ASSESSMENT OF WHOLE BLOOD FOR ANTIBODY-DEPENDENT CELL-MEDIATED CYTOTOXICITY IN KIDNEY PATIENTS

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Summary

A test using whole blood for antibody-dependent cell-mediated cytotoxicity has been assessed in kidney patients.

The antibody-coated target system comprised lymphocytes isolated from a normal donor sensitised with rabbit anti-human lymphocyte serum.

Comparison of results obtained with whole blood and isolated lymphocytes from the same patients shows that the whole blood test amplifies the differences between normal subjects, dialysis and transplant patients. It probably combines the effects of depressed lymphocyte function and lymphopenia and thus represents a more physiological in vitro correlate for in vivo immune function.

Introduction

Antibody-dependent cell-mediated cytotoxicity (ADCC) is mediated by cells carrying receptors for the Fc portion of IgG. The term K cell has been restricted to non-glass-adherent, non-phagocytic cells devoid of T or B cell markers. A number of antibody-coated targets are lysed exclusively by K cells (human lymphocytes or lymphoblastoid lines sensitised by antilymphocyte antibodies, Chang cells and some tumour cells).1

Quantitation of K cells can be achieved by various indices including: 1) K cell activity or effector cell function defined by the cytotoxic reaction of a constant number of lymphocytes; 2) K cell capacity, obtained by correction for peripheral blood lymphocyte counts, and 3) cytotoxicity of a constant volume of whole blood2,3,4. Depressed effector cell function has already been demonstrated in kidney allograft recipients5.

In the present work a test using whole blood against the same antibody-coated target system was assessed in order to correlate it with the performances of isolated lymphocytes as well as with the effects of immunosuppression.
Patients and Methods

Twenty-five normal subjects, 24 chronic uraemic patients on haemodialysis and 28 kidney allograft recipients were tested. The immunosuppressive regimen included azathioprine (2 mg/kg), prednisolone or methylprednisolone. Solu-medrol® dosage was increased up to 1000 mg IV/day for 3 to 5 days at the time of rejection. No ALG was administered.

The method for evaluation of effector cell function has been described in detail previously. Briefly, 51Cr-labelled target-lymphocytes from a normal donor are sensitised with a constant dilution (10^-3) of rabbit antihuman lymphocyte serum (ALS), washed 3 times to remove unfixed antibodies and mixed in 0.4 ml Beckman microtubes either with whole blood or with lymphocytes isolated from heparinised blood by the Ficoll-Hypaque gradient method. Isolated lymphocytes are set up at the following concentrations per test tube: 20.10^4 (effector to target cell ratio 40 : 1), 10.10^4 (E/T 20 : 1), 5.10^4 (E/T 10 : 1) and 2.5 10^4 (E/T 5 : 1). Details concerning the whole blood test will be given elsewhere. Briefly, 50 µl of heparinised whole blood from the same donor are tested simultaneously. After washing, 200 µl of medium are added to the packed cells. The mixture of effector and target cells is incubated for 4 hours at 37°C. Controls include minimum (from target cells alone) and maximum release (after lysis of targets by distilled water). Specific 51Cr release is calculated as follows:

\[
\frac{\text{Experimental Release} - \text{Minimum Release}}{\text{Maximum Release} - \text{Minimum Release}} \times 100
\]

The differences between the three experimental groups were tested by variance or covariance analysis.

Results

Effect of Removal of Phagocytic Cells by Carbonyl Iron Powder

Whole blood from 13 patients was incubated for 30 minutes with carbonyl iron powder. Cytotoxic activity remained unmodified (mean % specific release ± SEM before = 30.3 ± 4.5; after = 32.9 ± 4.7).

Comparison Between Performances of Whole Blood and Isolated Lymphocytes (Figure 1)

Mean cytotoxic levels were linearly correlated to log. number of effector lymphocytes. Some individuals showed a slight decrease of activity at the highest cellular concentration (40 : 1). Due to difficulties in isolating a sufficient number of lymphocytes in immunosuppressed patients, higher E/T ratios were not tested. The differences between the three groups of patients are statistically significant (0.001<p<0.01) but the use of whole blood makes the significance much higher (p<0.001) with reduction of the overlapping between the three groups and decrease of the variations existing within the immunosuppressed patients.
Figure 1. Comparison between effector cell function (cytotoxicity level of isolated lymphocytes at 4 different effector to target cell ratios) and cytotoxicity of 50 μl of whole blood. Each point represents the mean ± SEM. Covariance analysis used for isolated lymphocyte tests showed a significant difference between the titration curves of the 3 groups of patients (F = 5.92 (2.315 DF); 0.001 < p < 0.01).
Differences between whole blood assays analysed by variance analysis were highly significant: normal versus dialysis patients (F = 21.24 (1.90 DF); p < 0.001) and dialysis versus transplant patients (F = 33.55 (1.90 DF); p < 0.001)

The main explanation for this effect can be found in lymphocyte count variations. Uraemic and immunosuppressed patients have a combination of lymphopenia and cellular defects as detected by the effector cell function test.

When cytotoxicity of whole blood (Figure 2) is plotted against the number of lymphocytes/mm³, no significant relationship appears within each group of patients but when the 3 groups of patients are pooled together, a statistically significant relationship is observed (p < 0.05).

*Serial Evaluation During Immunosuppression* (Figure 3)

Use of high doses of steroids during the early course of transplantation as well as in rejection is accompanied by a marked decrease and even suppression of the cytotoxicity of whole blood (and almost to the same degree, of the effector cell function test). From our preliminary data, this effect is immediate and withdrawal of drugs after transplant removal is followed by prompt and full restoration of the activity, as compared with the pretransplant level. In this
Figure 2. Linear relationship between cytotoxicity of 50 μl of whole blood and lymphocyte counts. Level of significance is not reached within each group of patients (p > 0.05) but it is after pooling all patients together (F = 5.33 (1.80 DF); 0.02 < p < 0.05)

Figure 3. Effect of immunosuppression: high steroid therapy suppresses the cytotoxicity of whole blood; drug withdrawal is associated with its restoration
study no clearcut difference between high and low doses of steroids was observed. One patient receiving low prednisolone dosage without azathioprine, because of chronic hepatitis, had a relatively high cytotoxic level (39.0%).

Discussion

Beside the practical interest of using small amounts of blood and avoiding laborious separation techniques, this test has several theoretical implications. Separation techniques remove most of the granulocytes and possibly modify cell populations. Whole blood would represent thus a better in vitro correlate for the in vivo immune status. Polymorphonuclear leukocytes can mediate ADCC with particular antibody-coated targets. beside lymphopenia, corticosteroids induce neutrophilic leukocytosis. It was thus important to rule out the possible role of phagocytic cells in immunosuppressed patients. The test may possibly be influenced by plasma factors which are probably partly removed during separation procedures. However their role was previously shown to be minor in clinical transplantation.

Defective capacity to lyse antibody-coated targets in uraemia and drug-induced immunosuppression, combined with lymphopenia, probably explains the amplifying effect obtained with whole blood.

K cell depression induced by azathioprine takes several months to become significant and is probably related to K cell depletion. It is of smaller magnitude than the depression obtained with steroids, which are probably responsible for the early depression observed in transplant subjects.

The mechanisms of steroid action are still obscure. A direct effect at pharmacological concentrations is unlikely, although such concentrations can inhibit in vitro generation of human cytotoxic T cells. Redistribution of recirculating lymphocytes to the bone marrow with predominant T cell lymphopenia, have been recently observed. From our preliminary results, K cell modification probably occurs later than the early T cell depression. Whether this effect is due to redistribution or to K cell precursor depletion requires further investigation and the use of whole blood can be an adjuvant tool for this purpose.

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References

Open Discussion

ROWINSKI (Poland) What were the effector cells in your assay? Did you use the whole blood of the transplant patient?

DUPONT Yes.

ROWINSKI So actually what you measured was the potentiation of cell mediated cytotoxicity in ADCC assay more or less, because regularly in ADCC assay you are using the effector cells from non-immunised individuals.

DUPONT Yes, but we assume here that we measured a non-specific immunity, and of course I agree with you that this test could detect some CML activity against a random target. Most of the patients do not show very much activity compared with the controls.

HÄYRY (Helsinki) I think that I should clarify this situation a little bit for those of our colleagues who are not primarily immunologists. We are now dealing with a different type of cell as compared with the previous paper. In the previous paper the effector cell was directed against the transplant antigens of the graft donor, and was most likely a T-lymphocyte. In this paper we are dealing with a non-specific effector cell (K cell). By using immunology as a tool, this speaker is measuring the activity of a given type of cell able to perform the ADCC function. So these are two different things. As a matter of fact, you got a linear correlation between the number of lymphocytes and your K-cell activity in the blood, right? I wonder, couldn’t you have arrived at the same conclusions just by counting the number of lymphocytes in the blood and making the correlations with them?

DUPONT Yes! You mean that it is too sophisticated a way to count the lymphocytes?

HÄYRY I mean that you are using a fine and rather complicated method to
get fairly simple information. Am I right?

DUPONT  Well, when you compare the performance of isolated lymphocytes you always see a correlation between the whole blood test and the isolated lymphocyte test, but what is in my opinion interesting is that the whole blood test is really more discriminating and it amplifies the difference between different subjects. That is, I think, the whole point.

LEGRAIN (Paris)  Have you performed any tests on patients receiving ALG?

DUPONT  No.