RESEARCH NOTE

Influence of Dietary n-6 and n-3 Lipids upon the Development of Pulmonary Granulomas Induced by *Schistosoma mansoni* Eggs

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Pulmonary granuloma development was studied in mice injected with $2x10^3$ *Schistosoma mansoni* eggs through the main tail vein. Mice were divided into three groups and fed with a laboratory manipulated diet differing among them only in the source of fatty acids. Control group (CT) was fed with a soya oil diet; n-6 deficient animals (low n-6 group) received a supplemented coconut fat diet; and the third group, high n-3, was fed with a cod liver oil diet. The expected anti-inflammatory actions associated with essential fatty acids deficiency was noticed in our system. When compared with CT, animals fed with the n-6 deficient diet presented significantly reduced pulmonary granulomas 32 days after egg injection. Mice fed with high n-3 diet had pulmonary granulomas reduced as soon as 16 days post egg injection. Mean granuloma measurements for CT at days 8, 16 and 32 were, in $10^3 \mu m^2$, respectively 7.52 ± 5.92, 32.70 ± 18.09, 32.22 ± 19.87; for n-6 deficient animals 17.65 ± 18.85, 36.14 ± 29.14, 24.45 ± 14.06; and for high n-3 group were 11.60 ± 12.67, 9.97 ± 12.34, 25.54 ± 16.66.

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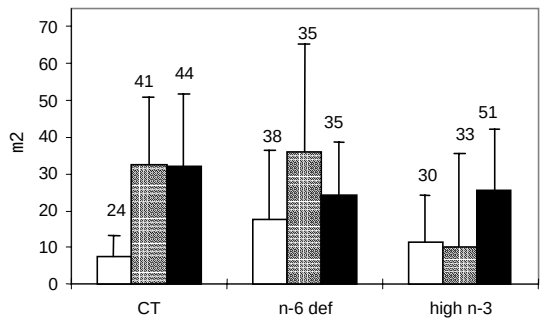
The evolution of inflammatory processes is influenced by the lipidic composition of the diet. In the past decades abundant data have been generated reporting the anti-inflammatory actions of n-6 deficiency and n-3 overload. It is noteworthy the effects of essential fatty acid deficient diets (EFAD diets) upon the development of autoimmune diseases such as murine glomerulonephritis (ER Hurd et al. 1981 *J Clin Invest 67*: 476-485), rheumatoid arthritis (JM Kremer et al. 1987 *Ann Int Med 106*: 497-503), psoriasis (SB Bittiner et al. 1988 *Lancet 1*: 378-380) and multiple sclerosis (C Bates et al. 1989 *J Neurol Neurosurg Psyc 52*: 18-22). Despite such studies, the mechanisms that support the antiinflammatory actions of these fats are not fully understood. Fatty acids generate potent modulators of cellular immune functions as eicosanoids, diacylglicerol, inositol phosphates and lyso-alkyl-phosphstidyl-choline and when produced at limiting ammounts may interfere with the physiological immune response (reviwed by P Yaqoob & PC Calder 1993 *J Biochem 25*: 1705-1714). On the other hand it is well described in the literature that altered patterns of cytokine profiles are obtained in EFAD states (S Endres et al. 1989 *New Eng J Med 320*: 265-271, PC Calder & EA Newsholme 1992 *Mediat Inflamm 1*: 107-112). Membrane fluidity changes with repercussions on the cell activity can also be expected in EFAD states (RR Brenner 1984 *Prog Lipid Res 23*: 69-96).

The EFAD status can be achieved by two different protocols: the first one by depriving the organism of n-6 fatty acids, and the second by increasing the intake of n-3 fatty acids. Schistosomiasis pathology is associated with the development of inflammatory processes that are set up around tissue trapped parasite eggs. Inside the eggs, miracidia will synthesize and liberate soluble antigenic molecules that will trigger granuloma formation. In an attempt to early down modulate *S. mansoni* induced pulmonary granuloma, female NIH Swiss mice were fed for 12 weeks on isoproteic and isocaloric diets containing different fat sources *ad libidum*. It has been shown in the literature that this time is enough to induce the EFAD state (JB Lefkowith 1990 *J Immunol 145*: 1523-1529). Soya oil, that is particularly rich in n-6 fatty acids, was used to supplement the control diet, coconut fat, that is almost devoid of linoleic acid was added to the diet n-6 deficient and cod liver oil was added to high n-3 diet because of its high content of eicosapentanoate. Complete composition of the diets is depicted in Table. After this period mice were injected, through the main tail vein, with sterile PBS, pH 7.4, containing $2x10^3$ *S. mansoni* eggs, LE strain. These eggs were obtained from infected Swiss mice liver. Animals

were killed 8, 16, and 32 days post injection by cervical dislocation; lungs were excised and inflated with 4% PBS-formaldehyde to undertake histopathological analysis. Lung samples were stained with hematoxylin-eosin and Giemsa. Measurements of pulmonary granuloma were done by means of the image analiser Kontron elektronik and the software KS3000. Granulomas with central, visible eggs in eight semi consecutive histological sections, separated one from another by at least 30 mm, were circulated with the mouse apparattus and the images were captured. In order to get more accurate results, each granuloma was measured three times and the mean was used for statistical analysis. Comparisions between groups were done using T test with the aid of the computer program Excel (Microsoft).

Measurements of pulmonary granuloma areas (Figure) showed that n-6 deficiency did not alter granuloma size till day 32 when the expected inhibitory effect was noticed (granuloma reduction of 24% when compared with CT). High n-3 diet leaded to a significant decrease in the size of pulmonary granulomas noticed as soon as 16 days post injection (a reduction of 72% when compared with CT) that was mantained at the subsequent time (21% of reduction in relation to control group).

Studies have shown that mononuclear cells from human volunteers fed with western fish oil concentrate supplemented diets produce less IL-1 and TNF-a when compared with controls (S Endres 1996 *Lipids 31* suppl: S239-S242). Besides, the inhibitory effect of high n-3 diets or its main components - eicosapentaenoic and docosahexaenoic acid - upon lymphocyte proliferation is well established (AH Merril Jr 1989 *Nut Rev 47*: 161-169). These facts, in conjunction with a diminished production of the pro-inflammatory molecule leukotriene B4, may be the base for a smaller tissue reactivity resulting in granulomas that down modulate earlier.



Pulmonary granuloma measurements of mice fed control (CT), n-6 deficient (n-6 def) or n-3 enriched diets (high n-3). C57BL/6 mice were inoculated with $2x10^3$ *Schistosoma mansoni* eggs through the main tail vein. Eight (□), 16 (▩), or 40 (■) days after egg injection two to three animals were killed and the lungs excised. Bars represent mean ± S.D. of measurements. Numbers above bars stand for the number of measured granulomas.

TABLE

Percent composition of the diets offered to the animals

Diets	Control	n-6 deficient	high n-3
Components (%) [a]			
Corn starch [b]	62.6	62.6	62.6
Casein	20.0	20.0	20.0
Mineral mixture [c]	5.0	5.0	5.0
Soya oil	5.0	–	–
Coconut fat	–	5.0	–
Cod liver oil	–	–	5.0
Vitamin mixture [c]	1.0	1.0	1.0
Cellulose	1.0	1.0	1.0
DL-methionine	0.4	0.4	0.4

a: E vitamin (0.1g/kg) and hydroxytoluene butylate (0.05g/kg) were added to prevent diet oxidation; *b*: Maizena, Refinações de Milho Brasil, São Paulo, Brazil; *c*: done in accordance with AOAC, 1980 (Association of Official Analytical Chemists). In *Official Methods of Analysis of the AOAC*, 13th ed., AOAC, Washington, DC.