

Serial study of C reactive protein concentrations in cardiac allograft recipients

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SUMMARY C reactive protein (CRP) concentrations were measured serially in 38 patients after cardiac transplantation. Three of 28 patients (11%) had raised values before transplantation. After transplantation, most patients showed rises in CRP concentrations associated with transplant surgery which became normal by day 7. Thereafter, 75/274 samples (28%) from 18/38 patients (47%) had raised CRP values. Most of the rises in CRP concentration were associated with infection (78%), which in most cases was bacterial. No correlation was found between CRP values and acute cardiac rejection assessed histologically on serial endomyocardial biopsy.

The clinical course of patients receiving allografts is complicated by the induction of a variety of iatrogenic abnormalities. For example, the potent immunosuppressive effects of steroids or cytotoxic drugs such as cyclosporin A and antithymocyte globulin may result in increased susceptibility to infection or neoplasia.¹ The administration of heterologous antithymocyte globulin may also induce serum sickness like reactions in some patients and in combination with other immunosuppressive drugs may result in reactivation or potentiation of herpes viral infections.^{2,3}

Recent investigations in cardiac allograft recipients have shown that many patients develop immunological disturbances including de novo generation of circulating immune complexes and pronounced C4 hypocomplementaemia.⁴ Bacterial and herpes viral antigens as well as antithymocyte globulin have been implicated as constituents of these circulating immune complexes.^{5,6} In addition, many patients transiently produce a range of antiheart antibodies.⁷ None of these variables, however, has been shown to correlate with acute cardiac rejection.

In the present study concentrations of the acute phase reactant C reactive protein (CRP) in heart transplant recipients were examined. This work was performed to determine whether measurement of CRP values would aid the identification of intercur-

rent infections, the differentiation between bacterial and viral infections, and the diagnosis of acute cardiac rejection.

Material and methods

Thirty eight patients with either terminal ischaemic heart disease or cardiomyopathy receiving cardiac allografts at Papworth Hospital, Papworth-Everard, Cambridgeshire, were studied. Patients' ages ranged from 19 to 52 years, with a mean age of 40.4 years. Blood samples were taken before transplantation and serially thereafter. The samples were taken either daily or weekly for the first six weeks, then less often for variable periods up to 6 months. The samples were stored at -70°C until assayed. Three groups of patients were studied: group 1 (n = 15) received either equine antithymocyte globulin (0.25-1.0 g/day) (Upjohn Company, Kalamazoo, Michigan) or rabbit antithymocyte globulin (50-200 mg/day) (kindly supplied by Dr CP Bieber, Stamford Medical Centre); group 2 (n = 5) received cyclosporin A (Sandoz Ltd) (18-10 mg/kg/day over five weeks); group 3 (n = 25) received both antithymocyte globulin and cyclosporin A. The antithymocyte globulin in this group was given for the first 10 days only and to control later rejection episodes if required.

Blood samples were also obtained from normal donors (n = 50) attending the Blood Transfusion Service in Cambridge and were processed similarly.

INFECTIONS

Eight patients had bacterial infections caused by

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streptococci, klebsiella, pseudomonas, listeria, or micrococci with two unidentified infections. Seven patients had viral infections caused by herpes simplex virus, cytomegalovirus, or mumps. One patient had pulmonary aspergillus infection and one patient had disseminated toxoplasmosis.

CARDIAC REJECTION

The presence and severity of rejection of transplanted hearts were estimated by noting changes in daily electrocardiogram voltages and by endomyocardial biopsy. The latter was normally undertaken at intervals of about 10 days. The presence of rejection was confirmed if histological examination of the biopsy showed cellular infiltration or myofibrillar disruption or both.

CRP DETERMINATIONS

CRP concentrations were measured on a Beckman ICS Auto Analyser II, using CRP kits supplied by Beckman Instruments. CRP values in 50 normal human serum samples were consistently below 3 mg/100 ml. This value was taken as the upper limit of normal.

Table 1 *C reactive protein (CRP) concentrations in heart transplant recipients during the first week after transplant*

| Post transplant day | No of patients | CRP* Median value (range) |
|---------------------|----------------|---------------------------|
| Pre-transplant† | 25 | 0.7 (<0.6-2.2) |
| 1 | 11 | 6.4 (<0.6-14.2) |
| 2 | 9 | 12.7 (<0.6-15.0) |
| 3 | 5 | 9.9 (<0.6-21.6) |
| 4 | 8 | 7.5 (<0.6-14.5) |
| 5 | 7 | 2.6 (<0.6-2.6) |
| 6 | 7 | 2.0 (<0.6-6.7) |
| 7 | 9 | 1.5 (<0.6-3.9) |

*Values expressed as mg/100 ml.

†Pre-transplant values excluding three patients with grossly raised CRP concentrations.

Table 2 *C reactive protein (CRP) rises in relation to infection in heart transplant recipients*

| | No positive/no tested (%)* | | |
|-------------------------------|----------------------------|---------------|--|
| | Antithymocyte globulin | Cyclosporin A | Cyclosporin A + antithymocyte globulin |
| Total† | | | |
| Samples | 61/201 (30) | 0/18 (0) | 14/55 (26) |
| Patients | 9/18 (50) | 0/5 (0) | 9/15 (60) |
| Associated with infection | | | |
| Samples | 48/201 (24) | 0/18 (0) | 3/55 (6) |
| Patients | 8/18 (44) | 0/5 (0) | 2/15 (13) |
| Not associated with infection | | | |
| Samples | 13/201 (7) | 0/18 (0) | 11/55 (20) |
| Patients | 6/18 (33) | 0/5 (0) | 8/15 (53) |

*Treatment groups comprise antithymocyte globulin (n = 18), cyclosporin A (n = 5), and cyclosporin A + antithymocyte globulin (n = 15).

†Excluding measurements taken during first seven days.

STATISTICS

The results were analysed by the Mann-Whitney U rank test.

Results

CRP VALUES BEFORE TRANSPLANTATION

Pretransplant samples were available from 28 patients. With the exception of three patients who had high concentrations (9.8, 7.0, 11.5 mg/100 ml), values in these samples were low or unmeasurable. A median value of 0.72 mg/100 ml (range <0.6-11.50) was obtained for all 28 patients, though when the three patients with high values were excluded the median value was 0.70 mg/100 ml (range <0.6-2.17). Two of these three patients were successfully transplanted and were still alive at one year. The third patient, described previously,⁸ developed toxoplasmosis after transplantation and died on day 42.

CRP VALUES AFTER TRANSPLANTATION

Ninety nine of 321 samples (31%) from 28/38 (74%) patients had raised CRP values after transplantation. In groups 1, 2, and 3, 76/228 (33%), 5/29 (17%), and 18/64 (28%) samples respectively had increased concentrations.

CRP INCREASES ASSOCIATED WITH TRANSPLANTATION

Daily samples were available from a few patients during the first seven days after transplant. CRP measurements over this period showed a sharp rise, usually within 48 h of operation, which peaked at days 2-3 (Table 1). The CRP concentrations were almost back to pretransplant values by day 7.

RELATION BETWEEN CRP VALUES AND INFECTION

Analysis of the relation between CRP values and

Table 3 C reactive protein (CRP) concentrations during different types of known infective episodes in heart transplant recipients

| Type of infection* | No of patients with raised CRP values/no tested (%)† | No of samples with raised CRP values/no tested (%)† | CRP concentration‡ Median value (range) |
|--------------------|--|---|---|
| Bacterial | 7/8 | 21/24 | 4.6 (<0.6–24.0) |
| Viral | 3/6 | 3/10 | 0.7 (<0.6–7.1) |
| Fungal | 1/1 | 1/1 | 5.3 |
| Toxoplasmal | 1/1 | 8/9 | 8.8 (2.9–14.1) |
| Total | 12/16 (75) | 33/44 (75%) | 5.3 (<0.6–24.0) |

*See methods section for details.

†Samples taken during the infection period were considered raised if they exceeded 3 mg/100 ml.

‡Values expressed as mg/100 ml.

infection was performed on data obtained from day 8 onwards to avoid incorporating the rise in CRP normally associated with transplant surgery during the first week after transplant. The results were analysed by comparing the incidence of CRP positivity in samples taken during recognised clinical infections with samples obtained at other times.

A total of 274 samples from 38 patients were tested (Table 2). Over half the patients receiving antithymocyte globulin alone showed rises in CRP concentration at some time after day 7, with about a third of the samples having high values. Five patients receiving only cyclosporin A showed no rises in CRP. Of the samples with raised CRP values in group 1 48/61 (79%) were associated with infection. In contrast, only 3/14 (6%) samples with raised CRP values were associated with infection in group 3. The CRP data were further analysed in relation to the type of infection (Table 3). Raised CRP values were found more consistently in patients with known bacterial infections than in those with viral infections. CRP values were also raised in the patient with an aspergillus infection and in another with toxoplasmosis.

CRP VALUES AND ACUTE CARDIAC REJECTION

The association between CRP concentration and acute rejection was analysed by comparing CRP values in samples taken the same day as a cardiac

biopsy (Table 4). About half the biopsies showed some evidence of acute rejection. The rank values of CRP of the biopsy positive and biopsy negative groups were not significantly different. Similar results were obtained when the biopsy positive group was split into three grades of rejection severity and each grade compared with the biopsy negative group.

Although the results in Table 4 indicate a lack of correlation between CRP values and cardiac rejection, the association of increased CRP concentration with infection may have obscured a relation with rejection. The data were thus edited as far as possible to exclude CRP rises associated with recognised infection, and then analysed in relation to the cardiac biopsy results. Thirteen samples with raised CRP concentrations unassociated with infection gave a median value of 4.4 mg/100 ml (range 2.2–12.0). Six of 13 biopsies taken at the same time were positive for rejection (46%), with a median value of 5.4 mg/100 ml (range 3.1–12.0). The biopsy negative group had a median value of 4.3 mg/100 ml (range 2.2–6.6). The CRP values were conversely examined in all samples with a corresponding biopsy showing rejection. The results showed that 8/37 (22%) samples had raised CRP concentrations, with a median value of 0.7 mg/100 ml (range <0.6–11.8). These results indicate that even when clinical infections are eliminated from the data, raised CRP values do not correlate with rejection. Similarly, when only positive biopsies are considered, median CRP values are low.

Table 4 C reactive protein (CRP) values and acute rejection in 30 heart transplant recipients

| Biopsy result* | No of biopsies | CRP concentration‡ Median value (range) |
|----------------|----------------|---|
| Negative | 59 | 0.9 (<0.6–20.9) |
| Positive† | 61 | 0.8 (<0.6–11.8) |
| Grade 1 | 40 | 0.8 (<0.6–11.8) |
| Grade 2 | 13 | 1.8 (<0.6–5.8) |
| Grade 3 | 8 | 0.8 (<0.6–4.0) |

*Rejection was assessed histologically on serial endomyocardial biopsies.

†Grade 1, 2, and 3 indicate minimal, moderate, and severe rejection respectively.

‡CRP values (mg/100 ml) in sera taken on same day as the cardiac biopsy.

Discussion

Much information has emerged recently on the role of CRP in disease.⁹ For example, CRP opsonises pathogenic bacteria, interacts with various products of inflammation, and fixes human complement.^{10 11} CRP concentrations are normally low¹² but rise predictably two to three days after various forms of operation^{10 13 14} and fall rapidly over the next two days. This pattern was also found in the heart transplant recipients studied here.

CRP concentrations also rise dramatically in patients with bacterial infections,^{9,10} but only modestly, if at all, in most viral infections.¹⁵⁻¹⁷ One recent report, however, has shown that CRP values rise substantially in patients with measles, though only when the rash appears or if the disease is complicated by pneumonia.¹⁸ CRP titres have also been found to be raised during rubella viral exanthem.¹⁹ In heart transplant recipients the rises in CRP values were mostly associated with the presence of infection in patients receiving antithymocyte globulin only (Table 2). Patients receiving cyclosporin A only had no rises in CRP, however, while those receiving both antithymocyte globulin and cyclosporin A gave a negative association with clinical infection. The explanation of this result is not clear. It is possible that infection was present sub-clinically in these patients. Certainly, cyclosporin A in low doses induces fewer infections, with a lower incidence and severity of acute rejection compared with antilymphocyte serum or antithymocyte globulin,²⁰ and this seems to be the case in the patients in this study. This suggests that the rises in CRP concentration in group 3 were due either to infection rendered sub-clinical by cyclosporin A treatment or to some other cause of inflammation.

Analysis of the type of clinical infection found in heart transplant recipients showed that the incidence and degree of raised CRP values were much higher during episodes of bacterial infection than viral infection (Table 3). It is not certain, however, whether the three instances of increased CRP values associated with herpes virus infection were the consequence of such infection or a coincidence. Certainly, analysis of circulating immune complex components in these patients suggests the presence of subclinical bacterial infection.⁶ Increases in CRP seen in the patient with aspergillus infection are consistent with the known ability of CRP to bind aspergillus extracts.¹⁰ It is also of interest to note that CRP values were consistently high in the patient with fatal toxoplasmosis.

In addition to intercurrent infections, heart transplant recipients undergo episodes of acute rejection of varying severity. The use of endomyocardial biopsy allows the diagnosis of rejection to be made on a histological basis and the degree of rejection to be graded. A recent report presented evidence supporting correlation between acute rejection of renal allografts and CRP titres.²¹ CRP values were associated in 35/38 renal rejection episodes, with raised CRP concentrations occurring on average 4-4 days before the rejection episode. Analysis of CRP values in the heart transplant recipients did not, however, show any clear cut association with cardiac rejection.

The reasons for the differences between the two types of transplant patients are not clear. They could be related to use of renal function indices versus cardiac biopsy as the method of diagnosing rejection, or to more severe inflammatory reactions occurring during kidney rejection. Alternatively, the presence of infection both clinical and sub-clinical could obscure any relation between CRP values and rejection. Even after editing the data to exclude periods of clinical infection, however, no relation between CRP values and acute rejection was evident. These observations are similar to those made in bone marrow transplant recipients, in whom increases in CRP concentrations correlated with infection and not with graft versus host disease.²²

The present data thus indicate that measurement of CRP concentrations would be useful in discriminating between infection and symptoms of acute cardiac rejection.

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