# REGULATION OF THE INTENSITY OF DELAYED-TIME HYPERSENSITIVITY TO SHEEP'S RED BLOOD CELLS BY THE K REGION OF THE MAJOR HISTOCOMPATIBILITY SYSTEM IN MICE

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The major H-2 histocompatibility gene complex in mice plays an exclusive role in the regulation of interaction between different populations of T cells [6, 10-13], including interaction between cells responsible for delayed-type hypersensitivity (DTH) and T suppressors [7, 10].

The writers previously developed a model of regulation of intensity of the DTH reaction to sheep's red blood cells (SRBC) by suppressor cells [1]. Preliminary experiments showed that interaction between suppressors and effectors of DTH is under the control of the H-2 complex; the absence of a suppressor effect in an allogeneic system is not due to rejection of the donor's cells.

The object of the present investigation was to determine the region of the H-2 complex which restricts this interaction.

## EXPERIMENTAL METHODS

Mice of the strains CBA (H-$2^k$), C57BL/6 (H-$2^b$), (CBA×C57BL/6)$F_1$ (H-$2^{k/b}$), A/Sn (H-$2^a$), ASW (H-$2^s$), ATL (H-$2^{t_1}$), BALB/c (H-$2^b$), B10.A (H-$2^a$), B10. HTT (H-$2^{t_3}$) and C57BL/6-M505 (H-$2^{ba}$) mutants were used. The suppressors were activated by injecting the mice intraperitoneally with $6 \times 10^9$ SRBC [1]. To induce DTH, an optimal dose of SRBC was injected intravenously into the mice: CBA and (CBA × C57BL/6)$F_1$ mice received $10^5$ SRBC; A/Sn, ASW, ATL, and BALB/c received $2 \times 10^5$ SRBC; C57BL/6, M505, B10.A, and B10.HTT received $10^6$ SRBC. The intensity of the DTH reaction was determined was determined by skin tests [1, 5]. On the 4th day after sensitization $10^8$ SRBC in 40 $\mu$l sterile physiological saline were injected into the footpad of the right hind limb, intradermally. The thickness of the footpads of both limbs was measured 24 h later by means of a type MK 0-25 engineers' micrometer, and the intensity of the reaction was judged from the difference.

The suppressor effect of spleen cells of mice treated with $6 \times 10^9$ SRBC was tested by transfer [1]. For this purpose, the mice were killed 5 days later, and a washed cell suspension was injected intravenously into intact recipients in a dose of $10^8$ cells. Immediately after transfer of the cells the recipients were sensitized by an optimal dose of SRBC. The efficiency of interaction of DTH suppressors and effectors from donors and recipients with a similar or different H-2 haplotype was estimated as the percentage suppression of intensity of the recipients' skin reactions.

In experiments with passive transfer of DTH, mouse spleen cells obtained on the 4th day after sensitization were injected intravenously into intact recipients in a dose of $10^8$. The recipients were given an intradermal reacting injection of SRBC 5-30 min later. The skin tests were read after 24 h.

## EXPERIMENTAL RESULTS

The percentage suppression of DTH when the individual regions of the H-2 complex of the donors of the suppressors coincided with those of the sensitized recipients is shown in Table 1.

As Table 1 shows, transfer of spleen cells from animals receiving a massive dose of antigen led to disturbance of DTH formation in syngeneic recipients (group 1) but had no effect on DTH formation in allogeneic recipients incompatible for the whole H-2 complex (group 2). Effective interaction between suppressors and effectors was observed also in a

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TABLE 1. Role of Regions of H-2 Complex in Restriction of Interaction between DTH Suppressors and Effectors

| Group of animals | Donors of suppressor cells | Sensitized recipients | No. of recipients | Identity of regions of H-2 complex of donors and recipients | | | | | | | | | Percent suppression |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | K | I—A | I—B | I—J | I—E | I—C | S | G | D | |
| 1 | CBA | CBA | 15 | + | + | + | + | + | + | + | + | + | 93 |
| 2 | CBA | C57BL/6 | 16 | — | — | — | — | — | — | — | — | — | 0 |
| 3 | (CBA×C57BL/6) $F_1$ | C57BL/6 | 12 | +/— | +/— | +/— | +/— | +/— | +/— | +/— | +/— | +/— | 95 |
| 4 | B10.A | A/Sn | 12 | + | + | + | + | + | + | + | + | + | 94 |
| 5 | ATL | ASW | 11 | + | — | — | — | — | — | — | — | — | 81 |
| 6 | CBA | A/Sn | 12 | + | + | + | + | — | — | — | — | + | 87 |
| 7 | ASW | B10.HTT | 5 | + | + | + | + | — | — | — | — | + | 92 |
| 8 | ATL | B10.HTT | 5 | + | — | — | — | + | + | + | + | + | 97 |
| 9 | M505 | C57BL/6 | 12 | — | + | + | + | + | + | + | + | + | 0 |
| 10 | ATL | CBA | 3 | — | + | + | + | + | + | + | + | — | 0 |
| 11 | ATL | B10A | 3 | — | + | + | + | — | — | — | — | — | 0 |
| 12 | BALB/c | A/Sn | 12 | — | — | — | — | — | + | + | + | + | 0 |

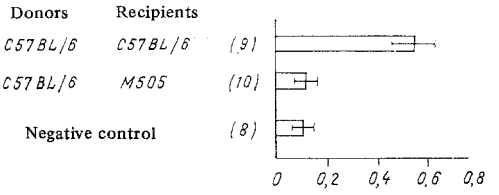


Fig. 1. Efficiency of passive transfer of DTH by spleen cells of sensitized donors to intact recipients. Horizontal axis – intensity of skin tests (in mm); number of mice in group shown in parentheses.

semiallogeneic system, on transfer of $F_1$ spleen cells with the haplotype of both parents to one of the parents (group 3), or in an allogeneic system, on transfer of allogeneic suppressor cells (B10.A) to recipients of the same H-2 haplotypes (A/Sn; group 4).

When mice with different H-2 haplotypes were used, suppression of DTH was observed only in the case of identity of the K-region, either alone or in combination with other regions of the H-2 complex (groups 5-8). The role of the K region in genetic restriction of interaction between DTH suppressors and effectors was exhibited particularly clearly in the experiments with mice (ASW and ATL) with the same basis (group 5) and with mutant (M505-C57BL/6) lines (group 9). In the first case coincidence of only the K region of the H-2 complex led to suppression of DTH, and in the second case absence of identity only for this region of the H-2 complex made transfer of suppressors ineffective. If the I and D regions coincided no suppression of DTH was observed.

It can be concluded from these results that the opinion which has evolved on the obligatory role of the I region and, more precisely, of I-I in the regulation of suppressor function is not valid for all types of suppressor cells. The results of the present experiments show that among suppressors there are some cells whose function is controlled by the K region of the H-2 complex. Data have recently been published to show that, unlike the suppressor factors described previously, containing products of the I-J and I-C regions [3, 7, 9], the suppressor factor regulating the intensity of contact sensitivity of DNFB [8] contains a product of the K or D region.

The role of the K region in regulation of interaction between suppressors and precursors of DTH effectors in the case of sensitization of SRBC, in the writers' opinion, sheds some light on the results of experiments to study adoptive transfer of DTH (Fig. 1). Transfer of DTH effectors, as Fig. 1 shows, evokes a high level of skin reactions in syngeneic recipients. Meanwhile transfer of DTH is impossible from mutants differing from the original strain by the K region of their H-2 complex. Consequently, interaction between DTH effectors and macrophages presenting the erythrocytic antigen also is restricted by the K region of the H-2 complex. Most probably interaction of this type between suppressors and DTH effectors is restricted by the same region of the H-2 complex whose product is involved in presentation of the antigen.

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# DELAYED TYPE AUTOIMMUNE REACTION AFTER LIGATION OF THE VASCULAR BUNDLE OF THE TESTIS

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The ability of autoantigens of the spermatogenic epithelium to induce autoimmunity after injury to the blood-testis barrier has not been proved [1, 3, 6]. However, injury to the testis after ligation of the vascular bundle supplying the organ is usually attributed predominantly to the after-effects of hypoxia, and autoimmune processes are disregarded [2, 13]. Nevertheless, there is evidence in the literature of a humoral response to testicular antigens after crushing of individual components of the testicular vascular bundle [7, 9, 14]. The development of cellular immunity under these circumstances can be judged by the infiltration of monocytes found in some cases into the testicular tissue [2, 5], but only in one investigation [7] was it assessed by the rosette formation test with spermatozoa.

The object of this investigation was to study the delayed type immune reaction to autoantigens of the testis after ligation of its vascular bundle.

## EXPERIMENTAL METHOD

Experiments were carried out on 35 noninbred mature male rats weighing 150-300 g. All painful manipulations including euthanasia were carried out under ether anesthesia. The vascular bundle of the testis was crushed by a ligature applied unilaterally for 40 min, which was repeated from 1 to 5 times at intervals of 1-30 days. The results were assessed after 7, 30, or 50-100 days. The difference in weight of the experimental and contralateral (intact) testes, expressed as a percentage of the weight of the contralateral testis, was used as an index of testicular atrophy.

Cellular immunity was assessed by the test of inhibition of adhesion of peritoneal exudate cells to glass in the presence of antigen (the adhesion inhibition test – AIT) [10]. The degree of a positive response was judged from the percentage of nonadherent cells after incubation of the cell suspension with antigen. The AIT of a mixture (1:1) of peritoneal cells with lymphocytes from the inguinal lymph node, the regional node for the experimental testis, also was investigated. The supernatant of a homogenate of intact autologous testis with a protein concentration of 2.5-3 mg/ml served as the autoantigen. Peritoneal cells were obtained without preliminary injection of irritants into the peritoneal cavity.

The experimental animals were divided into five groups: 1) ligation once, 2) twice, 3) three times, 4) ligation five times with an interval of 7 days, and 5) ligation five times with an interval of 1 day. The animsl were killed not less than 25 days after the first ligation (except in two cases), and in groups 4 and 5 they were killed after at least 50 days. The controls were intact animals and also: a) adhesion of cells without antigen; b) adhesion in the presence of bovine serum albumin (BSA) in a concentration of 2.5-5 mg/ml.

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