IMMUNOLOGICAL TOLERANCE ESTABLISHED IN PLACE OF INCOMPATIBILITY OF HOMOGRAFTS IN DOGS

A. V. Puza

Scientific Research Laboratory of the Surgical Clinic (Director, Professor Ya. Knyazovitski)
P. I. Shafarik University Medical Institute, Koshitse, Czechoslovakia
(Presented by Active Member, AMN, SSSR, A. V. Lebedinskii)
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At the present time, incompatibility (an immunological phenomenon) represents the greatest obstacle to the use of clinical homografting [1, 5, 20, 21]. It is therefore of the utmost importance to seek for methods of suppressing this response.

In 1953 Hasek [13, 14, 15] and Billingham, Brent and Medawar [10] independently published reports showing how tissue incompatibility could be overcome experimentally. Hasek called the phenomenon immunological approximation, and Medawar referred to it as acquired immunological tolerance. Following Hasek, we attempted to produce immunological tolerance to heterologous cellular antigens in rats [23], and later we measured their immunoadaptive period [22]. The results showed that immunological tolerance may also be induced in dogs.

Results of Experiments on the Acquirement of Tolerance in Dogs

No. of dog	Age of Recipient, days. At time of blood Replacement	Amount of blood given (in %)	Length of life of graft (in days)	Remarks
1	2	220	221	
2	2	250	606[1]	81[2] HK
3	3	170	606[1]	81[2]
4	3	350	94	
5	3	160	376	
6	3	210	377	
7	3	230	600[1]	426[2] HK
8	3	260	606[1]	
9	7	200	446	
10	7	200	198	144[2]
11	7	340	260	181[2]
12	11	200	283	221[2]
13	13	300	121	56[2]
14	13	200	121	56[2]

[1]Animal under observation until this time.
[2]Homografted skin passes through a "crisis," during which the hair falls out.
Note. HK indicates homograft of kidney.

Hasek [10] showed that the extent to which immunological tolerance develops depends not only on the antigenic relationship between donor and recipient, but also on the amount of antigen injected. This was also shown by Rhaba and his co-workers [17], who emphasized that in experimental embryonic parabiosis it is more effective to replace a large amount of blood than to inject large amounts of donor blood into the recipient embryo.

In our studies we used blood replacement; the amount of antigen introduced was the same as was given in the method of embryonic parabiosis, and also corresponded to the clinical method. This modification of the technique of complete blood replacement was worked out by I. Mikhailovskii [6] and P. M. Chepov [7], and has been described

in detail by O. S. Glozman and A. P. Kasatkina [2]. By this means we have been able to induce a high degree of specific immunological tolerance to skin homografts taken from the primary blood donors [24, 27]. We have also measured the immunoadaptive period in dogs [22].

The object of the present investigation has been to make prolonged observations on the condition of skin grafts in dogs in which a specific tolerance has been induced.

We have also tested our working hypothesis of the "individual universal approximation", which states that dogs who are tolerant to skin fragments for a long period are also tolerant to grafts of a different organ, the kidney.

Skin homograft on a dog 606 days after operation. The graft is completely covered with hair of a distinctive color.

EXPERIMENTAL METHOD

The experiments were performed on dogs, because in them, morphogenesis continues for several days after birth. Following the suggestion of P. F. Zdrodovskii [4] concerning the development of reactivity, we have supposed that new-born puppies are capable of immunological adaptation to homologous cell antigens.

We used dogs of various ages, weights, and sex from different litters. In most of the experiments the recipients were puppies of a few days old. Donors of blood and grafts were adult dogs unrelated to the recipients. Immunological tolerance was induced by complete blood replacement, by the method we have described previously [28]. The hair color of the donor differed from that of the recipient, and the skin fragment was transplanted in such a way that the donor hair grew in the reverse direction to that of the recipient.

A homograft of the kidney was made into the neck, and its vessels were joined to the carotid artery and to the internal jugular vein. The ureters were brought out onto the skin of the neck. Both recipient kidneys were removed less than three weeks after the homograft had been made.

EXPERIMENTAL RESULTS

In the first two weeks after birth, i.e. during the period in which immunity was being acquired, fourteen dogs were given a complete exchange of blood, during which period they received 1.7-3.5 times their own blood volume. The results of the grafting experiments are shown in the Table.

It can be seen from the Table that in the experimental animals the homografts were preserved for over three months. Four of the dogs have remained under observation for more than one and a half years, up to the present time. In some of the skin fragments, after a certain time, a crisis occurred in which some hair fell out. However, histological studies showed that even after the crisis the fragments remained viable, and continued to function. The Figure shows a skin graft in dog No. 8, which has survived for 606 days, until the present time. In this case the graft may be regarded as permanent. In all the grafts, cutaneous sensitivity was restored.

Into dogs Nos. 2 and 7, in which the skin fragments had taken permanently, we transplanted a kidney from the same donor. In dog No. 7, the kidney has survived, and has functioned until the present time. Both of its own kidneys have been removed, and it has lived for 162 days, and in two months its weight has increased by 1 kg. Tests have shown that the grafted kidney is functional.

In dog No. 2, the kidney graft was unsuccessful. It ceased functioning after 22 days, and typical signs of tissue incompatibility developed. Apparently insufficient tolerance had developed by the time the graft was made.

The results of these experiments therefore confirm our previous opinion [22, 24] that complete blood replacement with two or more times the animal's own volume of blood, during the first three days after birth, may induce a high degree of immunological tolerance, which is shown by the prolonged and stable taking of homografts. When a marked tolerance does develop, changes occur, the chief of which is a loss of hair.

The results obtained correspond with those of P. M. Chepov [8], who induced tolerance in adult dogs by means of plasmophoresis.

Our results on the induction of immunological tolerance by complete blood exchange have been confirmed by several authors [9, 11, 12]. In our opinion, they provide model experiments which make it possible to study the complex phenomena of tissue incompatibility in higher mammals.

These experiments have also shown that after blood replacement, the animals acquire a tolerance not only to skin, but also to other organs from the same donor (in our experiments, the kidney). Gombos and his co-workers [12] also came to the same conclusion; they studied the morphology and function of renal grafts. Jacina and his co-workers [18] made functional studies of renal homografts, and their results are in line with ours, and support our conclusions.

Experiments on the induction of immunological tolerance in dogs are of interest to clinicians. Marish [19] grafted skin onto patients whose blood had been completely replaced on account of erythroblastosis fetalis. The skin homografts were taken from primary blood donors. In some cases, the cutaneous grafts remained viable for two months. Therefore, in man, by a complete replacement, it is possible to induce tolerance, although it is not well shown, and does not make it possible to produce permanent homografts.

SUMMARY

Experiments on 14 dogs demonstrated that complete blood replacement within the first days after birth induces immunological tolerance to skin homografts which differs in each individual case. The animals become tolerant not only to skin, but also to other organs, in this instance the kidney, which continues to function after being grafted. Immunological tolerance is specific, i. e. the recipients acquire tolerance to the tissues and organs of the primary blood donor only. Experiments on immunological affinity in dogs may serve as a convenient model for a study of tissue incompatibility as affecting homografting in the higher mammals.

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