JACC Vol. 21, No. 1
January 1993:171–6

Coronary Sinus Sampling of Cytokines After Heart Transplantation: Evidence for Macrophage Activation and Interleukin-4 Production Within the Graft

ALISTAIR FYFE, MBBS, FRCP(C), PAUL DALY, MD, FRCP(C),
LUKE GALLIGAN, MBBS, FRACP, LIDIA PIRC, RN, CHRISTOPHER FEINDEL, MD, FRCS(C),
CARL CARDELLA, MD, FRCP(C)
Toronto, Ontario, Canada

Objectives. This study was undertaken to evaluate the organ-specific release of cytokines after heart transplantation and to assess any correlation with transplant rejection. This cytokine profile should document the relative activation of mononuclear cell subsets within the graft.

Background. Up to 60% of mononuclear cells infiltrating the cardiac allograft during rejection are macrophages, but their role is undetermined. The T lymphocytes are activated, but the activity of specific T cell subsets is not known. We sought to assess for the first time in humans the in vivo activation of mononuclear cell subsets by measuring coronary sinus cytokine levels after heart transplantation.

Methods. Paired superior vena cava and coronary sinus serum samples were assayed for interleukin (IL)-2, IL-4 and IL-6, soluble IL-2 receptors, tumor necrosis factor-alpha and neopterin in 10 patients at the time of 49 routine endomyocardial biopsy procedures. All cytokine measurements were made by using enzyme-linked immunosorbent assay; neopterin was measured by using radioimmunoassay.

Results. Interleukin-2 levels were not detectable (<0.8 U/ml) in either the superior vena cava or the coronary sinus in the presence or absence of rejection. Interleukin-2 receptor levels were uniformly elevated to 1,283 U/ml in the superior vena cava and to 1,232 U/ml in the coronary sinus, with no correlation with rejection severity. Interleukin-4 levels were consistently higher in coronary sinus serum than in peripheral blood (229 vs. 61 pg/ml, $p < 0.0005$), but there was no relation with rejection. Interleukin-6 levels were higher in the coronary sinus than in the superior vena cava (200 vs. 120 pg/ml, $p < 0.05$). Tumor necrosis factor-alpha showed consistently elevated levels in coronary sinus serum (68 vs. 17 pg/ml, $p < 0.0005$), with no relation with rejection. Neopterin, which is produced only by activated macrophages, was also consistently elevated in the coronary sinus (2.5 vs. 2.2 nmol, $p = 0.08$).

Conclusions. The cardiac allograft is a major source of cytokines after heart transplantation. The cytokine profile allows the activity of subsets of the mononuclear cell infiltrate to be investigated. Elevated coronary sinus activity of the macrophage-specific metabolite neopterin suggests macrophage activation within the allograft. This possibility is supported by elevated coronary sinus levels of tumor necrosis factor-alpha and IL-6. The T lymphocytes are activated, as evidenced by high soluble IL-2 receptor levels, but IL-2 production was suppressed by conventional immunosuppressive therapy. Coronary sinus IL-4 levels represent T helper-2 cell activation within the graft despite immunosuppression. We could find no temporal relation between the coronary sinus or superior vena cava cytokine concentration or profile and severity of rejection on concurrent biopsy studies.

(J Am Coll Cardiol 1993;21:171–6)

Cellular elements within an allograft actively involved in rejection express interleukin (IL)-2 receptors and secrete interleukins and other cytokines into surrounding tissues and serum. Measurement of serum interleukins and soluble or cellular interleukin receptors after transplantation have been used as a noninvasive marker of graft rejection in an effort to quantitate immune activation. Immunologic markers currently under investigation as potential rejection predictors include mononuclear cell number and subtype from both the peripheral blood and organ effluent (1), soluble (2) and cellular (3) IL-2 receptors, interleukin-2 (4) and IL-6 (5). The measurement of peripheral blood soluble IL-2 receptor levels as a marker of rejection has been restricted by a lack of sensitivity and specificity, particularly during active infection (2,3,6).

In addition to their use as clinical tools, measurement of cytokine levels from allograft effluent may provide fundamental insights into the importance of the various cellular elements in the rejection process. The relative role of T

From the Divisions of Cardiology, Cardiovascular Surgery and Nephrology, The Toronto Hospital, 200 Elizabeth Street, Toronto, Ontario, Canada M5G 2C4.

Manuscript received February 7, 1992; revised manuscript received June 29, 1992, accepted July 7, 1992.

Address for correspondence: Alistair Fyfe, MBBS, FRCP(C), Division of Cardiology, University of California, Los Angeles School of Medicine, 47-123 Centre for Health Sciences, 10833 Le Conte Avenue, Los Angeles, California 90024-1679.

0735-1097/93/$6.00

lymphocytes may be assessed by measuring their interleukin products, that is, IL-2 and IL-2 receptors. The activation of two distinct T helper lymphocyte subsets may be assessed on the basis of differences in cytokine profile. IL-2 and interferon-gamma are produced by the T helper-1 cell and IL-4, IL-5 and IL-6 are produced by a subclass designated T helper-2 cells (7,8). The presence and activity of the IL-4–producing T helper-2 subclone in human transplant recipients remain undetermined. Monocytes/macrophages are important in rejection as antigen-presenting cells and release monokines such as tumor necrosis factor-alpha IL-6 and neopterin. Neopterin is released only by activated macrophages, usually secondary to the influence of interferon-gamma released from activated T lymphocytes (9) and is therefore specific for macrophage activation.

Our study was undertaken to assess the activity of cell subsets within the cardiac allograft after transplantation and to explore the relation between such cellular activity and rejection. The concentrations of IL-2, IL-2 receptors, IL-4, IL-6, tumor necrosis factor-alpha and neopterin were determined in the coronary sinus, which receives 75% of the venous drainage from the myocardium. Levels were compared with those obtained from the superior vena cava at the time of routine myocardial biopsy after heart transplantation. The levels obtained were compared with the grade of rejection in endomyocardial biopsy samples taken at the same time.

We were able to identify macrophage activation within the allograft because of release of its specific cytokine neopterin. This activation was confirmed by elevated coronary sinus concentrations of IL-6 and tumor necrosis factor-alpha. The T lymphocyte activation was evident with elevated soluble IL-2 receptor levels in the coronary sinus; IL-2 production within the graft was not seen. The presence of T helper-2 lymphocyte activity within the graft was suggested by elevated coronary sinus levels of IL-4. We were unable to document any consistent elevation in the cytokine profile that correlated with rejection severity.

Methods

Sampling procedures and assays. Under a protocol approved by the Research With Human Subjects Review Board on January 15, 1990 and after written, informed consent was obtained from each patient, paired coronary sinus and superior vena cava blood samples were taken at the time of 85 routine myocardial biopsy procedures. From these samples, 40 were selected for batch analysis from patients monitored through an episode of rejection of varying severity during which serial sampling had been performed. A total of 10 patients were represented by this sample. All patients were maintained on a triple immunosuppressive regimen of cyclosporine, prednisone and azathioprine. Four patients were recruited during the initial posttransplantation phase and were receiving rabbit antithymocyte serum as induction therapy for the 1st 7 to 10 days. At least two studies were available for all patients, one at baseline and one during an episode of rejection. In two patients, 10 serial studies were performed at weekly intervals. Two patients had rejection >6 months after transplantation; one had a rejection episode >2 years after transplantation and the other received antithymocyte globulin for severe persistent rejection 6 months after transplantation.

With use of an internal jugular approach, a 7F multipurpose catheter and long-angled biopsy sheath (Cordis) were advanced into the coronary sinus under fluoroscopic guidance. The catheter was advanced to the level of midcoronary sinus to minimize reflux of right atrial blood. Injection of contrast medium confirmed catheter position, and cineangiography was undertaken to aid localization of the coronary sinus at subsequent studies. In this position, 50 ml of blood was collected. The catheter was then withdrawn to the superior vena cava and an additional 50 ml of blood was collected. Six routine right ventricular endomyocardial biopsy samples were then taken and assessed by a pathologist who had no knowledge of the results of the coronary sinus sampling. Serum was frozen at −20°C until batch analysis.

Concentrations of IL-2, IL-2 receptor, IL-4, tumor necrosis factor-alpha and IL-6 were assayed by using an enzyme-linked immunosorbent assay (ELISA, T Cell Sciences) and neopterin was assayed by radioimmunoassay (10). Rejection was assessed with the standardized criteria of the International Society for Heart Transplantation (11), and the presence or absence of rejection was considered with regard to need for treatment.

Statistical analyses. We hypothesized that if cytokines were being produced within the allograft, there should be a significant difference in cytokine concentration measured from the coronary sinus compared with that obtained during simultaneous sampling from the superior vena cava. However, because recirculation of the cytokine may, influence the concentration at either site, the simultaneous estimations were regarded as dependent variables. Hence, a paired *t* test was used to compare coronary sinus with superior vena cava cytokine concentrations in each patient, with significance defined as $p < 0.05$.

We further hypothesized that the cytokine concentration measured in the coronary sinus or superior vena cava or the difference between these two concentrations (as a measure of organ-specific release of cytokine) may show a correlation with the severity of rejection as determined on myocardial biopsy. We compared the cytokine concentrations measured in the absence of rejection with those measured during an episode of rejection in each patient. To allow for variations in cytokine concentrations within a patient over time, a repeated measures analysis of variance was used, with significance defined as $p < 0.05$.

Results

Successful entry and sampling of coronary sinus blood occurred in 85 of 93 attempts. No immediate or delayed complications were seen.

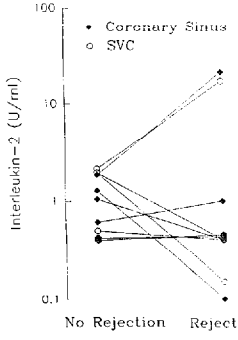


Figure 1. There is a lack of relation between coronary sinus and superior vena cava (SVC) interleukin-2 levels and rejection severity as seen from serial studies in seven patients. (Note the logarithmic scale.)

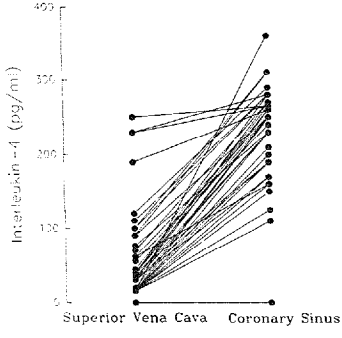


Figure 2. Paired coronary sinus and superior vena cava samples showing consistent interleukin-4 elevations in the coronary sinus ($p = 0.0001$).

Cytokine activity levels in the coronary sinus and superior vena cava. The results of cytokine activity determinations in both the superior vena cava and the coronary sinus are shown in Table 1. No IL-2 activity was detectable in either the coronary sinus or superior vena cava in patients receiving immunosuppressive therapy. There was no significant difference between coronary sinus and superior vena cava levels. The presence of rejection on biopsy did not correlate with a significant increase in either coronary sinus or superior vena cava IL-2 levels (Fig. 1).

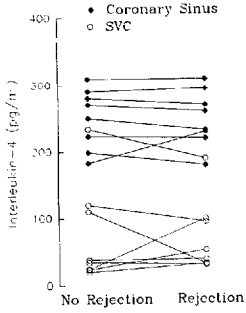
Soluble IL-2 receptor levels were similar in the coronary sinus (1,283 U/ml) and superior vena cava (1,232 U/ml) (<450 U/ml are found in normal volunteers). The four patients on antithymocyte serum therapy had IL-2 receptor levels of 1,857 ± 784 U/ml in the coronary sinus and 1,956 ± 888 U/ml in the superior vena cava. Those patients not receiving antithymocyte therapy had IL-2 receptor levels of 541 ± 176 U/ml in the coronary sinus and levels of 535 ± 197 U/ml in the superior vena cava ($p < 0.0005$ vs. antithymocyte therapy). There was no correlation between coronary sinus or superior vena cava IL-2 receptor levels and rejection severity.

Interleukin-4 activity is significantly elevated in the coronary sinus compared with peripheral levels (229 vs. 61 pg/ml, $p = 0.0001$) (Table 1). Activity was not suppressed in any patient during induction or maintenance immunosuppression. In no paired sample was the peripheral IL-4 level elevated compared with that in the coronary sinus (Fig. 2). We could find no relation between IL-4 levels and rejection severity, although there remained a consistent gradient between coronary sinus and peripheral IL-4 levels (Fig. 3).

Interleukin-6 activity in the coronary sinus (200 pg/ml) was significantly greater than venous activity (120 pg/ml, $p = 0.05$). In only 5 of 40 samples was the peripheral IL-6 activity higher than that seen in the coronary sinus (Fig. 4). No correlation with the presence of rejection could be found for IL-6.

Levels of tumor necrosis factor-alpha were higher in the coronary sinus than in the superior vena cava (68 vs. 17 pg/ml, $p = 0.0002$). In no instance was the peripheral level higher than the coronary sinus level (Fig. 5). In 12 of 40 samples, tumor necrosis factor-alpha was not detected in the peripheral sample, but was seen in the coronary sinus

Figure 3. The lack of correlation between interleukin-4 activity in the coronary sinus and superior vena cava (SVC) and biopsy grade of rejection are shown in eight patients with serial studies.



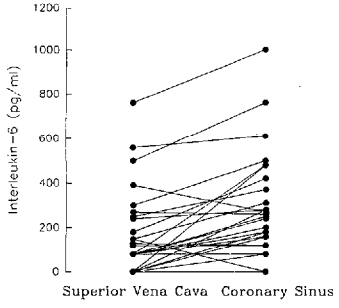


Figure 4. Paired coronary sinus and superior vena cava interleukin-6 estimations showing elevated coronary sinus activity ($p = 0.05$).

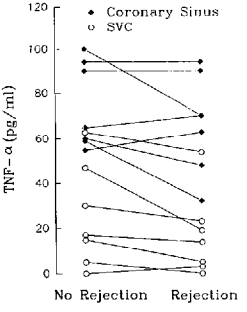


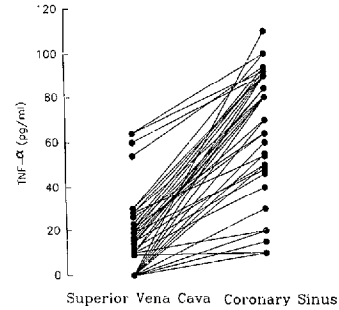
Figure 6. Coronary sinus and superior vena cava (SVC) tumor necrosis factor-alpha (TNF-α) activity showing no relation to rejection severity in seven patients with serial data.

sample. No correlation with rejection severity was found (Fig. 6).

Macrophage-specific metabolite neopterin levels were higher in the coronary sinus than in peripheral blood (2.5 vs. 2.2 nmol, $p = 0.08$) (Table 1). In only 2 of 40 samples was the coronary sinus neopterin concentration significantly lower than the peripheral level. No correlation to rejection severity was observed.

In one patient from whom samples were available from two serial episodes of rejection, there was a 2-week delay in biopsy evidence of rejection after elevated coronary sinus IL-6 levels in one episode and a 1-week delay for evidence of a second episode of moderate rejection occurring 4 weeks later (Fig. 7).

Figure 5. Paired coronary sinus and superior vena cava samples showing consistent elevations of coronary sinus tumor necrosis factor-alpha (TNF-α) ($p = 0.0002$).



Discussion

Coronary sinus sampling provides cardiac-specific venous effluent. This technique has proved useful in assessing myocardial metabolic and sympathetic activity. This study represents the first assessment of human in vivo allograft venous effluent sampling of cytokines to document immune activation within the transplanted heart. The concentration gradient between coronary sinus and peripheral blood should correlate with local cytokine production but will be influenced by 1) penetration from the interstitial space to the vascular space (spillover); 2) coronary blood flow; and 3) the serum clearance of the measured cytokine. Coronary sinus sampling was undertaken before biopsy to prevent artificial elevations in cytokines as a result of the mechanical trauma of the biopsy procedure. Care was taken to obtain samples at

Table 1. Venous and Coronary Sinus Cytokines After Heart Transplantation: Results From 40 Samples Taken at the Time of Endomyocardial Biopsy

	SVC	CS	p Value
IL-2 (U/ml)	2 ± 8	2 ± 5	NS
IL-2R (U/ml)	1,283 ± 937	1,232 ± 851	NS
IL-4 (pg/ml)	61 ± 63	229 ± 64	0.0001
IL-6 (pg/ml)	120 ± 180	200 ± 230	0.05
TNF-α (pg/ml)	17 ± 18	68 ± 27	0.0002
Neopterin (nmol)	2.2 ± 0.9	2.5 ± 1.3	0.08

Data are presented as mean value ± SD. CS = coronary sinus; IL = interleukin; IL-2R = soluble interleukin-2 receptor; SVC = superior vena cava; TNF-α = tumor necrosis factor-alpha.

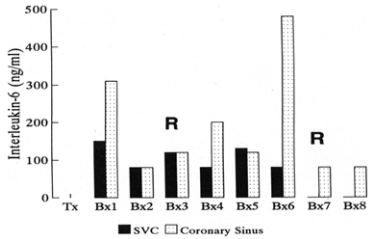


Figure 7. Serial weekly coronary sinus and superior vena cava (SVC) interleukin-6 estimations showing elevations of coronary sinus activity 2 weeks before the first rejection episode (R) and 1 week before the second episode. Bx = biopsy at weekly intervals; Tx = time of heart transplantation.

the level of the midcoronary sinus to avoid dilution of the sample with refluxing blood from the right atrium.

Ability of coronary sinus sampling to confirm site of cytokine production. In this study, the potential of coronary sinus sampling to confirm the site of cytokine production was established. For example, in 12 of 40 paired tumor necrosis factor-alpha samples, venous activity was undetectable, whereas activity in the coronary sinus measured 10 to 110 pg/ml. Previous studies (1,2) have been unable to find a relation between venous tumor necrosis factor-alpha levels and cardiac rejection. Similarly, specificity for a cardiac source of the cytokines is improved by considering the coronary sinus-superior vena cava gradient. All 28 estimations with elevated venous tumor necrosis factor-alpha levels had a positive coronary sinus-superior vena cava gradient, indicating a cardiac source.

Lymphocyte activation in cardiac allografts. In the samples assessed, coronary sinus sampling was unable to detect IL-2 activity, despite immune activation as shown by elevated IL-2 receptor levels. There was no clear evidence of IL-2 or IL-2 receptor production within the graft, although a long half-life of the soluble receptor in serum may account for the lack of a gradient between the coronary sinus and superior vena cava. The mean serum concentration of 1,323 U/ml is similar to previously published data (6). The inability to establish a significant elevation in soluble IL-2 receptor levels above baseline with an episode of rejection may be explained by patients in our study who were undergoing therapy with antilymphocyte preparations, which are known to cause significant baseline elevations in serum IL-2 receptor (13).

Interleukin-6 is produced in the allograft, as evidenced by significantly higher coronary sinus concentrations. This production may represent activation of two cell types already known to be present: macrophages with simultaneous production of tumor necrosis factor-alpha and neopterin, or T helper-2 cells with production of IL-4. Other potential sources of IL-6 include endothelial cells in the vessel wall, which are a major interface for immune activity (14). Interleukin 6 is nonspecific in indicating cell activation. The time course of cytokine production seen in the patient described in Figure 7 is compatible with macrophage activation occurring before lymphocyte activation and biopsy evidence of rejection. This may be important in predicting very early stages of immune activation, but a larger group of patients would be required to establish the time course of coronary sinus production. Interleukin-6 has been labeled as an "immune hormone" because of its ability to cause distant effects, such as fever, induction of acute phase proteins and induction of B cell differentiation (15). This syndrome is typical of symptomatic acute rejection. Long-term elevation of IL-6 levels in mice has been associated with the development of B cell neoplasias (16), raising the possibility that long-term elevation of IL-6 may be responsible for lymphomas after transplantation. Preliminary evidence (17) suggests that long-term elevation of IL-6 may be associated with the development of transplant atherosclerosis in the graft.

The specific elevations of coronary sinus IL-4 strongly suggest the presence of the T helper-2 lymphocyte subset within the graft, and this report independently confirms preliminary evidence for the presence and activation of this T lymphocyte subset in human transplant recipients. In murine models this T helper subset was not suppressed by conventional immunosuppressive therapy (18), a finding confirmed by the elevated levels in the coronary sinus in our group of patients receiving such therapy. Elevated IL-4 levels do not correlate with rejection, but a more complex relation should be sought. There is recent evidence that IL-4 may have an immunomodulating effect and that antibodies to IL-4 can produce acute rejection in rat allografts (Bolling SF, personal communication, October 1990).

Monocyte/macrophage activation in cardiac allografts. Cells of the monocyte/macrophage class constitute 30% to 60% of graft-infiltrating cells as detected by immunofluorescence in an autopsy study (19) of hearts explanted for fulminant rejection. The presence of macrophage activation in lesser degrees of rejection has been suggested by the presence of its major cytokine tumor necrosis factor-alpha in both serum (20) and tissue (16,21). There are alternate sources of small amounts of tumor necrosis factor-alpha (22) and therefore production of tumor necrosis factor-alpha does not indicate specific mononuclear subset activation.

Serum levels of neopterin were measured in both the coronary sinus and superior vena cava because neopterin is produced only by activated macrophages (23). The elevation of coronary sinus neopterin levels compared with superior vena cava concentrations confirms macrophage activation within the graft and suggests that macrophages may also be the source of the tumor necrosis factor-alpha and IL-6. The long half-life of neopterin in serum (70 to 200 min), particularly in patients with renal impairment (23), may explain the elevated superior vena cava concentrations and lack of a

more significant gradient between the coronary sinus and superior vena cava.

The significance of long-term macrophage activation as seen in patients weeks to months after transplantation is unclear. A complex relation with macrophage activation modulating or preceding rejection is suggested by the cytokine profile in one patient (Fig. 7) and remains to be confirmed. Elevations in serum neopterin (24) and IL-6 (5) have been shown to precede biopsy evidence of rejection by up to 3 days. Coronary sinus sampling should allow the in vivo assessment of interactions among cytokines. In vitro and in mice, IL-6 has been shown to inhibit the formation of tumor necrosis factor-alpha (25); this did not appear to be the case in our patients because tumor necrosis factor-alpha levels were elevated in the coronary sinus in all patients. Tumor necrosis factor-alpha is a known potent inducer of IL-6 (26); this effect may predominate because simultaneous elevations of both cytokines were seen in our series. In vitro, the production of tumor necrosis factor-alpha is inhibited by corticosteroid therapy but not by cyclosporine (27). This was not apparent in our patients, who were all receiving triple immunosuppressive therapy.

The patients included in this study were selected from a larger group because of the presence of a rejection episode early or late in the course of transplantation. It is possible that elevations of IL-4 or IL-6, even in the absence of concurrent biopsy rejection, may identify a group of patients with a greater level of baseline immune activation who are at increased risk for recurrent rejection.

Conclusions. Coronary sinus sampling of cytokines provides the ability to measure graft- and cell-specific immune activation. It allows the site of production of cytokines to be established, confirms the presence and activation of macrophages within a cardiac allograft and suggests the presence and activity of the T helper-2 subset within the graft. It provides independent confirmation for the production of cytokines within the graft, as suggested by cytokine gene transcription studies in biopsy material (28). Further study of immune activation markers from organ effluent should provide insights into clinical immunobiology, mechanisms of graft rejection and in vivo assessment of immunosuppressive regimens.

References

1. Holzinger C, Zuckerman A, Seitelberger R, Laufer G, Laczkovics A, Wolner E. Monitoring of mononuclear cells from coronary sinus and right atrium blood in patients following heart transplantation (abstr). J Heart Transplant 1990;9:63A.
2. Roodman ST, Miller LW, Tsai CC. Role of interleukin 2 receptors in immunologic monitoring following cardiac transplantation. Transplantation 1988;45:1050–4.
3. Fieguth HG, Haverich A, Hadam M, Kemnitz J, Dammnehayn L. Correlation of interleukin-2 receptor positive circulating lymphocytes and acute cardiac rejection. Transplant Proc 1989;21:2517–9.
4. Sunder-Plabmann G, Stockenhuber F, Balcke P. Serum interleukin 2 activity in renal graft recipients. Transplant Proc 1988;20:387–9.
5. Van Oers MHJ, Van Der Heyden APAM, Aarden LA. Interleukin 6 in serum and urine of renal transplant recipients. Clin Exp Immunol 1988; 71:314–9.
6. De Maria R, Zuccelli GC, Masinis S, et al. Nonspecific increase of interleukin-2 receptor serum levels during immune events in heart transplantation. Transplant Proc 1989;21:440–1.
7. Cherwinski HM, Schumacher JH, Brown KD, Mosmann TR. Two types of murine helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays and monoclonal antibodies. J Exp Med 1987;166:1229–35.
8. Swain SL, McKenzie DT, Weinberg AD, Hancock W. Characterization of T helper 1 and 2 cell subsets in normal mice. J Immunol 1988;141:3445–55.
9. Smillie AE, Rigby RJ, Petrie JJB. Monitoring the response to anti-rejection therapy with serum neopterin. Transplant Proc 1989;21:1869–70.
10. Werner ER, Bichler A, Daxenbichler G, et al. Determination of neopterin in serum and urine. Clin Chem 1987;33:62–6.
11. Billingham ME, Cary NRB, Hammond ME, et al. A working formula for the standardization of nomenclature in the diagnosis of heart and lung rejection: heart rejection study group. J Heart Transplant 1990;9:587–92.
12. Kobashigawa J, Stevenson LW, Loutc H, et al. Elevated serum tumor necrosis factor is not useful for monitoring cardiac transplant rejection (abstr). J Am Coll Cardiol 1991;17:273A.
13. Colvin RB, Fuler TC, MacKeen I, Kung PC, Ip SH, Cosimi AB. Plasma interleukin 2 receptor levels in renal allograft recipients. Clin Immunol Immunopathol 1987;43:273–4.
14. Libby P, Salomon RN, Payne DD, Schoen FJ, Pober JS. Functions of vascular wall cells related to development of transplantation-associated coronary arteriosclerosis. Transplant Proc 1989;21:3677–84.
15. Le J, Vilcek J. Biology of disease: interleukin 6. A multifunctional cytokine regulating immune reactions and acute phase protein response. Lab Invest 1989;61:588–602.
16. Suematsu S, Matsuda T, Aozasa K, et al. IgG_1 plasmacytosis in interleukin 6 transgenic mice. Proc Natl Acad Sci USA 1989;86:7547–51.
17. Leavy JA, Marboe CC, Barr ML, Rose EA, Brett J, Stern D. Myocardial interleukin-6: a marker for accelerated graft atherosclerosis following cardiac transplantation (abstr). Circulation 1991;84(suppl II):II-486.
18. Heeg K, Gillis S, Wagner H. IL-4 bypasses the immune suppressive effect of cyclosporine A during the in vitro induction of murine cytotoxic T lymphocytes. J Immunol 1988;141:2330–4.
19. Zerbe TR. Contrast of mediators: identification of cells and cytokines in human heart tissue with acute rejection and myocarditis. Transplant Proc 1989;21:443–4.
20. Lowry RP, Blais D. Tumour necrosis factor-α in rejecting rat cardiac allografts. Transplant Proc 1988;20:245–6.
21. Arbustine E, Grasso M, Diegoli M, et al. Expression of tumor necrosis factor in human acute cardiac rejection. Am J Pathol 1991;139:709–15.
22. Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: IL 6 and related molecules (IL 1 and TNF). FASEB 1990;4: 2860–7.
23. Autlizky WE, Tilg H, Niederwieser D, et al. Comparison of serum neopterin levels and urinary neopterin excretion in renal allograft recipients. Clin Nephrol 1988;29:248–52.
24. Havel M, Laczkovics A, Teufelsbauer H, Muller MM, Wolner E. Neopterin as a new marker to detect rejection after heart transplantation. J Heart Transplant 1989;8:167–70.
25. Aderka D, Le J, Vilcek J. IL-6 inhibits lipopolysaccharide induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. J Immunol 1989;143:3517–23.
26. Kishimoto T. The biology of interleukin-6. Blood 1989;74:1–10.
27. Dupont E, Schandene L, Denys C, Crusiax A, Wybran J. Assessment of production of tumor necrosis factor-α under the influence of immunosuppressive drugs. Transplant Proc 1989;21:70–1.
28. Dallman MJ, Larsen CP, Morris PJ. Cytokine gene transcription in vascularised organ grafts: analysis using semiquantitative polymerase chain reaction. J Exp Med 1991;174:493–6.