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Interleukin 2 production by peripheral blood lymphocytes in allograft recipients during acute rejection episodes

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Interleukin 2 production by peripheral blood lymphocytes in allograft recipients during acute rejection episodes. In this study we investigate the relationship between the Interleukin 2 (IL-2) yield produced by kidney allograft recipient's peripheral blood lymphocytes (PBL) under lectin stimulation and the occurrence of acute rejection episodes. PBL were harvested prospectively before grafting, after grafting in steady-state period, and at the onset of acute rejection episodes. In addition, we tested retrospectively the ability of PBL of recipients engrafted for more than 1 yr to produce IL-2. IL-2 levels were assessed on the IL-2-dependent CTL-L2 murine cell line. Our data show: *1)* before grafting, hemodialysed patients ($N = 14$) produced normal IL-2 yield compared with healthy donors ($N = 21$); *2)* the IL-2 secretion of PBL of recipients with good graft function ($N = 18$) is decreased markedly during roughly the first 12 months following transplantation ($P < 0.01$); *3)* when acute rejection crisis occurred during this time period ($N = 24$), a sharp and highly significant increment ($P < 0.01$) in lectin-induced IL-2 production of recipient's PBL was seen. After 1 yr, the capacity to secrete IL-2 upon lectin stimulation tends to be restored. Finally, our data correlate rejection and high PBL-IL-2 secretion clearly at a time when recipients with well-functioning grafts have markedly impaired IL-2 secretion.

La production d'Interleukine 2 par des lymphocytes sanguins périphériques de receveurs d'allogreffe au cours d'épisodes de rejet aigu. Nous avons étudié la relation entre la production d'Interleukine 2 (IL-2) par des lymphocytes sanguins périphériques (PBL) de receveurs d'allogreffe rénale lors d'une stimulation par une lectine, et lors de la survenue d'un épisode de rejet aigu. Les PBL étaient recueillis prospectivement avant la greffe, après la greffe lors d'un épisode de stabilité, et au maximum des épisodes de rejet aigu. En outre, nous avons testé rétrospectivement la capacité des PBL des donneurs greffés depuis plus d'un an à produire de l'IL-2. Nos données montrent: *1)* qu'avant la greffe, les hémodialysés ($N = 14$) produisaient une IL-2 normale par rapport à des donneurs sains ($N = 21$); *2)* que la sécrétion d'IL-2 par les PBL de receveurs dont la fonction du greffon était bonne ($N = 18$) diminuait de façon marquée au cours des 12 premiers mois après la transplantation ($P < 0.01$); *3)* et que lorsqu'une crise de rejet aigu se produisait pendant cette période ($N = 24$), il existait une augmentation prononcée et très significative ($P < 0.01$) de la production d'IL-2 lectine-induite par les PBL des receveurs. Au bout d'une année, la capacité de sécréter de l'IL-2 lors d'une stimulation par une lectine tendait à être restaurée. Enfin, nos données établissent clairement une corrélation entre le rejet et la sécrétion élevée de PBL-IL-2 lorsque les receveurs dont les greffons fonctionnent bien ont une sécrétion d'IL-2 très altérée.

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Interleukin 2 (IL-2) is a 15 KD glycoprotein [1] produced mainly by helper T cells [2] under antigen and monokine [3] stimulation. Antigen or lectin-activated T lymphocytes acquire a surface membrane receptor for IL-2 (RIL-2), and the IL-2-RIL-2 interaction induces T cell mitosis [4]. Since IL-2 is required for the expansion of activated T lymphocytes, it is probably involved in the cellular process leading to graft rejection that occurs after host lymphocyte activation by donor antigens. Although lymphocytes infiltrating the rejected allograft are the ultimate effector cells [5], peripheral blood lymphocytes (PBL) can recognize antigen and may be cytotoxic to donor target cells [6], therefore giving information on immune events linked to graft rejection. Moreover, PBL of the helper phenotype have been shown to transfer rejection capacity [7], suggesting that lymphokine-secreting cells—and therefore lymphocytes per se—may play an important role in triggering rejection.

This study investigated the ability of PBL from kidney transplant recipients to produce IL-2 when stimulated in vitro by mitogens. Blood samples were drawn prospectively from patients immediately before grafting, at various times after grafting when in a steady-state condition, and immediately before treatment of unambiguous acute rejection episodes. PBL harvested before grafting were found to produce normal levels of IL-2, while PBL of grafted recipients with stable renal function yielded low levels of IL-2. In contrast, rejecting recipients' PBL produced very high levels of IL-2 when stimulated by lectin.

Methods

Prospective study

Twenty-five patients engrafted for less than 12 months were studied (the period when 88% of the acute rejection episodes occurred), 23 first cadaveric and 2 living-related graft recipients. Twenty-one healthy individuals served as controls. Blood samples were systematically drawn before transplantation and at various times after grafting when patients showed good renal function. In addition, samples were obtained prospectively in all recipients who underwent an obvious acute rejection crisis ($N = 24$) at the very time of diagnosis and before any treatment of the episode. Rejection treatment consisted of anti-lymphocyte globulin (ALG) (Institut Merieux, Lyon, France)

for seven days with 1 mg/kg/day of prednisone (CS) for the first week, tapered to 15 mg/day by decreasing the CS doses by 10 mg per week. The rejection episodes were diagnosed on the basis of increased blood creatinine (>50 μmole/liter), graft enlargement and local tenderness in the absence of any alternative diagnosis. Samples obtained from patients with episodes of renal failure that could not be clearly attributed to rejection, for example, infectious or toxic episodes, were discarded.

All patients were systematically transfused before and at the time of grafting as described previously [8]. All received horse ALG for the first 2 weeks following transplantation at a dose required to keep their SRBC-T rosetting PBL between 5 and 15%. Blood samples were not drawn during ALG treatment to avoid the specific effect of ALG. Prednisone was given at 1 mg/kg/day for 2 weeks after grafting and tapered to 20 mg/day at the end of the second month. Azathioprine (AZA) was administered at 2 to 3 mg/kg, according to the white blood cell count. After the second month, ten recipients received cyclosporin A (5 to 6 mg/kg/day) with no additional immunosuppressive agents, according to a randomized trial comparing CS alone to a conventional regimen, specifically CS + AZA.

Rejecting or steady-state patients received non-statistically different doses of steroids at the time of bleeding, 22 ± 16 vs. 18 ± 24 mg/day, respectively.

Retrospective study

To assess IL-2 production by PBL of recipients with long-term, well-functioning grafts, 18 blood samples of recipients engrafted for a period ranging from 1 to 10 years were obtained. All recipients were under conventional CS + AZA treatment.

Culture medium and lymphocytes

Culture medium (CM) was RPMI 1640 (GIBCO) supplemented with 10% agammaglobulinemic human serum (CTS, Lille, France) and gentamicin (10 μg/ml). PBL were obtained from heparinized blood by sedimentation on Ficoll-hypaque, washed twice, and frozen in liquid nitrogen until used. Subsequently, PBL were checked routinely for viability (>90% required), and adjusted at the concentration required for the following tests.

IL-2 production

Optimal parameters (concentration of cells/ml, PHA dose, culture vessels, serum concentration and kinetics of IL-2 production) allowing reproducible IL-2 production of lectin-stimulated cells were assessed before starting the experiments and led to the following method: PBL were cultured in 96 well tissue culture plates (Nunc, Roskilde, Denmark) (0.2 ml/well) at 4.10^6 ml^{-1} in 0.1 ml of CM supplemented with 0.5% v/v PHA-P (Difco, Glasgow, Scotland). Cultures were set up in duplicate, and 48 hr later 75 μl of supernatant were harvested from each well, pooled, and kept at 4°C until assayed for IL-2.

IL-2 assay

The amount of IL-2 contained in the supernatant of PHA-stimulated PBL was assayed on the CTL-L2 IL-2-dependent cell line. Briefly, CTL-L2 (5000 per well) were incubated in Nunc tissue culture microplates (0.2 ml/well) in the presence of log 2 dilutions of either a reference IL-2-source containing 1 U/ml of IL-2 or supernatants of PHA-stimulated PBL of controls and patients. The same IL-2 standard containing 1 U/ml according to Bertoglio et al [9] was tested along with supernatants in all experiments, allowing the comparison between the various titers of IL-2 obtained. After 48 hr of culture, 0.25 μCi ^{3}(H)TdR (Amersham, United Kingdom; SA 23 Ci/mM) was added to each well. The cells were harvested 4 hr later with a Skatron multisample harvester and counted for radioactivity. Results are given in units of IL-2/ml, as defined previously [9], as well as in the average amount of ^{3}(H)TdR incorporated by CTL-L2 (expressed as cpm) in the presence of the PBL supernatants at each of the dilutions tested (1/4 to 1/128).

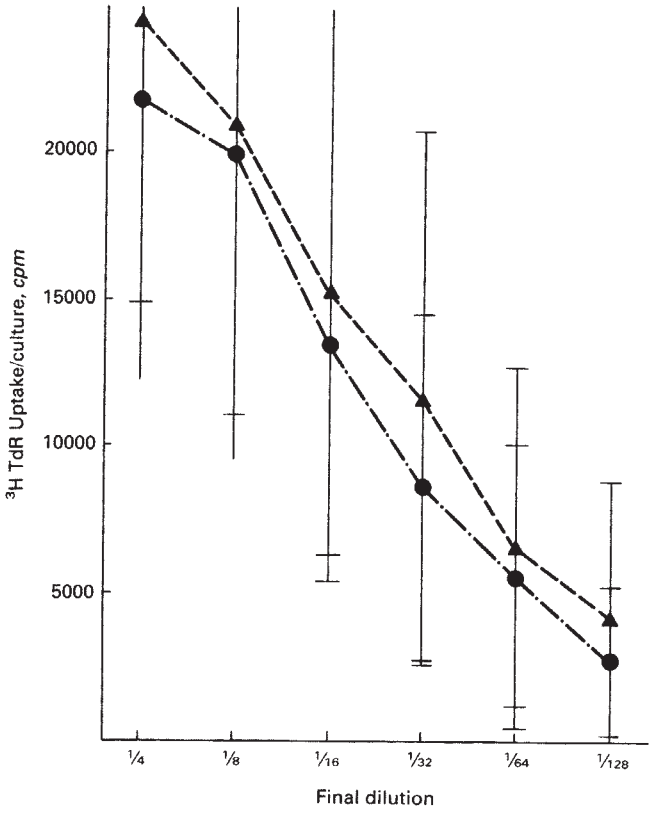

Fig. 1. *IL-2 yields of PHA-stimulated PBL from patients tested before grafting (▲, N = 14) and from healthy individuals (●, N = 21).* (Difference not significant).

Statistical analysis

The absolute values of ^{3}(H)TdR incorporation in CPM obtained after CTL-L2 incubation with the supernatants at serial log 2 dilutions were compared, using the Student and Wilcoxon tests. When the values could be paired (pre-graft and various post-graft values obtained from PBL of an individual in the same experiment), comparisons were made using the paired Student's *t* test.

Results

Lectin-induced IL-2 production of PBL of healthy individuals and pre-graft (hemodialyzed) patients

Figure 1 shows the IL-2 yield under standard conditions in 21 normal healthy donors and 14 hemodialyzed patients before

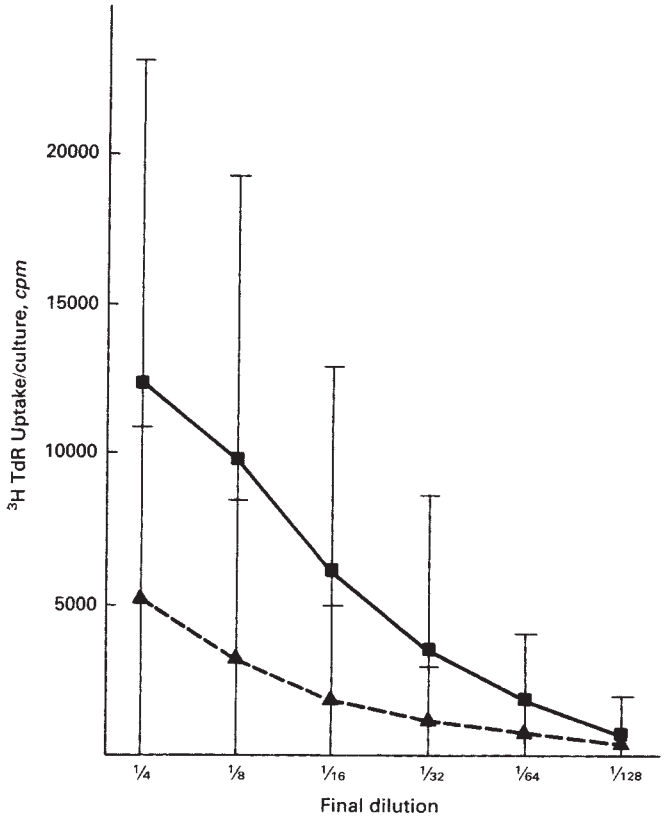

Fig. 2. *IL-2 yields of PHA-stimulated PBL of recipients tested either in a steady-state period (▲, N = 8) or at the time of an acute rejection episode (■, N = 24) ($P < 0.01$).*

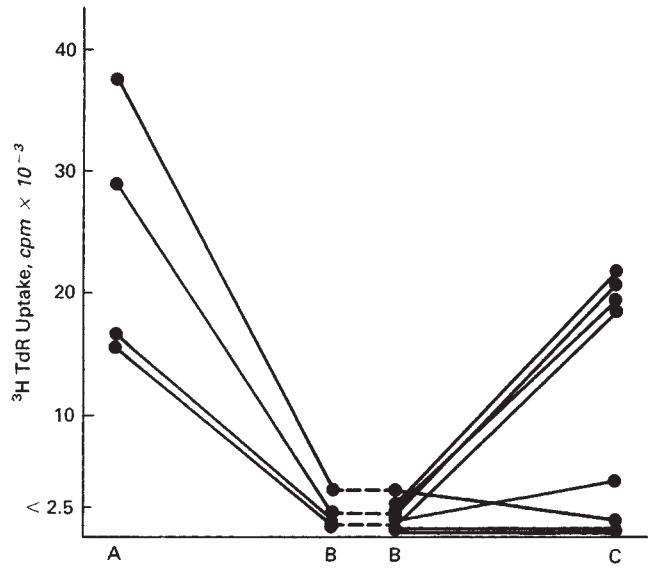

Fig. 3. *IL-2 production of recipients' PBL harvested serially at various times: A, before grafting; B, steady-state; C, at the onset of an acute rejection.* All samples tested in a same experiment. *^{3}H TdR incorporation of CTL-L2 cultured in PHA-induced conditioned medium tested at 1:4 dilution. $P < 0.05$ between A and C and between B and C values (Wilcoxon paired test).

grafting. The average amount of IL-2 produced did not differ significantly.

Lectin-induced IL-2 production of kidney allograft recipients

Twenty-four clearly individualized rejection episodes were studied and compared with control individuals and non-rejecting kidney-graft recipients, as well as with homologous pre-graft values. A highly significant ($P < 0.01$) difference was noted in the lectin-induced IL-2 yields (assessed either by comparison of the cpm obtained from CTL-L2 stimulated by the tested supernatants or by comparison of the U/ml of IL-2 produced; data not shown). Indeed, although PBL of all recipients taken as a single group produced significantly lower IL-2 levels than those of either healthy controls ($P < 0.01$) or an homologous sample taken before grafting ($P < 0.01$), PBL of recipients who were acutely rejecting their graft produced significantly more ($P < 0.01$) IL-2 than those of recipients with non-rejecting grafts studied at equivalent time periods after grafting (Fig. 2). It is important to point out that the average intake of CS and AZA did not differ between clinically stable patients and those rejecting their grafts, since blood samples were taken before treatment for the rejection. Ten patients taking cyclosporin A were tested for PHA-induced IL-2 production during a period of stable graft function. They produced amounts of IL-2 similar to those of recipients treated with CS and AZA who also had good graft function; therefore, the values concerning both these types of steady-state recipients were pooled.

Furthermore, serial blood samples covering the steady-state and rejection periods were studied in some patients. In these instances, care was taken to test the various samples of the same patient in the same experiments. Again, the IL-2 produced by PBL harvested at these different times showed a significantly sharp increase at the time of rejection (Fig. 3) ($P < 0.05$, paired t test).

IL-2 production in long-term kidney graft recipients

Although the PHA-stimulated PBL of the above recipients yielded low IL-2, note that they were all studied within a year following transplantation. To determine whether the low IL-2 production was permanent, patients, all of whom had good graft function, were tested at further intervals, >1 year. Figure 4 indicates that, interestingly, PBL of such long-term recipients with well-functioning grafts were capable of producing nearly normal amounts of IL-2 when stimulated by PHA under standard conditions, thus showing that the IL-2 production of kidney recipients tends to be restored with time.

Discussion

Our data show: *1)* there is a normal IL-2 yield from hemodialyzed patients' PBL; *2)* the capacity for IL-2 secretion of PBL of recipients with good graft function is grossly impaired during the first 12 months following transplantation; *3)* when a rejection crisis occurred during this time period, there was a sharp and highly significant increment in lectin-induced IL-2 production of recipient's PBL; *4)* in long-term, well-functioning graft recipients (>2 years), the capacity of PBL to secrete IL-2 upon lectin stimulation tends to be restored.

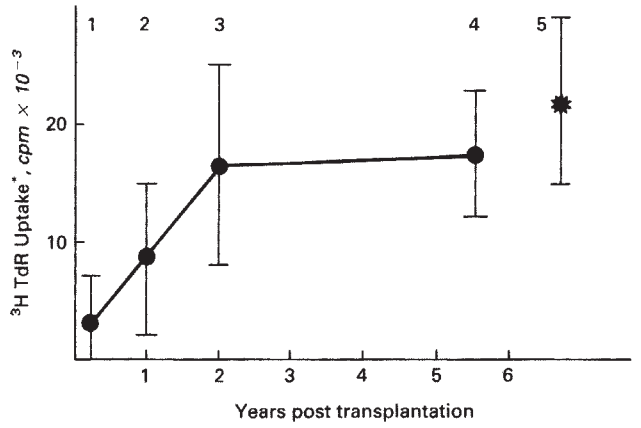

Fig. 4. *Time restoration of IL-2 production capacity of kidney recipients' PBL.* Values ($\bar{X}$ ± SD) of patients tested at 1, 1 to 3 months (N = 19); 2, 3 to 12 months (N = 18); 3, 1 to 2 years (N = 11); and 4 after 2 years (N = 12). Values obtained in healthy individuals (N = 21) are given in 5. *Same as Fig. 3.

The very low IL-2 secretion by PHA-stimulated PBL of kidney recipients after grafting contrasts with the normal IL-2 production capacity of PBL of these patients harvested immediately before grafting and suggests that immunosuppressive therapy (CS and AZA) is involved. CS are known to impair the IL-2 production of lymphocytes in vitro dramatically [10]; however, PBL of non-grafted patients, for example, those with systemic lupus erythematosus treated with CS, do not exhibit lower IL-2 production [11]. Moreover, it would seem that prolonged CS treatment can result in "escape" from the IL-2 secretion blockade. Caution is required, therefore, if one indicates that CS alone is responsible in this IL-2-secretion defect after transplantation. AZA may be involved also, although this possibility has yet to be studied in vitro or in vivo. Other explanations for the low IL-2 level in the supernatants of PHA-stimulated PBL of these recipients may involve adaptive regulation of immune function, for example, suppressor cells. In addition, the relationship of impaired endogeneous production of IL-2 to a decrease in NK cells that bear IL-2 receptors [12] perhaps should be addressed since it has been shown that natural killer (NK) effector functions are decreased dramatically in the first year following an allograft also [13].

The patients with acute rejection episode produced high levels of IL-2 compared to those of non-rejecting recipients ($P < 0.01$), although at the time of testing the average level of immunosuppressive drug intake was similar in both groups. In several cases, serial blood samples were collected before rejection, at the time of rejection, and several weeks after rejection (data not shown). These data confirmed that high IL-2 production occurred only at the very time of the rejection episode.

This increase in IL-2 level associated with rejection may be due either to an increase in the T lymphocytes able to secrete IL-2 under activation ($T4^+$, primarily) or to an increase of the IL-2 production by a given activated lymphocyte at the time of rejection (laboratory analysis in progress). In addition, it is not clear whether the increased IL-2 production is the cause or the effect of rejection, and samples must be drawn systematically at close intervals after transplantation to determine the answer. Although the absence of a radioimmunoassay for IL-2 makes it difficult to monitor the IL-2 level in the blood, the recognition of an association between high PBL capacity to produce IL-2 after PHA activation and graft rejection may bring new possibilities of monitoring recipient immune responses in the near future.

Our data do not clarify the possible role of this increased IL-2 production by PBL in triggering or in sustaining the cellular events underlying rejection. Increased IL-2 production may only reflect, at the peripheral level, the increase of activated $T4^+$ cells, which has been shown in animal models to transfer rejection capacity in immuno-incompetent allograft recipients [7]. However, it is likely that these cells are activated by the allogeneic stimuli within the graft. In this case, they may play a role in recruiting and amplifying the cellular immune response against the graft [5], since recent findings have shown an IL-2 requirement for allograft rejection in rodents [14].

The interpretation of the data is complicated by the observation that the PBL capacity of IL-2 production is not decreased in recipients with good renal function years after grafting. It may be hypothesized that, at that time, adaptive mechanisms such as anti-idiotypic antibodies [15, 16] and specific suppressor cells [17] inhibit (at the anti-donor clonal level) the specific response against graft antigens. On the other hand, IL-2 secretion may be restored years after transplantation simply because of a reduction in immunosuppressant dose. Finally, although further studies are still required to clarify the predictive values as well as the relevance of high IL-2 production by PBL in the mechanism of rejection, our data correlate rejection and high PBL IL-2-secretion clearly at a time when recipients with well-functioning grafts have markedly impaired secretion of this lymphokine.

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