Changes in Concentrations of C-Reactive Protein in Serum after Kidney or Heart Transplantation

Frederick Van Lente,¹ William Castellani,¹ and Lenox B. Abbott²

C-reactive protein (CRP) concentrations were serially determined in serum after kidney or heart transplantation. The initial postsurgical CRP response in these patients was compared with that of control patients undergoing related procedures but not subjected to immunosuppressive therapy. Immunosuppression clearly depressed the postsurgical CRP response in transplant recipients. The effect is greatest with the administration of cyclosporine. In addition, we found serial CRP determinations to be a sensitive indicator of renal but not cardiac allograft rejection. The specificity of CRP as such a predictor was affected by non-rejection-based inflammation. We conclude that serial determination of CRP, interpreted by the extent by which its concentration increases between sequential samples, may be a useful adjunct to biochemical monitoring of renal transplants, but a similar approach to monitoring heart transplants is not possible.

Additional Keyphrases: immunosuppressive drugs · cyclosporine · enzyme immunoassay · acute-phase proteins

The concentration of C-reactive protein (CRP), a sensitive acute-phase reactant, is nonspecifically increased in serum in many inflammatory conditions. This response evidently (1, 2) is a result of interleukin-1 production by mononuclear phagocytic cells and, as such, is an indirect index of this aspect of the immune response.

The extent of the increase in response to the inflammation of graft rejection is of interest, because such measurements might be useful for detecting and monitoring rejection episodes. This would be a specific application; CRP assay is already used generally in monitoring postsurgical complications (3). Unfortunately, reports differ as to whether serial CRP determinations allow one to distinguish graft-rejection processes after renal or cardiac transplantation (4–7), which would seem to invalidate CRP assay as a means of monitoring all organ allografts. However, these previous studies of CRP after transplantation are not clear-cut. The effect of concurrent immunosuppressive therapy may be variable, and the drug regimen is not always documented. Several different methods have been used to determine CRP, making difficult any direct intercomparison of studies. The criteria of effectiveness of serial CRP determinations differ considerably among studies, and are not always clearly stated.

Here we report the effect of immunosuppression on CRP concentrations in serum. We also assessed the effectiveness of serial assays of CRP for predicting rejection episodes after two different organ allograft procedures: transplants of vital kidney from a relative, and cardiac transplants. We find that immunosuppression depresses the acute-phase CRP response, and that perhaps such assays are less efficient for monitoring cardiac transplantation than for renal transplantation.

Materials and Methods

We determined CRP by homogeneous enzyme immunoassay (ELISA) with reagents from Syva Co., Palo Alto, CA 94303, and a RA-1000 random access analyser (Technicon Instruments Corp., Tarrytown, NY 10591). Instrument settings were:

1. IA Table 18
2. Type 4
3. % smp Vol 8
4. Filter 3 (405 nm)
5. Delay, s 0 30
6. 2 Rgt Vol, % 35
7. Units mg/dL
8. Unit Fac 1.0000
9. Decimal Pt 1
10. IA Type 0
11. 2nd Rgt Delay, s 0 15

CRP reagents and standards were prepared according to the manufacturer's protocol. Working antibody–substrate Reagent A (1st Rgt) was prepared by diluting Reagent A ninefold with CRP CRP buffer solution just before use. We used an identical procedure to prepare working enzyme Reagent B (2nd Rgt). Calibrators were used as supplied in the reagent kit.

Assay standardization was performed before each run. The usable range of the assay was 0.0 to 130.0 mg/L. Within-run and day-to-day CVs were respectively 1.7% (mean = 59.4 mg/L, SD = 0.674 mg/L, n = 30) and 4.5% (mean = 28.3 mg/L, SD = 1.28 mg/L, n = 26). Comparison with a rate nephelometric method (Beckman ICs; Beckman Instruments, Brea, CA 92621) yielded a correlation coefficient of 0.99, a slope of 0.962, and a y-intercept of 2.71 mg/L (n = 40).

Creatinine, serum urea nitrogen, total protein, albumin, aspartate aminotransferase, and alanine aminotransferase were also determined in the RA-1000, with reagents from Technicon, according to the manufacturer's protocol.

We studied 44 patients (28 men and 16 women; mean age 33.6 years), admitted consecutively for either renal or heart allograft procedures. Twenty-six patients received kidneys from living, related donors, with from one haplotype to identical HLA match; 18 patients received heart transplants. Immunosuppressive therapy (see Results) was begun just before surgery and was continued throughout the study period.

Acute renal allograft rejection was established by the clinical decision to begin bolus treatment with methylprednisolone, which, in turn, was based on decreased allo-

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Received November 25, 1985; accepted January 13, 1986.
graft function as determined by systemic and renal-function factors: i.e., increased serum creatinine, decreased urine output, renal perfusion scan, and renal biopsy findings. That a cardiac allograft was being rejected was established by periodic endomyocardial biopsies. Clinically significant rejection episodes were characterized by biopsy findings consistent with moderate to severe acute rejection, which prompted bolus therapy with methylprednisolone.

The nonimmunosuppressed control groups were 22 patients receiving cardiac artery bypass grafts and 36 patients undergoing either donor nephrectomy, renal autotransplantation, or simple nephrectomy.

Serum samples were obtained serially from all patients, starting the day before surgery and continuing until day of discharge. The length of hospitalization varied from three to five weeks. Samples were frozen at −20 °C until analysis.

Because data were found not to be normally distributed, we used the nonparametric Wilcoxon Rank Sum Test for statistical analysis. Predictive value analysis was performed as described by Galen and Gambino (9). We used the chi-square test to determine the significance of sensitivity, specificity, and predictive values.

Results

CRP concentrations increase as a result of surgical trauma (2, 9). Table 1 compares the CRP response of patients after transplant surgery with that of nonimmunosuppressed patients after closely related surgical procedures. From the second to the fourth postoperative day the difference between transplant patients and controls is statistically significant (p < 0.01). The CRP response is markedly depressed by standard immunosuppressive therapy after either kidney or heart transplant surgery. On the fourth postoperative day, the mean concentration of CRP in control patients was threefold that seen after cardiac transplants. Mean CRP concentrations after renal allografts were as little as one-tenth of that of the controls; and for nine of 26 kidney recipients the peak CRP value was <20 mg/L after surgery. The significantly higher (Wilcoxon Rank Sum Test, p < 0.01) peak CRP concentrations in patients after cardiac transplantation than after renal transplantation may reflect the more extensive surgical trauma associated with cardiac transplantation. CRP values peaked two to three (mean = 2.5) days after surgery in both patient groups, which is not significantly different from that seen in cardiac controls (mean = 3.2 days) or renal controls (mean = 2.9 days).

After renal transplantation, patients were treated with either anti-lymphocyte globulin–azathioprine–prednisone or cyclosporine–prednisone immunosuppression protocols. The immediate postoperative CRP response (Table 2) is clearly lower in the cyclosporine-treated patients, to a statistically significant degree on the third and fourth postoperative days. Cyclosporine has a somewhat greater suppressive effect on the CRP response than does the combination of anti-lymphocyte globulin and azathioprine. No similar comparison was possible for heart-transplant patients, all of whom received the same cyclosporine–prednisone treatment.

We monitored patients for the onset of clinically significant allograft rejection, starting four to five days after surgery. During the period of study, 10 of the 26 kidney recipients had a total of 15 rejections. Of the 18 heart recipients, endomyocardial biopsy findings in 11 were consistent with moderate to severe acute rejection, and these patients were treated with bolus methylprednisolone; in all, 78 biopsies were performed, with 15 considered positive for clinically significant allograft rejection.

We assessed the utility of serial measurements of CRP in serum, after the postoperative peak, for predicting the onset of acute rejection in the presence of immunosuppression. Rejection before this point could not be evaluated, but occurred in only one patient. Our criterion for evaluating changes in sequential CRP values was as follows: An increase in CRP >5.0 mg/L between successive samples was considered positive if the final concentration exceeded either 20.0 or 30.0 mg/L. Sensitivity was determined by the presence of a positive CRP increase before a documented rejection episode divided by the number of rejection episodes.

As seen in Table 3, a CRP increase to >30.0 mg/L is sensitive and specific for indicating acute rejection after renal transplantation. The increase in CRP occurred as much as four days earlier than clinical recognition of rejection. Increasing the CRP threshold from 20 to 30 mg/L had a negligible effect on the lead time between CRP increase and clinical recognition of rejection. Specificity was based on the absence of a CRP increase in the 16 kidney recipients who did not experience rejection during the period

<table>
<thead>
<tr>
<th>Table 1. Postoperative CRP Concentrations (mg/L): Transplant Recipients vs Controls</th>
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<tbody>
<tr>
<td><strong>Post-operative day</strong></td>
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<tr>
<td><strong>Cardiac</strong></td>
</tr>
<tr>
<td>0</td>
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<tr>
<td>1</td>
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<tr>
<td>2</td>
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*a, b, c Significantly different from controls at * *p < 0.05, * *p < 0.01 (Wilcoxon Rank Sum Test), or not significantly different (* *p > 0.10).
Table 3. Predictive Value of CRP Increase* for Onset of Allograft Rejection

<table>
<thead>
<tr>
<th>CRP Increase, mg/L</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive Value</th>
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<tbody>
<tr>
<td>Kidney transplants</td>
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<tr>
<td>&gt;20.0</td>
<td>53.3</td>
<td>76.6</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>(8/15)</td>
<td>(47/83)</td>
<td>(8/24)</td>
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<tr>
<td>&gt;30.0</td>
<td>67.7</td>
<td>80.2</td>
<td>99.1</td>
</tr>
<tr>
<td></td>
<td>(14/15)</td>
<td>(13/15)</td>
<td>(14/16)</td>
</tr>
<tr>
<td>Heart transplants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;20.0</td>
<td>53.3</td>
<td>74.8</td>
<td>97.0</td>
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<tr>
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<td>(8/15)</td>
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*Positive change in CRP >5.0 mg/L between sequential samples 0-4 days before clinical recognition of rejection and initiation of treatment. **No. of "positive" CRP increases/no. of transplant rejections. *Chi square test.

of study. The sum of false-positive and true-negative results changed with this change in CRP criteria, owing to a variation in the number of false positives per individual patient in the rejection-free group. Attempts to improve specificity by increasing the CRP threshold values decreased sensitivity unacceptably.

Successful treatment with methylprednisolone promptly decreased the concentrations of CRP to <20 mg/L within four days in 12 of 15 acute renal-rejection episodes. Three cases were characterized by continued rejection and CRP concentrations that remained >50 mg/L.

In contrast to the findings in kidney recipients, CRP increases after cardiac transplantation had less sensitivity, specificity, and predictive value for the onset of rejection. Attempts to improve predictive value by adjusting CRP threshold values were unsuccessful. We conclude that the sensitivity and specificity of C-reactive protein for predicting acute rejection episodes is different in the two groups studied.

False-positive increases in CRP were observed in both groups of transplant recipients. Six of 11 false-positive CRP increases to >30 mg/L could be associated with a clinically evident inflammatory episode not related to graft rejection: two operations for correction of complications not related to the organ graft, three episodes of bacterial infection, and one reaction to administration of anti-lymphocyte globulin. CRP peaks during these episodes (30-300 mg/L) were greater than those seen during allograft rejection (7-160 mg/L), possibly because of high-dose steroid therapy initiated during the latter. We were not able to associate the remaining false-positive increases in CRP with a clinically observed event.

After renal transplantation, positive (>20 mg/L) CRP increases occurred 0-4 days before a positive change in the concentration of creatinine in serum. We found no correlation between CRP and either alanine or aspartate aminotransferase or creatine kinase after heart transplantation. The correlation between concentrations of CRP and either total protein or albumin was not significant in either group.

Discussion

Azathioprine, anti-lymphocyte globulin, cyclosporine, and corticosteroids exhibit a variety of immunosuppressive actions (10-12), including lymphocyte toxicity and interleukin-2 inhibition; their effect on interleukin-1 is still some-what unclear. As we report here, there is a major effect of immunosuppressive therapy on the extent of the postsurgical response of CRP concentrations, observed after both kidney and heart transplantation. Although the effect was more pronounced after cyclosporine therapy, all immunosuppression regimens appear capable of affecting the magnitude of the CRP response. However, we could not identify a quantitative relationship between the concentrations of CRP and whole-blood cyclosporine after cardiac transplantation surgery.

Serial CRP determinations were reasonably sensitive for renal, but not heart, allograft rejection. This indicates a continued capacity for CRP production in response to an appropriate immune stimulus, even in the presence of immunosuppression. Significant postoperative nonrejection complications, including a repeated operation or infection, were also associated with clear increases in CRP and were considered to be false positives in this study. These findings are consistent with an incomplete inhibition of the acute-phase response by immunosuppressive drugs.

The lower sensitivity of CRP for cardiac allograft rejection is puzzling, although consistent with an earlier report (7). Perhaps the effect of immunosuppression is greater on the immune response in cardiac allograft rejection, or the degree of inflammation associated with cardiac rejection may be less. Or perhaps the determination of the presence of rejection by regular biopsies after cardiac transplantation is a much more sensitive means of determining the presence of rejection than the clinical assessment following renal transplantation, which may decrease test sensitivity (13). In any event, monitoring CRP does not add useful information for the management of cardiac allograft rejection.

The ability of CRP to signal the onset of clinically significant renal allograft rejection appears useful in the management of kidney transplant patients, but must be balanced against the possibility of false-positive CRP increases. It would be deleterious to treat infections with additional immunosuppressants.

The CRP assay described here can be easily included in a renal transplant monitoring profile, which might include CRP; creatinine, blood urea nitrogen, albumin, and electrolytes. The inclusion of CRP provides the means for earlier prediction of renal allograft rejection, and creatinine, albumin, urea nitrogen, and electrolytes confirm the assessment of allograft function. Unfortunately, this approach cannot be as successfully applied to heart transplantation. The efficiency of CRP for predicting allograft rejection cannot be assumed to be similar in all organ systems; in fact, significant increases in CRP may indicate graft success rather than rejection following liver transplantation (14). The successful application of a biochemical rejection marker is probably both organ and marker dependent.

We gratefully acknowledge the gift of C-reactive protein reagents from Syva Co. and the technical assistance provided by Mr. Gary Kinn.

References