Early prediction of acute rejection after inbred rat kidney transplantation using macrophage migration inhibition test

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Abstract

In order to formulate an early diagnostic method for acute rejection after kidney transplantation, macrophage migration inhibition test (MIT) was carried out with lapse of time after inbred rat kidney allotransplantation. The mean survival time of rat kidney allograft was found to be 7.07 +/- 1.34 days. On the other hand, in the group treated with rabbit anti-rat lymphocyte serum (ALS) the mean survival time was lengthened to 14.15 +/- 2.14 days (p less than 0.05). The corresponding antigen used for MIT was prepared with donor kidney by ultrasonication, and its protein concentration at 180 mug/ml was the most optimal as not to elicit non-specific inhibition of macrophages. In the control group, activity of macrophage inhibitory factor (MIF activity) turned positive 3 days after the transplantation, and it became strongly positive by 5 or 7 days at the period when rejection crisis appeared frequently. ALS-treated group showed a lower MIF activity than the control group (p less than 0.05) and on 7-12 days before rejection crisis appeared frequently, MIF activity became strongly positive. These findings suggest that this MIT is simple and will be proved to be useful in predicting the acute rejection as well as in controlling the immunosuppression.

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EARLY PREDICTION OF ACUTE REJECTION AFTER INBRED RAT KIDNEY TRANSPLANTATION USING MACROPHAGE MIGRATION INHIBITION TEST

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Abstract In order to formulate an early diagnostic method for acute rejection after kidney transplantation, macrophage migration inhibition test (MIT) was carried out with lapse of time after inbred rat kidney allotransplantation. The mean survival time of rat kidney allograft was found to be 7.07 ± 1.34 days. On the other hand, in the group treated with rabbit anti-rat lymphocyte serum (ALS) the mean survival time was lengthened to 14.15 ± 2.14 days (p<0.05). The corresponding antigen used for MIT was prepared with donor kidney by ultrasonication, and its protein concentration at 180 pg/ml was the most optimal as not to elicit non-specific inhibition of macrophages. In the control group, activity of macrophage inhibitory factor (MIF activity) turned positive 3 days after the transplantation, and it became strongly positive by 5 or 7 days at the period when rejection crisis appeared frequently. ALS-treated group showed a lower MIF activity than the control group (p<0.05) and on 7-12 days before rejection crisis appeared frequently, MIF activity became strongly positive. These findings suggest that this MIT is simple and will be proved to be useful in predicting the acute rejection as well as in controlling the immunosuppression.

Early prediction of acute rejection at the time of human kidney transplantation is a major problem for obtaining a stable and longterm functional kidney graft. Up to now the clinical diagnosis of acute rejection has been mainly based on biochemical tests of renal functions. Such multiple, indirect measurements can not be of a considerable help because they reveal positive after the severe damages of kidney graft. However, it is now generally accepted that allograft rejection is primarily a cell-mediated immune response (1, 2, 3), so that a more direct, in vitro immunological diagnostic method (4-7) would be desirable.

Of the several in vitro tests being used for measurements of the cell-mediated immunity, MIT has been demonstrated to be related to delayed hypersensitivity by George and Vaughan (8), David (9-11), Bloom and Bennett (12, 13) and others.

Al-Askari et al. (14, 15) have demonstrated that activity of leucocyte inhibitory factor (LIF activity) reflects well the transplantation immunity, espe-
cially the allograft rejection-reaction. However, there have been few reports about experimental or clinical study on MIT as an immunological diagnostic parameter for rejection-reaction, and there remain in this MIT many problems concerning technological error, and non-specific inhibition by excess antigen.

In view of this, a study was made first on the optimal concentration of antigen in order to eliminate the non-specific inhibition of macrophages by excess antigen. Next, using the kidney transplantation model developed by Sun Lee (16) and Sakai (17) with close-bred rats, macrophage migration inhibition activity (MIF activity) was measured with lapse of time after transplantation. As a result it was demonstrated that the time of acute rejection of transplanted kidney graft and MIF activity correlate so well that the measuring of MIF activity is found to be a quite useful method in predicting the rejection at an early stage.

In addition, for the purpose to study the influences of immunosuppressive agents on MIF activity, ALS was injected into animals after rat kidney transplantation to see its effect on the survival time of the graft as well as changes in the MIF activity.

**MATERIALS AND METHODS**

1. **Animals**: Rats weighing about 200g were used and they were a close-bred strain obtained from a commercial supplier. Using a R-antigen incompatible pair each time (MP rat as a donor; Wistar rat as a recipient), and in every instance males were used in consideration of sex linked loci.

2. **Rat kidney transplantation**:
   a. **Anesthesia**: The animals were anesthetized by an ether inhalation combined with oxygen gas sufficiently.
   b. **Harvesting the left donor kidney**: After opening abdomen and retracting intestines laterally to the right side, the left kidney was mobilized while preserving the perirenal fat. The dessection was carried down to the aorta and vena cava. The right renal artery, superior mesenteric artery and lumbar arteries were ligated with 2-0 silk. A 2-0 silk ligature was placed loosely around the aorta below the left renal bifurcation, and a small vascular clamp was applied to the aorta just below the celiac artery. The left kidney was perfused with 3ml 4°C-haparinized saline via the aorta, which entered below the ligation with a No. 27 needle.

   Following perfusion, the ligature was pulled tight and tied. Thus an aorta segment with left renal artery was obtained by sharp division of the aorta immediately below the clamp and the tie. An elliptic excision of the vena cava was made, the right ureter was traced to bladder neck, and the right ureter was ligated and was dissected below the ligature. As soon as the kidney was removed from the donor animal, it was placed in cold saline.

   c. **Rat kidney transplantation (Fig. 1)**: As soon as the abdomen was opened,
right nephrectomy was performed. The aorta and vena cava below the renal bifurcation were made free from the fibrous structures and were both completely clamped longitudinally with a small Satinsky type vascular clamp, in order to connect the donor's renal artery-aortic segment and renal vein-inferior vena cava segment in end to side fashion to the recipient's aorta and inferior vena cava, with 8-0 monofilament nylon suture under stereoscopic microscope, "Nikon". Both host's and donor's bladders were transected at their middle portion and end to end anastomosis was performed. Left nephrectomy was done immediately or on the fifth postoperative day.

Fig. 1. Photograph of rat kidney transplantation.

Total ischemic time varied between 42-53 min, and total operation time between 70-90 min. Antibiotics were orally administered for 3 postoperative days. Measurements of MIF activity were performed on 0, 1, 3, 5, 10, 14 and 21 postoperative days.

3. Lymphocyte preparation: Two ml defibrinated blood was obtained by cardiac puncture from recipient and viable recipient lymphocytes were purified by a Ficoll-Conray gradient separation technique. Cell viability was determined by the 1% trypan-blue and cell count was quantitated on a Bürker-Türk hemocytometer. Approximately $1.2 \times 10^6$ lymphocytes per ml can be obtained from 2 ml blood.

4. Antigen preparation: Resected right kidney of donor was used for antigen source. It was adjusted to 1g and minced by scissors. After adding 5 ml medium TC-199 and ultrasonication at 20Kc, 7 φ tip, 150 mA for 15 min, and centrifuging at 3,000 rpm for 30 min, the supernatant was stored at $-20^\circ$C as crude antigen. Protein concentration of this supernatant was measured by spectrophotometry. For the purpose to eliminate nonspecific inhibition of macrophages
by excess antigen and to determine an optimal antigen concentration, medium TC-199 was added to this supernatant to dilute it to 2-, 4-, 10-, 20-, 60- and 100-fold dilutions, and the migration area of macrophage at each diluted concentration was calculated.

5. Harvesting macrophages: Macrophages were obtained from guinea pigs (300-500g in weight), injected intraperitoneally with sterile liquid paraffin 4-6 days before testing. The abdominal cavity was opened, and irrigated with Hank's balanced solution. Such irrigated solutions containing macrophages were centrifuged twice at 800 rpm for 5 min, the oil phase was aspirated, and the remnant cells were then suspended in medium TC-199, and adjusted to 2-5×10⁸ cells/ml.

6. Macrophage migration inhibition test (MIT): By mixing normal guinea-pig macrophages with test lymphocytes proportion of 10 to one, the mixture was put into a hematocrit tube (Terumo, length 75mm, diameter 1.45-1.65mm, one end closed) under a negative pressure. After centrifuging the tube at 800 rpm for 5 min, the tube was cut at the boundary of the supernatant and the cell layer. Four tubes of cell layer were fixed with silicon grease on the round cover-glass placed on the bottom of a 1 ml-Mackness-type petri dish. Three such groups of petri dishes were prepared as follows: 1) macrophages + medium TC-199, 2) macrophages + medium TC-199 + sensitized lymphocytes, and 3) macrophages + medium TC-199 + antigen + sensitized lymphocytes. These petri dishes were covered with a square cover-glass and incubated in the 5% CO₂ gas incubator for 24 hr.

Measurements of migration area were taken as shown in Fig. 2. Namely, by selecting the time when macrophages are actively moving and when there is no non-specific inhibition of macrophages due to excess antigen, the long and the short diameters of the ellipse are measured under a microscope and the product of these diameters is taken as the area of migration, and the ratio of the area of migration in the presence of antigen to the area of migration in the absence of antigen is taken as the percentage of migration index as in the following formula:

\[
\text{MI\%} = \frac{\text{area of migration in the presence of antigen}}{\text{area of migration in the absence of antigen}} \times 100
\]

7. Preparation, in vitro assay and administration of rabbit anti-rat lymphocyte serum (RARLS, ALS): Following the method of Ono (18), the mesenterial, axillary, and submandibular lymph nodes are taken from Wister rat, then sectioned finely, added with 5ml Hanks' solution and left standing for 30min. These are passed through the stainless steel mesh, and centrifuged at 1,000 rpm for 10 min, and a single cell suspension (0.5×10⁹ lymphocytes/ml) is prepared. This cell suspension is injected intravenously into rabbits once a week for 4 consecutive weeks to immunize with total of 1.6×10⁹ lymphocytes, and in the fifth week blood is aspirated from the animals by cardiac puncture, inactivated at 56°C for 30 min, and stored at -20°C until use.
Fig. 2. Photomicrographs of macrophage migrating fans from the capillary tube. (a) medium alone; (b) medium containing sensitized lymphocytes; (c) (b) plus corresponding antigen. 61.8% inhibition is seen in the lowest fan. (×20)

Lymphoagglutinin titer was determined by placing 0.025 ml serially diluted sera with the same volume of a lymph node lymphocyte ($2 \times 10^7$ per ml) suspension. The mixture was made in a microtiter plate. Lymphoagglutination was
read after 2.5 hr's incubation at 37°C and determined by the double dilution tube method. Lymphocyte suspensions \((1 \times 10^7 \text{ per ml})\) of 0.1 ml were mixed with 0.1 ml undiluted or diluted ALS in each tube and incubated at 37°C for 60 min. Lymphoagglutination test was considered as positive only if more than half of the cells were clamped. Lymphocytotoxicity was measured by the Terasaki's microcytotoxicity test [19].

ALS was administered intraperitoneally 1 ml each immediately, 3 days and 5 days after transplantation.

8. Observation and determination of the acute rejection reaction of rat kidney transplant:

The diagnosis of acute rejection is done by careful and daily observation of clinical signs (oliguria or anuria, anorexia, loss of weight, loss of activity, vomiting, etc.) of transplanted rat. Autopsy is always carried out and the allografted kidney is fixed in formalin solution, sectioned, stained with hematoxylin-eosin, and observed under a microscope.

RESULTS

1. Determination of optimal antigen concentration:

The protein concentration of supernatant as crude antigen proved to be 360 mg/dl by spectrophotometric measurements. As shown in Fig. 3, when the protein concentration of antigen containing solution is 36 μg–180 μg/ml, there is no non-specific inhibition of macrophages, but when it is over 360 μg/ml, there can be observed non-specific inhibition of macrophages due to excess antigen.

Therefore, the highest, nontoxic concentration of antigen proved to be 180 μg per ml in protein content.

2. The in vitro activity of ALS:

The serum titers \(\text{in vitro}\) gave the lymphoagglutinin titer of 1 : 1,000 and the lymphocytotoxic titer of 1 : 1,024.

3. Survival time of kidney allograft:

As shown in Table 1, in the control group consisted of 14 animals, all expired by uremia due to acute rejection up to the tenth postoperative day, their mean survival time being 7.07 ± 1.34 days.

In contrast, in the 20 cases injected with 1 ml each of ALS intraperitoneally, which was given immediately after transplantation and on the third and fifth postoperative day, their mean survival time was significantly \((p<0.05)\) lengthened up to 14.4 ± 2.12 days as compared with animals of non-treated group.

4. Changes of MIF activity in untreated group:

Table 2 gives the changes of MIF activity as measured with lapse of time after transplantation. The MIF activity began to become positive from the third postoperative day, on the fifth day when there appeared uremic signs and...
Prediction of Acute Rejection

![Graph](image.png)

Fig. 3. Nonspecific effect on macrophage migration by various antigen concentration.

Table 1: Comparison of rat kidney allograft survival between untreated group and ALS treated group

<table>
<thead>
<tr>
<th>No.</th>
<th>Survival day</th>
<th>Mean survival day ± S.D.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group 14</td>
<td>5, 5, 5, 6, 7, 7, 7, 7, 7, 7, 8, 8, 10, 10, 9, 10, 11, 11, 12, 12, 13, 13,</td>
<td>7.07 ± 1.34</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>ALS treated group 20</td>
<td>14, 14, 14, 14, 14, 15, 15, 17, 17, 21 &lt;, 21 &lt;, 21 &lt;</td>
<td>14.4 ± 2.12</td>
<td></td>
</tr>
</tbody>
</table>

acute rejection reaction, it was 56.67 ± 7.63%, at the peak of strong MIF activity on the seventh day it was 59.41 ± 1.55%, and by the tenth day there could be seen a tendency of slight recovery.

5. Changes of MIF activity in ALS-treated group:

Table 3 shows changes of MIF activity after transplantation. By the third postoperative day MIF activity becomes positive, by the seventh day it is as strong as 65.50 ± 4.98%, by the 14th day when the signs of uremic death due to rejection appear frequently, MIF activity rises slightly to 73.88 ± 6.28%, and by the 21st day it recovers up to the borderline.

Fig. 4 illustrates the comparison of changes in MIF activity between the untreated group and ALS-treated group. In the pre-rejection period (untreated group on the third postoperative day, and ALS-treated group on the seventh...
TABLE 2  CHANGES OF MIGRATION INDEX AFTER RAT KIDNEY ALLOGRAFT-UNTREATED GROUP (14 CASES)

<table>
<thead>
<tr>
<th>Posttransplantation (day)</th>
<th>Mean migration index ± S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>106.25±11.17</td>
</tr>
<tr>
<td>3</td>
<td>65.04±12.45</td>
</tr>
<tr>
<td>5</td>
<td>56.57±7.63</td>
</tr>
<tr>
<td>7</td>
<td>59.41±1.55</td>
</tr>
<tr>
<td>10</td>
<td>65.75±12.99</td>
</tr>
</tbody>
</table>

TABLE 3  CHANGES OF MIGRATION INDEX AFTER RAT KIDNEY ALLOGRAFT TREATED WITH ALS* (20 CASES)

<table>
<thead>
<tr>
<th>Posttransplantation (day)</th>
<th>Mean migration index ± S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>106.50±10.31</td>
</tr>
<tr>
<td>3</td>
<td>84.98±5.78</td>
</tr>
<tr>
<td>5</td>
<td>71.85±7.48</td>
</tr>
<tr>
<td>7</td>
<td>65.50±4.98</td>
</tr>
<tr>
<td>10</td>
<td>71.53±6.17</td>
</tr>
<tr>
<td>14</td>
<td>73.88±6.28</td>
</tr>
<tr>
<td>21</td>
<td>79.40±4.80</td>
</tr>
</tbody>
</table>

*Rabbit antirat lymphocyte serum

postoperative day) the MIF activity has begun to become already positive in both groups, and even in the rejection period (control group on 5th–7th postoperative day and ALS-treated group on 10th–14th day) there is demonstrated a distinctly strong MIF activity. In the ALS-treated group the MIF activity on the fifth, seventh and tenth days after transplantation shows a significant difference (p<0.05), being slightly lower than that of the untreated group.

6. Histology of rat kidney allograft:

Histologically, there can be observed a slight infiltration of round cells into the interstitium (Fig. 5-a) by the third postoperative day, by the seventh day a strong infiltration of round cells and edema of the interstitium can be recognized, and the degeneration and swelling of glomeruli and renal tubules become marked, presenting a typical rejection pattern (Fig. 5-b, Fig. 5-c). Fig. 5-d shows histopathological picture of transplanted kidney of ALS-treated group, which are sacrificed on the 21st postoperative day, revealing the hyaline degeneration of glomeruli and fibrosis of the interstitium and indicating already a complete rejection picture. There can be seen no tendency that the rejection pattern in nontreated control group is severer than that in ALS-treated group.
DISCUSSION

In recent years rat histocompatibility antigens have been discovered one after another, and now there are known Ag-B by Palm et al. (20), R-1 factor by Bogden et al. (21), and R-antigen by Aizawa (22). At the same time, by the development of microsurgical technique of Jacobson (23) it has become possible to perform vascular anastomosis in size of 1-4 mm, and by the establishment of rat kidney transplantation technique of Sun Lee (16) and Sakai et al. (17), immunological analysis of transplanted organ has made a great advance.

In the rat kidney transplantation the intensity of rejection reaction and the survival time depends on the qualitative and quantititative differences of the histocompatibility between donor and recipient. According to Murray (24)
Fig. 5. Histological pictures of rat allografted kidney (HE) a) third postoperative day (untreated group) b) c), seven postoperative day (untreated group) d) 21st postoperative day (ALS-treated group)
the survival time of rat transplantation in a pair having different Ag-B locus is 6.8 days in average, and by Guttman (25, 26) it is reported to be 7-8 days.

There is no report on the rat kidney transplantation in an R-antigen incompatible combination, and Kanda (27) states that the survival time of rat cardiac allograft (R-antigen incompatible combination) is 6.8 ± 0.1-7.8 ± 0.4 days.

In our present rat kidney transplantation in which donor was MP rat and recipient was Wistar rat, the survival time was 7.07 ± 1.34 days because of an R-antigen incompatible pair, and all the animals died of acute rejection within 10 days.

For the migration inhibition test there are two methods known, namely, the macrophage migration inhibition test (MIT) in which guinea pig macrophages are used as the indicator cells, and the leukocyte migration inhibition test (LIT) in which polymorphonuclear leukocytes (PMN) are used as the indicator cells as developed by Sjoborg and Bendixen (28, 29). In practice, the degree of cell-mediated immunity are determined by MIF activity which is calculated by the wandering area ratio (% migration index) between the antigen-added group and non-added group.

LIT is feasible to manipulate, because it uses buffy coat, and has an advantage in that it is sensitive to the crude semisoluble antigen generally obtained from tumor or organ. For this reason, Smith (1969) (30), Weeke (1970) (31), Falk (1972) (32), Dormont (1972) (33), and Wood (1973) (34) used LIT for the prediction of rejection in their kidney transplantation, and Eddleston (1971) (34) et al. used it in the diagnosis of rejection in hepatic transplantation, and studied the efficacy of LIT.

However, reproducible results cannot be attained, because it is impossible to maintain a certain fixed percentage between PMN and sensitized lymphocytes, and there is the discrepancy between MIF and LIF (36, 37). In the MIT used in the present experiment, normal guinea-pig macrophages and sensitized lymphocytes could be constantly mixed in a capillary tube at the ratio of 10:1, and the percent migration index was calculated after culturing for 24 hr. David et al. (11) stated that sensitized lymphocytes in a capillary tube were sufficient at 5% of the mixture.

As for the antigen source ordinarily used in MIT or LIT, there have been reported donor leukocyte or lymphocyte preparations (32, 38), donor spleen extracts (32, 33), donor kidney extracts (31), and foetal kidney extracts (30, 31). As a rule, leukocyte or lymphocyte preparation is used in the case of living donor, and lymph node or spleen extracts in the case of cadaver donor.

Falk et al. (32) used the spleen extract or donor lymphocyte preparation as antigen where the typing of HL-A antisera has previously been done in the case
of cadaver donor, and in the case where 2-3 HL-A histoincompatibility coexist, the most sensitive LIF activity is said to be observed.

The most important problem to bear in mind in executing this MIT seems to be the concentration of antigen, particularly the avoidance of non-specific inhibition by excess antigen.

Relative to the antigen concentration it is commonly represented by common protein concentration, as reported (30-35, 38) in the concentration ranges of 100 μg/ml-2 mg/ml.

In the case where crude semisoluble antigens such as kidney extract and spleen extract are used, Wood (34) et al. state that the optimal antigen concentration would be 50-100 μg/ml. Falk et al. (32) adjust the concentration of donor lymphocytes to $1 \times 10^8$ cells/ml, which is subjected to ultrasonication, and the supernatant thus obtained is stored at -20°C before use. Turnipseed (38) et al. adjust the number of donor lymphocytes to $25 \times 10^6$ cells, subjected to freeze-thawing at $-30°C$ repeatedly 7 times, and the cell suspension so obtained is used as the antigen-containing solution, and state that as for protein concentration 0.8-1 mg/ml would be the optimal antigen concentration.

With crude semisoluble antigen derived from donor kidney which we used in the present experiment, the protein concentration of 180 μg/ml, coinciding with the protein concentration as pointed out by Smith (30), Wood (34), and Week (31), seems to be the optimal antigen concentration at which no non-specific inhibition is induced.

The next important problems would be the relationship between MIF activity and rejection period, especially the mutual relationship during the pre-rejection period.

Eidemiller et al. (39) measured LIF activity with lapse of time after transplantation of rat cardiac allograft, and stated that percent migration index was down to 70-75% already on the fifth day, and by the 7th day when cardiac arrest occurred frequently, the activity became positive. They emphasize LIT to be effective in early prediction of rejection.

As a result of the present experiments, MIF activity already turned positive even during the pre-rejection period of the third and fifth days. From these findings, this MIT would serve as a useful immunological method in the early prediction of rejection.

In the application of LIT to human kidney transplantation, the positive rate of LIF activity in the rejection crisis was 100% by Wood (34), 100% by Falk (32), 95% by Dormont (33), and 67% by Weeke (31), indicating that LIF activity as determined by LIT has a close mutual relationship with rejection crisis. In contrast, the positive rate of LIF activity during the pre-rejection period is said to be as low as 13.3% by Dormont (33) and 16.7% by Falk.
Judging from these reports and the results of the present experiment, MIT seems to reflect the extent of the cellular-immunity during the pre-rejection period more sensitively and accurately than LIT does. And, on the basis of the present findings, it is intended to apply MIT to human kidney transplantation in the near future and the mutual relationship of pre-rejection period and rejection crisis to MI activity would be studied further.

In human kidney transplantation by which the immunosuppressive agents are usually administered, there would be a strong influence on MIF activity by various immunosuppressive agents.

Dormont (33) states that with the maintenance dose less than 0.5 mg/kg of azathiopurine, there can be hardly seen any drug effect on MIF activity, and that a strong MIF activity appears at the time of rejection crisis. However, in the case of a large dose of corticosteroid, for example, in the administration of 0.2-0.4 mg/kg of betamethasone, MIF activity is said to be suppressed quickly within a short period of time, and it recovers to normal usually within 7 days and in most cases within 1-3 days. Therefore, it is essential to conduct MIT always prior to the administration of large dose of corticosteroid to avoid the misjudgment.

There are few reports on the effect of heterologous antilymphocyte serum (ALS) in MIF activity, and on the basis of in vitro experiments Raulov et al. (40) state that ALS suppresses the production of MIF which is released from sensitized lymphocytes, while it does not affect MIF at all once it has been produced. Considering these findings it is to be recommended that ALS or ALG needs to be administered prior to the transplantation.

In the present experiment, ALS administered at an early stage after transplantation suppressed well the MIF activity, but it revealed MIF activity turned positive at the pre-rejection and rejection periods, and also was effective in prolonging the survival of rat kidney allograft.

Judging from these facts, the MIF activity became positive at the early stage of acute rejection even under the influences of various immunosuppressive agents, and increase in parallel with the increase of cell-mediated immune response.

Therefore, it may be concluded that MIF activity can grasp accurately and reflect the impending rejection or rejection crisis after kidney transplantation.

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REFERENCES

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