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Abstract

We applied unidirectional MLC test to renal allograft in dogs, and investigated the correlation between the growth rates of MLC reaction and the intensity of rejection of the kidney transplants or the postoperative renal function. It was concluded that the grade of rejection became three plus (+ + +) when the rate of blastformation was more than 18 %, while it became one plus when the rate was less than 15 %. The rate of blast. formation was closely correlated with the strength of rejection of kidney transplants. However, the postoperative renal function was not always correlated with the mixed lymphocyte reaction.

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TISSUE TYPING BY UNIDIRECTIONAL MIXED LYMPHOCYTE CULTURE III. THE RELATIONSHIP OF *IN VITRO* LYMPHOCYTE COMPATIBILITY TO RENAL ALLOGRAFT REJECTION

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In our previous reports (1, 2), it has already been demonstrated that the blastformation rates of unidirectional mixed lymphocyte cultures (MLC), using the supersonicated cell homogenate of the potential donor, reflect the histocompatibility of mice. Further, we applied this unidirectional MLC test to skin allografts in dogs, and verified that the growth rate of MLC was inversely proportional to the median survival time of skin allografts (3).

There are few reports which show the relationship between the unidirectional MLC test and the rejection of kidney transplant. In view of this we have investigated to see whether or not such a relationship exists. MIWA (4) of our laboratory has presented a report indicating such a correlation in canine renal allotransplantation.

In the present experiment we supplemented 7 pairs and examined the relationship between the growth rates of MLC and the strength of rejection of kidney transplants. In addition, we examined the relationship between the blastformation rates and the postoperative renal function. This paper describes our findings of such a study.

METERIALS AND METHODS

1. *Renal allograft*: Mongrel dogs, picked at random of either sex and weighing 12 to 18 kg, were used. A pair of dogs were anesthetized with pentobarbital sodium (30 mg/kg), and subjected to bilateral nephrectomy. Renal allografts were exchanged between them. Immediately after the excision, the left kidney of the prospective donor was perfused at room temperature with lactated Ringer's solution, each liter of which contained 50mg of heparin sodium and 1g of procain hydrochloride, under a pressure of 120 cm of water, until the venous effluent became clear. The kidney was then transplanted in the opposite iliac fossa of the prospective recipient by an anastomosis of the renal artery and vein to

external iliac artery and common iliac vein, respectively, in an end-to-end fashion with the use of Nakayama ring. In all instances, the interval between ligation of renal vessels and revascularization was 20 to 60 minutes. The ureter was implanted into the anterior bladder. To prevent the kidney from moving about and to avoid the ureter and renal vessels from twisting and kinking, the capsule of the kidney was sutured in the iliac fossa. All the animals received 0.5g of streptomycin intramuscularly for 7 days after the operative procedures. After the allotransplantation, urine production, blood urea nitrogen levels, white blood cell counts, and other hematologic conditions were checked every other day. These animals were sacrificed under anesthesia 7 days later and the transplanted kidney was examined grossly and microscopically. Animals died of technical failure were excluded from the series. Autopsies were performed on each animal and histological preparations were made from the kidney transplant, liver, spleen, stomach, small intestine, heart, lung, lymph nodes and bone marrow.

The degree of rejection is graded according to the standard criteria by CALNE (5). It is as follows: one plus (+)··· minimal cellular infiltrate; two plus (++)··· moderate cellular infitrate and/or moderate vescular changes, more than 50% viable cortical tissue remaining; three plus (+++)··· severe cellular infiltrate and/or vascular changes, less than 50% viable cortical tissue remaining. On the other hand, postoperative renal function was classified into three grades by urinalysis and BUN level, that is, good, fair and poor.

2. *Preparation of lymphocytes and MLC*: The methods employed here were identical with those described in our previous reports (3, 4). Briefly, preceding the renal allograft, the pure suspension of lymphocytes was obtained from the peripheral blood of each dog. One ml of lymphocyte suspension (4×10^4 cells/ml) of prospective recipient was mixed with the homogenate of supersonicated lymphocytes of prospective donor in the ratio of 1 : 1 (v/v). Autologous single culture was performed as a control. Phytohemagglutinin (PHA-M) solution was added to culture medium in the concentration of 1 per cent (v/v). The cells were cultured at 37°C for 72 hours, and then the percentage of blastformation was assessed morphologically according to the same classification as described by MIWA (4).

RESULTS

As shown in Table 1, the rate of blastformation in MLC ranges from 10.0% to 28.2% while the rate in single culture is 6.9%. The grade of rejection is given as follows: 13 cases proved to be (+++), 3 cases (++), 6 cases (+). In comparing the rates of blastformation, all (+++) pairs show the rate to be well over 18% (22.5% in average), (++) pairs 15–18% (16.2%), (+) pairs 10–15% (12.3%). It can be said that the allograft reaction of kidney transplant is strong when the rate of blastformation is over 18%, and that the reaction is moderate when the rate is less than 15%. On the other hand, in our comparison of the postoperative renal

TABLE 1. RELATIONSHIP BETWEEN THE RATE OF BLASTFORMATION AND THE GRADE OF REJECTION OF RENAL ALLOGRAFTS

Recipient No.	Donor No.	Grade of rejection	Rate of blastformation	Postoperative renal function
38	39	(+++)	20.6	fair
44	45	(+++)	26.6	poor
48	49	(+++)	22.8	poor
50	51	(+++)	20.4	good
52	53	(+++)	19.4	good
54	55	(+++)	26.0	good
59	58	(+++)	28.2	fair
60	61	(+++)	21.8	good
63	62	(+++)	27.3	poor
64	65	(+++)	19.5	poor
65	64	(+++)	18.9	poor
67	66	(+++)	19.3	poor
69	68	(+++)	20.1	poor
56	57	(++)	17.4	good
58	59	(++)	16.2	good
68	69	(++)	15.1	good
39	38	(+)	11.6	good
40	41	(+)	10.0	poor
42	43	(+)	12.5	good
61	60	(+)	11.6	good
62	63	(+)	13.5	good
66	67	(+)	14.6	poor
Auto.	—	—	6.9	—

function with the rate of blastformation or the grade of rejection, we find that the function is poor in 7 cases of the 13 with (+++) rejection while it is good in 4 of them. In the 6 pairs of (+) rejection, 4 cases show good renal function but two cases poor function. Generally, the renal function tends to be poor when the rate of blastformation is high or the allograft reaction is strong, and it is good when the rate is low or the reaction is moderate. However, the postoperative renal function is not always correlated with the rate of blastformation.

DISCUSSION

It is considered that the MLC test is useful in detecting the antigen difference between the donor and the recipient as a whole. There are

many reports showing the correlation between the growth rate of MLC and the intensity of rejection, but the MLC reaction is two-way method in the majority of them. To select the most suitable donor, it is obviously desirable to measure only the antigenicity of the donor to the recipient. One-way stimulation in the MLC is the method that manifests the antigenicity of the donor to the recipient. BACH *et al* (6). devised one-way stimulation by treating the cells of one individual (potential donor) with mitomycin C. And they (7) demonstrated the correlation between the degree of stimulation in MLC and the skin graft survival in man. In addition to their reports, there are some reports (8, 9) in which one-way stimulation is accomplished by mitomycin C treatment or x-irradiation.

However, HUEMER (8) suggested that the mitomycin treatment proved to be not completely effective. ELVES (10) reported similar findings. Further, he reported that considerable evidence of cell damage was seen in cultures using mitomycin technique, but it was not seen in the x-irradiation method. In any case, it seems that mitomycin C technique for inactivating the lymphocyte of prospective donor poses many difficulties which are not present in x-ray method.

On the contrary, our supersonication method of obtaining stimulator cells is very simple and the destruction of cells is completely effective. Besides the antigenicity of destroyed cell homogenate is sufficient (1). Even with dog renal allograft we have found that the unidirectional mixed lymphocyte culture supplemented with PHA, a method of our own device, is applicable. For the purpose to attain a longer survival of dog renal allograft, in the test of supernonicated lymphocytes of prospective donor as antigen against viable lymphocytes of prospective recipient it is desirable to have the blastformation of less than 15% and any lymphocyte group of over 20% blastformation should be avoided. We prefer to employ this one-way stimulation method for tissue typing of renal allograft in man as well as for the study of the relationship between the lymphocyte compatibility and serotyping or the relationship between the former and the kidney-graft survival.

CONCLUSION

We applied unidirectional MLC test to renal allograft in dogs, and investigated the correlation between the growth rates of MLC reaction and the intensity of rejection of the kidney transplants or the postoperative renal function. It was concluded that the grade of rejection became three plus (+++) when the rate of blastformation was more than 18%, while

it became one plus when the rate was less than 15%. The rate of blastformation was closely correlated with the strength of rejection of kidney transplants. However, the postoperative renal function was not always correlated with the mixed lymphocyte reaction.

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