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Histocompatibility antigens and cell maturation in the mouse

Antígenos de Histocompatibilidad y Diferenciación en el Ratón

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H-2 ANTIGENS CELL MATURATION DIFFERENTIAL ANTIGENICITY

The presence or absence of H-2 antigens on various tissues and organs of the mouse have been analyzed from the early 1960 by using two different criteria: the absorption of isoantibodies and the induction of isoimmune reactions (1).

The results obtained lead to the conclusion that most of the tissues, if not all of them, present H-2 antigenicity. Moreover, there is some agreement on the quantity of H-2 antigens in the different tissues. Thus, the spleen is the organ presenting the highest amount followed by lymph node, liver, thymus, lung, adrenal gland, gut, kidney and salivary glands. Erythrocytes, heart, brain, testis and skeletal muscle are tissues with low H-2 antigenicity (for a review, see 2).

However, the analysis of the data show some differences among the authors. Thus, Pizarro *et al.* (3) give to the liver and spleen the same H-2 antigenicity while Amos *et al.* (4), Schlesinger (5) and Basch and Stetson (6) present values for the liver that are half the amount found in the spleen. These discrepancies, and many others in other tissues from different laboratories, are basically explained by the use of different techniques in measuring the hemagglutinins and/or the presence of inhibitors able to suppress the *in vivo* sensitizing ability, specially in the liver (2). It has also been discussed that the anomalous behaviour of different tissues may be related to the lack of antigenicity of "soluble" fractions (1).

In the present study, the H-2 antigenicity is investigated in four different tissues from one strain of mice and using the same *in vitro* experimental conditions (preparation of the sample, absorption of the antiserum and titration of the hemagglutinins). The only variable introduced is the age of the mice. The results point to a differential antigenicity of the tissues tested as a function of the age of the mouse.

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MATERIAL AND METHODS

Chemicals: Dextran (Macrodex) was purchased from Pharmacia A-B, Stockholm. All other compounds were product of Sigma Chemical Co. and were of reagent grade.

Animals: ASnell and ASwiss mice, bred and maintained in our laboratory, were used. The animals were kept

on a natural light-and-dark schedule and fed ad *libitum* until 2 hours before the experiment, when their food, but not their water, was withdrawn. As it has been previously shown that the expression of H-2 antigens is not dependent of the sex of mice (7), experimental groups were prepared including both sexes.

Antiserum: ASwiss anti ASnell serum (H-2^s anti H-2^a) was produced in ASwiss female mice by weekly intraperitoneal injections of a mixed homogenate obtained from liver and spleen (first injection) or from spleen only (second and third injectioons) of ASnell mice. Seven days after the last injection, the animals were bled from the tail vein. Blood from several donors was pooled, the serum collected after the clot had remained at 37°C for 1 hour and aliquots of 0.2 ml stored at 30°C. This antiserum contained activity against the H-2 alloantigenic specificities 4(D), 8(H) and 11(K) as it has previously been demonstrated by Hoecker *et al.* (8).

Homogenates: ASnell mice from 15 days to 7 months of age were killed by cervical dislocation. Parotid glands, spleen, liver and kidney were quickly dissected free from adhering fat and lymph nodes, rinsed in sterile saline, blotted and chilled on ice. The tissues were finely minced with curved scissors until they could be passed easily through a Pasteur pippete, transferred to small centrifuge tubes and washed three times with sterile saline, by centrifuging at 650 × g for 5 min. at 4°C. Supernatants were discarded and the remaining pellets were adjusted to 50±3 mg wet weight and kept on ice until used for absorption of the antiserum. Determination of H-2 antigenicity on each organ were done in triplicate at the different ages tested.

Quantitative absorption: The pellets were resuspended with 0.2 ml of the specific antiserum diluted 1:8 v/v with 2% dextran in saline. Original titer of the antisera used was 1:2084. Absorption was performed by incubation in a shaking water bath for 1 hour at 37°C. At the end of this period the suspensions were spun down at 3500 × g for 20 min. at 4°C, the supernatants saved and kept on ice. Specific hemagglutinins in the absorbed antiserum were determined according to the modification of Hoecker *et al.* (8) of the dextran-human serum method of Gorer *et al.* (9), using a microtiter plate. Briefly, 0.025 ml of the supernatant were diluted in an equal volume of 2% dextran, and thereafter serially in 2% dextran. For the agglutination, 2% red blood cells suspended in 2% bovine serum albumin in saline was added to each dilution and to appropriate controls. To avoid non-specific agglutination 5% of mice normal serum pooled from several strains was previously added to the red blood cells suspension.

Each experiment always included a negative control for the absorption (a tissue from the strain of mice in which the antiserum had been produced). Parotid gland and spleen from ASwiss mice were used routinelly for this purpose. Another negative control for the hemagglutination was a red blood cell suspension from ASwiss mice.

RESULTS AND DISCUSSION

In order to know the appropriate amount of homogenate to be used in the absorption experiments and considering that the spleen is the organ reported to have the highest H-2 antigenicity (2), different amounts of spleen homogenate were tested against a constant amount of specific antiserum. Results of these experiments are shown in Table 1. Clearly enough, 75 mg (or more) of the spleen homogenate completely absorb the activity of the specific antiserum at the dilution tested. On the contrary, 50 mg produce an absorption with a remaining titer that is convenient for comparative purpose. Homogenates of parotid glads or spleen from ASwiss mice do not change the original titer of the specific antiserum, showing absence of non-specific absorption. Based on these data, 50 mg wet weight of each homogenate was used for the absorption experiments.

TABLE 1

Relationship between increasing amounts of spleen homogenate and H-2 antigens content

Spleen Homogenates mg, wet weight	*Remaining titer*
50	46
60	16
≥ 75	no titer

The spleen homogenates were prepared from ASnell mice 2 months old. Incubation of the homogenates with the specific antiserum and determination of hemagglutinins in the supernatant after absorption were performed as described under Material and Methods. As negative controls, homogenates of parotids and spleen from ASwiss mice were prepared and treated as the spleen homogenates from ASnell mice. Original antiseum titer was 1:2048. Titer after absorption with parotids or spleen ASwiss mice was 1:2048.

Figure 1 shows the results obtained when specific antiserum was absorbed with parotid, kidney, liver or spleen homogenates. The remaining titer after absorption with the parotid or kidney show no variation at the different ages tested, the supernatant from kidney presenting always a titer higher than that for the parotids. On the contrary, the titer remaining after absorption with liver or spleen decreases with the age of mice up to 2-4 months of age and increases again without reaching initial levels, at 7 months of age. Between 2 and 4 months of age, the liver and the spleen show the lowest remaining titer, that is, the highest absorbing capacity. Again, liver presents always more titer in the supernatant than spleen.

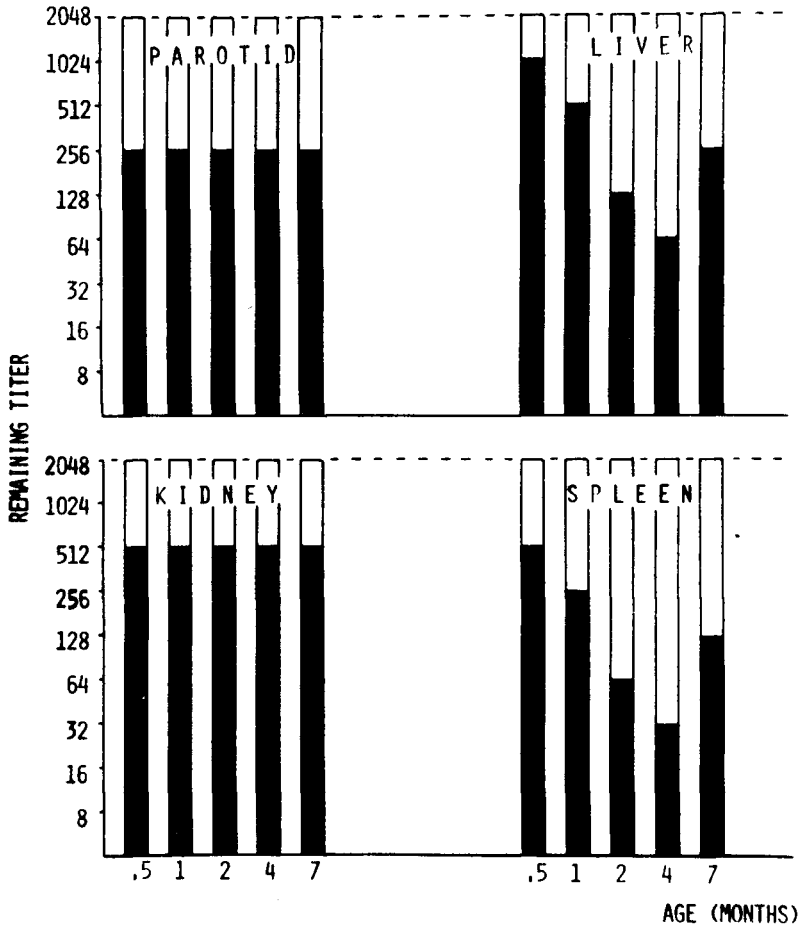

Fig. 1. Expression of H-2 antigens in adults and developing ASnell mice. Parotid (50 mg), liver (53 mg), kidney (53 mg) and spleen (52 mg) from ASnell mice at the age indicated in the abscisa were homogenized and incubated with the specific antiserum as described under Material and Methods. In the ordinate the remaining titer obtained after absorption of the specific antiserum with each homogenate, measured by hemagglutination, are indicated. As negative controls, homogenates of parotids (50 mg) and spleen (50 mg) from ASwiss mice were prepared, and treated as the spleen homogenates from ASnell mice. Original antiserum titer was 1:2048. Titer after absorption with parotids or spleen from ASwiss mice was 1:2048.

When these results are expressed as percentage of absorption of anti H-2 antigen from the original antiserum (Figure 2), it is apparent that the H-2 antigen concentration does not change as a function of the age in parotid gland and kidney of ASnell mice. On the contrary, drastic variations in the expression of H-2 antigens with the age of mice is observed when spleen or liver are used.

Confirming previous observations (2), the spleen seems to be the organ presenting the highest H-2 antigenicity from 2 months of age. However, at 15 days and 2 month of age, the H-2 expression in the spleen is in the same order of magnitude, or even lower, than the parotid and/or kidney. The importance of this findings is evident when considering the wide use of spleen as the organ having the highest H-2 antigen content, in comparative studies with other tissues (2). Then, the variation in the H-2 expression of the spleen as a function of age should be considered when this organ is used for comparative purposes. Similar precautions should be taken when using the spleen

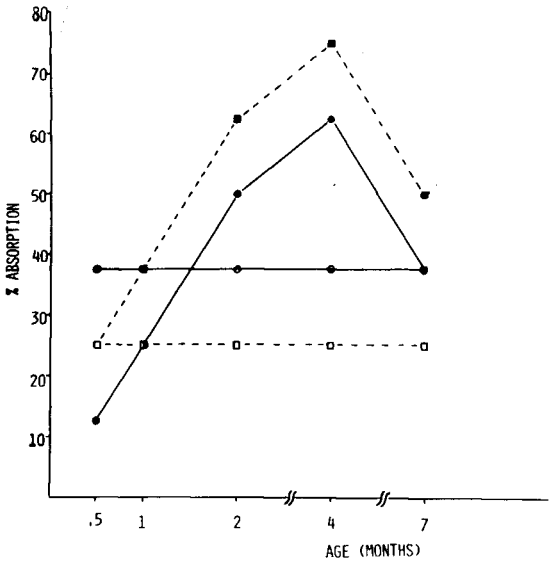

Fig. 2. Relative absorption of anti H-2 by parotid glands (o——o), liver (●——●), kidney (□----□) and spleen (■---■) of ASnell mice, as a function of age. Preparation of homogenates, absorption with the specific antiserum, assay system for the hemagglutinins, original antiserum titer and controls were as described under Fig. 1.

and liver as a source of H-2 antigens for preparation of specific antiserum. According with the data presented in Figure 2, mice of 2-4 months old should be the most appropriate for this purpose.

On the other hand, it is known that the acini of the rat submandibular gland are not fully differentiated ar birth but attain their adult structure during the first six weeks of postnatal development (10). It is also known that spleen shows an explosive growth during the first two weeks of extrauterine life in the mouse (11). However, there is a different behaviour between these organs. The salivary gland is tied to a specialized secretory process which is going to reach an appropriate level of function after the fourth week of life. On the contrary, the spleen shows a biphasic pattern of growth: the first due to a rapid expansion of the erythropoietic tissue and the second mainly indebted to the development of the lymphoid fraction with a relative detention of erythropoiesis (11).

Thus, it is atractive to postulate that the different patterns of H-2 antigenicity observed in this work is connected with this two-step mechanism of differentiation showed by liver and spleen. It is now important to further study this hypothesis by assaying the H-2 antigens of these organs in the intrauterine life and by testing their absorption capacity of the specific antiserum at different protein concentrations, to avoid saturation phenomena.

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