Transplant Int (1992) 5 [Suppl 1]: S228–S230

TRANSPLANT
International

Phenotype of endomyocardial biopsy-derived T-lymphocyte cultures and chronic rejection after heart transplantation

K. Groeneveld[1], A. H. M. M. Balk[2], A. J. Ouwehand[2], E. H. M. Loonen[1], M. vd Linden[2], S. Strikwerda[2], B. Mochtar[2], N. H. P. M. Jutte[1], and W. Weimar[1]

[1] Department of Internal Medicine I, Erasmus University Rotterdam, Rotterdam, The Netherlands
[2] Thorax Center, University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands

Abstract. Chronic rejection (CR) is a major problem in long-term survival in heart transplantation. We analysed whether the occurrence of CR correlates with the incidence of acute rejections (AR) or with characteristics of endomyocardial biopsy-derived cell cultures. CR was diagnosed by annual angiography and defined as all coronary vascular changes. One year after transplantation 24 of the 63 patients had CR (38%). The incidence of AR in CR + and CR − patients was comparable. The patients in both groups had similar individual median percentages of EMB-yielding cell cultures. During the first year the CR − patients had more cultures in which at least 60% of the cells were CD4 + T cells (50% vs 37%, $P = 0.05$), due to a stronger CD4 predominance in the first 6 months. In the second year the CD4 predominance in the patients diagnosed as CR + after 1 year tended to be higher ($P = 0.08$). The patients had comparable percentages of cultures predominated by CD8 + T cells, γδ T cells or NK cells, irrespective of the time interval. These results might indicate that CD4 + T lymphocytes play a dual role in the aetiology of CR.

Key words: Chronic rejection – Heart transplantation – CD4 + T cells

Introduction

The long-term survival of heart transplant recipients is limited by the development of accelerated coronary artery disease. Knowledge about the aetiology and treatment of this disease, commonly referred to as chronic rejection (CR), is limited [1]. Libby et al. describe a model in which CR was the result of a cellular immunological reaction in the graft arteries [4]. In this model the major cell type involved was the host CD4 + helper T cell.

Offprint requests to: Kees Groeneveld, Ph. D., Department of Internal Medicine I, Bd 299, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

After heart transplantation endomyocardial biopsies (EMB) are taken at regular intervals to diagnose acute rejection. We analysed whether the development of CR is related to the occurrence of acute rejection episodes. We also compared patients with and without CR with respect to growth patterns and phenotypic composition of cells grown from EMB [6, 10] to analyse whether the occurrence of CR is correlated with any of these characteristics.

Materials and methods

Patients

We studied 63 consecutive cardiac allograft recipients transplanted between January 1988 and February 1990. The median age of the patients was 50 years, with a range of 19 to 59 years. All patients had received preoperative blood transfusions and all received cyclosporine and low-dose prednisone as maintenance immunosuppression. In the early post-transplantation period serial EMBs were obtained at weekly intervals. Later EMBs were taken less frequently. The rejection grade was assessed according to the criteria of Billingham. Only is cases of infiltrate with myocyte necrosis (grade 2) was antirejection therapy instituted. During the first year pTx we received 12 to 23 EMBs from each patient (median 15 EMBs). In the second year pTx 2–5 EMBs per patient were obtained.

Diagnosis of CR was assessed by annual coronary angiography and defined as the presence of all coronary vascular changes, including minor wall irregularities of the epicardial branches and the intramyocardial branches. All coronary angiographies were scored by consensus of two of us (AHMM B and M vd L).

Culture method and phenotypic analysis

Endomyocardial biopsies were cultured as described by Ouwehand et al. [6]. Surface differentiation antigens of the EMB-derived cultures were analysed as described before [6]. A phenotypic marker was defined as predominant when at least 60% of the cells were positive.

Statistics

Unless stated otherwise data were analysed by the Mann-Whitney U test.

Table 1. Median individual percentage of CD4 or CD8 predominance ($\geq 60\%$ of the cells) in T-cell cultures from CR + and CR − patients derived from EMBs taken during different time intervals

Time interval	Predominant phenotype	Median % of cultures (number of patients)		
		CR + patients	CR − patients	*P*-value
First year	CD4	37 (24)	50 (39)	0.05
	CD8	33 (24)	25 (39)	0.60
0–180 days	CD4	44 (24)	59 (39)	0.06
	CD8	27 (24)	20 (39)	0.54
180–360 days	CD4	10 (24)	33 (35)	0.80
	CD8	50 (24)	33 (35)	0.58
Until first acute rejection[a]	CD4	50 (17)	50 (27)	0.32
	CD8	20 (17)	0 (28)	0.10

[a] rejection biopsy included

Results

Patients

One year after transplantation, 24 of the 63 patients studied (38%) were diagnosed as having CR. The median age of these 24 CR + patients (50 years, range 17–59 years) was similar to that of the CR − patients (50 years, range 17–58 years).

During the first year post-transplantation the number of EMBs obtained from CR + and CR − patients were comparable: CR + patients median number of EMBs, 15 (range 13–23); CR − patients, 16 (range 12–23). After 12 months, 17 CR + patients (71%) and 29 CR − patients (74%) had had one or more periods of acute rejection. The number of acute rejection episodes was not related to the development of CR.

Cell cultures

Lymphocyte cultures (to a minimum of 10^6 cells) could be established from at least one EMB from all patients. After 1 year the CR + and CR − patients had comparable median percentages of biopsies yielding T-cell cultures: CR + patients; 43%; CR − patients, 33%; $P = 0.45$. The percentage growth from biopsies of CR + and CR − patients at different time intervals during this first year was also highly comparable.

In the first year the CR − patients had a higher percentage of cultures in which at least 60% of the cells were CD4 + T cells (CR − patients, median percentage 50%; CR + patients, 37%; $P = 0.05$) (Table 1). This difference in CD4 predominance was mainly caused by the EMB obtained in the first half year ($P = 0.06$), since in the second half year CD4 + T cells predominated in CR + and CR − patients in equal percentages ($P = 0.80$). CR + patients tended to have a higher percentage of CD8 + T-cell-dominated cultures from EMBs obtained before the first acute rejection episode ($P = 0.10$). In other time intervals the percentages of cultures dominated by CD8 + T cells in both patient groups were comparable. There were no differences between CR + and CR − patients with respect to the predominance of NK or γδ cells.

Second year

Eleven CR + patients and 15 CR − patients could be studied for a second year. Two of the 15 patients negative for CR after 1 year developed CR during the second year. The 13 CR − patients and the two newly diagnosed CR + patients did not differ with respect to the characteristics of the graft infiltrating cells. This might very well be due to the small number of patients and small number of EMBs obtained in the second year. Five of the 11 patients diagnosed as having CR after 1 year had at least one acute rejection during the second year, compared with only one of the 13 patients who at the end of the second year were still CR − ($P = 0.17$, Fisher test).

The 11 CR + patients were compared with the 13 CR − patients concerning the cultures derived from EMBs obtained during the second year. The patient groups had comparable median percentages of cell growth and predominance of CD8 + T cells. There were no differences in the predomination of either NK cells or γδ T cells. During the second year the 11 CR + patients tended to have a higher percentage of cultures dominated by CD4 + T cells ($P = 0.08$). We, therefore, also analysed the CD4 predominance in these 11 CR + and 13 CR − patients in the first year (Fig. 1). During the first 6 months the CR − patients tended to have a higher median percentage of CD4-dominated cultures ($P = 0.09$). This difference diminished in the second half year ($P = 0.88$).

Discussion

We analysed whether the development of chronic rejection can be related to the occurrence of acute rejection episodes or to characteristics of EMB-derived cultures of graft infiltrating cells.

In our patient group the occurrence of acute rejection episodes was not related to the development of CR. The percentage rejectors in both groups was similar, and a relationship between the number of acute rejection episodes and the development of CR was not found. These results are in agreement with those reported by other groups [2, 5]. However, Uretsky et al. [8] demonstrated a relationship between the occurrence of major acute rejection episodes during the first year after transplantation and the subsequent development of CR. These conflicting results might be explained by differences in the definitions of both acute rejection and CR.

With the exception of predominance of CD4 + T cells, the CR + and CR − patients were highly comparable when data concerning growth patterns and phenotypic composition of graft infiltrating cells for the complete first year after transplantation were analysed. The patient groups had highly similar percentages of lymphocyte growth from EMBs irrespective of the time interval. Kaufman et al. [3] demonstrated an association between lymphocyte growth from EMBs taken during the first 3 months post-transplantation and the subsequent development of CR. In our study the CR + and CR − patients had highly similar percentage growth from EMBs obtained during the first 90 days (data not shown). These

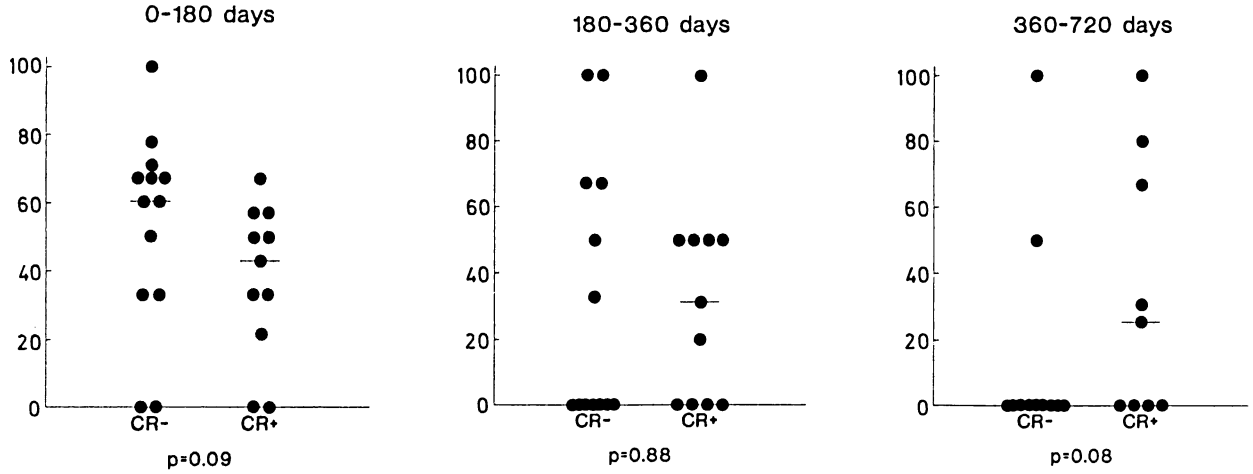

Fig. 1. Individual percentages of T-cell cultures from endomyocardial biopsies (EMB) in which CD4+ T cells predominated (≥ 60% of the cells). EMBs were taken at different time intervals pTx from 11 patients with CR at 1 year after transplantation and 13 patients without CR at 2 years after transplantation. Median percentages are indicated

conflicting results might be caused by differences in the growth medium, or by differences in the definitions of both cell growth and CR.

The CR+ and CR− patient groups had comparable percentages of γδ T-cell-dominated cultures from EMBs obtained before the diagnosis of CR. The presence of γδ T cells seems not to be related to CR, since Vaessen et al. [9] showed that in the years after diagnosis of CR the EMB-derived cultures from CR+ and CR− patients also yielded γδ T cells in comparable frequencies.

The most pronounced difference between CR+ and CR− patients was found in the predominance of CD4+ cells (Table 1). During the first half year the CR− patients had a higher percentage of CD4-dominated cultures, while in the second half year no differences between the two groups were found (Table 1). In the second year post-transplantation the CR+ patients showed a higher median percentage of CD4-dominated cultures compared with the patients who remained free from CR during the first 2 years (Fig. 1). The change in time of this difference between CR+ and CR− patients was due to a decline in CD4-dominated cultures in the CR− patients.

The higher CD4 predominance in the patients having CR is in agreement with the model described by Libby et al. [4], in which CD4+ helper T cells interacting with foreign HLA class-II antigens are thought to play an important role. Histological evidence for the importance of class-II antigens in relation to CR has come from Salomon et al. [7], who demonstrated high class-II expression on endothelial cells in arteriosclerosis lesions from human cardiac allografts. We do not have an explanation for the stronger CD4 predominance in the cultures from the CR− patients during the first 6 months after transplantation. One might speculate that the patients diagnosed as having CR at the end of the first year developed CR because of the lack of graft infiltrating CD4+ T cells.

In conclusion, analysis of heart allograft infiltrating cells in relation to CR does not lead to the definition of a factor with prognostic value for the development of CR. The differences between CR+ and CR− patients in the predominance of CD4+ T cells in EMB-derived cultures and the change with time of this difference might indicate that CD4+ T cells play a dual role in the aetiology of chronic rejection.

Acknowledgment. This work was supported by grant 89.055 from the Netherlands Heart Foundation.

References

1. Balk AHMM, Weimar W (1992) Chronic heart graft rejection in the clinical setting. In: Paul LC, Solez K (eds) Organ transplantation: long term results. Marcel Dekker Inc, New York
2. Gao SZ, Schroeder JS, Alderman EL, Hunt SA, Silverman JF, Wiederhold V, Stinson EB (1987) Clinical and laboratory correlates of accelerated coronary artery disease in the cardiac transplant recipient. Circulation 76 [Suppl V]: V56–61
3. Kaufman CL, Zeevi A, Kormos RL, Zerbe TR, Keenan RJ, Uretsky BF, Griffith BP, Hardesty RL, Duquesnoy RJ (1990) Propagation of infiltrating lymphocytes and graft coronary disease in cardiac transplant recipients. Hum Immunol 28: 228–236
4. Libby P, Salomon RN, Payne DD, Schoen FJ, Pober JS (1989) Functions of vascular wall cells related to development of transplantation-associated coronary arteriosclerosis. Transplant Proc 4: 3677–3684
5. Narrod J, Kormos R, Armitage J, Hardesty R, Ladowski J, Griffith B (1989) Acute rejection and coronary artery disease in long-term survivors of heart transplantation. J Heart Transplant 8: 418–421
6. Ouwehand AJ, Vaessen LMB, Baan CC, Jutte NHPM, Balk AHMM, Essed CE, Bos E, Claas FHJ, Weimar W (1991) Alloreactive lymphoid infiltrates in human heart transplants: loss of class II directed cytotoxicity more than three months after transplantation. Hum Immunol 30: 50–59
7. Salomon RN, Hughes CCW, Schoen FJ, Payne DD, Pober JS, Libby P (1991) Human coronary transplantation-associated arteriosclerosis: evidence for a chronic immune reaction to activated graft endothelial cells. Am J Pathol 138: 791–798
8. Uretsky BF, Murali S, Reddy PS, Rabin B, Lee A, Griffith BP, Hardesty RL, Trento A, Bahnson HT (1987) Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisone. Circulation 4: 827–834
9. Vaessen LMB, Ouwehand AJ, Baan CC, Jutte NHPM, Balk AHMM, Claas FHJ, Weimar W (1991) Phenotypic and functional analysis of T cell receptor γδ-bearing cells isolated from human heart allografts. J Immunol 147: 846–850
10. Zeevi A, Fung JJ, Zerbe TR, Kaufman C, Rabin BS, Griffith BP, Hardesty RL, Duquesnoy RJ (1986) Allospecificity of activated T cells grown from endomyocardial biopsies from heart transplant patients. Transplantation 41: 620–626