

FAILURE OF RENAL HOMOGRAFT PROTECTION WITH RNA PRETREATMENT¹

Nine years ago Groth et al. (3) reported from our laboratories that infusion of canine renal and hepatic homografts with solutions containing RNA of either donor-specific or third-party origin caused significant prolongation of transplant function and recipient survival in about one-fourth of the experiments. This was apparent confirmation of earlier claims by Jolley et al. (4) and others (1, 7). Since then, similar results have been described with animal RNA or even yeast RNA by some investigators (5, 6, 9, 11) but not by all (2). Because of the potential importance of such graft pretreatment, we have repeated our original investigations and have not been able to confirm them.

The RNA was prepared with modifications of a standard technique (10) from fresh third-party mongrel dog spleens that were passed through a meat grinder. Aliquots were placed in glass vials and quick-frozen in a Dry Ice-acetone mixture. The frozen samples were kept at -20°C until the extraction procedure. The frozen spleen was homogenized with cold Tris-EDTA-buffered saline, 1% mercaptoethanol, and redistilled phenol. After centrifugation the aqueous layer was reextracted with phenol, and then precipitated with ice-cold absolute ethanol. The mixture was kept at -20°C overnight. The precipitate was dissolved in Tris-EDTA-buffered saline and reprecipitated with absolute ethanol to remove any remaining phenol. The final precipitate showed the characteristic ultraviolet absorption for RNA with a mean 280- to 260-nm ratio for all samples of 1:1.99. The protein content was less than 0.1%. RNA was quantitatively measured by the Orcinol procedure for pentose. The RNA was added to lactated Ringer's solution in

amounts of 10 mg/100 ml and the pH was adjusted to 7.45 before perfusion into the kidney grafts.

Renal transplantation in mongrel dogs was the test model. A donor animal gave one kidney to a test recipient and the other kidney to a control. In two of the 14 experiments and in three of the controls, the organs were perfused with 400 ml of cold lactated Ringer's solution for about 70 min using a commercial renal preservation unit (Waters Instruments, Inc., Rochester, Minnesota). The amount of RNA added to the perfusate in the experimental group averaged 106.2 mg. In the other 12 experimental kidneys, approximately the same amount of RNA was

TABLE 1. Effect of RNA infusion on renal homograft survival values given in mean days \pm SD

| | Control | Experimental |
|----------|------------------|---------------|
| No. | 14 | 14 |
| Survival | 11.64 \pm 2.92 | 9.5 \pm 2.2 |

added to 200 ml of lactated Ringer's solution and used to slowly infuse the organs over a 20-min period. These kidneys as well as those infused with unmodified lactated Ringer's solution were stored in the refrigerator at 10°C for an additional hr in the same way as described by Groth et al. (3).

Animals that died in less than 5 days were excluded from statistical computations since intussusception, respiratory complications, and technical imperfections were the usual causes of early death. This necessitated eliminating about 30% of the recipients, almost equally divided between the test and control animals.

The survival of animals that lived at least 5 days was the same statistically with or without RNA in the infusate (Table 1). The actual survival was somewhat longer in the control series, but the difference was not significant.

Histopathological examination of the control and RNA infused kidneys revealed no differences.

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This investigation thus failed to confirm a potentially promising observation. It was not apparent why the results were different in 1975 and 1976 than in 1967 and 1968. Possibilities include a difference in the quality or quantity of RNA used, or more likely some systematic artifact in laboratory procedure or animal care that was present in our laboratory at an earlier time but not today. However, the best possibility is that our original observations led us to invalid conclusions. More recently, it has been better appreciated that mongrel dog recipients not infrequently have long survival with no treatment at all (8). The fortuitous inclusion of a few such long-term surviving recipients in the test groups but not in the controls of our previous RNA work could thus have been misinterpreted.

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