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ABSORPTION ANALYSIS OF H-2D AND K ANTIGENS ON SPERMATOZOA

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SUMMARY

The presence of H-2D and K antigens on mouse spermatozoa has been investigated by absorption followed by testing on the proper target lymphocytes. It is concluded that, in addition to Ia antigens, H-2D and K antigens are indeed expressed on mouse sperm cells.

INTRODUCTION

The presence of H-2 region determined antigens on spermatozoa has been amply demonstrated by absorption of anti-H-2 sera (Vojtiskova, 1969; Vojtiskova *et al.*, 1969; Vojtiskova & Pokorna, 1972), presensitization of mice to subsequent skin grafts (Vojtiskova *et al.*, 1969), direct cytotoxicity (Goldberg *et al.*, 1970; Johnson & Edidin, 1972), antiglobulin techniques (Erickson, 1972; Tsuzuka *et al.*, 1972), immunofluorescence (Vojtiskova *et al.*, 1969; Erickson, 1972) and immunoelectronmicroscopy (Vojtiskova *et al.*, 1974). Quantitative studies utilizing radio-labelled reagents suggested that the amount of H-2 antigen present on the surface of spermatozoa was about one-tenth that present on the surface of spleen cells (on a per cell basis) (Erickson, 1972; Tsuzuku *et al.*, 1972). However, the discovery of Ia antigens determined by the H-2 region (reviewed in Shreffler & David, 1975) and the demonstration that Ia antigens were present on spermatozoa (Hämmerling *et al.*, 1974) raised the possibility that the H-2 region determined antigens on spermatozoa might be only Ia antigens. Most of the antisera used to detect H-2 region antigens were raised in strain combinations which might have resulted in anti-Ia specificities being present. For instance, the monospecific anti-H-2.4 used by Vojtiskova & Pokorna (1972) was raised in (C57B1/10 × DBA/1)F_1 hybrids against B10.A and could have contained anti-Ia 1,1 components as well. On the other hand, Vojtiskova *et al.* (1969) used a C57B1/10 anti-A strain leukaemia sera—most tumour lines have not contained Ia

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specificities so that this antisera which reacted with spermatozoa may have only had anti-H-2D and K specificities. Furthermore, the absorption studies of Vojtiskova and coworkers (Vojtiskova, 1969; Vojtiskova *et al.*, 1969; Vojtiskova & Pokorna, 1972) utilized erythrocytes as target cells (haemagglutination was being tested). Erythrocytes do not contain Ia antigens so the decrease in haemagglutination reactivity of anti-H-2 region sera by absorption with spermatozoa again suggests that spermatozoa contain H-2D and K antigens. However, H-2 (D and K) antigens could not be detected on spermatozoa by immunoprecipitation (Vitetta *et al.*, 1975). This negative result might be due to the low level of H-2D and K antigens present on spermatozoa or to the unusual structural features of the spermatozoal plasma membrane. We have re-investigated the presence of H-2D and K region antigenic specificities on spermatozoa by absorption of appropriate, specific antisera.

MATERIALS AND METHODS

Sera

Three antisera were used in these studies: (1) anti-H-2D^2: (B10.D2 × A)F_1 anti-B10.A(2R). This sera detects only the private specificity of H-2^b(2) and was the gift of D. C. Shreffler and C. S. David; (2) anti-H-2D^4: (B10 × AKR.M)F_1 anti-B10.A. This sera detects only the private specificity of H-2^d(4) and was also the gift of D. C. Shreffler and C. S. David; (3) anti-H-2K^d: (B10.A × A)F_1 anti-B10.D2. This sera weakly detects Ia.8 (titre 1 : 32) but reacts strongly with H-2 (titre 1 : 1024), and was a gift of D. H. Sachs.

Animals

129/Sv (H-2^{bc}) and BALB/c (H-2^d) males provided sperm for absorptions and target lymphocytes with their respective sera. A/Orleans (H-2^a) males provided spermatozoa for control absorptions with all three sera.

Cell preparation

Spermatozoa were prepared from epididymides and ducti deferentes of mice killed by exsanguination (to decrease red blood cell contamination) by a slicing and soaking technique as previously described (Betlach & Erickson, 1973), but in phosphate buffered saline. They were washed once and suspended in Hanks' balanced salt solution containing 4% heat inactivated fetal calf serum IPT (immune precipitate tested) (Gibco). They were counted in a haemocytometer and cell contamination was noted. There was less than 1% contamination with epithelial or other cells while erythrocytic contamination ranged from 1–2%.

Lymphocytes from mesenteric lymph nodes were prepared in phosphate buffered saline and resuspended in Hanks' balanced salt solution containing 4% heat inactivated fetal calf serum IPT.

Absorption and testing of sera

The indicated numbers of absorbing spermatozoa were pelleted in microfuge tubes and the indicated aliquot and dilution of antisera added. The cells were resuspended and the mixture left at 0°C for 1 hr. The cells were then again pelleted and the supernatant sera tested in ^{51}Cr-release or trypan blue staining cytotoxicity tests. Lymphocytes were labelled

with carrier free sodium ^{51}chromate (200 μCi/10^7 cells) by incubation for 45 min at 37°C in Hanks' with 4% fetal calf serum IPT, washed three times in this media, and 50 μl containing about 2×10^5 cells were incubated with 50 μl of the antisera diluted in the same medium for 15 min at 20°C. This incubation was followed by addition of 50 μl of appropriately diluted rabbit serum, absorbed on mouse lymphocytes (Goldberg *et al.*, 1971), as a source of complement and incubated for 30 min at 37°C. At the end of the reaction, 1·5 ml cold phosphate buffered saline was added, the cells spun at 1000 *g* for 15 min at room temperature, and 1 ml of the supernatant counted in an Intertechnique gamma counter. The maximal release was the difference in counts between the highest dilution of antibody with complement, or a frozen cell control, and complement alone controls. Alternatively, 20 μl of a fresh trypan blue solution (0·4%) was added after the incubation with complement and the percentage of dead (stained) cells determined.

RESULTS AND DISCUSSION

The anti-H-2D^2 sera had a 50% (of maximal) lysis titre of about 1:300 on 129/Sv lymph node lymphocytes. When 120 μl of a 1:100 dilution were absorbed with 3×10^6 target sperm (129/Sv), the activity was reduced by 30% while 15×10^6 target sperm removed all the antibody activity. Controls of target erythrocytes (5×10^5) and spermatozoa isogeneic to the antibody producer had little or no effect on the antibody activity (Table 1). A second D-end serum was tested by the trypan blue cytotoxicity test. As seen in Table 2, all the reactivity could be readily removed by absorption with target spermatozoa while target erythrocytes equal to or greater than the maximal number contaminating the sperm had

TABLE 1. Absorption of anti-H-2D^2 serum by 129/Sv spermatozoa and erythrocytes

	Percentage of maximal release after absorption with			
Unabsorbed	3×10^6 129/Sv sperm	15×10^6 129/Sv sperm	15×10^6 A/or sperm	5×10^5 129/Sv r.b.c.
65	44	0	80	59

Of the anti-H-2D^2 serum 120 μl of a 1/100 dilution were absorbed with the indicated number of cells for 1 hr at 0°C. Then, the supernatant was tested for activity on 129/Sv lymph node lymphocytes by ^{51}Cr release test. Data are expressed as percentage of freeze-thaw release.

TABLE 2. Absorption of anti-H-2D^4 serum by BALB/C spermatozoa or erythrocytes

Dilution of sera	Cytotoxic index (% of max. killing above complement controls) after absorption with			
	Unabsorbed	8×10^7 spermatozoa	10^6 rbc	5×10^6 rbc
1/100	100	52	100	80
1/200	100	10	80	75
1/400	86	6	72	72
1/800	50	0	72	50

Of anti-H-2D^4 serum 110 μl were absorbed with the indicated number of cells for 1 hr at 0°C, then the supernatant was serially diluted (and one tube undiluted) for testing with BALB/C lymphocytes in the trypan blue cytotoxicity test.

TABLE 3. Absorption of anti-H-2K^d serum by BALB/C sperm and erythrocytes

Percentage of maximal release after absorption with			
Unabsorbed	2×10^6 BALB/C sperm	10×10^6 BALB/C sperm	10×10^6 A/or sperm
100	59	0	99

Of the anti-H-2K^d serum 110 μl of a 1/100 dilution were absorbed with the indicated number of cells, for 1 hr at 0°C. Then, the supernatant was tested for activity of BALB/C lymph node lymphocytes, by ^{51}Cr release test. Data are expressed as percentage of maximum release.

little or no effect. Similarly (Table 3), the activity of the anti-H-2K^d sera could readily be absorbed with 10^7 target sperm (BALB/C) while 2×10^6 target sperm partially absorbed the sera and 10^7 control sperm (A/Or) had little or no effect.

These results show that the titre of anti-H-2D^2 and anti-H-2D^4 and anti-H-2K^d (a serum which contains some weak reactivity to Ia antigens, however) sera were readily decreased by target spermatozoa, while not by spermatozoa isologous to the antibody producing strain. The significant decrease in the activity of the anti-H-2K^d sera after absorption with 2×10^6 spermatozoa (Table 3) (which would have only contained, at most, 2×10^4 epithelial and other cells and, at most 4×10^4 erythrocytes) and the failure of 5×10^5 erythrocytes to absorb the anti-H-2D^2 sera (Table 1) while 15×10^6 sperm, which would have contained at most $4{\cdot}5 \times 10^5$ erythrocytes, removed all the antibody activity (and a similar negative effect with red blood cells on the anti-H-2D^4 sera) argue strongly that spermatozoa, and not other cells, absorbed the antibody.

There are a variety of data to suggest that H-2K and D antigens are present on murine spermatozoa (see Introduction); only immunoprecipitation data seemed to be against the possibility (Vitetta *et al.*, 1975). However, the latter authors state that the sensitivity of their technique is such that H-2D and K antigens would only be detected if present at 10% or more of the level per splenocyte. However, the quantitative estimates of H-2 on spermatozoa (Erickson, 1972; Tsuzuku *et al.*, 1972) suggested that there was only about 10% as much H-2 region antigen per spermatozoa as per lymphocyte. Furthermore, the negative immunoprecipitation results do not take into account the special features of the spermatozoal plasma membrane (Johnson, 1975) which may greatly affect the rate and extent of detergent solubilization of membrane antigens.

These results in regards to H-2 antigens on spermatozoa, are remarkably similar to recent results in studies of HL-A antigens on human spermatozoa. There is evidence for antigens equivalent to Ia antigens in man and both these and the classical HL-A antigens are found on spermatozoa (reviewed in Erickson, 1976). Thus it appears that the antigenic components of the major histocompatibility locus are expressed on spermatozoa in mice and men.

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