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Monitoring of cardiac graft recipients: comparison of in vivo activated, committed T lymphocytes in peripheral blood and in the graft

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Abstract. The proliferative and cytotoxic capacity of peripheral blood lymphocytes (PBL) and the cytotoxic activity of lymphocytes propagated from endomyocardial biopsies (EMB) towards donor cells was used to identify in vivo activated, committed T cells. A series of 39 PBL samples and 38 EMB simultaneously taken from 20 patients after heart transplantation was cultured in interleukin 2 (IL-2) conditioned medium. The cytotoxic capacity of these cultures against donor cells was tested in a 4-h chromium-51 release assay. From a comparable patient group, 224 samples were evaluated for donor reactivity by a primed lymphocyte test (PLT). Analysis showed that PBL cultures hardly ever contained committed cytotoxic T lymphocytes (cCTL, 2/39) or committed proliferative T lymphocytes (cPTL, 1/224). In contrast, significantly more EMB cultures (17/38, $P < 0.001$, χ^2 test) demonstrated donor-directed cytotoxicity. This was especially found during rejection (11/17 vs 6/21 without rejection, $P = 0.05$). These results show that after heart transplantation, committed cells are mainly found in the graft.

Key words: Heart transplantation – Rejection monitoring – Committed T lymphocytes

Despite previous attempts to correlate morphologic and phenotypic changes of T lymphocytes in the peripheral blood lymphocytes (PBL) with allograft rejection, histologic evaluation of endomyocardial biopsies (EMB) remains the only reliable method for the diagnosis of cardiac rejection. Results of diagnostic studies in PBL were conflicting and did not provide information about the function and specificity of these T cells [2, 3, 7]. We have recently demonstrated that the lymphocyte growth obtained from EMB cultured in interleukin (IL-2) conditioned medium correlates with the histologic rejection grade [5] and that the majority of the in vivo activated, committed T lymphocytes showed cytotoxicity against donor antigen-committed T lymphocytes [1]. In this report, we analyzed whether the presence of committed T lymphocytes in PBL and their proliferative or cytotoxic ability to react against donor cells was associated with rejection episodes. The specific cytotoxicity of PBL cultures was compared with cytotoxic reactivity patterns from lymphocytes isolated from EMB obtained at the same time.

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Patients and methods

Patients were diagnosed by histologic criteria according to Billingham for acute rejection in EMB. All cardiac transplant recipients received cyclosporin A (CsA) and low-dose steroids as immunosuppressive therapy.

We analyzed 39 (Ficoll-Hypaque isolated) PBL and 38 EMB samples simultaneously taken from 20 heart transplant patients. The PBL and EMB samples were expanded in IL-2 containing medium and tested in a 4-h chromium-51 release assay for cytotoxic reactivity as reported previously [5]. As targets we used donor B-LCL, third party B-LCL (Epstein-Barr virus transformed B-lymphocyte cell lines) and lymphokine-activated killer (LAK) and natural killer (NK) sensitive target cell line K562 to detect committed cytotoxic T lymphocytes (cCTL) in the cultures. Cold target inhibition studies with K562 were performed to confirm the allospecificity of the cCTL derived from the cultured PBL. A fixed number of cold K562 (2.5×10^4) was added to 2.5×10^3 hot K562 or donor B-LCL cells. Control values were established by adding unrelated cold PHA blasts to hot target cells.

From a comparable patient group we tested 20 pretransplant and 224 post-transplant PBL samples with a standard primed lymphocyte test (PLT) [9] against donor and third party spleen cells to detect cPTL in PBL.

Results

We grew sufficient cells from 19/38 EMB (10 patients) and from all PBL ($n = 39$, 20 patients) for the analysis of donor-directed cytotoxicity.

All cultured PBL samples showed LAK-like nonspecific cytotoxicity since the cultures killed K562 and vari-

Table 1. Cultures containing committed cytotoxic T lymphocytes in peripheral blood lymphocytes (PBL) and endomyocardial biopsies (EMB) in relation to periods of acute rejection (AR)

	Number of cultures (%)		
	AR^-	AR^+	P value[a]
PBL	0/23 (0)	2/16 (12)	n.s.
EMB	6/21 (28)	11/17 (65)	0.05

[a] χ^2 test

ous B-LCL. In 2/16 (12%) PBL cultures taken at the time of or followed by acute rejection, we identified cCTL after cold target inhibition with K562 (Table 1). Significantly more (11/17, 65%) of the corresponding EMB cultures were cytotoxic against donor antigens ($P < 0.01$, χ^2 test). The EMB cultures did not lyse the NK-sensitive cell line K562. None of the 23 PBL cultures, taken during periods of immunologic quiescence, contained cCTL. In contrast, we observed donor-directed cytotoxicity in 6/21 (28%) of the EMB cultures ($P = 0.02$) taken in the absence of rejection. This proved to be significantly lower than was found during acute rejection (6/21 vs 11/17, $P = 0.05$).

In cases in which there was histologic evidence of rejection, 1/40 of the PBL samples showed a significant proliferative response when stimulated with donor cells compared with pretransplant samples. None of the other PBL samples ($n = 184$, no rejection) demonstrated primed lymphocyte responses against donor antigens. Third party stimulator cells did not induce proliferation of the PBL in the PLT.

Discussion

We demonstrated that the in vitro proliferative or cytotoxic capacity of PBL cultures towards donor antigens was negligible even at the time of or prior to an acute rejection. A significantly higher proportion of the corresponding EMB cultures showed cytotoxicity against donor antigens. The number of EMB cultures containing cCTL was correlated with histologic diagnosis of rejection. These data clearly demonstrate that committed lymphocytes are only seldom present and indicate that committed alloreactive cells have a preference for the allograft. Similar results obtained with limiting dilution analysis of alloreactive precursor cytotoxic T lymphocytes (pCTL) were reported from human cardiac graft infiltrating cells [8] and cCTL in mouse sponge grafts [4]. Another recent report suggested that the increased frequencies of circulating donor-specific pCTL were associated in a predictive manner with rejection. However, the measurement of pCTL in PBL had no predictive value as no difference was found between clinically relevant (myocytolysis) and irrelevant (infiltrate only) rejection episodes [6]. We conclude that the immune status of the PBL is no reflection of the histologic and functional changes in the EMB. Therefore, monitoring of the PBL for the diagnosis of rejection in heart transplant recipients remains an illusion.

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