# ISOLATION AND SOME CHARACTERISTICS OF H-2 TRANSPLANTATION ANTIGEN IN MICE

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Details are given of the isolation of H-2 transplantation antigen from the spleen of BALB/c mice in a soluble form. The antigen was identified by exhaustion of the activity of the specific isoantiserum, purified by gel filtration, and its approximate molecular weight determined. The isolated antigen is biologically active and it induces the appearance of specific transplantation immunity, detectable by the secondary response test, and also of humoral isoantibodies detectable by a cytotoxic test in vitro.

Transplantation antigens are substances of protein or glycoprotein nature responsible for induction of transplantation immunity, and they are located on the surface membrane of all cells of the body [4, 9, 10, 13]. They are synthesized by tissue compatibility genes. It is only recently that satisfactory methods of obtaining transplantation antigens in a soluble form, including from human material, have been developed [7, 11, 14]. The study of soluble transplantation antigens is of particular importance at the present time because of the increased tolerance of soluble proteins purified from complexes of aggregated protein molecules [8].

In the investigation described below the properties of a complex of transplantation antigens determined by the H-2 locus in mice were investigated.

## EXPERIMENTAL METHOD

Mice of lines BALB/c, C57BL/6, and C3H/Sn (from the Nursery of the Academy of Medical Sciences of the USSR) were used. Anti-BALB/c isoantiserum was obtained by six weekly intraperitoneal injections of BALB/c mouse spleen cells into C57BL/6 mice in a dose of half a spleen per mouse. Antigen was obtained from the spleens of BALB/c mice 14 days after injection of cells of a Friend's leukemia; the mean weight of the spleens was 1.5 g. The initial insoluble antigenic complex was isolated by incubation of spleen cells in hypotonic (0.1 M) NaCl solution by the method of Davies [8]. The soluble antigen fraction was obtained by digestion of the original insoluble complex with papain [2, 5]. The supernatant fraction of the material treated with papain, after centrifugation at 130,000 g, was used as the soluble antigen. The supernatant was dialyzed against 0.002 M tris buffer, the protein content was determined by Lowry's method, and the material was preserved by the addition of thymol.

Activity of the transplantation antigen was measured in a cytotoxic text in vitro [1] by exhaustion of the activity of anti-BALB/c antiserum of C57BL/6 mice in a dilution giving 80% of dead cells with the test material. The unit of antigenic activity was the quantity of protein in the highest dilution of antigen giving a decrease of cytotoxic activity from 80 to 50% of dead cells. The specific antigenic activity was determined as the number of antigenic units per mg protein. Before investigation of the antigenic activity of the material, NaCl was added to it to an isotonic concentration.

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TABLE 1. Specific Antigenic Activity of Transplantation Antigen in the Course of Its Isolation and Purification

| Antigen | Concentration of antigen inhibiting activity of antiserum (in mg/ml) | Number of antigenic units per mg protein |
|---|---|---|
| Insoluble | 0.3 | 133 |
| Soluble | 0.4 | 100 |
| First protein peak of G-75 eluate | 0.2 | 200 |
| Second protein peak of R-300 eluate | 0.05 | 800 |

TABLE 2. Induction of Secondary Response to Graft of Donor's Line after Injection of Transplantation Antigen

| Donor of graft | Dose of antigen (in mg) | Mode of injection | Number of animals | Mean time of survival of graft |
|---|---|---|---|---|
| BALB/c | – | – | 10 | 10.6 ±0.17 |
| BALB/c | 1 | Subcutaneously | 8 | 7.7 ±0.17 |
| BALB/c | 0.5 | Subcutaneously | 7 | 9.4 ±0.2 |
| C3H/Sn | – | – | 10 | 10.7 ±0.3 |
| C3H/Sn | 1 | Subcutaneously | 8 | 10.5 ±0.3 |
| BALB/c | 1 | Intravenously | 8 | 10.7 ±0.2 |
| BALB/c | 10 | Intravenously | 8 | 10.9 ±0.17 |

TABLE 3. Formation of Cytotoxic Isoantibodies against Lymphocytes of Donor's Line after Injection of Transplantation Antigen

| Dilution of antiserum | Percentage of dead cells | Cytotoxic index |
|---|---|---|
| 1:10 | 93 | 0.9 |
| 1:20 | 61 | 0.51 |
| 1:40 | 36 | 0.18 |
| 1:80 | 29 | 0.1 |

Soluble antigen (200 mg protein) was applied to a Sephadex G-75 column (2 × 100 cm, 0.02 M tris buffer, pH 7.4 [5]), which was preliminarily calibrated with human serum albumin. Eluted peaks of ultraviolet absorption at 280 mμ were collected separately and their antigenic activity determined. A 10-mg sample of the protein fraction eluted from the Sephadex G-75 column in the first peak was applied to a column of Biogel R-300, 1 ×20 cm, in the same buffer [6], and the positions of the protein were determined relative to human serum albumin eluted from the same column.

Different amounts of protein of the soluble unpurified antigen were injected intravenously and subcutaneously into C57BL/6 mice 4 days before transplantation of skin from the tail of BALB/c or C3H/Sn mice by Medawar's method in Egorov's modification [3]. One group of C57BL/6 mice received a subcutaneous injection of 1 mg soluble antigen on the first and 14th days; blood was taken from them on the 21st day and tested for the presence of humoral isoantibodies in a cytotoxic test against lymphocytes of BALB/c mice.

## EXPERIMENTAL RESULTS

The protein content of the insoluble antigen was about 1% of the moist weight of the spleens used. The protein content of the supernatant after centrifugation at 130,000 g, i.e., of the soluble antigen, was 20% of the insoluble complex.

The various fractions of transplantation antigen in the course of its isolation and purification were diluted serially, and each dilution of antigen was tested by exhaustion of the cytotoxic activity of anti-BALB/c antiserum from C57BL/6 mice (Table 1). The exhaustion of activity of the antiserum was specific, for activity of the anti-C57BL/6 antiserum from BALB/c mice did not disappear after absorption with the antigen.

For the exhaustion test with each sample of antiserum 0.025 ml of antigen was used and the unit of activity was taken to be the protein content of antigen depressing activity of the antiserum in the reaction medium of one sample. The standard antiserum dilution was 1:80.

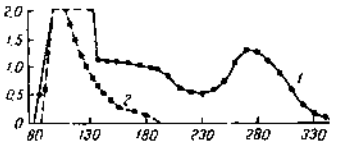


Fig. 1

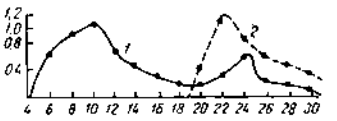


Fig. 2

Fig. 1. Elution of soluble antigen on Sephadex G-75 column: 1) antigen; 2) human serum albumin. Ordinate, absorption at 280 mμ; abscissa, volume of eluate (in ml).

Fig. 2. Elution of antigen on Biogel R-300 column. Legend as in Fig. 1.

After elution of soluble antigen from the Sephadex G-75 column (Table 1), the antigenic activity was found in the first protein peak which was eluted in approximately the same position as human serum albumin (Fig. 1).

No antigenic activity was found in the second protein peak, at least in a concentration of 2 mg/ml, which included soluble papain present as an impurity. The antigenic activity of the eluate after chromatography on a Biogel R-300 column was found in the second protein peak, the specific activity of which was 8 times higher than the activity of the initial soluble antigen. No activity was found in the first protein peak at least in a concentration of 2 mg/ml. The position of elution of the active fraction corresponded approximately to the character of elution of human serum albumin, indicating that their molecular weights are closely similar: approximately 60,000-70,000 (Fig. 2).

It can be seen in Table 2 that unpurified soluble transplantation antigen, possessing an activity of 100 units/mg in the exhaustion test, can induce transplantation immunity against a graft from the donor BALB/c line in a minimal dose of 0.5 mg by subcutaneous injection ($P < 0.01$) 4 days before transplantation. However, a graft taken from a C3H/Sn mouse was rejected at the same time as in the control despite several common features of antigenic specificity between lines BALB/c and C3H/Sn. After intravenous injection in a dose of 1 or 10 mg 4 days before transplantation, the grafts were rejected exactly as in the controls.

The formation of cytotoxic isoantibodies in response to two injections of soluble antigen in a dose of 1 mg is shown in Table 3. Antibodies were formed in a titer of 1:40 (cytotoxic index in the zone of confidence not less than 0.15 [1]).

The results described in this paper indicate that transplantation antigen determined by the H-2 locus can be identified in BALB/c mice both in vitro and in vivo, and they agree on the whole with the findings of Davies [6], Manson et al. [12], et al. The biological activity of the transplantation antigens requires further investigation.

## LITERATURE CITED

1. B. D. Bronde, Byull. Éksperim. Biol. i Med., No. 5, 64 (1964).
2. A. Ya. Kul'berg and I. A. Tarkhanova, Vopr. Med. Khimii, No. 5, 52 (1961).
3. N. N. Medvedev and I. K. Egorov, Genetika, No. 8, 86 (1966).
4. A. A. Yarilin, Byull. Éksperim. Biol. i Med., No. 9, 109 (1969).
5. D. Davies, Immunology, 11, 115 (1966).
6. D. A. L. Davies, Transplantation, 5, 31 (1967).
7. D. A. L. Davies et al., Transplantation, 6, 571 (1968).
8. D. Dresser, Immunology, 5, 378 (1962).
9. G. Haughton, Transplantation, 4, 238 (1966).
10. B. O. Kahan and R. A. Reisfeld, Science, 164, 514 (1969).
11. B. O. Kahan et al., Proc. Nat. Acad. Sci. (Washington), 61, 897 (1968).
12. L. Manson et al., Ann. New York Acad. Sci., 120, 251 (1964).
13. B. Medawar, J. Anat. (London), 78, 176 (1944).
14. A. Sanderson and J. Batchelor, Nature, 219, 184 (1968).