INCREASED INTERLEUKIN II PRODUCTION DURING KIDNEY ALLOGRAFT REJECTION

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Summary
We have studied the lectin-induced production of Interleukin II (IL-2) by peripheral blood leucocytes (PBL) obtained from recipients of kidney grafts. Pre-graft values (haemodialysed patients) are similar to those of healthy individuals. After grafting and during the first year the IL-2 levels produced by PBL from recipients with well functioning grafts are very low (p<0.001).

Years after grafting the IL-2 yields tend towards restoration. At the onset of an acute rejection the IL-2 production rises significantly. These data show that an increase in IL-2 production may play a role in the rejection process.

Introduction
Interleukin II (IL-2) is a factor required for T lymphoblast growth [1] and thus a key factor for the development of the normal cellular immune response. This 15 KD molecule is produced by T-lymphocytes under lectin or antigen stimulation and acts via a 50 KD membrane receptor, present on activated T lymphocytes [2], as well as on Natural Killer cells [3]. It has been shown that IL-2 plays a role in rejection of heart allografts in rats [4] and recently we have used this growth factor to expand and study T lymphocyte clones extracted from human rejected kidney grafts.

In this paper we show that although peripheral blood lymphocytes (PBL) of dialysed patients can produce normal amounts of IL-2 under phytohaemagglutinin (PHA) stimulation, after grafting there is a dramatic decrease in PHA induced IL-2 yields. In contrast, during acute rejection, the lectin induced IL-2 production is significantly increased.

Patients, material and methods

Patients Heparinised blood samples were systematically and prospectively obtained immediately before grafting (n=14) and after grafting either during a
steady period of good graft function (n=18) or at the onset of an acute rejection crisis (n=24) before any specific treatment of the episode. In addition, single blood samples were obtained from long-term recipients of well-functioning grafts (1 to >10 years) to assess the IL-2 yields at a time of minimal immunosuppression. All patients were systematically transfused before and during grafting and their immunosuppressive treatment is described elsewhere [5]. Blood samples from patients under anti-thymocyte serum were not used to avoid bias. Finally PBL were obtained from 21 healthy individuals as controls.

Materials and methods (1) IL-2 production Briefly, PBL were obtained on ficoll hypaque gradients, washed twice and frozen until used. On the day of the experiment, thawed PBL (4x10^5 in 0.1ml) were stimulated with PHA-P (Difco) at 0.5% (v/v) in Greiner tissue-culture microplates (0.2ml/well). Culture medium was RPMI 1640 supplemented with 10% (v/v) human serum. After 49 hours of culture (37°C, 5% CO_2) 0.1ml of supernatant of each culture well was harvested, pooled by duplicates and kept at 4°C until assayed for IL-2.

(2) IL-2 assays IL-2 contents of the supernatants were tested on the IL-2-dependent CTL-L-2 murine cell line as previously indicated [5]. Results are given in mean ± SD of CPM obtained from 5x10^3 CTL-L-2 cells cultured 18 hours in the presence of supernatants (diluted fourfold) and pulsed four hours with 0.25μCi of tritiated thymidine. Statistical analysis was by the Fischer 't' test or the Wilcoxon paired test.

Results

IL-2 yields of PBL obtained from recipients before and after grafting in patients with good graft function (Figure 1)

IL-2 yields of pre-graft PBL samples were similar to those from normal individuals indicating that haemodialysed patients can produce normal levels of this lymphokine upon appropriate stimulation.

After grafting, PBL from kidney recipients produced significantly lower levels of IL-2. This profound defect in IL-2 production persisted until the end of the first year after grafting and tended to return to normal after two years.

IL-2 yields in patients undergoing acute rejection episodes

All rejection crises studied (n=24) occurred within the first year after grafting and thus during a period of very low (or nil) IL-2 production in non-rejecting patients. Figure 1 shows that PBL of rejecting patients produced significantly higher amounts of IL-2 (p<0.01) than non-rejecting ones.

Data obtained from eight patients in whom all the serial samples (i.e. pre-graft and post-graft samples, including rejection samples) could be studied, also showed a dramatic and significant drop of the PBL IL-2 yield after grafting followed by a sharp and significant increase of IL-2 production at the onset of a rejection crisis (Figure 2).
Figure 1. IL-2 levels produced by PBL of kidney recipients. Kinetics and increase during rejection episodes. Results are given in CPM obtained from CTL-L-2 cells cultured in the presence of PHA-stimulated PBL supernatants diluted fourfold. Rejection values have been located at the average time of the rejection episodes.

Figure 2. IL-2 yield of PBL of kidney recipients undergoing acute rejection episodes.
Discussion

We have shown that after grafting PBL from recipients of well-functioning kidneys yield very low levels of IL-2. It is likely that this defect is due to the high immunosuppressive drug regimen used in these patients, since corticosteroids, as well as cyclosporine have been shown, in vitro, to dramatically impair IL-2 production [6]. However, IL-2 production of patients with systematic lupus erythematosus did not correlate to their corticosteroid treatment [7]. This discrepancy may be related to differences in the dosage of the drug. Interestingly Natural Killer cell (NK) activity is also grossly impaired in kidney recipients [8] with roughly similar kinetics and as NK cells bear IL-2 receptors the two (IL-2–NK) phenomena may be linked.

Patients undergoing acute rejection have PBL which produce much more IL-2 on PHA stimulation than those obtained from recipients of well-functioning grafts. Obviously they are not 'high IL-2 producers' since, as shown in Figure 2, they have low IL-2 yields during the steady state period. This increased IL-2 yield cannot be explained by variation in the immunosuppressive regimen and is likely to be related to the rejection process. This IL-2 yield may be a poor reflection of higher lymphokine production by lymphocytes invading the rejected graft.

Finally, the restored IL-2 production in long-term well functioning kidney recipients (which can be explained by the lower immunosuppression) suggests that normal IL-2 production by itself is not involved in the rejection process. However, it has now been proved that such recipients have developed clonal regulatory mechanisms (i.e. involving suppression of clones specifically committed against the donor antigens [9]) which could explain why, at that time, normal IL-2 producing capacity may not have a deleterious influence in this context.

References

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