DELAYED-TYPE HYPERSENSITIVITY TO WEAK HISTOCOMPATIBILITY ANTIGENS AND ITS GENETIC RESTRICTION

I. V. Lyadova and L. N. Fontalin

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Convincing evidence has recently been obtained of the involvement of delayed-type hypersensitivity (DTH) effectors in the rejection of foreign transplants [5, 8, 9]. However, the problem still continues to be debated [4, 7, 14]. In particular, it is not clear whether correlation exists between DTH formation and rejection of the graft in incompatibility relative to so-called "weak" (not coded by the major histocompatibility system) antigens [10]. Genetic restriction of DTH to "weak" antigens likewise has received little study.

The aim of this investigation was to study the DTH formation and the rate of rejection of allografts in response to strong (H-2) and "weak" (non-H-2) histocompatibility antigens, and also genetic H-2 restriction of DTH to non-H-2 antigens.

EXPERIMENTAL METHOD

The following animals were used in the experiments: male and female mice of inbred lines CBA/CaLacSto (CBA), C57BL/6YSto (B6), DBA/2YSto (DBA/2), obtained from the Stolbovaya Nursery, Academy of Medical Sciences of the USSR, and mice of congeneic lines C57BL/10SnY (B10), B10.BR/Y (B10BR), C3H/SnY (C3H), C3H.SW/Y (C3H.SW), B10.D2/SnY (B10.D2) and B10.RIII (71NS)/SnY (B10.RIII), generously presented by the Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, and the Experimental Animals Department of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

The DTH reaction was induced by the method developed by the writers previously [3]. A suspension of allogeneic spleen cells, obtained by the usual method, described in detail previously [3], was used as the alloantigens. Mice were sensitized by subcutaneous injection of allogeneic spleen cells in a volume of 0.2 ml in a dose of 10^7 (for sensitizing CBA mice) or $2 \cdot 10^7$ (for sensitizing B6 and DBA/2 mice). The reacting injection of antigen was given 5 days later. For this purpose, $5 \cdot 10^6$ allogeneic spleen cells in a volume of 0.05 ml were injected subcutaneously into the right hind limb of the mouse, and the same number of syngeneic spleen cells was injected into the left hind limb. The reaction was read 24 h later by measuring the thickness of the limbs by means of an MK 0-25 mm micrometer with an accuracy of 0.01 mm. All the experiments included one group of mice which received only the reacting injection of antigen without preliminary sensitization (negative control).

An allogeneic skin graft was transplanted by the method described previously [1].

The numerical results were subjected to statistical analysis by Student's t test. Arithmetic mean values and confidence intervals at the $p = 0.05$ level are given in Fig. 1.

EXPERIMENTAL RESULTS

To investigate the DTH reaction to different alloantigens, B6 mice (experiments of series A) and CBA mice (experiments of series B) were used as recipients. Spleen cells from mice of different lines (C3H, C3H.SW, B10, B10.BR), differing from the recipients with respect

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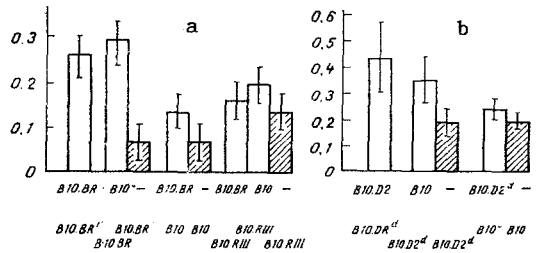

Fig. 1. H-2 restriction of DTH to non-H-2-antigens. Horizontal axis: top row denotes group of animals, bottom row denotes donors during testing; vertical axis — level of DTH reaction (in mm). Unshaded columns — experiment, shaded — negative control. a) Recipients of CBA (H-2^k) line; b) recipients of DBA/2 (H-2^d) line. Each group consisted of no fewer than six mice.

to H-2- and (or) non-H-2-antigens, served as the antigens. H-2 haplotypes, differences between recipients and donors with respect to non-H-2-antigens, and the results of the investigations are given in Table 1. The intensity of DTH is shown as the difference between levels of response in the experimental group and in the corresponding negative control.

It will be clear from Table 1 that the level of response to a combination of H-2- and non-H-2-antigens, consisting mainly of non-H-2-antigens, and to non-H-2-antigens alone, was virtually identical (A = 1, 2, 3; B = 1, 2, 3)。 These results are in agreement with data in the literature, according to which DTH to non-H-2-antigens is not lower, and may sometimes even be higher, than DTH to H-2 antigens [10, 12]. The response to non-H-2-antigens alone was weak, only a little above the negative control level (A = 4, B = 4).

When the same combinations of recipients and donors the rate of rejection of the allografts was determined. Grafts incompatible mainly for non-H-2-antigens in B6 recipients were rejected faster than grafts incompatible mainly for H-2-antigens (A = 2, 3, $p < 0.001$). The time of rejection of grafts incompatible mainly for H-2-antigens and of grafts incompatible only for non-H-2-antigens in CBA recipients did not differ statistically significantly. The results are in agreement with data in the literature showing a cumulative effect during rejection relative to several transplantation antigens [2, 5]. In the case when recipients and donors differed only for single non-H-2-antigens rejection took place more slowly, and was chronic in type, correlating with an extremely low DTH level in the mice of the corresponding groups (A-4, B-4). Thus the parallel investigation of the intensity of the DTH reaction and the rate of rejection of skin allografts in two lines of recipients in response to different alloantigens showed that correlation exists between these parameters of immunity. The results are also evidence that so-called "weak" histocompatibility antigens are in fact not weak: incompatibility for several such antigens may lead to an immune response comparable in strength with that to antigens coded by the major histocompatibility system (MHCS).

In experiments studying the restriction of DTH to non-H-2-angigens, different combinations of lines of mice were used. In the experiments of series A DTH was investigated in CBA mice to non-H-2-antigens (genetic basis) of B10 mice (Fig. 1a). Mice sensitized with, and given a reacting injection of B10.BR mouse spleen cells, with the same H-2 haplotype as the recipients (H-2^k), but differing from them with respect to non-H-2-antigens (group 1), served as the positive control. Mice of the experimental groups at the sensitization or reaction stage were injected with these same non-H-2-antigens, but in association with H-2 haplotype foreign for the recipient (spleen cells of mice of various congeneic lines obtained on the basis of B10 were injected). Mice of one experimental group were sensitized by B10 cells with the H-2^b haplotype and were given a reacting injection of B10.BR (H-2^k) mouse spleen cells. As Fig. 1 shows, the level of the reaction in this group was the same as in the positive control (group 2). Thus at the sensitization stage replacement of B10.BR spleen cells by B10 spleen cells did not prevent DTH formation to non-H-2-antigens. Mice of other experimental groups were sensitized by B10.BR spleen cells, and given a reacting injection of B10 (group 4) or B10.-RIII (group 6) spleen cells, with H-2^b and H-2^r haplotypes respectively. The DTH reaction was significantly weaker in these groups than in the positive control group ($p < 0.001$ and $p < 0.01$). The intensity of the DTH reaction to non-H-2-antigens also was reduced in CBA

TABLE 1. DTH Reaction and Length of Survival of Skin Allografts in Response to Strong and Weak Histocompatibility Antigens

Series of experiments	Group No.	Recipients	Donors	Differences between recipients and donors		DTH, mm	Number of mice in group	Length of survival of graft, days	Number of mice in group
				with respect to H-2 antigens	with respect to non-H-2-antigens				
A	1	$B6^b$	$C3H^k$	+	$+^1$	0.22±0,05	11	13,3±0,1	8
	2	$B6^b$	B10. BR^k	+	$\pm^2$	0.16±0,05	9	16,0±0,1	7
	3	$B6^b$	C3H. SW^b	—	$+^1$	0,20±0,03	17	13,6±0,1	7
	4	$B6^b$	$B10^b$	—	$\pm^2$	0,09±0,03	9	22,2±0,1	6
B	1	$CBAk$	$B10^b$	+	$+^3$	0,34±0,05	20	15,2±0,5	6
	2	$CBAk$	C3H SW^b	—	$\pm^4$	0,39±0,06	9	12,7±0,3	7
	3	$CBAk$	B10 BR^k	—	$+^3$	0,26±0,05	21	19,5±3,5	6
	4	CBA^k	$C3H^b$	—	$\pm^4$	0,08±0,04	20	24,6±2,3	9

Legend. $+^1$) Differences between recipients and donors mainly with respect to non-H-2-antigens: H-1, H-3, H-7, H-8, H-9, H-13; $\pm^2$) the same, with respect to non-H-2-antigens only: H-9; $+^3$) the same, with respect mainly to non-H-2-antigens: H-1, H-3, H-7, H-8, H-9, H-12; $\pm^4$) the same, with respect to non-H-2-antigens only: H-9, H-12, H-13.

mice sensitized with B10 alloantigens and receiving a reacting injection of B10.RIII alloantigens (group 7, $p < 0.05$). Thus in groups in which the reacting injection consisted of cells expressing non-H-2-antigens and products of the H-2 haplotype, "foreign" for the recipient, the DTH level was lowered. Similar results were obtained also in the experiments of series B on DBA/2 recipients (haplotype $H\text{-}2^d$, Fig. 1b).

The results showed differences in recognition of the antigen by T-cells at the sensitization and reaction stages of DTH: at the sensitization stage non-H-2-antigens were recognized independently of the H-2 haplotype of the donors' cells, whereas recognition of the same antigens at the reaction stage required compatibility of the recipient and the donor's cells with respect to H-2 molecules.

These results can be explained in terms of the concept of restriction of cell cooperation. It can be tentatively suggested that at the sensitization stage non-H-2-antigens are processed by the host's A-cells, as a result of which DTH effectors are formed and recognize foreign non-H-2-antigens in context with their "own" H-2. The possibility cannot be ruled out that DTH effectors restricted for the donor's H-2 molecules may be formed during sensitization. However, their discovery, for reasons which will be clear (interference due to the strong reaction to foreign H-2) is difficult. Mechanisms of recognition of non-H-2-antigens at the reaction stage are evidently different. Data on restriction of the effector phase of DTH can be explained on the grounds that non-H-2-antigens are recognized by DTH effectors without compulsory processing by macrophages. This view is in agreement with that expressed by Owens et al. [11].

There are only a few publications devoted to the study of genetic restriction of DTH to non-H-2-antigens. Absence of restriction of the afferent phase of DTH to non-H-2-antigens has been demonstrated [13]. Other workers [15] observed partial H-2 restriction of both phases of DTH to non-H-2-antigens. The somewhat contradictory nature of our own results and those obtained by the workers cited above can be explained on the grounds that during the development of DTH several subpopulations of effector cells arise, which differ in the genetic restriction of their response. This hypothesis is based on data [12] obtained recently during the study of the cytotoxic response to non-H-2-antigens.

Our own results are not only of theoretical, but also of great practical importance. They are evidence in support of the need for selection of a donor with respect both to strong (HLA) and to weak histocompatibility loci, and also that during repeated transplantations compatibility for MHCS antigens (HLA) may increase the risk of graft rejection on account of weak transplantation antigens.

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CHANGES IN SOME IMMUNOLOGIC AND BIOCHEMICAL PARAMETERS INDUCED IN GERMFREE ANIMALS BY T-ACTIVIN

V. Ya. Arion, A. V. Karaulov,
Yu. I. Khromenkov, I. V. Sanina,
A. K. Tagirova, Yu. A. Baryshkov,
and M. M. Intizarov

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The microflora has a considerable influence on the formation of physiological and immune reactions and on metabolic functions of the host organism [9, 11]. The use of germfree animals as models of immunodeficiency states has assumed great importance in connection with the study of the mechanisms of action of immunomodulating agents.

Several investigations aimed at studying the effect of the microbial factor on the defensive systems of the body have been undertaken on germfree animals [4, 13]. So far, however, the state of activity of the enzymes of xenobiotic metabolism and the state of immunoreactivity, including natural cytotoxicity, have received little study. The immunomodulator T-activin is known to have a many-sided influence on functioning of the T-system of immunity [1]. This preparation has been successfully used in the treatment of various immunodeficiency states [5].

The aim of this investigation was to study the effect of the thymus preparation T-activin on activity of enzymes of xenobiotic metabolism (EXM) and on cell-mediated cytotoxicity — two important mechanisms of defense of the body which realize their action without antigenic stimuli.

EXPERIMENTAL METHOD

Noninbred guinea pigs were obtained from the Central Laboratory Animals Nursery, Academy of Medical Sciences of the USSR. Young germfree guinea pigs were obtained by hysterotomy, using the gnotobiotic operating isolator system of the Research Laboratory of Experimental Biology and Medicine, Academy of Medical Sciences of the USSR. The animals were reared up to the age of 8 days with observance of the rules of a germfree technique in soft plastic isolators

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