Effect of Diet on Adjuvant-Induced Disease and Mitogenic Responses of Fisher Rats

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Abstract

Fisher rats from an inbred colony, when fed on a salt-free high-protein diet, developed only a mild arthritis after adjuvant injection. Their spleen cells failed to respond in vitro to concanavalin A (a T-cell mitogen), although they possessed a B-cell function of plaque formation to sheep red blood cells. When a full salt supplement was included in the diet, or magnesium or copper or zinc was included in the drinking water, adjuvant-induced arthritis was severe and the response to the T-cell mitogen was restored. The above results suggest that these trace elements may stabilize or activate certain cell populations needed for some immune responses in rats.

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Rats of our Fisher inbred strain have recently showed only a mild response to the development of adjuvant-induced disease (AID) after administration of Freund's adjuvant (FA), and failed to respond to T-cell mitogens. The cause of this failure was thought to be the type of food they were eating. Other investigators had previously reported that AID was strongly inhibited when young rats were fed a copper-deficient diet for 2 months [1], or when rats were fed for short periods a high carbohydrate diet deficient in copper, magnesium or zinc or in the three trace elements together [2]. In the present study, we have examined the effect of diet on the induction of AID in rats, and on the mitogenic response of untreated rat spleen cells to concanavalin-A (CON-A) and to immune plaque formation (PFC) to sheep red blood cells (SRBC) as an evaluation of T and B-cell function, respectively.

Groups of 10 Fisher inbred rats of either sex (175–200 g) were fed a commercial diet and given free access to water. Standard commercial diet composed of 20% protein, 8% cellulose, 3% fat, 0.8% calcium, and 0.7% phosphates with a full salt supplement. In some experiments, the salt supplement was completely omitted, whereas in others it was replaced by copper sulfate (0.02 g/l), magnesium sulfate (3 g/l), or zinc sulfate (0.6 g/l) in their drinking water.

After at least 2 weeks on each diet, 8 rats from each group were injected intradermally into the base of the

tail with 0.6 mg desiccated *Mycobacterium butyricum* suspended in 0.1 ml liquid paraffin. The onset of arthritis was followed over 4 weeks, and mean arthritic scores (on an arbitrary scale) were allocated.

The spleen cells were removed and cultured in RPMI 1640 (Serva) with or without 2.5% AB human serum and 10⁻⁵M 2-mercaptoethanol (2-ME), as described elsewhere [3]. Concanavalin-A (Sigma) was added to cultures in different concentrations. The response to CON-A was evaluated by the incorporation of tritiated thymidine (3H-TdR) into cells. For the immune plaque formation, 1 ml of 5% SRBC in salt solution (BSS, Serva) was injected intraperitoneally into

rats, and 5 and 6 days later the number of plaque-forming cells (direct and indirect) were counted, according to the method of Jerne and Nordin [4] modified as follows: into small plastic tubes kept at 50°C, were placed 0.15 ml agar (0.5% in BSS with DEAE-dextran), 25 µl SRBC (1/8 in BSS), 25 µl of spleen cells (diluted in BSS), and Finally 25 µl of complement (1/8 in BSS) alone or with anti IgG final dilution in C 1/40 (Rabbit anti-Rat IgG, Bionetics, USA). The contents of the tube was emptied into a plastic Petri dish, covered with a cover slip (24 × 24 mm) and incubated for 3–4 hat 37 °C.

Rats fed a salt-free diet developed a mild arthritis in contrast to rats fed full-salt or salt-free diet supplement with magnesium, copper, or zinc (fig. 1), all of

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which developed severe arthritis 15–17 days after the administration of FA.

The above results show that trace elements added to the diet of rats play a role in the induction of AID. Copper is involved in several biochemical processes [5], some of which, like prostaglandin and collagen biosynthesis, are closely linked with inflammation. Copper deficiency increases the severity of carrageen-an-induced edema in rat paws [1] in contrast to the production of AID, which is strongly inhibited [2]. Magnesium deficiency partially inhibits the dextran inflammation and the development of AID in rats. Zinc deficiency has no effect on dextran inflammation, but it inhibits the development of AID [2].

The adjuvant-induced disease which is considered to be a comparable animal model for rheumatoid arthritis in man has been extensively studied, not only as an immune disease, but also to evaluate different anti-inflammatory and immunosuppressive drugs. AID is believed to be a disease of cell-mediated immunity resulting from the disseminated mycobacteri-al antigens present in FA [6, 7]. However, our previous work [8], and the work of others [9], point out that in AID a latent virus might be involved. West [2] suggests that the absence of certain trace elements from the rat diet probably results in the killing of lymphocytes, and the prevention of their multiplication or interference with receptors on the cell membranes, thus affecting the induction of AID. Our results confirm the finding that certain trace elements assist in the induction of AID. This fact can be alternatively interpreted that a certain population of cells may be activated or stabilized by trace elements, i.e. cells that harbor or have a receptor for a latent virus, which in turn is activated by Freund's adjuvant.

The mitogenic response to different concentrations of CON-A splenocytes from normal rats fed with different diets is given in figure 2a-c, the results being representative of four similar experiments. When 2-ME is added to the culture medium, there is an increase of incorporation of 3H-TdR at low CON-A concentrations only in rats fed no magnesium (fig. 2a). In the other cases (fig. 2b, c), the two curves, with and without 2-ME, are similar. 2-ME has been found to stabilize the cell membrane [10], to facilitate the uptake of cystine, and to partly replace certain accessory cells in in vitro cultures [11].

Our results show that, when the trace elements Mg⁺⁺, Zn⁺⁺ or Cu⁺⁺ are added to the animal diet, certain regulatory cell populations are stabilized.

12 15 17 19 21
Time after adjuvant injection, days
0 2 A 2

Fig. 1. Effect of diet on the development and severity of AID in Fisher rats. A = Diet with full salt supplement; Δ = salt-free diet with added Mg, Cu, or Zn; $\square$ = salt-free diet. The arthritic scores are expressed as mean $\pm$ SEM.

12 16 24 0 12 16 2
Concentration of CON-A, $\mu\text{g/ml}$

Fig. 2. Effect of diet on the in vitro response of splenocytes to CON-A (see text). Filled circles without 2-ME; open circles with 2-ME. a Salt-free diet, b Salt-free diet + Mg, Cu, or Zn. c Full salt supplement diet. Stabilization of the cell membrane has also been suggested by Bach [12] as one of the possible actions of zinc in mice. The above cell population in animals fed diets without the addition of trace elements can be bypassed in cultures with 2-ME and low mitogen concentrations. The need for certain adherent cells in low CON-A concentrations has been reported earlier [13] for spleen cell cultures in mice. Splenocytes in serum-free cultures from animals fed without additional

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Mg $^{++}$ do not respond at all to CON-A (the cells lyse; results not shown), contrary to those in cultures from animals fed a balanced diet [14].

A surprising result was that the presence or the absence of magnesium salts did not affect significantly the number of PFC found in spleens of animals immunized with SRBC, a thymus-dependent antigen. The average number of PFC per spleen in groups of 10 rats fed salt-free or full salt diet was $40,000 \pm 20,000$ for IgM and $75,000 \pm 35,000$ for IgG. This contrasts with zinc deficiency [15, 16] in mice where antibody production to SRBC is drastically diminished.

This means that magnesium has a different effect from that of zinc in humoral responses of the rat, or that the salt-free diet had some traces of Mg^{++} , which in turn resulted in different degrees of sensitivity to trace element deficiency in humoral and cellular responses.

Taken together, our results suggest that a certain cell population that affects T-cell rather than B-cell responses, is sensitive to trace element deficiency, and that this population directly or indirectly is involved in the induction of AID in rats.

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