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Discrepancy between mRNA expression and production of IL-2 and IL-4 by cultured graft infiltrating cells propagated from endomyocardial biopsies

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Abstract We studied whether acute rejection correlated with the cytokine production pattern and mRNA expression of interleukin-2 (IL-2) and interleukin-4 (IL-4) in lymphocyte cultures derived from endomyocardial biopsies (EMB) that were stimulated with B cell lines of donor origin. Unstimulated biopsy cultures neither expressed mRNA nor produced IL-2 or IL-4. All stimulated biopsy cultures contained mRNA transcripts for IL-2 and IL-4. In contrast, we found different IL-2 and IL-4 production patterns. Within the first 90 days after heart transplantation (HTx), higher levels of IL-4 were measured in cultures derived from EMB with myocytolysis than in cultures from EMB without signs of myocytolysis. More than 90 days after HTx, this phenomenon was reversed and more IL-4 was produced in cultures derived from EMB without myocytolysis. These differences were not detected for IL-2 production.

Key words Acute rejection
Myocytolysis · Cytokines
Endomyocardial biopsies · Clinical heart transplantation

Introduction

In mice, $CD4^+$ helper T cell subsets can be divided based on their cytokine production pattern [5]. The key differences are the secretion of interleukin (IL-2) and IFNγ by Th1 cells, and IL-4, IL-5, IL-6 and IL-10 by Th2 cells. A third subset, Th0 cells, secrete a mixture of these cytokines, i.e. IL-2, IFNγ, IL-4, IL-5 and IL-10. In the present study we investigated the production of IL-2 (Th1 and Th0) and IL-4 (Th2 and Th0) in bulk cultures derived from endomyocardial biopsies (EMB) taken after clinical heart transplantation (HTx). We studied whether differences in IL-2 and IL-4 mRNA expression and/or production could be correlated with acute rejection as histologically diagnosed in EMB.

Materials and methods

All patients received cyclosporin A and low-dose prednisone as maintenance immunosuppression. EMB were taken regularly for histological diagnosis of rejection [1]. We obtained 21 EMB from 12 patients. Histological analysis demonstrated signs of myocytolysis in nine EMB. EMB-derived cell cultures were established in IL-2-containing medium as described before [4]. The cells were extensively washed and 5×10^4 cells per well were incubated for 24 h in culture medium (RPMI 1640-DM and 10% pooled, heat inactivated human serum) in the absence of exogenous IL-2. Thereafter, 5×10^4 irradiated (60 Gy) and washed Epstein Barr virus (EBV) transformed B cells (B-LCL) of donor origin were added per well. After 20 h, culture supernatants were harvested to measure cytokine production by an ELISA [IL-2: detection range 15–1000 pg/ml (Immunotech, Marseille, France); IL-4: detection range 10–450 pg/ml (CLB, Amsterdam, The Netherlands)]. At the same time, the remaining cell pellets were snap-frozen for mRNA analysis by reverse transcriptase PCR (Gibco BRL, USA). β-actin mRNA was used as a positive control for successful RNA extraction. Unstimulated cells, treated in

the same way as stimulated cells, served as control. The results were compared using the two-tailed Mann-Whitney *U* test.

Results and discussion

Unstimulated cultures neither expressed mRNA for IL-2 and IL-4 nor produced these cytokines. After stimulation with donor B-LCL, all EMB-derived cultures demonstrated mRNA transcripts for IL-2 and IL-4. A different pattern was found for cytokine production. After stimulation, nine cultures produced both IL-2 and IL-4. These cultures consisted either of a Th0 population or a mixture of Th1- and Th2-secreting cells. The simultaneous occurrence of both Th1 and Th2 cells has also been described by Lowry and coworkers [3] for murine heart allografts. Five cultures demonstrated a Th1-like cytokine pattern because only IL-2 was produced, and one revealed a Th2-like pattern by producing only IL-4. The remaining six cultures did not produce detectable levels of IL-2 or IL-4. This coincided with the absence of proliferation after stimulation with donor cells in a primed lymphocyte test (data not shown). A discrepancy between mRNA expression and production of cytokines is also described by Dallman et al. [2] and can be explained by a regulating factor at the posttranscriptional level.

EMB-derived cultures were divided into two groups. The first group consisted of EMB taken during the first 90 days after HTx, in which most acute rejections occur, the second group consisted of EMB taken more than 90 days after HTx. There were no significant differences within these groups in the production of IL-2 between cultures from EMB with or without myocytolysis (Table 1). Also, no significant differences in IL-2 production were found when we compared EMB with myocytolysis taken early and late after HTx, nor when we compared the EMB without myocytolysis taken during these time periods. In contrast, in mRNA isolated directly from the solid EMB, transcripts of IL-2 were significantly more often found in EMB with myocytolysis than without myocytolysis. To measure the production of the different cytokines, lymphocytes have to be propagated from the EMB and cultured in the presence of ± 30 units IL-2. These culture conditions may change the cytokine production pattern of the cells. Different culture conditions have to be tested to study possible culture artifacts. In addition, since most patients are carriers of EBV, IL-2 might be produced by EBV-specific T cells, if these were present in the cultures.

Table 1 Interleukin (IL)-2 and IL-4 production in cultured endomyocardial biopsies (EMB) stimulated with donor B-LCL in EMB without and with myocytolysis early and late after clinical heart transplantation HTx

Days	No myocytolysis		Myocytolysis		*P*-value
	Range (pg/ml)	Median	Range (pg/ml)	Median	
IL-2					
≤ 90	15–359	55.5	15–212	151	0.28
> 90	15–392	129.5	15–257	33.5	0.23
IL-4					
≤ 90	10–36	10	10–218	42	< 0.095
> 90	10–450	16.5	10–18	10	0.18

Different results were found for the production of IL-4. Early after transplantation, higher IL-4 levels were measured in cultures from EMB with signs of myocytolysis compared to EMB without myocytolysis ($0.056 < P < 0.095$; Table 1). Later after transplantation, this phenomenon tended to be reversed. Concerning biopsies with myocytolysis, significantly more IL-4 was produced in cultures derived from EMB taken early after HTx than later ($0.032 < P < 0.056$).

In conclusion, a discrepancy was found between mRNA expression and production of IL-2 and IL-4 in EMB cultures stimulated with B-LCL of donor origin. mRNA transcripts of these cytokines were always induced after stimulation with donor antigen. In addition, a changing pattern of IL-4 but not of IL-2 production was found that was dependent on the time posttransplantation and state of rejection.

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