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Preparation of HY Antibody in Female Mice as a Model for Sex Preselection

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ABSTRACT

The aim of this sutdy is to prepare Histocompatability Y(HY) antibody in female mouse at the first phase (current study), and to separate mouse embryos with regard to their sex, to be able to obtain offsprings of disired sex, by means of HY antibody, at the second phase. Totally 421 BALB/C inbred mice were used. Antibodies were produced in females by intraperitonial injections of spleen and testis cells of neonatal male mice. The sera of females were tested by five different immunological techniques, as follows: Double gel diffusion, Counter Immunoelectrophoresis, Plain electrophoresis, Immunofluorescence and Cytotoxicity test. The first two techniques showed no positive results, but the remaining three methods proved the existence of HY antibody in sera. In electrophoresis, an increase in gamma-globulin and total globulins index, over albumin, was detected in injected mice sera. In immunofluorescence assay HY antigen was revealed on the surface of the mouse testis and spleen cells. Cytotoxicity test has been the most suitable method for detecting HY antibody. Also, the possibility to differentiate X-bearing and Y-bearing embryos could be used in prevention of X-linked diseases as well as in population control.

INTRODUCTION

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A male-specific Histocompatability-Y (HY) antigen was found by skin isografts from inbred mice (1). Only females rejected grafts of males, but all other grafts in different sex combinations were accepted. Many scientists considered HY antigen, the most important product of sex determining genes. It has been shown that the HY antigen is detectable in preimplantation mouse embryos (2,7).

The aim of this study is to prepare HY antibody in female mouse at the first phase (current study), and to separate mouse embryos, with regard to their sex, to be able to obtain off springs of desired sex, by means of HY antibody at the second phase. Once applied to man, the consequences would be tremendous. The ability to differentiate between X-bearing and Y-bearing embryos could be used in prevention of X-linked diseases as well as in population control.

MATERIALS AND METHODS

Totally 421 BALB/C inbred mice, bred for over 80 generations in "Razi Institute" were used.

Preparation of Antisera

Antibodies were produced in adult female mice sera by injections of male spleen and testis cells from 362 neonatal mice (Fig.1:A,B,C,D). The spleen and testis cells were dissociated by a glass homogeniser in saline solution (Fig. 1: E,F). The suspensions were filtered through 200-mesh screen (Fig.1: G) in order to remove connective tissue. They were then centrifuged, and resuspended in saline, after which they were ready for injection. Weekly injections with fresh suspension were carried out intra-peritonially on 30 adult females for six weeks. A booster injection was carried out at week 9 (Fig.1: H).

Detection of Antibody

Females were bled in week 10; the sera were then separated by centrifugation at 3000 rpm for 30 minutes and frozen at -80°C. The sera were then tested by five immunological techniques as follows:

1) Double gel diffusion; 2) CounterImmuno-electrophoresis;3) Plain Electrophoresis; 4) Immunofluorescence; 5) Cytotoxicity test.

RESULTS AND DISCUSSION

The first two techniques i.e. Double gel diffusion and Counter Immunoelec-trophoresis showed no positive results, but the remaining three methods proved the existence of HY antibody in female mice sera.

Electrophoresis

An increase in gamma-globulin and total globulines index over albumin, were detected (Table 1 and Fig.2) in sera of mice injected by testis and spleen cells, compared pared to control mice, the former being higher. This is consistent with other findings (4,6).

Immunofluorescence

An indirect immunofluorescence assay with Fluorescein Isothiocyanate (FITC) conjugation mouse immunoglobulins revealed HY antigen on the surfaces of the mouse testis and spleen cells, by green fluorescent radiations. These radiations were not observed around control cells.

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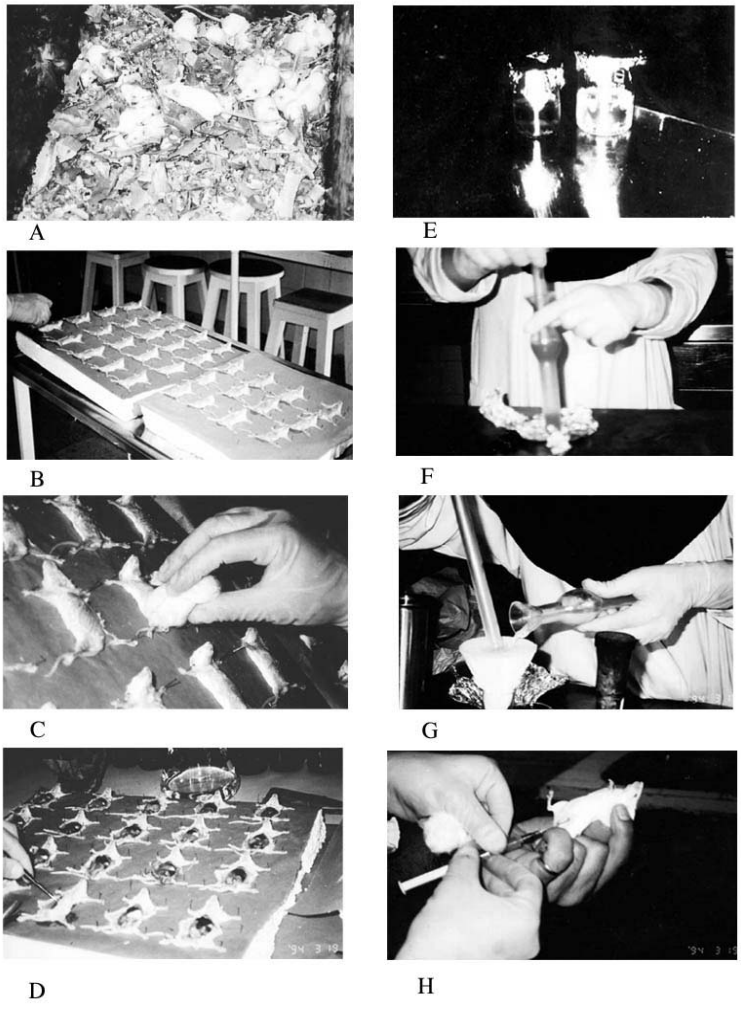
Albumin Rel%		Control	Anti Spleen	Anti Testis
Globulins	Alpha 1	4.6	3.7	4.3
	Alpha 2	7.8	8.3	8.1
	Beta	8.8	6.2	5.7
	Gamma	19.2	23.9	25.3
	Total	59.6	57.8	56.6

Fig.1. **Preparation of antisara**



Cytotoxicity Test

Cytotoxicity was considered the most appropriate test, and was based on the Scheide (6) method, also used by others (5), with some modifications. Our findings were consistent with the findings in a molecular survey on HY antigen (8). The components used for the test (Fig. 3) were as follows:

a) The suspensions of HY antigen prepared from testis and spleen cells of 4 neonatal male inbred mice, [while in some other studies (4, 6) used male mice dermal tissue cells] as antigen source.

b) The undiluted and serially diluted (1/2, 1/4, 1/10, 1/100) antisera obtained from blood of interperitonially injected adult females under investigation.

c) Complement for the cytotoxicity test, taken form rabbit serum.

Equal volumes (0.05 ml) of suspension a (containing 5×10^6 cell/ml as a source of antigen), undiluted and serially diluted antibody b and rabbit serum complement c, were incubated together in two stages at room temperature for an hour.

The viability of cells were determined after addition of 0.16% trypan blue solution and investigated under inverted microscope.

The rate of cell lysis was almost positively related to the concentration of HY antibody in each test. In control groups, as well as in other tests, where any components of a, b or c were excluded, the results were all negative.

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Fig. 2. **An increase in gamma – globulin against a decrease in albumin**

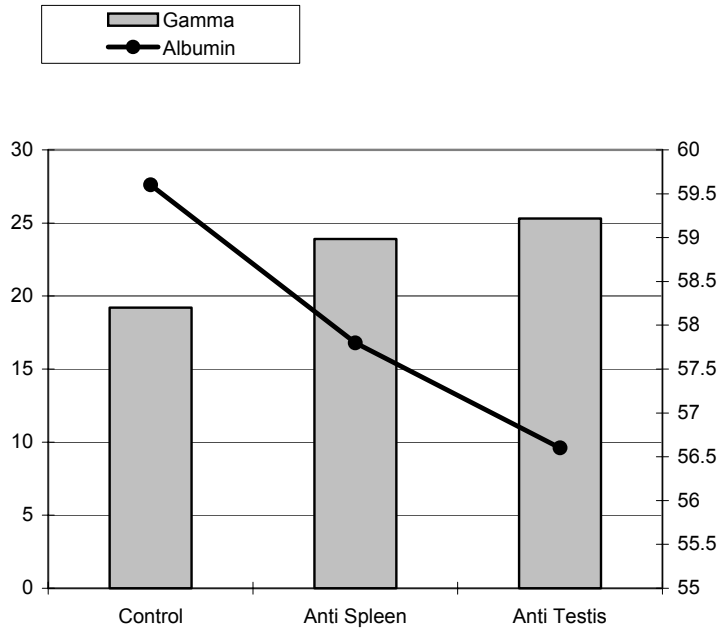
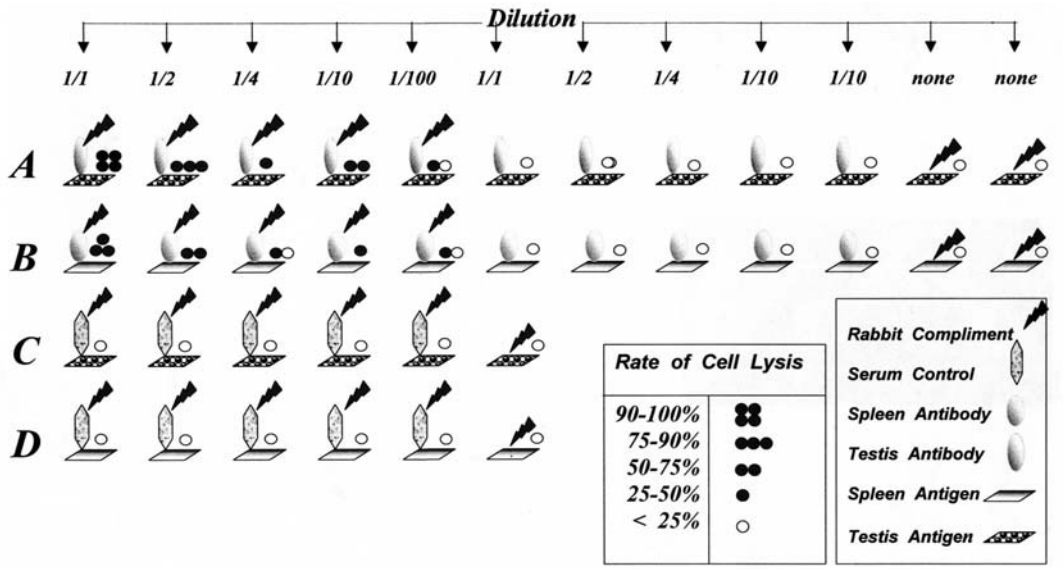


Fig. 3. **Antigen-Antibody complexes and serum controls in Cytotoxicity Test**



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