EFFECTS OF ANTILYMPHOCYTE SERUM ON HYPERACUTE RENAL REJECTION IN GOATS*

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SUMMARY

An experiment is described whereby the suppressive action of antilymphocyte serum on the suspected immunological response in exteriorized goat kidneys, produced by specifically sensitized thoracic duct cells, was tested. The results suggest that in this system, the specific lymphocytic reaction mechanism is inhibited by antilymphocyte serum and is more in accord with the 'blindfolding' theory than any other.

In previous publications[1,2] an experiment was described which showed the effect of specifically sensitized allogeneic thoracic duct cells (TDC) on exteriorized kidneys of goats. These cells produced a hyperacute interruption of renal blood flow and urine production. The fact that this reaction occurred only when specifically sensitized TDC were used, suggested that it was an immunological phenomenon. In order to ascertain whether this was in fact the case the effect of antilymphocyte serum (ALS) on the kidneys was investigated, since it is known that ALS suppresses cell-mediated immune responses. The problem is of general interest, because the mode of action of ALS is not yet completely defined and also because the method used could provide evidence on whether ALS can act on the efferent limb of the immune response without killing the effector cells involved.

MATERIALS AND METHODS

Animals

Unselected adult goats bought from farmers were used for these experiments. They weighed 30 - 40 kg. ALS donors were female New Zealand White rabbits weighing 2·5 - 3 kg each.

Methods

Preparation of ALS. ALS was prepared in New Zealand White rabbits using TDC from different goats by the method of Levey and Medawar.[3] Complement was destroyed in the ALS by heating the sera at 56°C for 30 minutes. The sera were sterilized by millipore filtration and stored at −20°C until use. Lymphocytotoxic titres were determined.

Cytotoxicity measurements. A detailed description of cytotoxicity measurement has been given in a previous publication.[2] Basically, a constant amount of washed goat-TDC were incubated in serially 2-fold dilutions of decomplemented serum in Hanks's solution. Fresh rabbit serum was used as complement source. The rabbit serum had previously been absorbed with a washed erythrocyte pool from 5 or more goats.

The proportion of viable cells was estimated by the standard trypan blue exclusion test.

Exteriorization of the kidneys. The method has been described by Loubser *et al.*[1] This method provides functioning exteriorized kidneys of an experimental animal (original skin donor) in which perfusion studies can easily be done and subsequent effects accurately evaluated.

TDC. As has been described in a previous publication, the thoracic ducts of specifically sensitized goats (skin graft recipients) were cannulated 10 days after the second set of skin grafts had been transplanted. When about 10^9 TDC were collected at 4°C with continuous heparinization the lymph was centrifuged at 322 × G for 45 minutes. The supernatant fluid was removed and the cells were treated in one of the following ways:

(*i*) They were resuspended in Ringer's lactate to a final volume of 250 ml.

(*ii*) The cells were resuspended in not more than 30 ml of Ringer's lactate; 12 ml of ALS (volume arbitrarily chosen) was added and the suspension was left to incubate at room temperature 25 ± 2°C for 30 minutes. Thereafter the volume of the suspension was increased to 250 ml by adding Ringer's lactate.

(*iii*) The cells were again resuspended in not more than 30 ml of Ringer's lactate; 12 ml of ALS was added and the suspension was incubated at room temperature for 30 minutes. The cells were then washed once in Ringer's lactate and resuspended in 250 ml of fresh Ringer's lactate. The supernatant fluid of this suspension did not show the presence of any residual protein when tested by electrophoresis.

In both the incubation procedures the suspensions were agitated repeatedly to ensure maximal mixing of the cells with the ALS.

Perfusion of exteriorized kidneys. As described in a previous publication,[2] the silastic tubes connecting the exteriorized kidneys were clamped and after the blood had been washed out of the kidney with cold Ringer's lactate, the cells were perfused through the kidney for about 10 - 15 minutes. If the perfusion did not stop during this procedure the clamps were removed and the blood circulation through the kidneys was re-established.

RESULTS

Two batches of ALS were used in these experiments. One batch had no lymphocytotoxic titre when tested against TDC obtained from a panel of goats, and the other batch had a cytotoxic titre of 1:128.

Not one of the batches of ALS caused an increase of stained cells *in vitro* in a complement-free system, i.e. before and after the incubation of TDC and ALS. The percentage of stained cells in the perfusate was not increased when comparisons were made before and after perfusion of the kidneys. In all cases the percentage of viable non-stained cells never decreased below 97%.

When one of the kidneys of the original skin donor was perfused with the specifically sensitized allogeneic TDC, resuspended in the isotonic solution, the perfusion could not be completed in 6 out of 12 cases and the flow of urine stopped. In the remainder of the cases, subsequent blood flow was interrupted within 5 - 15 minutes, and again urine flow ceased. Blood flow could not be re-established in spite of attempts to flush the kidneys with

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Rheomacrodex or other isotonic solutions. The immunological characteristics of this reaction were proved by the necessary controls in previous experiments.[2]

In contrast, when the other kidney of the same goat was perfused, previous incubation of the TDC with ALS prevented this blockage of the renal vasculature. This occurred even when the incubated cells had been washed free of the remaining circulating ALS after the incubation period. In both cases the perfusion did not cause a cessation of the flow through the kidney, nor did blood flow stop after it had been re-established. Repetition of this procedure after ½-hour caused no obvious defect.

DISCUSSION

The most important observation is that both a lymphocytotoxic and a non-lymphocytotoxic ALS is able to block the hyperacute reaction in the kidney without any evidence of the killing of the specifically sensitized TDC. This occurred with one batch of ALS when cells were examined *in vitro* and in the presence of complement, as well as in all complement-free systems using either batch of ALS. The percentage of stained cells remained unchanged during the incubation of the TDC with ALS, and there was no increase in the percentage of stained cells in the perfusate after perfusion of the kidneys.

These results suggest that in this system, the specific lymphocyte reaction mechanism or response is inhibited by ALS, and is more in accord with the 'blind-folding' theory than any other.

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