DONOR SPECIFIC SUPPRESSOR CELLS IN ASSOCIATION WITH REJECTION OF A RENAL ALLOGRAFT

J B Dossetor, E M Liburd, T Kovithavongs, M R Higgins, V Pazderka

University of Alberta, Edmonton, Canada

Summary

Details are presented of three aspects of immunological monitoring in a single instance of severe irreversible rejection of a renal allograft. Rejection is associated with strong evidence of lymphocyte mediated cytotoxicity (LMC). During this period reduced responsiveness of recipient cells to stimulation in vitro by donor cells is observed; this is more reduced than is responsiveness to random cells. Donor-specific suppressor cells also develop, as detected in two systems.

It is postulated that the data are evidence that competition exists between different aggressive and suppressive subpopulations of recipient lymphocytes.

Introduction

We have published evidence of two donor-specific phenomena which develop in peripheral blood lymphocytes (PBL) of renal allograft recipients (R) and may relate to tolerance induction [1]:

(a) Decrease in recipient PBL in vitro responsiveness to donor cell stimulation, a phenomenon which we have termed reduced donor-specific killer cell inducibility. It is detected by a two-step assay: in the first part R cells are stimulated by mitomycin treated donor cells (Dm) for six days in mixed lymphocyte culture (MLC); residual cells are then tested for cell mediated lympholysis (CML) against PHA-treated donor cell targets (51 chromium labelled) in a 16 hour CML assay. This MLC→CML system is symbolised as (R/Dm).

(b) Demonstration of donor-specific suppression by post-transplant R cells (mitomycin treated) on the capacity of donor cells (Dm) to induce killer cells in a third party responder PBL population (X). In this MLC→CML system the responder PBL of individual X are stimulated by Dm cells in the primary MLC in the presence of small numbers of recipient cells, Rm; the initial cell proportions are 1:1.5:0.25. After six days in MLC only X cells are alive. These residual X cells are then tested against donor PHA-blasts in a 16 hour CML assay. This MLC→CML...
reaction is symbolised \((X / D_m + R_m)\).

More recently, knowing that pretransplant recipient cells nearly always react well to stimulation by donor cells, it has been possible to reduce this three cell system to two cells. Pretransplant recipient cells, \(R(-T)\), are used as responders. These are collected in large numbers prior to transplant and stored in liquid nitrogen. Subsequently reclaimed \(R(-T)\) cells are stimulated by donor cells, \(D_m\), and the effect of small numbers of different samples of post-transplant recipient cells, \(R_m(+T)\), are studied for their possible suppressive effect on donor-directed CML. This modified suppressor system is symbolised as \((R(-T)/D_m + R_m(+T))\).

In earlier studies there were few rejections and none were irreversible. In one rejection episode [2] suppressor cells seemed to have disappeared during rejection, but this was shown to be probably due to the deleterious effect (on recipient lymphocytes) of the large doses of Solumedrol.

It is obviously of great importance to know what happens to these phenomena during rejection to determine their clinical significance. A detailed study of an instance of irreversible rejection is the subject of this report.

Methods

Details of in vitro assays for recipient CML inducibility and recipient suppressor cells have been previously described [1] as, also, has the assay for in vivo generated killer cells, termed lymphocyte mediated cytotoxicity or LMC [3]. LMC is the equivalent of 'direct' CML of some authors who use the term 'indirect' CML for the in vitro induced killer cells.

Case Report

Mrs VBOU, a 58 year old lady, had chronic renal failure from longstanding analgesic abuse. She had been maintained for four months by haemodialysis before cadaver-donor transplantation late in 1978. Early allograft function was good and serum creatinine was reduced rapidly to normal. Standard doses of azathioprine and prednisone (but not ALG) were used. Prior to transplantation humoral crossmatch tests were negative against T and B cells as, also, was the cell mediated crossmatch (LMC).

It is evident from the clinical course in the lower panel of Figures 1, 2 and 3 that rejection started about day seven and was complete and irreversible by about day 15. The allograft was left in place until day 51 but renal function did not return despite 11g of iv Solumedrol. This period was complicated by severe leucopenia and E. Coli were cultured from the blood on one occasion. She did not need dialysis during the period immediately after renal allograft excision as her original kidneys were able to keep serum creatinine at about 4mg/dl.

Figure 1 shows that LMC became minimally positive on day three and was very strongly positive on day ten. This coincided with the peak of irreversible rejection. Thereafter LMC was normal and only became marginally positive again during the period immediately after allograft excision when, presumably, residual killer cells, no longer being absorbed by the allograft, were more plentiful in the blood stream.
Figure 1. Changes in activity of lymphocyte mediated cytotoxicity (LMC) in irreversible rejection (upper panel); immunosuppressive medication and clinical events (lower panel).

Figure 2 shows changes in the ability of recipient cells, taken pretransplant and at various time points after transplantation, to form killer cells to donor cell stimulation, (R/D_m), in comparison with their reactivity to unrelated random normal cells, (R/X_m). The ratios of these two degrees of responsiveness is shown by the heavy solid line. There was a greater reduction in recipient PBL responsiveness to donor stimulation than to the random cell. This might be due to several factors: D-responsive cells have been induced into becoming killer cells in vivo and uncommitted cells of that clone are thereby depleted; or, donor-specific suppressor cells are developing in the PBL and inhibiting responses to donor. The fall in responsiveness in control (R/X_m) assays is probable evidence for non-specific effects of immunosuppression, infection and poor general health on lymphocyte responsiveness.

Figure 3 shows that donor-specific suppressor cells were developing while the allograft was in place despite evidence of strong LMC assays and irreversible rejection. This was shown in both donor-specific assays (where either a randomly selected normal, X, or the recipient’s own pretransplant stored cells, R(-T), were used as the responder population). It is of particular importance to note that
Figure 2. Sequential changes in responsiveness of recipient’s cells to killer cell induction by donor cells compared to a random individual, X. The heavy line is the ratio of changes in these two parameters.

Recipients cells, \( R_m \), had no effect on the important control \( (X/Y_m + R_m) \). Without this control the other effects would have to be attributed to non-specific deleterious affects being mediated by \( R_m \) cells. (Similar numbers of recipient lymphocytes were used in all these in vitro assays notwithstanding the severe leukopenia at certain times).
Figure 3. Sequential changes in capacity of mitomycin treated recipient cells, $R_m$, to suppress anti-donor killer cell induction in $X$ or $R(-T)$ cells; and inability of $R_m$ cells to inhibit killer cell induction to another random cell, $Y$. (For further explanation see text)

Discussion

Several populations of recipient cells must be activated by allograft implantation. In successful grafts the balance of influences must be in favour of donor-specific suppressor cells of T lymphocyte origin (unpublished data). We conjecture that donor-specific immunological tolerance is thereby achieved, permitting reduction in immunosuppressive medication to acceptable levels. In unsuccessful grafts
cytotoxic cells dominate the clinical scene but, as shown here, the phenomena of donor-specific reduced R responsiveness and Rm mediated suppression can also be developing in other subsets of PBL. Clearly more data are needed as well as means to measure these conflicting forces more accurately.

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References


Open Discussion

JEEKEL (Maastricht) When you use your control studies, you replace the Dm by Ym and in certain stage also the Rm by Ym cells. Were those Ym cells taken from recipients of kidneys?

DOSETOR No the X, Y and Z in this instance are people around the laboratory whose cells are taken periodically and frozen down and available for use at a later date.

STENZEL (New York) I wonder if you have measured proliferation during the in vivo development of the cytotoxic lymphocytes (CTL) in the early as well as the late stages? The development of suppressor cells seems very coincident with the immunosuppression. I wonder if you have the opportunity of studying these assays in patients who are not transplanted but were immunosuppressed for other reasons?

DOSETOR In your first question were you referring to the CML induction in vivo, spontaneous blastogenesis or were you referring to the correlation between CML induction and MLC response in vitro? There is a crude correlation but not a good correlation there. In quite a number of instances a high degree of stimulation can occur in the MLC and very few killer cells be induced. To your second question, the only group of patients that we have looked at was five patients studied very extensively who had had blood transfusions while on dialysis. And in these we were unable to demonstrate any suppressor cell activity. The problem is that we do not know what the antigen is to which this suppression is developing. It does not appear to be related to HLA and therefore one would really need the specific donor of the blood transfusion to do a valid test. Using a panel of 5 normal people around the place as a panel of target cells we were unable to show suppressor cells developing as result of blood transfusion.

WOODS (Leicester) Have you had the opportunity to look at the effect of reci-
pient serum in this assay. Do you think that serum factors could be blocking suppressor cell activity in vivo in the patient who was rejecting, but had both good suppressor cell activity and positive lymphocyte mediated cytotoxicity?

DOSSETOR We haven’t looked at that. The cells are washed more than once in the course of all these procedures, so we believe that serum factors are not present on the surface of the cells as far as one can tell. But you can’t exclude that completely. We have not looked at the effect of adding serum to this system.