

## LYMPHOCYTE SPONTANEOUS BLASTOGENESIS AS A MONITOR OF RENAL ALLOGRAFT REJECTION

Hidegori Kanda, Tadashi Uemura, Seiji Kunikata,  
Takeshi Matsuura, Takahiro Akiyama and Takashi Kurita

*From the Department of Urology, Kinki University School of Medicine*

Spontaneous blastogenesis (SB) of peripheral blood lymphocytes was studied by determining protein synthesis using  $^3\text{H}$ -leucine to establish an immunological monitoring method after renal transplantation. In acute rejection, the SB level was twice as high as those in ATN and in the quiescent state. A rise in SB level comparable to that in rejection was observed in patients with infection. The SB level was continuously determined postoperatively in eight patients undergoing renal transplantation. Of the eight patients, three showed acute rejection four times in total. Elevation of SB level was simultaneously observed at each rejection episode. Rejection was not noted in any of the other five patients. False positive elevation of SB level was observed five times. The cause of the false positive changes was unknown in three cases and due to infection in two cases. Elevation of SB level is considered to be nonspecific and represents total lymphocyte activity. Due to its simple procedure and quick results, this method should provide a useful clinical parameter of rejection.

**Key words:** Renal transplantation, Spontaneous blastogenesis, Allograft rejection

### INTRODUCTION

It is desirable to establish a method by which the diagnosis of rejection can be confirmed prior to development of actual tissue damage. Allograft rejection after renal transplantation is caused by an immunological response ascribed to allo-antigens and T cell-mediated immunity acts mainly in this immune reactivity. Antigen reactive cells may appear in the peripheral blood before any clinical signs of rejection. Therefore, rejection can be detected early by monitoring the lymphocyte behavior. Spontaneous blastogenesis (SB) of lymphocytes, determined by DNA synthesis from  $^3\text{H}$ -thymidine uptake, has been reported useful for detection of impending rejection<sup>4,6,8,12</sup>. However, protein is synthesized far earlier than DNA<sup>5,9</sup>, so that the detection of protein synthesis, as an indicator of SB, may permit early diagnosis of rejection. The present study was designed to develop an early prediction of allograft rejection. SB of peripheral blood lymphocytes was studied by determining the lymphocyte protein synthesis by addition of  $^3\text{H}$ -leucine. The

clinical usefulness of this method was investigated in eight patients with renal transplants.

### PATIENTS AND METHODS

*Patients and control subjects.* SB was studied under various conditions, including six healthy controls, eight patients with chronic renal failure before renal transplantation, three patients in ATN, four patients in acute rejection, 11 patients in a quiescent state after transplantation, and four patients with infections. The postoperative change of blastogenic activity of lymphocytes was studied in four posttransplant recipients. Eight patients undergoing renal transplantation (six cases from living related donors and two cases from cadaver donors) were monitored postoperatively at intervals of two to five days for four weeks.

*Immunosuppression.* The standard immunosuppressive regimen was based on the use of ciclosporin (Cs) and prednisolone. The dosage of Cs was started with a daily dose of 12 mg/kg body weight and gradually tapered down to 4 mg/kg/d in 4 weeks. Prednisolone was started at 60

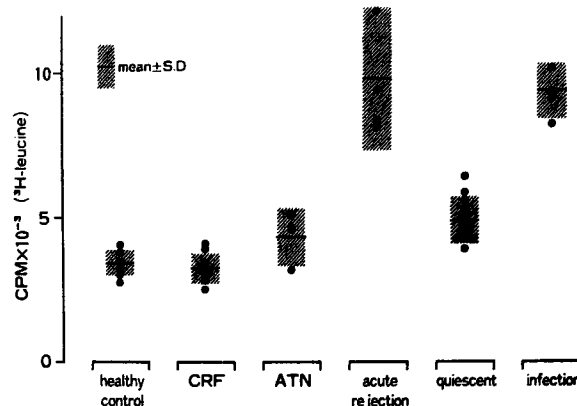


Fig. 1. Blastogenic activity of lymphocytes under various conditions. Activity during acute rejection is about twice as high as that in ATN or quiescent state. Patients with infections also showed high activity.

mg/d and tapered to 20 mg/d in 4 weeks. Rejections were treated with methylprednisolone 250 mg/d for three days. The diagnosis of rejection was based upon clinical criteria, mainly a rise in serum creatinine.

**Blastogenesis assay.** Lymphocytes were isolated from 5 ml of human heparinized peripheral blood through density gradient centrifugation using Ficoll-Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden), washed three times, and suspended in leucine-free RPMI-1640 medium (Flow Laboratories, North Ryde, Australia) to prepare a final concentration of  $1.5 \times 10^6$  cells/ml. The lymphocytes ( $3 \times 10^5$  cells) were incubated with  $1 \mu\text{Ci}$  of  $^3\text{H}$ -leucine (New England Nuclear NET-1352, Boston, 6 Ci/mmol) in quadruplicate in microplates (Corning 25860, NY) at  $37^\circ\text{C}$  for an hour in a 5%  $\text{CO}_2$  incubator. One hour later, the cells were harvested with a semiautomatic multiple cell harvester. The filter strips were dried from which the uptake of  $^3\text{H}$ -leucine was counted in a liquid scintillation system, and the blastogenic activity was expressed as counts per minute (cpm).

## RESULTS

**Blastogenic activity of lymphocytes under various conditions.** Fig. 1 shows the SB under different conditions. The activity

was found to be  $3606 \pm 465$  (mean  $\pm$  SD) cpm in normal controls,  $3772 \pm 506$  cpm in chronic renal failure patients prior to grafting,  $4342 \pm 982$  cpm during acute tubular necrosis following grafting,  $9756 \pm 2518$  cpm during acute rejection,  $4852 \pm 817$  cpm in quiescent subjects, and  $9325 \pm 986$  cpm in patients with infections. No difference was found between healthy controls and chronic renal failure patients. Activity during acute rejection was about twice as high as that in ATN or quiescent states. Patients with infections showed activity as high as that found in patients with acute rejection.

**Blastogenic activity in recipients.** Table 1 lists the postoperative changes in SB. Case 2 showed no rejection, with stable SB. In the other four cases, the activity was about two times the normal level in acute rejection.

Table 1. Postoperative changes in SB. The activity is about two times normal in acute rejection.

| Case | ATN  | Quiescent | Rejection | Quiescent |
|------|------|-----------|-----------|-----------|
| 1    | —    | 4211      | 8090      | 4172      |
| 2    | 3234 | 4492      | no        | 4132      |
| 3    | —    | 6452      | 13418     | 5832      |
| 4    | 5105 | 5214      | 8112      | 4495      |
| 5    | 2091 | 2920      | 5249      | 2444      |

cpm

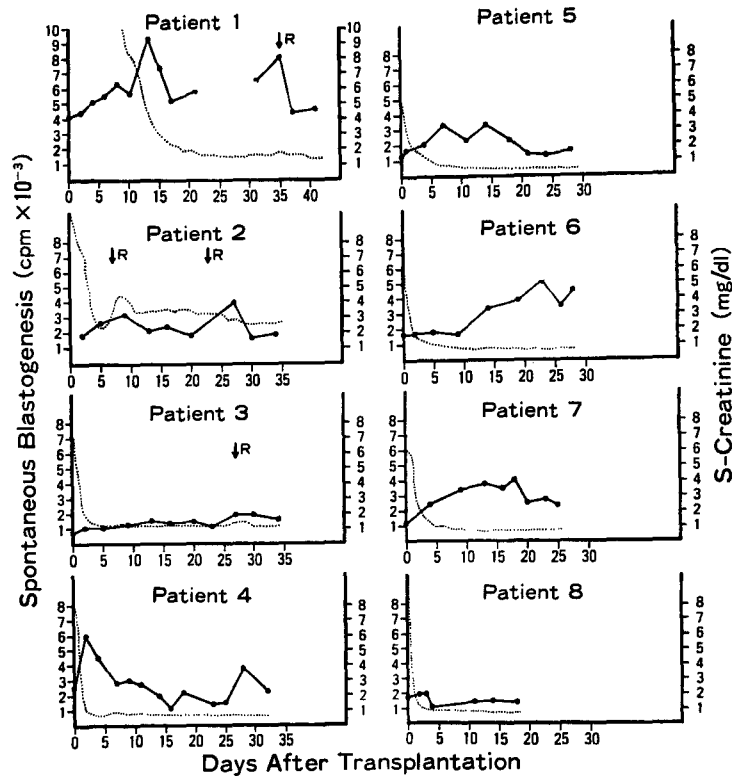


Fig. 2. Individual SB curves in eight renal transplant patients. SB curves are represented by the solid dots and solid line. Serum creatinine level is shown by the dotted line. R indicates acute rejection. Three patients (1,2,3) show acute rejection four times in total. SB level elevates simultaneously each rejection episode. The other five patients (4,5,6,7,8) show no rejection. False positive elevation is seen in 6 patients (1,2,4,5,6,7).

tion, and then rapidly returned to the original values during the quiescent state before rejection. These measurements were obtained from blood collected at the time of clinical diagnosis of acute rejection.

*Postoperative changes in SB and clinical course.* Fig. 2 shows the individual SB curves in eight renal transplant patients with different clinical courses. Of the eight patients, three showed acute rejection four times in total. The SB level elevated simultaneously during each rejection episode. The other five patients had favorable postoperative courses and showed no rejection.

In Patient 1, blastogenic activity elevated on the 13th postoperative day. However, this elevation was not interpreted as

a sign of acute rejection on the basis of other parameters and clinical findings. Activity then lowered and entered a stable state with diuresis. SB monitoring was interrupted for ten days during the course. SB activity was found to be slightly enhanced on the 31st day and to be markedly high on the 35th day. The serum creatinine level rose on the 34th day and reached a peak on the 35th day. The urine FDP level began to rise on the 28th day and reached a peak on the 34th day. A clinical diagnosis of rejection was made on the 35th day.

In Patient 2, SB level slightly rose on the 5th postoperative day and peaked on the 8th day. The serum creatinine level began to rise on the 6th day. A clinical diagnosis of rejection was made on the

7th day. The SB value returned to the original level by pulse therapy with methylprednisolone, but the serum creatinine level was not improved. Needle biopsy of the graft was conducted on the 23rd day, and a diagnosis of acute rejection of the vascular type was made on the basis of histopathological findings. The SB level determined on the 25th day was high. Acute rejection could be detected by SB monitoring.

In Patient 3, the SB level elevated on the 27th day, when a clinical diagnosis of acute rejection was made.

In Patient 4, the SB level elevated on the 28th day, but no signs of acute rejection were observed in other parameters of clinical findings. The elevated level was regarded as false positive.

In Patient 5, the SB level peaked on the 6th and 13th days. The cause of the peak on the 6th day was not known. The peak on the 13th day was attributed to an elevation of blastogenic activity caused by infection since leukocytosis and fever up were observed and a diagnosis of pneumonia was made on the basis of chest X-ray examination.

In Patient 6, although SB level elevated from the 10th day, the possibility of rejection or infection was denied. The cause of elevation of SB level was unknown.

In Patient 7, leukocytosis was observed from the 13th day. A diagnosis of wound infection was made. Antibiotics were administered, and the change was normalized on the 20th day. The SB level gradually rose after transplantation, reached a peak on the 17th day, and dropped on the 20th day. The elevation of SB level was attributed to infection in this patient.

In Patient 8, no rejection was observed. Elevation of SB level was not detected.

#### DISCUSSION

Rejection is a complication of renal transplantation which is unavoidable to some extent and one of the important factors determining the prognosis of renal allograft. The most important point in treatment for rejection is to detect the onset of rejection at an early stage. Since

rejection is an immunological reaction aimed at eliminating foreign cells, serial immunological examination of peripheral blood lymphocytes after transplantation would be useful to predict the onset of rejection even when patients are under immunosuppressive treatment.

In the present study, we examined spontaneous blastogenesis (SB) of peripheral blood lymphocytes for immunological monitoring after renal transplantation. Many investigators have reported that the SB of lymphocytes is related to allograft rejection and is useful for immunological monitoring after transplantation. We attempted to use protein synthesis as an indicator of SB and studied its clinical usefulness.

In acute rejection, the SB level was twice as high as that found in patients in ATN or in the quiescent state. Thus acute rejection, in particular rejection during ATN, could be diagnosed by the present method. However, it may be difficult for this method to discriminate between rejection and infection, because a high SB level is also found in patients with infection. Discrimination might be achieved by clinical findings.

In our present clinical study, acute rejection was observed four times in three patients, and the SB level was elevated simultaneously with each rejection episode. SB level was judged to rise slightly before the elevation of serum creatinine level. The SB level rose several days after transplantation in Patients 1, 4 and 5. This change is thought to be an effect of the operation and/or various drugs administered. In Patients 4 and 6, the cause of the high SB level during the 3rd and 4th postoperative weeks was unknown. The elevations of SB level were thought to be false positive changes. Changes caused by the non-specific reaction of peripheral blood lymphocytes such as SB are modified by histocompatibility in transplantation, individual immunological ability, immunosuppressive agents, effects of operation, infection and other factors. Therefore, diagnosis of acute rejection may include false positive or negative results. It has

been reported that the elevation of SB level was not attributed to rejection but to an increase in myeloblastic activity as a result of the administration of large doses of steroids<sup>9</sup>. In the present study, however, pulse therapy with steroids did not induce elevation of SB level but instead tended to lower it.

No detailed examination was done to elucidate what SB represents. It is said that the number of circulating T cells increases in acute rejection<sup>2</sup>. In particular, activated T cells play a major role in the reaction. It is also said that the proportion of cytotoxic killer cells is large in cellular rejection<sup>10</sup> while B cells make up the majority, and the proportion of cytotoxic T cells is very small in vascular rejection<sup>11</sup>. In Patient 2, however, the first rejection was thought to be cellular rejection, but the second is vascular rejection. SB level rose during both rejection episodes in this patient. There is a hypothesis that SB represents natural killer cells since the elevation of SB level is non-specific<sup>7,11</sup>. In the present study, the SB level was elevated not only in rejection but also in infection. Therefore, blastogenic activity may increase when some immunological mechanisms function, and the SB level may represent total lymphocyte activity. To establish immunological monitoring after renal transplantation and to elucidate the mechanism of acute rejection, studies using various new monoclonal antibodies are now being conducted. Further studies are required to determine the identity of the cells responsible for spontaneous blastogenesis.

In conclusion, the procedure of the present method is simple. No special materials are needed except for the radioisotope, and the result is obtained in only about three hours. We believe that this method permits serial examinations at short time intervals and provides a useful parameter for detecting acute rejection in its early stage.

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## 和文抄録

### Spontaneous Blastogenesis による腎移植患者の免疫学的モニタリング

近畿大学医学部泌尿器科学教室 (主任: 栗田 孝教授)

神田 英憲, 植村 匡志, 国方 聖司, 松浦 健

秋山 隆弘, 栗田 孝

腎移植後の免疫学的モニタリング法の1つとして、末血リンパ球の spontaneous blastogenesis (SB) を  $^3\text{H}$ -leucine を用い、タンパク合成から検討した。急性拒絶反応において SB は、ATN 期あるいは安定期の2倍の高値を示した。また、感染症でも急性拒絶反応と同程度に SB の上昇を認めた。8例の腎移植患者で術後連続して SB を測定し、うち3例に計4回急性

拒絶反応を認め、そのすべてに一致して SB の上昇を認めた。他の5例には拒絶反応を認めなかった。本法は手技が簡単で、短時間で結果が判明することから、臨床における拒絶反応の parameter の1つとして有用と考えられた。

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