FINE-NEEDLE ASPIRATION CYTOLOGY IN THE
PREDICTION AND DIAGNOSIS OF ACUTE REJECTION
EPISODES IN MAN

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Summary

The rate of spontaneous proliferative activity of blood leucocytes, the presence of donor-directed cytotoxic cells in the circulation of transplant recipients and the fine-needle aspiration cytology of the transplant have been evaluated for the monitoring of acute rejection episodes in 8 recipients of cadaver kidney transplants. We could not find any correlation between the rate of proliferative activity or the appearance of cytotoxic cells in the recipients’ circulation on the one hand, and the onset of rejection on the other hand. A detectable blast response, consisting of lymphoblasts and plasmablasts could, however, be seen inside the kidney parenchyma during the early stages of rejection. We propose that the blast response could be used for the monitoring of rejection episodes in kidney transplant recipients.

Introduction

The deterioration of renal allograft function during the immediate post transplantation period is on most occasions due to rejection [1]. The need for an immunobiological test for rejection – independent of the clinical evaluation of the patient – is particularly urgent usually only in those cases where the graft has not resumed its function immediately. In order to become a useful diagnostic method, this type of test should be simple to perform, and the results of the test should be available in a few hours of time. The following assays fulfilling these criteria have been proposed: (i) Quantitation of spontaneous proliferative activity (SPA) in the blood [2]; (ii) Detection of (donor-directed) specific cytotoxic activity (SCA) in blood mononuclear leucocytes [3]; and (iii) The use of fine-needle aspiration biopsy (FNB) cytology to monitor the activation of the immune response inside the allograft parenchyma [4]. In this communication we have compared the FNB test with the other two tests, and also define some diagnostic criteria for positive FNB cytology.
Materials and Methods

Fine-needle aspiration biopsies from renal transplants and heparinised blood samples were obtained usually twice a week from eight cadaver kidney recipients during the first three post-operative weeks. The transplant function resumed in all cases immediately after the operation. The onset of rejection was defined as the day when the (initially declining) serum creatinine values first increased. Three of these patients underwent an acute irreversible rejection, on three occasions the rejection was reversed by intensive steroid therapy and on two occasions there was no clinical sign of rejection.

Fine-needle aspiration biopsies (FNB) were done with an 0.6 mm external diameter needle, and a sample of approximately 5 μl in size was pulled by suction into hepes-buffered RPMI-1640 tissue culture medium containing 0.5% human serum albumin. No complications from the biopsies were observed. Cytocentrifuged cell smears were made from the aspirates, and stained with May-Gruenwald-Giemsa (MGG). When possible, part of the aspirate was used for the quantitation of spontaneous proliferative activity.

Spontaneous proliferative activity (SPA) was quantitated via tritiated thymidine incorporation. Blood cells were washed three times with phosphate-buffered saline (PBS) and 2 x 10^5 cells in 200 μl of RPMI-1640 medium were plated per well in Falcon (Falcon Plastics, Los Angeles, Calif.) Microtest II tissue culture trays. The cultures were performed in a humidified atmosphere of 5% CO₂ in air. When possible, an equal aliquote of the aspirate material was similarly cultured. All cultures were immediately pulsed with 0.8 mCi/ml tritiated ³H-thymidine (³H-TdR, NEN Chemicals, Boston, Mass., sp. act. 1 mCi/ml), and harvested after 2 hours with a Scatron semi-automatic cell harvester (Flow Laboratories, Irvine, Scotland). The rate of incorporation was measured with a liquid scintillation counter.

Specific cytotoxic activity (SCA) to donor-derived target cells. On four occasions spleen cells were obtained from the cadaver donor, and 1 x 10^6 cells were plated per well in Falcon tissue culture plates in RPMI-1640 medium containing 5% pooled human AB-serum. During the first three days most of the cells died and after the first medium change only the adherent cells remained in the wells. Approximately 10^4 adherent cells were obtained per well, and they quickly elongated and resumed the shape of cultured macrophages. In further culturing 10 μCi/ml of sodium ⁵¹Cr chromate (The Radiochemical Centre, Amersham, England) was added into the culture medium. The macrophages which did not divide during the culture tolerated their radioactive environment well and could be used instantly as target cells in the cytotoxicity assays. Ficoll-Isopaque separated blood mononuclear leucocytes were added onto washed relevant (donor-derived) or irrelevant (HLA-non-identical) target cells, and a 4-hour cytotoxicity assay [3] was performed. The specific ⁵¹Cr release was expressed according to the formula on the following page —
Per cent specific release = \[
\frac{\text{Experimental release} - \text{spont. release}}{\text{Maximal release} - \text{spont. release}} \times 100
\]

The spontaneous release very seldom exceeded 10% of maximal release (Triton X-100).

**Results**

*Correlation Between Blood Cytotoxic Activity and the Onset of Rejection*

The cytotoxic activity of blood mononuclear leucocytes to donor-derived and irrelevant macrophages is illustrated in Figure 1. On all occasions a high cytotoxic activity was present to donor-derived target cells. Sometimes weak cytotoxic activity was displayed against irrelevant target cells as well. We could not clearly correlate the occurrence of specific cytotoxic activity to the onset

![Figure 1](image_url)

Figure 1. Cytotoxic activity of recipient's blood mononuclear leucocytes to donor (relevant) and irrelevant macrophages in the 4-hour cytotoxicity assay. The day of onset of rejection is indicated with a dashed line. Open circles: reversible rejection; crosses: no clinical evidence of rejection. In cases of no rejection, day 0 has been adjusted as 5th postoperative day.

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of rejection, as the cytotoxic activity had a broad profile extending over several days, and as a distinct cytotoxic activity was also displayed by patients with no evidence of rejection.

Correlation Between Spontaneous Proliferative Activity and the Onset of Rejection

This is illustrated in Figure 2. Both in the aspirate and in the blood a high and variable proliferative activity was present in cases of rejection as well as in cases with no clinical evidence of rejection. As we could not correlate the proliferative activity with the onset of the rejection episodes, we also attempted to analyse whether a local proliferative activity, presumably present inside the allograft parenchyma, could be detected by comparing the activity in the aspirate to the activity in the blood. As seen in Figure 2 although such a

![Graph](image)

**Figure 2.** Proliferative activity in the aspirate and the blood, and ratio between these two. The day of onset of rejection indicated with a dashed line. Closed circles: irreversible rejection; open circles: reversible rejection; crosses: no evidence of rejection. In cases of no rejection day 0 adjusted as the 5th postoperative day.
correlation could be established, it did not correlate with rejection, as patients with no clinical evidence of rejection displayed high ratios as well.

**Correlation Between Fine-needle Aspiration Cytology and the Onset of Rejection**

Blood is the major contaminant of fine-needle aspirates. It was, therefore, necessary to investigate which cell elements in the inflammatory infiltrate were different from those of blood, and could be used as signs of local immune activation. In another context we have reported a detailed analysis of the cellular infiltrates in rejecting human renal allografts (E v. Willebrand and P Häyry, submitted for publication). Some of these results are summarised in Table I.

<table>
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<th>TABLE I. Characteristics of the Cellular Infiltrate in Rejecting Human Renal Allografts and in the Blood of Allograft Recipients*</th>
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<td>RBC/WBC ratio</td>
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**DIFFERENTIAL DISTRIBUTION (%)**

- Lymphoblasts: 7 ± 4, 1 ± 1
- Plasmablasts + plasma cells: 5 ± 3, 1 ± 1
- Lymphocytes: 24 ± 4, 11 ± 1
- Monocytes: 19 ± 6, 11 ± 3
- Macrophages: 26 ± 9
- PMN: 18 ± 7, 75 ± 8

* Results compiled from v. Willebrand and Häyry (submitted for publication). Four cadaver kidneys were removed during acute irreversible rejection due to transplant rupture, perfused thoroughly to remove blood and disaggregated with collagenase-DNase. The differential counts were performed directly from cytacentrifuged cell smears of the initial dispersate. The low RBC/WBC ratio in the dispersate compared to that of the blood excluded any significant blood contamination of these specimens.

Except for the presence of large numbers of macrophages inside the rejecting kidney parenchyma, all differences from the blood were quantitative rather than qualitative. A seven-fold frequency of lymphoblasts and five-fold frequency of plasmablasts was characteristic for the inflammatory infiltrate. The presence of these two cell types was therefore used as main cytological criteria.

The relationship between the fine-needle aspiration finding and the onset of rejection is given in Figure 3. There was a definitive blast response taking place inside the kidney allograft, while no such response could be documented in the blood smears of the very same patients. The highest blast responses were recorded on the occasions of acute irreversible rejection. However, a definitive, though usually much weaker, blast response took place inside the kidney parenchyma also on occasions with no clinical evidence of rejection. Thus the correlation between the magnitude of the blast response and the outcome of the transplant is not definite.
Figure 3. Presence of blast cells and lymphocytes in the aspirate and in the blood. The day of onset of rejection is indicated with a dashed line. Closed circles: irreversible rejection; open circles: reversible rejection; crosses: no clinical evidence of rejection. In cases with no rejection, day 0 has been adjusted as the 5th postoperative day.

Discussion

Regardless of the use of immunosuppressive therapy, transplantation of a kidney allograft is always accompanied by activation of the immune response in the recipient. This is clearly reflected in the proliferative activity of WBC in the recipients' circulation. Although several of the cells incorporating tritiated thymidine under these conditions may be cells of the myeloid series [5,6], some of these cells are evidently lymphoblasts and lymphocytes, as demonstrated by previous autoradiography findings [6]. The immune activation also results in the generation of specific donor-directed cytotoxic cells which will be detected in the recipients' circulation during the immediate post-transplantation period. For obvious reasons it is, however, difficult to use these as criteria for rejection, because the same cytotoxic cells and high proliferative activity are also frequently seen in recipients with no evidence of rejection.
In experimental conditions allograft rejection is accompanied by blast cells and proliferative response inside the kidney allograft [7], and a vastly increased outflow of blast cells into the draining lymphatics [8,9]. As methods for the identification of the idiom is not possible to demonstrate the specificity of the proliferating blast cells. However, we consider it likely that the blast response taking place inside the kidney parenchyma most likely reflects immune activation towards the transplant, and have therefore suggested the detection of the blast response as a tool for monitoring of rejection episodes in transplant recipients.

One practical difficulty is linked to blood contamination of the aspirates. The evaluation of the intra-allograft response is especially difficult in those cases where a high blastogeneic activity is seen in the blood. This is frequently observed especially in patients with rheumatoid arthritis and possibly other autoimmune disorders. It should also be noted that the assay cannot be used in a quantitative fashion since, except in the most severe cases of rejection, the rate of the blast response does not correlate with the outcome of the kidney transplant. A considerable reduction of the transplant function may sometimes be seen even in cases when the blast response is only moderate, and blast responses of only slightly smaller magnitude are sometimes seen in kidneys displaying no clinical evidence of rejection.

Acknowledgments

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References


Open Discussion

BELL (Leicester) Do you think that we should really all be going around putting needles into kidneys like this? There could be quite a few kidneys in trouble. Secondly, there is no evidence that trying to diagnose rejection prior
to biochemical changes does any good.

HÄYRY As regards the diagnosis of rejection, it is usually so simple that you can do it without prior investigations.

BELL You must come and show us sometime.

HÄYRY About what you said as to whether it is dangerous or not. I have not seen any dangerous consequences of this technique.

Regarding very early diagnosis of rejection, I can assure you that it is very important, but it probably cannot be done in man.

LADENFÖGÖN (Copenhagen) Firstly, what is your criteria for onset of rejection? Is this increase in serum creatinine or some other thing? Secondly, how often is it possible for you by a fine-needle biopsy to detect a rejection before increase in serum creatinine? For if it is not possible to detect a rejection with this test before an increase in serum creatinine, it is safer and easier to use serum creatinine.

HÄYRY All these patients displayed in the slides have had a primary functioning kidney and so the onset of rejection was taken as the day when the serum creatinine was higher than the previous day, when it had been initially declining, and thereafter continued to increase. So this is the only selection of the material to make the diagnosis exact.

Can you see rejection before fall-off of transplant function? If you look at the slide very carefully you can see that if you look at the sum effect you can probably see rejection two days before the blood response. However, I want to make it quite clear that you cannot predict on the basis of this how severely the kidney is going to be damaged.