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L. Loonen
L. Vaessen
A. Balk
K. Groeneveld
B. Mochtar
N. Jutte
F. Claas
W. Weimar

Long-term survival of heart grafts in the presence of donor-specific cytotoxic T-cell precursors (CTLp) in the peripheral blood

E. H. M. Loonen (✉) · L. Vaessen
K. Groeneveld · N. Jutte · W. Weimar
Department Internal Medicine I,
University Hospital Rotterdam-Dijkzigt,
Dr Molewaterplein 40,
NL-3015 GD Rotterdam,
The Netherlands

A. Balk · B. Mochtar
Thorax Center,
University Hospital Rotterdam-Dijkzigt,
Rotterdam, The Netherlands

F. Claas
Department of Immunohaematology and
Bloodbank, University Hospital,
Leiden, The Netherlands

Abstract To monitor their immunological status we determined donor and third-party-specific cytotoxic T-cell precursor frequencies (CTLpf) in the peripheral blood of 15 heart transplant recipients. PBL samples were obtained at different time points before and after transplantation. Donor-specific CTLpf and third-party-specific CTLpf were within the same range for all samples (1–1489/10^6 cells). The donor-specific CTLpf were not different between patients who had never had an acute rejection (AR) and patients who had an acute rejection as diagnosed by endomyocardial biopsy (EMB). No difference was observed between donor-specific CTLpf of samples taken on the day of transplantation and those obtained between 3 months and 3 years after transplantation. There was also no relationship between the donor-specific CTLpf in the PBL and the culturing success of lymphocytes from EMB taken at the same time. CTLpf were in the same range both when cultures could be propagated from the graft and when no cells grew out. We conclude that long-term graft survival is possible in the presence of CTLpf in peripheral blood.

Key words Cytotoxic T-cell precursors · Heart transplantation Peripheral blood lymphocytes

Introduction

The cornerstone of rejection diagnosis remains histological examination of endomyocardial biopsy (EMB) which is both time consuming and invasive. Several attempts have been made to correlate immunological parameters in the peripheral blood with acute cellular rejection after organ transplantation. Herzog et al. [2] and Reader et al. [8] suggested the clinical value of limiting dilution analysis of PBL which is a sensitive and quantitative method for measuring the frequency of donor-reactive CTL. Reader et al. [8] found in a few cases a relationship between donor CTLpf and the immunological status of the graft. In contrast to Herzog et al. [2]. They found a low CTLpf in kidney transplant patients with well-functioning grafts more than 1 year after transplantation. We used this method to monitor the immunological situation after clinical heart transplantation by determining the donor-specific CTLpf in sequentially taken PBL samples.

Materials and methods

This report is based on 15 recipients of an orthotopic cardiac allograft. All patients had received preoperative blood transfusions and all received cyclosporin and low-dose prednisone as main-

tenance immunosuppression. PBL (75) samples were obtained from 15 patients at 5 different time points between 3 months and 3 years after transplantation. Pretransplantation samples were obtained from 8 patients. The rejection grade in EMB was assessed according to the criteria of Billingham et al. [1]. PBL samples were obtained concomitantly with the EMBs.

Isolation of peripheral blood mononuclear cells (PBMC)

PBMC were isolated from heparinized venous blood of the recipient by Ficoll Hypaque density gradient centrifugation. The cells were washed twice with Hank's balanced salt solution (Gibco) and resuspended in RPMI-1640 Dutch modification (Gibco) culture medium supplemented with 10% human serum, L-glutamine, penicillin and streptomycin.

Limiting dilution analysis

According to the method of Kaminski et al. [5], cultures were set up in 96-well round-bottomed microculture plates (Costar). All samples from each patient were measured in one assay. Graded numbers of responder cells were cultured in 24 replicates with 50000 irradiated (30 Gy) stimulator cells in a total volume of 0.2 ml. Stimulator cells were donor or third-party spleen cells. Third-party spleen cells had no HLA class I or class II match with donor or recipient. Incubation was for 10 days at 37 °C in a humid atmosphere containing 5% CO_2. Recombinant IL-2 (final concentration 5 U/ml) was added on day 3 and 6 and the wells were assayed for cytotoxicity on day 10. PHA blasts prepared from donor or third-party spleen cells were used as targets in a 4 h ^{51}Cr-release assay.

·sis of limiting dilution data

ıation of the frequency of a population was done by determin-ın of the number of negative wells. Microcultures were consi-ıred negative when the specific experimental lysis did not exceed 3 × SD above background. CTLpf and their 95% confidence were determined by the Jack-knife procedure for Maximum Likelihood. Frequency calculations were made using a computer program developed by Strijbosch et al. [9].

Results and discussion

Donor-specific CTLpf and third-party-specific CTLpf for all samples were within the same range (Table 1). All samples with a donor-specific CTLpf of $1/10^6$ were excluded from subsequent analysis. In total we measured 75 PBL samples. In 17 cases CTLpf were equal to $1/10^6$, leaving 58 CTLpf for analysis. The median donor CTLpf in PBL before transplantation was comparable with the CTLpf long after transplantation (median 439 days, range 72–895 days) (Table 1). The median donor-specific CTLpf in PBL from rejectors was not different from the median CTLpf in the PBL from non-rejectors (Table 1). Even at the time that an acute rejection was diagnosed CTLpf in PBL were within the same range as during a period with stable graft function (Fig. 1). These results are in agreement with those of Herzog et al. [2] but not with those of Reader et al. [8], and with previous studies reporting that monitoring of peripheral blood does not give information about intragraft events during rejection [2–4, 10]. From EMB taken concurrently with the PBL samples, in 17/42 cases T-cell cultures were established. We found no difference in CTLpf between blood samples

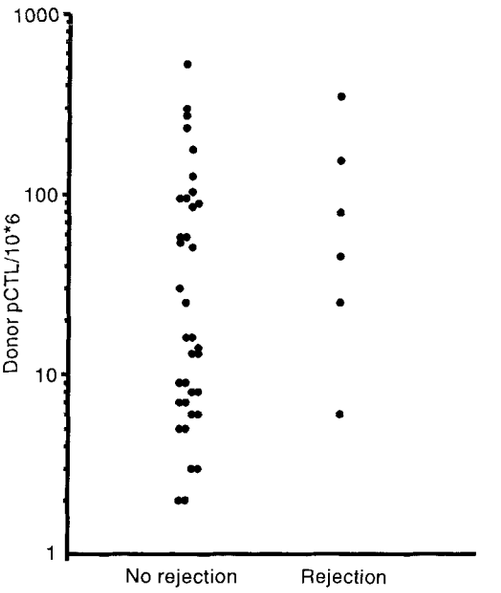

Fig. 1 The donor-specific CTLpf of PBL samples in relation to histological signs of rejection in EMB at the same time-point

Table 1 Donor and third-party CTLpf for all samples or subdivided into pre/post transplantation samples and samples from always (AR+) and never (AR−) rejecting patients

	Donor	Third-party	Donor-specific CTLpF [a]			
	($n=75$)	($n=75$)	Pre-Tx ($n=8$)	Post-Tx ($n=12$)	AR+ ($n=34$)	AR− ($n=10$)
Median	13 [b]	33	28	53	24	14
Range	1–1489 [b]	1–589	4–1489	2–217	2–522	3–95

n = number of samples
[a] only the frequencies $> 1/10^6$
[b] CTLp/10^6

taken simultaneously with growing or non-growing biopsies. In contrast, intragraft monitoring both in terms of establishing T-cell cultures from EMB and measuring their cytotoxicity [6] but especially in determining their avidity [7] was correlated with clinical events. We assume that the donor-reactive CTLp which we found in the PBL of patients with long-term heart survival will mature into CTL with low avidity for donor antigens as apparently they have no allograft-destroying potential.

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