THE USE OF C-REACTIVE PROTEIN IN THE DIAGNOSIS OF RENAL ALLOGRAFT REJECTION

M K Chan, R G Ye, B Jones, K K Wong, M K Li

SYNOPSIS

Serum C-reactive protein (C-RP) concentrations were monitored post-renal transplantation. In patients who received HLA-identical grafts without rejection, serum C-RP concentrations peaked on the second post-operative day and decreased to below 0.5mg/dl from the 4th post-operative day onwards. A > 40% rise in serum C-RP predicted 86.9% of rejection episodes. The sensitivity was increased to 95% if chronic vascular rejections were excluded. A rise in serum C-RP preceded a rise in serum creatinine by a mean of 0.95 days. Peak C-RP concentrations correlated with peak creatinine concentrations ($r = 0.60$ $p < 0.001$). The specificity of a > 40% rise in serum C-RP in diagnosing rejection was 95%. Several distinct patterns of serum C-RP changes were encountered and the significance of these were discussed. Serial measurements of serum C-RP is useful in the diagnosis and management of renal allograft rejection.

SING MED J. 1988; 29:145–149

INTRODUCTION

Many immunological tests have been proposed for the diagnosis of renal allograft rejection (1-4). Unfortunately, most of these cannot be performed routinely and are consequently of little value in the day-to-day management of patients. We have used quantitative measurements of C-reactive protein (C-RP) prospectively to assist the diagnosis of allograft rejection and our 1-year experience with this test is reported here.

PATIENTS AND METHODS

From the end of March 1985 to the beginning of April 1986, we routinely measured C-RP concentrations in renal transplant recipients. Serum C-RP concentrations were measured by rate nephelometry using the COBAS B10(R) centrifugal analyzer (Hoffman-La Roche, Basle, Switzerland). Anti-C-RP antiserum, nephelometric grade, was obtained from Unipath (Bedford, UK), and was used at a dilution of 1/100 in polyethylene glycol 6000, 4% in saline. The C-RP standard was from Behringwerke (Marburg, Germany), and was used at 0.5, 1.0, 3.0, 5.0, 7.0 and 10.0 mg/dl to obtain a calibration curve. The control serum (Behringwerke) and patients' sera were diluted 1/21 in saline for testing. Samples with C-RP values exceeding 10.0 mg/dl were re-tested at 1/210. During this period, there were 19 renal transplants (14 from living related donors and 5 from cadaveric donors). Immunosuppression consisted of azathioprine and low dose prednisolone in 15 patients and triple therapy (azathioprine, prednisolone and cyclosporin A) in 4 patients. Serum C-RP as well as creatinine concentrations were measured daily from the day of operation till the patient was discharged and at every follow up visit.

Rejection was diagnosed when there was a rise of serum creatinine of > 0.02 mmol/l for no obvious reasons such as obstructive uropathy. Fourteen biopsies of the allograft were performed in 11 patients. Rejection episodes were treated by intravenous methylprednisolone pulses which were followed by anti-thymocyte globulin on 3 occasions. Diagnosis of rejection was considered definite if there was histological evidence on renal biopsy and probable if there was a significant rise in serum creatinine which returned to normal with anti-rejection therapy. Altogether 23 episodes of rejection were encountered in 15 patients, 13 of whom had serial serum C-RP measurements from the day of operation and 2 were patients transplanted prior to March 1985.

RESULTS

Six patients received HLA identical kidneys and post-operatively no rejection episodes occurred within 2 weeks. The profile of their mean serum C-RP is shown in Figure 1. The mean serum C-RP rises immediately postoperatively to a peak value on the second day and falls to below 0.5 mg/dl from the fourth day onwards. There was no evidence of infections in these patients.

Table 1 shows the peak concentrations of serum C-RP and the corresponding values in serum creatinine in patients with definite rejection and in those with probable rejection episodes. The rise in serum C-RP concentration preceded the rise in serum creatinine concentration by 0.95 ± 0.37 days (mean ± s.e.m.). Only on 2 occasions did the rise in serum C-RP con-

Department of Medicine
Queen Mary Hospital
Hong Kong
M K Chan
R G Ye

Department of Pathology
Queen Mary Hospital
B Jones

Government Surgical Unit
Queen Mary Hospital
K K Wong

University Department of Surgery
Queen Mary Hospital
M K Li

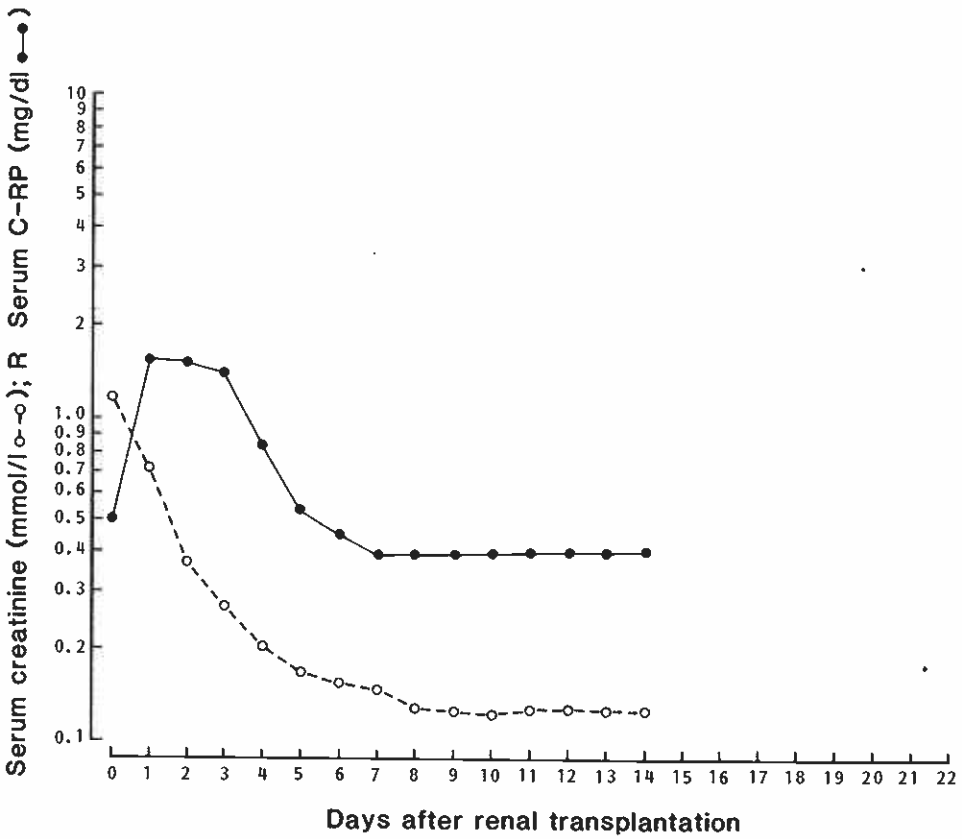

Fig. 1 Profile of C-reactive protein and serum creatinine after renal transplantation between identical siblings.

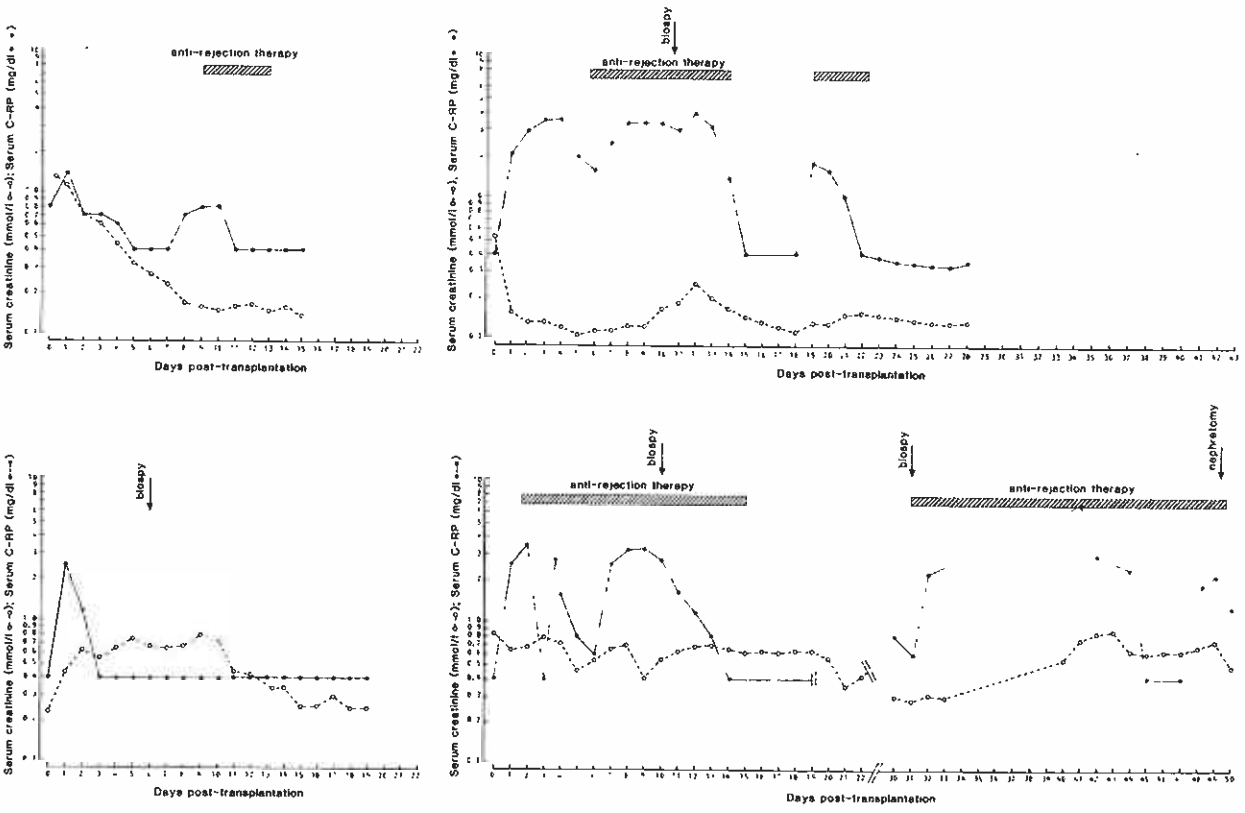

Fig. 2 Examples of different patterns of response in C-reactive protein in renal allograft recipients.
Left Upper: Acute reversible rejection
Left lower: Acute tubular necrosis in allograft
Right upper: Repeated rejection episodes
Right lower: Irreversible rejection

Table 1 Changes in serum C-Reactive protein and serum creatinine concentrations during renal allograft rejection

Rejection episode	Time (days) after transplant	Peak serum C-RP (mg/dl)	Duration (days) of C-RP elevation	Peak serum creatinine (mmol/l)	Duration (days) creatinine elevation	Time lag (days) between C-RP and creatinine	Transplant biopsy
1	183	7.0	7	0.534	8	0	Rej.
2	319	2.6	2	0.781	12	-1	Rej.
3	8	0.6	3	0.287	15	-2	N.D.
4	68	1.8	3	0.192	3	0	Rej.
5	49	1.8	2	0.500	8	0	N.D.
6	31	3.2	6	0.190	14	0	Rej.
7	6	3.5	8	0.253	10	-3	Rej.
8	18	1.8	4	0.160	10	-1	N.D.
9	2	3.5	3	0.912	10	-1	Rej.
10	8	0.7	2	0.215	12	-2	Rej.
11	7	0.7	11	0.130	14	3	Rej.
12	80	0.7	13	0.200	15	-3	Rej.
13	4	4.0	9	0.190	16	0	N.D.
14	11	0.7	1	0.280	12	-2	Rej.
15	7	2.3	10	0.200	13	2	N.D.
16	12	1.5	2	0.275	12	0	N.D.
17	9	0.8	2	0.170	4	-4	N.D.
18	6	0.9	6	0.180	6	-1	N.D.
19	10	3.3	11	0.690	17	-4	Rej.
20	30	7.1	16	0.878	**	0	Rej.
21	426	0.5	*	0.400	**	*	Rej.(chronic)
22	32	0.5	*	0.180	10	*	Rej.(chronic)
23	12	1.9	5	0.180	4	-2	N.D.
Mean ± S.E.M.	58±23	2.23±0.39	6.0±0.9	0.347±0.051	10.7±0.9	-0.95±0.39	

* not applicable

** never returned to pre-rejection values

centrations lag behind that of serum creatinine.

The peak serum C-RP concentrations correlate significantly with peak serum creatinine concentrations ($r = 0.60$, $p < 0.001$). The peak serum C-RP also correlates significantly with the time which serum C-RP takes to return to baseline values ($r = 0.46$, $p < 0.05$). The time taken for serum C-RP concentrations to return to baseline values correlates with that taken by serum creatinine concentrations to return to pre-rejection values ($r = 0.46$, $p < 0.05$). Somewhat surprisingly, peak serum creatinine concentrations do not correlate with the time serum creatinine concentrations take to return to pre-rejection values.

The sensitivity of a rise in serum C-RP concentration in the diagnosis of allograft rejection is calculated by the formula: total no. of episodes of (> 40%) rise in serum C-RP concentration/total no. of rejection episodes = 20/23 = 86.9%. If the two episodes of chronic vascular rejection (proven on biopsy) were excluded, the sensitivity increases to 20/21, i.e. 95%. The specificity of the test is given by the formula: no. of rejection episodes/no. of episodes of (> 40%) rise in serum C-RP = 20/21 = 95%. However, in the 1 case where a rise in serum C-RP was not followed by allograft rejection, the patient had severe acute pyelonephritis affecting the allograft resulting in E. Coli septicaemia.

Figure 2 shows the different patterns of changes in serum C-RP concentrations observed in our patients. Firstly, a rise in serum C-RP preceding a small rise in serum creatinine allograft biopsy confirms acute cellular rejection (left upper, fig 2). Secondly, an allograft which has acute tubular necrosis shows no appreciable function clinically. Serum C-RP profile is normal. A biopsy of the allograft confirms acute tubular necrosis and anti-rejection therapy is avoided (left lower, fig 2). Thirdly, serum C-RP rises again after an initial decrease. Anti-rejection therapy returns serum C-RP to baseline levels. At the same time serum creatinine concentration improves. A second smaller rise in C-RP is followed by significant deterioration in renal function, signaling the onset of another rejection (right upper, fig 2). Fourthly, serum C-RP concentrations rise intermittently despite anti-rejection therapy. The graft is lost as a result of irreversible rejection (right lower, fig 2).

DISCUSSION

To be of value to the clinician, the result of any test for the detection of allograft rejection must be available on the day blood is sampled. Rate nephelometry appears to be a simple and rapid method for quantitative measurements of serum C-RP concentrations. C-RP is an acute phase reactant(5) and its concentration in the serum rises in response to any tissue injury. This is evident from the fact that serum C-RP concentration rises immediately after renal transplantation. In the absence of rejection, serum C-RP concentrations return to normal baseline values after 4 days. Thus if serum C-RP concentrations remain persistently elevated after the fourth postoperative day, in the absence of any signs of gross infection, allograft rejection must be suspected even if the allograft may have suffered from primary non-function. We have shown that based on a > 40% rise in serum creatinine concentration, allograft rejection can be diagnosed in 86.9% of cases. Serum C-RP concentrations do not rise during chronic rejection. This is perhaps not unexpected as the protein is an acute phase reactant. When chronic rejection episodes are excluded from consideration, the sensitivity of the test increases to 95%. The only false positives occur with severe sepsis and as gross infections are often clinically obvious, such 'false positives' do not diminish the value of the test. It is worthy of note that a rise in serum C-RP heralds cellular rejection since it precedes increases in serum creatinine in many instances. It is also important to point out that upon anti-rejection therapy, serum C-RP concentration often returns towards baseline before serum creatinine does. The peak of serum C-RP concentration correlates with that of serum creatinine. Thus it reflects the severity of the rejection. That the peak serum creatinine does not correlate with the time taken for serum creatinine concentration to return to pre-rejection values implies that not all rejection episodes are completely reversible. The duration of rise in serum C-RP concentrations correlates with that of the increase in serum creatinine concentrations. Thus not only does a rise in serum C-RP provide an earlier diagnosis of rejection than does an increase in serum creatinine, persistent elevation in serum C-RP concentrations during anti-rejection therapy carries a grave prognosis for the outcome of the graft. Our results are in close agreement to those obtained by other investigators(6–10). The use of laser nephelometry(6) makes it possible to provide results within 24 hours of sample collection. We contradict the claim that C-RP is less effective in predicting the onset of very early rejection episodes which occur less than 7 days after renal transplantation(11) and agree that a persistently raised or intermittently raised serum C-RP despite anti-rejection therapy carries a grave prognosis(8). Serial serum C-RP monitoring is especially useful in the evaluation of renal allografts with primary non-function. Our documentation of serum C-RP profile in patients who receive HLA identical renal transplants without rejection and the observation that peak and duration of serum C-RP correlate with the severity of rejection while chronic rejection is not accompanied by a rise in serum C-RP concentrations(10) further define the role of serum C-RP monitoring in renal transplantation. Recently another acute phase reactant, serum amyloid A protein, has been shown to be even more sensitive than serum C-RP in predicting renal allograft rejection(9,12).

REFERENCE

1. Kerman RH, Geis WP: Total and active T cell dynamics in renal allograft recipients. Surgery 1976; 79:398–407.
2. Byfield PE, Barth CL, Brodie JA, et al: The use of circulating rosette-forming cells as a guide to determination of ATG dose (Dose-by-Rosette). Transplant Proc 1978; 10:627–31.
3. Thomas J, Thomas F, Edwards E, Hoffman S, Lee HM: Regulations of T cell mediated cytotoxic allograft response in human transplant recipients. Transplant Proc 1981; 13:1594–98.
4. Tanimura S, Taguchi Y, Kunori T, Satomi S, Kaneda I, Kasai M: Monitoring of in vitro blastogenic response in kidney transplant patients by flow cytometry. Transplant Proc 1985; 17:652–5.
5. Hurliman J, Thorbecke GJ, Hochwald GM: The liver as the site of C-reactive protein formation. J Exp Med 1966; 123:365–78.
6. Laurent P, Touraine JL, Malik MC, Zeller R, Traeger J: Monitoring of C-reactive protein after renal transplantation using a laser nephelometric method. Transplant Clin Immunol 1980; 12:175–81.
7. White J, Meyer E, Hardy MA: Prediction of onset and termination of renal allograft rejection by serum levels of C-reactive protien. Transplant Proc 1981; 13:682–5.
8. Groth J, Kaden J, Hoffmann P, Muller K, Mrochen H, Mebel M: C-reactive protein and clinical kidney transplantation. Nephron 1984; 37:236–39.
9. Maury CPJ, Teppo AM: Comparative study of serum amyloid-related protein SAA, C-reactive protein and beta-2-microglobulin as markers of renal allograft rejection. Clin Nephrol 1984; 22:284–92.
10. Lempert N, Freed B, Godlewski J, et al: C-reactive protein: An indicator of rejection episode severity in renal allograft recipients pre-treated with donor-specific transfusions. Transplant Proc 1985; 17:662–4.
11. Freed B, Walsh A, Pietrocola D, MacDowell R, Lempert N: Early detection of renal allograft rejection by serial monitoring of serum C-reactive protein. Transplantation 1984; 37:215–8.
12. Maury CPJ, Teppo AM, Eklund B, Ahonen J. Serum amyloid A protein: A sensitive indicator of renal allograft rejection in humans. Transplantation 1983; 36:501–4.