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Activities of adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) on undernourished and renourished rats' thymus

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Abstract

We studied the effect of administration of a low quality dietary protein, from weaning onwards, on the thymus of undernourished rats and the posterior effect of refeeding with a high quality dietary protein. Changes in thymus weight and the activity of Adenosine Deaminase (ADA) and Purine Nucleoside Phosphorylase (PNP) on thymus, were determined. Wistar rats were suckled in groups of 14–16 per dam since birth to weaning (23 days) to obtain undernutrition. At weaning, a group of 14–16 rats received pre-cooked maize flour (Protein content: 6.5%) for 18 days. One group was sacrificed (M) and the other rats were refed with the casein diet (Protein content: 20%) during 20 days (R). The age-matched control groups were fed stock diet since 40 (C40) and 60 (C60) days of age, respectively. At the end of the experimental period, body (Bw) and thymus weight were determined. ADA and PNP activities were determined in thymocyte suspensions. Highly significant differences in thymus weight—expressed as mg or mg/$Bw^{0.75}$—and the activity of ADA and PNP were observed in rats fed the experimental diet containing maize flour, when compared to the respective age-matched control. No statistical differences were observed between R and C60.The administration of a high quality dietary protein to undernourished weanling rats is capable to reverse the damage produced by the low quality dietary protein on thymus weight and ADA and PNP thymus activities.

Keywords: Adenosine deaminase; Purine nucleoside phosphorylase; Quality dietary protein; Renutrition; Thymus; Undernourished rats

1. Introduction

The studies on undernourished rats have shown that nutritional disorders produce serious effects on thymus and other lymphoid organs; the same was observed in human malnutrition [1–4].

Moreover, undernutrition induced during and limited to the suckling period is also critical for the complete development of the thymus and little is known about the effects of different natural proteins administered from weaning [4–6].

Some authors suggest that the activity of Adenosine Deaminase (ADA) and Purine Nucleoside Phosphorylase (PNP)-involved in purine metabolism—might affect lymphocyte T development and immune system, due to intracellular accumulation of toxic levels of deoxynucleotides. The purine degradation enzymes ADA and PNP are closely linked to the function and differentiation of lymphocytes as it was evidenced from the association of severe combined immunodeficiency diseases with inherited deficiencies of these enzymes. ADA functions in the purine catabolic pathway by catalyzing the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. PNP catalyzes the reversible phosphorolysis of inosine and deoxyinosine, the products of the ADA-catalyzed reaction, as well as of guanosine and deoxyguanosine to form hypoxanthine and guanine, respectively [7,8] (Figure 1).

Because cereals are used as weaning and basic foods for children during the first year of life in developing countries, in the present report we study the effect of oral administration of a low quality dietary protein (precooked maize flour) from weaning onwards and the posterior effect of a rehabilitation diet. For this purpose, changes in thymus weight and the activity of ADA and PNP on thymus were determined.

2. Material and methods

Wistar rats (closed colony from breeding unit kept at our department) were suckled in groups of 14–16 per dam since

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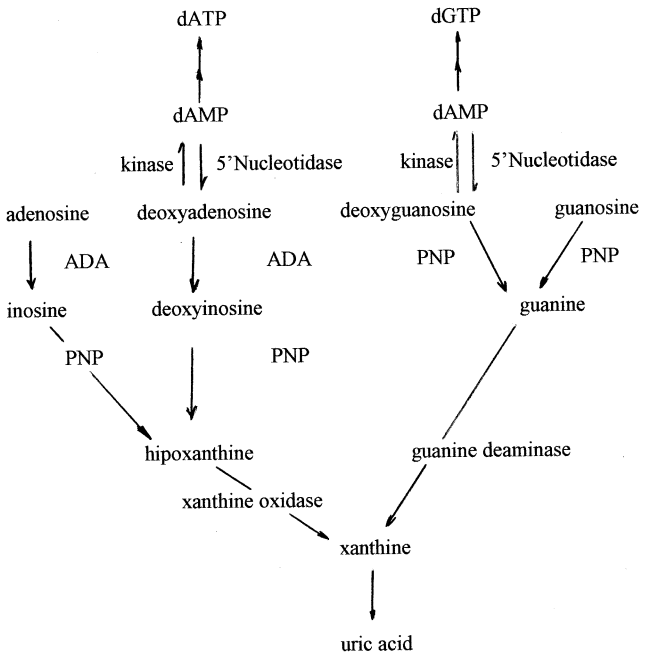

Fig. 1. Role of Adenosine Deaminase (ADA) and Purine Nucleoside Phosphorylase (PNP) in the deoxypurine metabolism [11].

birth to weaning (23 days) to obtain undernutrition, according to the standard procedure of McCance and Widdowson as previously described [9].

At weaning a group of 14–16 rats received an experimental diet containing as only source of protein pre-cooked maize flour (Protein content: 6.5%) (Biological value: 59.9) for 18 days. One group (n = 6–8 animals) was sacrificed (M) and the other rats were refed with the casein diet (R) (Protein content: 20%). Experimental isocaloric diets providing all the essential nutrients were prepared according to the recommendations of the American Institute of Nutrition, except the protein [10]. The age-matched control groups were fed stock diet (Cargill, protein content: 24.5%); one group was sacrificed at 40 days of age (C40) and the other at 60 days (C60).

During all the experiments the animals were housed individually in screen-bottom cages and exposed to a 12 hr light-darkness cycle (7.00 AM to 7.00 PM), room temperature was kept at 21°C ± 1.0. Water and diet were offered "ad libitum".

At the end of the experimental period, body weight (Bw) was determined and the animals were exsanguinated by venous puncture; thymus was removed, and organ weight (Tw) was determined; cells were released by teasing with forceps in potassium phosphate buffer, filtered through nylon wool and washed before use. Enzyme activities were determined in cell suspensions as was described previously [11,12].

The statistical analysis was performed using the Student's t test [13] to analyze the effect of the administration of a low quality dietary protein and the posterior renutrition.

Table 1
Thymus weight (means ± S.D.) and the activity of ADA and PNP (means ± S.D.) in experimental and control groups.[a]

Group	Tw (mg)	Tw (mg/Bw$^{0.75}$)	ADA μmol uric acid × 10^{-1}/ organ/W	PNP μmol uric acid × 10^{-1}/ organ/W
M	43.1 ± 17.8*	2.9 ± 1.0*	23.1 ± 9.4*	9.4 ± 4.0*
C40	384.1 ± 56.8	9.9 ± 1.6	8.2 ± 1.3	2.6 ± 0.5
R	409.0 ± 56.1	10.6 ± 1.4	10.6 ± 1.1	3.3 ± 1.5
C60	455.8 ± 35.6	8.8 ± 1.1	13.1 ± 2.0	4.6 ± 1.4

[a] 6–8 animals per group.
* $p < 0.01$ with respect to C40.

3. Results

Thymus weight (mg or mg/Bw$^{0.75}$) and the activity of ADA and PNP expressed as μmol uric acid × 10^{-1}/organ/W (where W = Tw (mg)/Bw(g)$^{0.75}$) are shown in Table 1.

Highly significant differences in thymus weight expressed as mg or mg/Bw$^{0.75}$ and the activity of ADA and PNP were observed in rats fed the experimental diet containing maize flour when compared to the respective age-matched control (C40).

Data point out that thymus weight and the activity of these thymic enzymes are severely affected by unbalanced diet with maize as only source of protein when it was administered from weaning onwards.

After 20 days of refeeding with casein diet (group R), activity of ADA and PNP decreased significantly when compared to M; thymus weight were higher than M. On the other hand, no statistical differences were observed between all the studied parameters in the renourished animals and its age-matched control (C60).

4. Discussion

In previous papers, we have demonstrated in experimental model that undernutrition, marginal, moderate and severe protein malnutrition at weaning, produce in rats loss of thymus weight, reduction in the number of thymocytes, diminished proportion of T cells presenting the antigenic determinant W3/13 and DNA content with concomitant increase in cell size and activity of ADA and PNP[1] [5,12, 14–16].

The results of this report seem to confirm that the nutritional stress provoked by the administration of a diet containing maize flour at a low concentration, from weaning onwards, affects the development of thymus expressed as loss of organ weight, with a concomitant increase in the

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activity of ADA and PNP. In the group fed maize diet all these values, were significantly different from both the values of the age-matched control group and those for undernourished rats at the time of weaning (ADA: 13.3 ± 2.8, PNP: 2.8 ± 0.9) [5]. The unbalanced diet due to the presence of lysine as the limiting aminoacid in the protein would induce the increase in ADA and PNP activities as an alternative mechanism to avoid the accumulation of high levels of deoxynucleotides, which would be toxic for T lymphocytes; the same behavior was observed when well-nourished rats were fed free protein diet from weaning onwards [12].

Previous reports have shown that the oral administration of casein diet (protein content: 20%)during 20 days was not enough to reverse the effect provoked by malnutrition and undernutrition on antigenic determinants [17]. On the other hand, in this paper we demonstrate that this recovery diet was capable to reverse the effect provoked by the oral administration of maize flour diet (low quality dietary protein) on the activity of ADA and PNP. Our work, performed "in vivo" reinforces our hypothesis related to diet as an important modifying factor that affects the activity of enzymes related to T lymphocyte development, suggesting a relationship between protein feeding and these thymic enzymes.

On the other hand, some authors have observed an increase of ADA activity in human serum in some adverse conditions such as in acquired immunodeficiency syndrome (AIDS), and they have pointed to serum ADA value as useful marker for monitoring the clinical progression of AIDS patients [18]. It is important to point out that there were observed immunological similarities between patients with AIDS and those with nutritional disorders as protein-calorie malnutrition [19,20]. For this reason and taking into account the present findings, it would be interesting to determine the relationship between the activity of ADA and PNP in thymocytes and serum in experimental models, in order to analyze and propose these biochemical parameters as potential and useful markers of nutritional status.

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