# SECONDARY IMMUNODEFICIENCY ASSOCIATED WITH THE USE OF ANTILYMPHOCYTIC SERUM

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A study of immune reactions of cellular and humoral types in the late stages after injection of antilymphocytic serum (ALS) showed that a state of deficiency of the humoral response to sheep's red blood cells is preserved in CBA mice. A second injection of ALS deepened the depression of the humoral immune response but had virtually no effect on survival of allografts. The results are discussed from the standpoint of the effect of ALS on immunity and the role of different mechanisms in the rejection reaction.

KEY WORDS: antilymphocytic serum; humoral immunity; cellular immunity.

Despite the severity of the depressive properties of antilymphocytic serum (ALS) in experiments in vivo and in vitro, this substance has not found wide clinical application. There are several reasons for this, especially the difficulty of obtaining ALS and testing its activity and also the presence of side effects [5-8]. There are also indications that during repeated use of ALS its immunodepressive activity weakens [13, 14]. The reason for this phenomenon has not been finally explained.

The object of this investigation was to study immunologic competence in the late stages after primary and repeated use of ALS in reactions of humoral and cellular immunity.

## EXPERIMENTAL METHOD

Experiments were carried out on CBA mice obtained from the "Stolovaya" Nursery, Academy of Medical Sciences of the USSR. ALS was isolated from the blood of rabbits immunized with mouse lymph node cells [9]. The titer of lymphocytotoxins in the antiserum was 1:512. Allografting of skin on CBA mice was carried out by the use of a skin graft from the tail of C57BL/6 or A mice [12]. The humoral immune response of the mice was determined on the 4th day after immunization with 0.5 ml of a 2% suspension of sheep's red blood cells (SRBC) in the local hemolysis test [10]. Statistical analysis of the results was carried out by the use of the nonparametric U criterion [4].

## EXPERIMENTAL RESULTS

The scheme of the experiments to study repeated use of ALS was as follows: skin from C57BL/6 mice was grafted on CBA mice and ALS was injected in a dose of 0.5 ml on the 2nd and 4th days after skin grafting, or the animals were immunized with SRBC after the second injection of ALS. The data on the formation of transplantation immunity following primary and readministration of ALS are given in Table 1.

It follows from Table 1 that during skin grafting the survival of the grafts following primary administration of ALS in two doses was 25.4 days (group 4). If the mice received further injections of ALS 1 or 2 months after rejection of these grafts, however, and allografting was carried out again with skin from mice of a different strain (line A), the grafts survived for a shorter time, namely 14.75 or 16.6 days respectively (groups 1 and 2). Rejection of the grafts took place at about the same times in animals not receiving the second course of ALS (group 3).

A somewhat different picture was observed when the effects of primary and secondary administration of ALS on humoral immunity were studied (Table 2). Repeated administration of ALS was found to induce considerable immunodepression of the humoral immune response (groups 1 and 2), roughly 10 times greater than

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TABLE 1. Survival of Skin Allografts in CBA Mice after Primary and Readministration of ALS

| Group | Primary injection of ALS | Interval | Repeated injection of ALS | Survival of grafts, days |
|---|---|---|---|---|
| 1 ($n$=8) | 0,5 ml twice | 1 month | Second and 4th days after transplantation, 0.5 ml each | 14,75 (13—16) $P_{1,4}<0,05$ |
| 2 ($n$=7) | Same | 2 » | Same | 16,6 (13—20) $P_{2,4}<0,05$ |
| 3 ($n$=6) | » » | 1 » | — | 13,6 (13—15) $P_{3,4}<0,05$ |
| 4 ($n$=7) | — | — | Second and 4th days after transplantation, 0.5 ml each | 25,14 (20—30) |

TABLE 2. Effect of Primary and Readministration of ALS on Humoral Immune Response

| Group* | Primary injection of ALS | Interval | Repeated injection of ALS | Number of antibody-forming cells in spleen |
|---|---|---|---|---|
| 1 | 0,5 ml twice | 1 month | 0,25 ml | 6 140 (400—14 000) $P_{1,3}<0,05$ |
| 2 | Same | 2 » | Same | 3 140 (300—6 200) $P_{2,3}<0,05$ |
| 3 | » » | 1 » | — | 19 222 (2 800—42 300) $P_{3,4}<0,05$ $P_{3,5}<0,05$ |
| 4 | — | — | 0,25 ml | 55 466 (31 700—101 700) $P_{4,5}<0,05$ |
| 5 | — | — | — | 118 400 (87 600—180 600) |

*Six or more animals in each group.

the immunodepressive activity shown by the same dose of ALS when administered the first time (group 4). It should also be noted that in mice receiving ALS a month previously ability to respond to SRBC was depressed more severely than in animals of the control group.

If the maintenance of the state of depression of the humoral immune response for a month after injection of ALS (Table 2) is compared with data showing the absence of depression of transplantation immunity (Table 1), it suggests that the late action of ALS on responses of humoral and cellular immunity is opposite in direction. Moreover, secondary administration of ALS aggravates the state of depression of humoral immunity and, at the same time, has no significant effect on the reaction of rejection of the new graft. The mechanism of "secondary ineffectiveness of ALS" in the reaction of transplantation immunity or the mechanism of "resistance" of reactions of rejection of a genetically new allograft to the action of repeated administration of ALS is not clear. The reason for the ineffectiveness of the secondary course of ALS during allografting may perhaps be production of antibodies reacting with H-2 antigens of C57BL/6 and A mice [3]. However, in these experiments rejection of allografts was not accelerated, as it ought to be if such cross-reacting antibodies were present [3].

It can be tentatively suggested that a definite role in the mechanism of the secondary ineffectiveness of ALS is played by antibodies against ALS proteins that were formed after the primary course, as has already been reported in the literature [5, 13, 14]. However, evidence against the leading role of such antigens is given by the depressive action of ALS on responses of humoral immunity.

Another possibility is that ALS, by its primary action on lymphocytes, induces a state of immunodepression of both the cellular and the humoral immune response, which is later replaced by a secondary functional deficiency of humoral immunity only.

The ineffectiveness of secondary administration of ALS during allografting may be connected with the fact that not only the state of cellular immunity (the degree of its depression), but also the state of humoral immunity plays a role in the mechanism of graft survival; the deficiency of humoral immunity, moreover, does not prolong the survival of the graft.

Nor can other explanations of the secondary ineffectiveness of ALS during allografting be ruled out, such as activation or "unleashing" of phylogenetically older rejection reactions, resting on immune and nonimmune bases, be ruled out [1, 2].

Repeated administration of ALS 1 or 2 months after the primary course thus inhibits the immune response to SRBC but does not affect survival of allografts. In the late stages after two courses of ALS the development of a functional secondary immunodeficiency of the humoral immune response is observed.

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