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ASSOCIATION BETWEEN IMMUNE RESPONSE AND BODY CONFORMATION IN THE MOUSE (*Mus musculus*)

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ABSTRACT

Humoral immune response (HIR) to sheep red blood cells (SRBC) and the behaviour of a transplantable mouse lymphoma (L-DGE) were investigated in four lines of mice (CBi^+, CBi^-, CBi/C and CBi/L) with different body conformations, and in the base population (CBi) from which these lines were derived.

CBi^+ (high weight-long tail) and CBi^- (low weight-short tail) mice, selected for a phenotypic correlation between body weight and tail length, had similar HIR to SRBC and tumor behavior (percentages of takes and tumor regression) and did not differ from the base population CBi. CBi/C (high weight-short tail) and CBi/L (low weight-long tail) mice, selected for negative phenotypic correlation, showed a significant difference in HIR to SRBC and in tumor behaviour between the two of them and with CBi. Ten days after antigenic challenge, CBi/C had the highest hemagglutinin titers (6.49) to SRBC and a great capacity of tumor rejection (97.2%); CBi/L had the lowest values of hemagglutinin (4.75) and 68% tumor regression.

CBi/C and CBi/L mice also differ in the amount of fat deposition, skeletal morphology, biochemical properties and bone quality of the femur. In view of these findings and the results of the present experiments it is suggested that in the antagonistic selection, genes that regulate growth, development and immune response including those on chromosome 17 were subjected to recombinations, though other chromosomes, could also be involved.

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INTRODUCTION

An association between H-2 haplotypes and body weight in divergently selected lines of mice was found by Bulfield (1980). Previously, Gregorová *et al.* (1977) observed that H-2 haplotypes were associated with different organ weights. In addition, genetically obese mice (C57BL/6J ob/ob) showed an altered immunity (Sheena and Meade, 1978). Moreover, Bennett (1975) and Gill *et al.* (1983) have found recessive genes affecting growth and development (T/t region), linked to the H-2 complex in the mouse.

The aim of this research was to study the humoral immune response (HIR) to sheep red blood cells (SRBC) and the behaviour of a transplantable mouse lymphoma (L-DGE) in four lines of mice with different conformations and in the base population from which these lines were derived.

MATERIALS AND METHODS

Animals

Adult mice of both sexes belonging to four lines selected for body conformation and the base population, as controls, were used. Body conformation was evaluated at 7 weeks of age by a quantitative index which combines body weight and tail length; these characters showed a genetic and phenotypic correlation ($r_G = 0.60$; $r_P = 0.56$). Lines CBi/C (high weight-short tail) and CBi/L (low weight-long tail) were selected against the phenotypic correlation between the two components of the selective index. Lines CBi^+ (high weight-long tail) and CBi^- (low weight-short tail) were generated from the agonistic selection. A detailed description of this selection scheme is given elsewhere (Di Masso *et al.*, in press).

Humoral immune response to SRBC

SRBC were placed in Alsever solution and washed three times with phosphate buffered saline (PBS), pH 7.2 before using. Mice were sensitized i.p. with 0.1 ml of a 50% SRBC suspension at day 0. Blood samples from the tail vein were obtained 7, 10 and 21 days after sensitization. Sera from each sample were inactivated at 56°C for 30 minutes and stored frozen at -20°C. Hemagglutinin titration was performed in round-bottomed well microtitre trays. The values were expressed as the ln of the inverse of the last dilution with positive agglutination.

Lymphoma DGE behaviour

L-DGE is a poorly differentiated immunoblastic type lymphoma; it appeared spontaneously in 1985 in a BALB mouse and is maintained by subcutaneous (s.c.) serial passages in syngeneic mice. It is inoculated s.c. by trocar in the right flank of the animals. Percentages of takes, tumor regression after a variable period of growth and lethality were recorded.

Statistical analysis

Chi square and Student's t tests were used for statistical evaluation (Snedecor and Cochran, 1967).

RESULTS

Humoral immune response to SRBC

CBi^+ and CBi^- lines showed no differences in hemagglutinin titers to SRBC 10 days after antigenic challenge, when compared to CBi (Table I). On the other hand, CBi/C and CBi/L mice significantly differed from CBi and from each other. The highest value was observed in CBi/C mice and the lowest one in CBi/L mice (Table I). CBi/C and CBi/L showed different kinetics of antibody production, CBi/C also being different from CBi, CBi^+ and CBi^- (Figure 1).

Table I - Humoral immune response to SRBC.

Line	n	Hemagglutinin titer[a]	
CBi^- (low weight-short tail)	11	5.668 ± 0.127	
CBi^+ (high weight-long tail)	12	5.976 ± 0.183	
CBi (base population)	12	5.541 ± 0.256	*
CBi/L (low weight-long tail)	14	4.748 ± 0.250	** *
CBi/C (high weight-short tail)	11	6.486 ± 0.252	

n: number of animals; a: mean ln value of hemagglutinin titer ± standard error; * $P < 0.05$; ** $P < 0.01$.

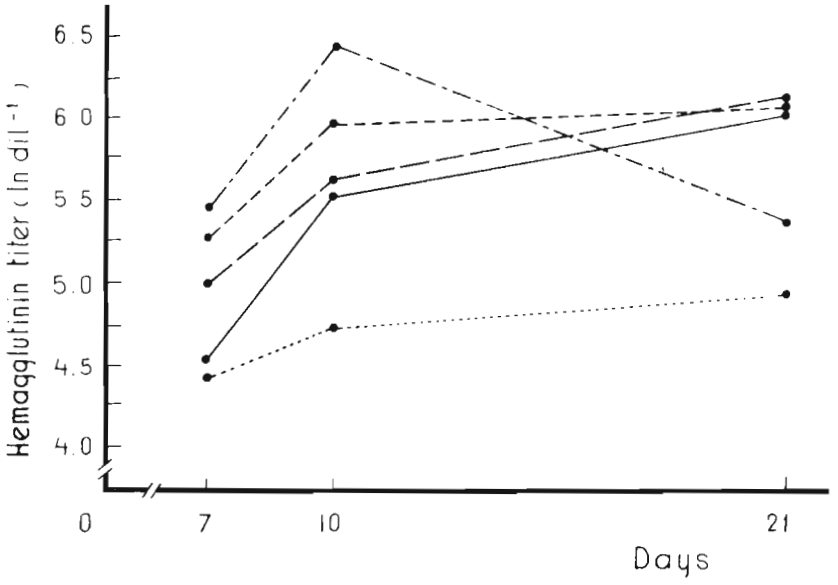

Figure 1 - Kinetics of hemagglutinin production. CBi: base population, CBi^+ and CBi^-: selected favoring the correlation between body weight and tail length, CBi/C and CBi/L: selected against the above mentioned correlation.

Lymphoma DGE behaviour

Neither the antagonistic nor the agonistic selection modified the percentages of takes of L-DGE. Similar percentages of takes were found in the four experimental groups and in the base population (Table II).

The percentages of tumor regression and lethality in CBi^+, CBi^- and CBi were not statistically different (Table II). However, in the lines which were selected against the correlation between body weight and tail length the capacity to reject the tumor was significantly modified. In both these lines (CBi/C and CBi/L) the percentages of tumor regression increased significantly with respect to the base population ($P < 0.001$ and $P < 0.01$, respectively) and in addition, CBi/L differed from CBi/C ($P < 0.001$). Percentages of lethality are given in Table II.

DISCUSSION

Humoral immune response to SRBC in the mouse reachs a peak of hemagglutinin production from seven to 10 days after the antigenic challenge; thereafter,

the agglutinin titers can decrease or persist at a high level and even increase slightly for as long as three months (Biozzi *et al.*, 1968). These patterns of HIR were observed in the different genotypes tested.

Table II - Lymphoma DGE behaviour.

Line	Takes (%)	Regression (%)	Lethality (%)
CBi^- (low weight-short tail)	97.0 (33/34)	33.3 (11/33)	64.7 (22/34)
CBi^+ (high weight-long tail)	94.7 (36/38)	13.9 (5/36)	81.6 (31/38)
CBi (base population)	93.3 (28/30)	32.1 (9/28)	63.3 (19/30)
CBi/L (low weight-long tail)	96.1 (50/52)	68.0 (34/50)	30.8 (16/52)
CBi/C (high weight-short tail)	81.8 (36/44)	97.2 (35/36)	2.3 (1/44)

** $P < 0.01$; *** $P < 0.001$.

Selection against the phenotypic correlation for body weight and tail length affected not only hemagglutinin titers but also the kinetics of the HIR to SRBC. At 10 days after antigenic challenge CBi/C mice were high responders when compared to CBi/L, both lines differing from the base population CBi. Meanwhile, the CBi^+ and CBi^- mice, originated from the agonistic selection, did not differ, either in terms of kinetics or the level of HIR to SRBC when compared to CBi.

The antitumoral response obtained in the different selected lines challenged with L-DGE was analogous with that obtained in the HIR. In the agonistic selection (CBi^+ and CBi^-) the percentages of tumor regression with respect to CBi mice were not modified. On the other hand, CBi/C and CBi/L mice (antagonistic selection) had an increased capacity of tumor rejection, with differences between them and also compared to CBi mice. Taking into account that the specific immune response is one

of the main mechanisms of the antitumoral response when tumors are immunogenic (Heberman, 1977; Old, 1981; Brodt, 1983), it is not surprising that similar results were obtained in both series of experiments.

Previously, it was found that CBi/C and CBi/L mice also differed in other biological characteristics such as fat deposition (Trumper *et al.*, 1989), skeletal morphometry (Di Masso *et al.*, personal communication) and biochemical properties and bone quality of the femur (Puche *et al.*, 1989). In view of these findings and the results obtained in the present experiments it is suggested that during antagonistic selection, genes that regulate growth, development and immune response were subjected to redistribution. Hence, CBi/C and CBi/L lines would be recombinant for body conformation and immune response.

It is known that the T region of chromosome 17 of the mouse, linked to the H-2 complex, plays a significant role in growth and development, sperm production and function (Dunn and Bennett, 1969) and genetic recombination (Klein and Hammerberg, 1977). Moreover, it is speculated that the T and H-2 loci share an important function such as cell-cell recognition at early and late phases of embryonic development respectively. Considering that one of the functions of t haplotypes is the suppression of recombination (Lyon *et al.*, 1979) over an extensive region of chromosome 17, including the H-2 region, the antagonistic selection for body conformation could have involved some genes in the t-complex linked to certain H-2 haplotypes. This would explain why different immune responses were concomitantly observed in new body conformations obtained by the antagonistic selection since immune response genes (Ir) are mapped between two histocompatibility loci (H-2K and S) of the H-2 complex (Gill *et al.*, 1978; Schwartz, 1986). Of course it is also possible that genes on other chromosomes are responsible for these effects.

If the afore mentioned association between body conformation and immune response is confirmed in other species it would have theoretical and practical importance in the field of developmental genetics.

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RESUMO

A resposta imune-humoral (HIR) para células vermelhas do sangue de carneiros (SRBC) e o comportamento de um linfoma transplantável de camundongos foram investigados em quatro linhagens

de camundongos (CBi^+, CBi^-, CBi/C e CBi/L) com diferentes estruturas corporais e nas populações base (CBi) das quais estas linhagens derivaram.

Camundongos CBi^+ (peso alto - rabo longo) e CBi^- (peso baixo - rabo curto), selecionados para uma correlação fenotípica entre peso corporal e comprimento do rabo, tiveram HIR e SRBC e comportamento tumoral (porcentagem de formação e regressão de tumores) similares e não diferem da população base CBi. Camundongos CBi/C (peso alto - rabo curto) e CBi/L (peso baixo - rabo longo), selecionados para correlações fenotípicas negativas, mostraram diferença significativa entre HIR e SRBC e no comportamento tumoral, entre eles e em relação a CBi. Dez dias após o contato antigênico, CBi/C teve os níveis mais altos de hemaglutinina (6,49) para SRBC e uma grande capacidade de rejeição de tumores (97,2%); CBi/L teve os menores níveis para hemaglutinina (4,75) e 68% de regressão de tumores.

Camundongos CBi/C e CBi/L também diferem na deposição de gordura, na morfologia do esqueleto, nas propriedades bioquímicas e na qualidade do fêmur. Em vista destes dados e resultados dos experimentos feitos, é sugerido que em seleção antagônica, genes que regulam o crescimento, desenvolvimento e resposta imune estão sujeitos a recombinações. É proposto que a redistribuição de genes aconteceu no cromossomo 17 onde genes que participaram no regulamento do crescimento e desenvolvimento (região T) e resposta imune (complexo H-2) estão próximos.

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