similar to that found in female mice; on the other hand, in prepubertally castrated female B/W mice that are given 5α -dihydrotestosterone (DHT) or testosterone (T), there is prolongation of life and less severe GN (7, 10). DHT or T, administered to noncastrated female B/W mice at 3 mo of age or castrated female B/W mice at 6 mo of age, significantly improved survival (11).

Although DHA and DHA-sulfate are commonly referred to as "adrenal androgens," this is incorrect, in the strictest sense, since there is no known intrinsic androgenic property of either steroid. At this time, however, we cannot exclude the possibility that DHA acts, in part, by way of conversion to potent androgens. But, there are cogent reasons to believe that DHA does not act singularly by way of conversion to androgen(s). Notably, the plasma levels of anti-dsDNA antibodies in B/W mice treated with T or DHT were similar to those found in nontreated animals (11, 12). Importantly, in DHA-treated mice, the levels of anti-dsDNA autoantibodies did not rise appreciably. Thus, we suggest that in the prevention of lupus, DHA acts, at least in part, in a manner independent of conversion to androgen.

In the present study, the mean weight of the mice was similar in both groups of animals. This is of considerable importance in view of the findings of studies conducted by Fernandes and associates (13, 14), who found that a reduction in dietary fat resulted in prolongation of life and a decrease in the propensity for autoimmune disease in New Zealand Black mice. They also found that the longevity of B/W and DBA/2f mice was prolonged by dietary restriction (15, 16). Furthermore,

in nonautoimmune strains of mice, it has been demonstrated that a diet enriched in salts and vitamins, but limited in calories and protein, so-called "underfeeding," slows the rate of aging and prolongs life (17). We do not believe that underfeeding was a factor in the beneficial effects observed in the DHA-treated mice as there were no significant differences in the weight or in the rate of weight gain of mice in either group.

DHA and DHA-sulfate are major secretory products of the human adrenal gland (18). Although DHA-sulfate is the principal precursor of placental estrogen biosynthesis (19), the biological role for either DHA or DHA-sulfate in men or nonpregnant women is unknown. Interestingly, DHA feeding prevents obesity (20–22), prevents tumor development (23, 24), ameliorates diabetes (25–27), and prevents autoimmune hemolytic anemia (28) in rodents. Irrespective of the exact mechanism(s) by which DHA-treatment is beneficial, the oral administration of DHA was effective in the prolongation of life in B/W mice destined to develop lupus erythematosus and in the prevention of the formation of anti-dsDNA autoantibodies.

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