QUANTITATIVE CHANGES IN URINARY LYMPHOCYTE EXCRETION AFTER RENAL ALLOTRANSPLANTATION

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The clinical picture of acute renal allograft rejection is often diffuse, and a number of post-transplant complications can mimic or mask it making early detection difficult. We have investigated the value of daily, quantitative measurement of the urinary excretion of erythrocytes, polymorphonuclear neutrophils, basophils, eosinophils, macrophages, lymphocytes and both large and small renal tubular cells as an aid in the diagnosis of acute rejection after kidney transplantation.

MATERIALS AND METHODS

After collection of a timed urinary specimen, the hourly excretion rate for erythrocytes and renal tubular cells was determined by counting the number of these cells per microliter uncentrifuged urine with the use of a Bürger-Türk chamber after staining with a diaminofluorescein-peroxide-phloxine stain (Prescott and Brodie, 1964). Granulocyte excretion was determined by the same technique, and the part of the total granulocyte count made up by eosinophils, basophils and polymorphonuclear neutrophils was estimated by examination of a May-Grünwald-Giemsa stained preparation of the urinary sediment. In order to obtain satisfactory differentiation, very short staining times were used: 15 seconds in May-Grünwald and 30 seconds in Giemsa stain. Macrophage and lymphocyte excretion was calculated by determining the per cent relationship between these cells and polymorphonuclear neutrophils in the stained smear and by multiplying this number by the excretion rate for polymorphonuclear neutrophils. This technique of measuring urinary cell excretion, including the criteria used for cell identification, will be described in more detail elsewhere (Spencer and Posborg Petersen, 1967).

Quantitative urinary cytological studies have now been carried out in 18 patients during at least the first 1 to 2 months immediately following renal allotransplantation. Bilateral nephrectomy of the patients' own kidneys was performed at the time of transplantation, or before, in all cases, and therefore the patients' own kidneys did not contribute to the post-transplant urinary sediment.

Twenty-four acute renal allograft rejection episodes were diagnosed using clinical and physiological criteria: the most commonly observed signs of rejection being fever, graft tenderness, decreased urine production, sodium retention and a decrease in creatinine clearance.

RESULTS

Information concerning the excretion of erythrocytes, polymorphonuclear neutrophils, basophils, large and small renal tubular cells and macrophages was of no help in making or confirming the diagnosis of rejection. No particular level or pattern of excretion was characteristic of rejection, and increased excretion was seen both with and without there being evidence of
rejection. The large, often bizarre-shaped renal tubular cells mentioned by Taft and Flax (Taft and Flax, 1966) were observed regularly in both the stained and the unstained sediment without it being possible to ascertain any relationship between the presence of these cells and rejection.

Eosinophil excretion was of some aid in rejection diagnosis as eosinophils were seen in association with 12 episodes. In two cases, they provided the only urinary sediment abnormality noted at the time of rejection. In only one patient, a case of post-transplant glomerulonephritis, were eosinophils present in the urine at a time when rejection was not taking place. Eosinophil excretion at the time of rejection varied from $8 \times 10^8$ to $5 \times 10^9$ cells per hour. There was no parallel between blood eosinophilia and urinary eosinophil excretion.

Lymphocyte excretion proved to be the most reliable sediment index of threatening renal allograft rejection. A sharp increase in the excretion of these cells to a value greater than $2 \times 10^4$ per hour was seen with 21 of the 24 rejection episodes. The three episodes where significant lymphocyturia was not seen were all diagnosed during the 2nd post-transplant day on the basis of fever and graft tenderness alone without graft function being affected, and therefore at a time when lymphocyte infiltration of the transplant probably had not taken place to any great extent. This was, in addition, at a time when there was still appreciable postoperative hematuria, and therefore a correction for lymphocyturia resulting from bleeding into the urinary tract was made. By knowing the number of erythrocytes per microliter urine, an estimate could be made of the number of microliters of blood present in the urine, and therefore the number of lymphocytes derived from this source.

Lymphocyte excretion was, on the other hand, definitely increased with every clear-cut rejection episode. Excretion was greater than $5 \times 10^3$ cells per hour at the time of all 15 moderate to severe rejection episodes, excretion in some cases being up to $5 \times 10^5$ lymphocytes per hour. Excretion between 2 to $5 \times 10^4$ lymphocytes per hour was found with 6 episodes of mild rejection. In one patient no rejection episodes were diagnosed and in this patient the level of lymphocyte excretion remained below the significant $2 \times 10^4$ cells per hour throughout the entire course.

Lymphocyte identification was not always easy. The differentiation of lymphocytes from small renal tubular cells and isolated nuclei, particularly those from squamous epithelial cells, presented difficulties. Strict identification criteria were therefore used. Only more or less round, very basophilic cells with a distinct nuclear and cell membrane and a thin rim of cytoplasm were counted as lymphocytes. In a study in normals (Spencer, 1966), such cells were only rarely seen and their excretion never exceeded $1 \times 10^3$ per hour. Although the presence of lymphocytes in the urine of patients after kidney transplantation is probably almost always a sign of rejection, $2 \times 10^4$ lymphocytes per hour has been used in the present study as the lower limit of significant lymphocyturia because lymphocyte excretion below this level is difficult to determine with certainty both because of problems of cell identification and of sampling errors.

Morphologically, lymphocytes could be separated into two groups: a small lymphocyte similar to the small lymphocyte of the peripheral blood and a large lymphocyte similar to the lymphoblast of immature lymph nodes and of the bone marrow. This latter cell may be identical with the cell found in lymph nodes draining allografts (André et al., 1963). It was the large lymphocyte that was typically found at the time of rejection.

On only one occasion was a lymphocyte excretion greater than $2 \times 10^4$ cells per hour not associated with a rejection episode. This was in a patient with an acute urinary tract infection with resultant massive excretion of polymorphonuclear neutrophils, and renal tubular cells. The urine was, in addition, collected through a flank drain and there was, therefore, an admixture of non-renal cells.
Significant lymphocyturia preceded by 1 or more days other signs of rejection in 5 of the 21 episodes. In the other 16, significant lymphocyturia appeared concomitantly with other clinical and laboratory signs of rejection. Lymphocyte excretion decreased promptly on the initiation of prednisone therapy and reached insignificant values in the course of 3 days to 3 weeks. In two patients, however, peak excretion occurred 3 and 4 days after prednisone therapy had been started.

Lymphocyte excretion has been studied in 2 patients with chronic rejection and in two with hyperacute rejection. Increased lymphocyte excretion could not be demonstrated in these patients.

The pattern of lymphocyte excretion with severe, moderate and mild rejection is illustrated in Figures 1, 2, and 3.

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**Fig. 1.** A 30-year-old man with medullary cystic kidney disease who received a kidney from his sister in April 1966. Urine production was initially good, but began to decrease on the 3rd post-transplant day. On the 4th day there was fever, graft tenderness, sodium retention and steroid therapy was started. Lymphocytes were first seen in the urine on the 3rd day, but significant excretion was not demonstrated until the 4th post-transplant day. A biopsy of the graft on the 7th day showed massive infiltration with mononuclear cells. Lymphocyte excretion was large and prolonged, becoming insignificant on the 17th day. As can be seen, there was an inverse relationship between lymphocyte excretion on the one hand and sodium and water excretion on the other. Signs of renewed rejection appeared on the 30th day and were associated with a second wave of lymphocyte excretion. Increased steroid was without effect and death occurred on the 35th post-transplant day.
Fig. 2. A 40-year-old man with chronic pyelonephritis who received a cadaver kidney in December 1965. Urine production began immediately after transplantation and totaled 8.8 liters in the first 24 hours. Graft function was good until the 6th post-transplant day when there was an acute fall in urinary and sodium excretion accompanied by fever, malaise and graft tenderness. Increased numbers of lymphocytes were detected in the urine for the first time on the 6th post-transplant day. Lymphocyturia began to decrease after initiation of steroid therapy and disappeared after 17 days. A second and third rejection episode were diagnosed on the 30th and 42nd post-transplant day. These two episodes were milder and were both associated with significant, but short-lived lymphocyte excretion. Increased lymphocyte excretion was with the third rejection episode the first sign of impending rejection preceding other signs by 2 days. The patient is now, 19 months later, in good health with a normally functioning graft.
Fig. 3. A 25-year-old woman with medullary cystic kidney disease who received a kidney from her sister in May 1966. The kidney transplant produced urine immediately and graft function was excellent throughout the post-transplant course. There was a mild, acute rejection 6 days after transplantation evidenced by sodium retention and eosinophilia. On the same day, urinary lymphocyte excretion was slightly elevated. All symptoms of rejection disappeared within a few days after steroid therapy was started. Significant lymphocyturia persisted for 6 days. There were no further rejection episodes and the patient is now, 13 months later, in good health with a normally functioning graft.

SUMMARY AND CONCLUSION

The urinary excretion of erythrocytes and large and small renal tubular cells determined directly and lymphocytes, eosinophils, basophils, polymorphonuclear neutrophils and macrophages determined indirectly has been studied quantitatively in 18 patients after renal allotransplantation.

The most constant and characteristic urinary sediment index of rejection was the excretion of more than $2 \times 10^4$ lymphocytes per hour. This was found with 21 of the 24 rejection episodes diagnosed in the 18 patients studied. Morphologically, the lymphocytes seen with rejection could be separated into two types: a small lymphocyte similar to the small lymphocyte of the peripheral blood and a large lymphocyte similar to the lymphoblast of immature lymph nodes. In 5 patients, significant urinary lymphocyte excretion clearly preceded other signs of rejection.

Excretion of the other cells studied bore no regular or predictable relationship to rejection with the exception of eosinophils which were found at the time of 12 of the 24 rejection episodes.
REFERENCES


Spencer, E. S. (1966): Unpublished data.
