Increased serum C-reactive protein (sCRP) is a sensitive marker of renal graft rejection. We describe the cases of two children with uncomplicated renal transplantation who had false-positive sCRP values on analyzers using rabbit anti-CRP but values within the reference range with anti-CRP from other animal species. Cross-reaction with heterophilic antibodies was suggested by clinical and biological signs of serum sickness and daily treatment with rabbit antilymphocyte globulin (ALG). The interference depended on the serum concentration of the cross-reactant and was removed by subtotal IgG adsorption to Protein A or Protein G or by immunoadsorption using rabbit ALG or total IgG in non-immune rabbit serum. Anti-rabbit IgG and IgM antibodies were detected in both patients. These are the first reported cases of cross-reaction with heterophilic antibodies in a turbidimetric CRP assay.

Effective management of patients receiving renal allografts requires early recognition of rejection episodes. The process of acute allograft rejection is mediated by an influx of immunoactivated cells associated with a complex cascade of lymphokine/cell interactions within the graft, producing stimulation of the inflammatory system (1–4). In addition to direct monitoring of renal function, a variety of putative markers of inflammatory responses (e.g., cytokines and acute-phase proteins) in the early postgraft period provide valuable information on the rejection process. Serum C-reactive protein (sCRP) can be assayed simply on a daily basis, and increases correlate well with episodes of renal graft rejection (5). sCRP may be routinely quantified by using a variety of turbidimetric and nephelometric methods with heterologous antibodies. Although these immunoassays are considered reliable, some false-positive results have been reported. High concentrations of bilirubin, lipids, and rheumatoid factors are known to interfere with many CRP assays (6–9). Monoclonal gammopathies, predominantly of the IgM type, give falsely high concentrations of sCRP by nonspecific interaction between the patient’s monoclonal immunoglobulin and the heterologous anti-CRP used for the assay (10–12) or the sCRP in vivo (11, 13). Furthermore, monoclonal and even polyclonal IgM may affect latex particle-based assays (14, 15). Specific cross-reactions in patients with heterophilic antibodies against the heterologous anti-analyte are increasingly observed, mainly with human anti-mouse antibodies (16). To our knowledge no such artifact has been described in automated immunoturbidimetric or immunonephelometric CRP assays.

We report the cases of two kidney recipients in whom sCRP was falsely increased because of interference by the patients’ anti-rabbit heterophilic antibodies in assays using rabbit anti-human CRP (anti-CRP).

Case Reports

Patient A, a 3-year-old boy with bilateral renal hypoplasia and end-stage renal failure, received a kidney allograft in November 1997. Immunosuppressive therapy consisted of prednisone, azathioprine, and rabbit polyclonal antilymphocyte globulin (ALG; Thymoglobuline®, Pasteur Merieux Connaught) administered daily from the first day postgraft; ALG was gradually replaced by cyclospor-
The postgraft profiles of the CRP values of the two patients were identical until day 11 (Fig. 1). However, between days 13 and 25 a discrepancy emerged, with increased sCRP values similar to the reference range (300 mg/L vs 27 mg/L with the Array on day 13). During this period the patient had no signs of graft rejection.

Patient B, a 5-year-old girl, had end-stage renal failure secondary to nephroblastoma. In February 1998, after 2 years of hemodialysis, she received a kidney graft from her father. Immunosuppressive treatment was identical to that described above. On the 10th day postgraft a urinary tract infection was suspected on the basis of fever (40 °C) and a culture of Enterococcus faecium in urine. Between days 11 and 20, a discrepancy in sCRP values similar to that in patient A was observed (90 mg/L with the Hitachi vs 27 mg/L with the Array on day 11). Serum sickness was suspected on day 13, with a reduction in complement factors (C3, 690 mg/L; C4 <100 mg/L; reference range, 90–340 mg/L), and undetectable hemolytic complement (CH50; reference range, 80–120%). By day 16, the presence of anti-rabbit immunoglobulins was documented. ALG treatment was suspended on the 10th day postgraft. Serum CRP values assayed with two different methods were identical until day 11 (Fig. 1). However, between days 13 and 25 a discrepancy emerged, with increased sCRP concentrations measured with the Hitachi 911 analyzer (Boehringer) and values within the reference range measured with the Beckman Array analyzer (66 mg/L with the Hitachi vs <5 mg/L with the Array on day 13). During this period the patient had no signs of graft rejection.

**Materials and Methods**

**Sample Collection**

Blood was drawn daily from the first day postgraft. After clotting, the sample was centrifuged, and sCRP was assayed immediately. The remaining serum obtained from patient A from day 13 to day 17 was pooled (pooled serum A), aliquoted, and stored at −20 °C for further investigations.

**CRP Measurements**

The following assays were performed: (a) an emergency nephelometric assay using goat anti-CRP on the Array analyzer (antibody and analyzer from Beckman); (b) a routine turbidimetric assay using rabbit anti-CRP (Dako) on the Hitachi 911 analyzer (Boehringer); and (c) assays of controlled CRP values by turbidimetry on the Turbitimer analyzer (Behringwerke) or Axon analyzer (Bayer Technicon), both of which use a rabbit anti-CRP (respectively, Behringwerke and Biomerieux), the Kone Pro analyzer, which uses a sheep anti-CRP (analyzer and antibody from Kone Instruments), and nephelometry with the BN II analyzer using a monoclonal mouse anti-CRP (analyzer and antibody from Behringwerke).

**Other Serum Protein Measurements**

Albumin and IgG were assayed by means of nephelometry using either goat (Array) or rabbit antiserum (BN II).

**Adsorption of Human IgG**

Pooled serum A (300 µL) was incubated for 5 min at 37 °C with 200 µL of fourfold diluted Protein G-Sepharose (Sigma) or Protein A-Sepharose (Sigma). After centrifugation (3000g, 5 min) the supernatant was collected and analyzed for CRP, IgG, and albumin. Serum dilution attributed to the addition of Protein G or Protein A was calculated from the ratio of the albumin concentration before and after treatment. Corrected CRP and IgG values were calculated using this ratio.

**ImmunoPrecipitation of Human Anti-Rabbit Antibodies**

Human heterophilic antibodies to rabbit IgG were immuno precipitated by adding either the putative rabbit immunogen (ALG) or total IgG in non-immune rabbit serum. Briefly, pooled serum A (160 µL) was incubated for 3 min at 37 °C with increasing volumes of ALG (5 g/L IgG) or 8 µL of non-immune rabbit serum and was then centrifuged (3000g, 5 min). CRP was determined in the supernatant, using rabbit anti-CRP on the Hitachi analyzer.

**Effect of Serum Dilution**

Pooled serum A was serially diluted in 0.15 mol/L NaCl (3:4, 2:3, 1:2, and 1:4), and the diluted CRP was assayed using rabbit anti-CRP on the Hitachi device. The percentage of interference at each dilution was estimated by multiplying the ratio (experimental value/theoretical value) by 100.

**Titration of Human Anti-Rabbit Antibodies**

Specific human anti-Thymoglobuline (i.e., anti-ALG) antibodies were determined by the supplier (Pasteur Merieux Connaught), using an ELISA method. Briefly, microtiter plates were coated with 100 µL per well of 2500-fold diluted Thymoglobuline (0.2 µg of rabbit IgG) and incubated first with 500-fold or 250-fold diluted serum (to screen for the respective heterophilic IgG and IgM antibodies) and then with goat anti-human IgG (or IgM) conjugated with horseradish peroxidase. A serum sample was considered positive for heterophilic antibodies when absorbance values exceeded the mean value of six negative serum controls and was scored (+), (++), or (+++) for values exceeding the mean + 3 SD, 6 SD, and 9 SD, respectively.

**Results**

The postgraft profiles of the CRP values of the two patients assessed with the Hitachi and Array analyzers...
are shown in Fig. 1. The two analyzers yielded similar values until day 7 after grafting. A sudden increase in Hitachi values occurred from days 13 and 11 in patients A (66 mg/L) and B (90 mg/L), respectively, whereas controlled Array values remained within the reference range in patient A (<5 mg/L) and slightly increased in patient B. Over the following days, this discrepancy between the two analyzers persisted, despite a gradual reduction in Hitachi values. In subsequent studies all serum samples obtained from patient A between days 13 and 17 were pooled. Values obtained for this pool with the two analyzers exhibited the same discrepancy (46 mg/L with the Hitachi vs <5 mg/L with the Array; Table 1). This increase in sCRP values on the Hitachi device was confirmed with two other analyzers also using rabbit anti-CRP (Turbitimer and Axon). In addition, the same increased values were found with serum sampled from patient B on day 11 on the three analyzers using rabbit anti-CRP. Values within the reference range were obtained for patients A and B with the three analyzers using non-rabbit anti-CRP, regardless of animal species, i.e., goat (Array), sheep (Kone Pro), and mouse (BN II).

After nonspecific subtotal IgG adsorption (Table 2) of pooled serum A using either Protein G-Sepharose or Protein A-Sepharose, the Hitachi and Turbitimer values matched the Array value. Surprisingly, however, the sCRP discrepancy between the Hitachi and Array analyzers was not observed with two other serum proteins (albumin and IgG), also measured with rabbit antisera (Table 2).

When aliquots of pooled serum A were incubated with increasing amounts of ALG (purified rabbit IgG), the increased Hitachi sCRP value gradually fell to the Array value after a minimal rabbit IgG concentration of 156 mg/L had been reached (Fig. 2). This was confirmed by similar findings when 50 mL/L total IgG in non-immune rabbit serum was used instead of ALG.

To evaluate the influence of the serum concentration of the putative cross-reactant on the increased Hitachi sCRP values, pooled serum A was serially diluted. Interference, evaluated as the percentage of the ratio between measured values on the Hitachi device and expected CRP values, gradually fell from 100% in the undiluted pool to 47% in the fourfold diluted pool. Because of the relatively low CRP value in pooled serum A (46 mg/L), it was not possible to study higher dilutions. Serum sickness was suspected in patient A on day 9 and later in patient B; therefore, the patients’ sera were tested for heterophilic anti-rabbit antibodies. Results for samples drawn from patients A and B on days 16 and 17, respectively, clearly demonstrated the presence of human anti-rabbit IgG of both the G and M isotypes. According to the supplier, the signals obtained were the highest given by the ELISA technique (greater than the mean of six negative sera + 9 SD) and corresponded to a score of +++.

**Discussion**

CRP is a well-established marker of rejection episodes after renal allografting (5). Graft function without complications is associated with an initial peak of sCRP on day 2 after transplantation and a gradual return to <20 mg/L by day 5. The rejection process is generally associated with an early rise in sCRP, except in patients receiving

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**Table 1. Serum CRP (mg/L) determination with antisera of different animal species.**

<table>
<thead>
<tr>
<th>Rabbit anti-CRP</th>
<th>Non-rabbit anti-CRP</th>
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<tbody>
<tr>
<td>Hitachi</td>
<td>Turbitimer</td>
</tr>
<tr>
<td>Axon</td>
<td>Kone Pro</td>
</tr>
<tr>
<td>Array</td>
<td>BN II</td>
</tr>
<tr>
<td>p.s.A</td>
<td>46</td>
</tr>
<tr>
<td>s.B</td>
<td>90</td>
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<tr>
<td></td>
<td>66</td>
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<tr>
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<td>138</td>
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</table>

*Sheep anti-CRP.*

*Goat anti-CRP.*

*Mouse anti-CRP.*

*p.s.A, pooled serum from patient A; s.B, serum drawn from patient B on day 11.
immunosuppression therapy based on high doses of cyclosporine A (17).

Roughly similar patterns were observed in the two cases reported here (Fig. 1). The initial peak in sCRP concentrations was observed between days 2 and 4, with a return towards reference values by day 5, indicating excellent primary graft function (5). However, from day 11 a discrepancy emerged between increased sCRP values on the Hitachi device (using rabbit antiserum) and reference or slightly raised values on the Array device (using goat antiserum). This discrepancy disappeared by day 25 in patient A and by day 19 in patient B, with the Hitachi values falling to the Array reference values.

No clinical or biological signs of inflammation, rejection, or monoclonal or polyclonal gammopathies were found, and only patient B had a suspected infection (urinary tract, between days 9 and 13). From day 13 onward, all individual or pooled samples from the two patients gave values within the reference range on the Array and other analyzers (Kone Pro and BN II) using non-rabbit anti-CRP (Table 1 and Fig. 1). In the same way, the unexplained increase in CRP obtained with the Hitachi device (from day 13 in patient A and day 11 in patient B) was confirmed on all daily and pooled samples on two other analyzers also using rabbit anti-CRP (Turbitimer and Axon).

Both patients were receiving daily infusions of total rabbit IgG in anti-human lymphocyte immune serum (Thymoglobuline) from the day of grafting to day 10 postgraft. In addition, serum sickness was suspected from day 10 in patient A and day 13 in patient B on the basis of clinical and biological signs (consumption of complement factors). This strongly suggested an immunization process against the putative rabbit IgG immunogen (rabbit ALG), and the subsequent rise in human anti-rabbit antibody (HARA) titer. A major limitation in first line use of heterologous polyclonal ALG is the frequency of human anti-animal immunoglobulin response. In 10–32% of recipients assigned to this treatment, a serum sickness appeared from the seventh day after the first administration (18–20). Similar findings have been reported with immunosuppressive mouse monoclonal antibodies (Orthoclone OKT3, Ortho Pharmaceutical), which induced a rise in human anti-mouse antibody titers because of the same immunization process (21, 22).

HARAs can form an artifactual immune complex by binding to iso/allotypic determinants expressed by rabbit anti-CRP antibodies. This hypothesis was confirmed by a normalization of the increased Hitachi CRP values when the bulk of human IgG was removed by Proteins G or A, and HARAs were specifically blocked by immunoadsorption with rabbit ALG or total IgG in non-immune rabbit serum (Table 2 and Fig. 2).

However, IgG and albumin concentrations assessed on a BN II device, also using rabbit antiserum, were within reference ranges. Concentration-dependent interference by HARAs is one possibility because the patient’s sample was prediluted 400-fold for the IgG and albumin assays and 21-fold for the CRP assay. In keeping with this possibility, a higher sCRP value (66 vs 46 mg/L) was found with the Turbitimer device, using only 11-fold diluted pooled serum A (Table 1), and the level of the cross-reaction fell from 100% to 47% when the same sample was prediluted fourfold. Unfortunately, in the albumin and IgG BN II nephelometric assays, it was not

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**Table 2. Interference with CRP determination of serum subtotal IgG release.**

<table>
<thead>
<tr>
<th></th>
<th>CRP, mg/L</th>
<th>IgG, g/L</th>
<th>Albumin, g/L</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hitachi</td>
<td>Turbitimer</td>
<td>Array</td>
</tr>
<tr>
<td>p.s.Aa</td>
<td>46</td>
<td>66</td>
<td>&lt;5</td>
</tr>
<tr>
<td>p.s.A + protein G</td>
<td>&lt;5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;5&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>p.s.A + protein A</td>
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<sup>a</sup> Pooled serum from patient A.
<sup>b</sup> Corrected values (measured × dilution factor).
<sup>c</sup> The dilution factor for each analyzer was calculated from the albumin ratios before and after addition of Protein A- and Protein G-Sepharose.

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Fig. 2. Effect of adding increasing quantities of purified rabbit IgG [rabbit ALG (Thymoglobuline)] on measured CRP values. Pooled serum from patient A was assayed on a Hitachi analyzer with rabbit anti-CRP.
possible to study the interference with <400-fold diluted serum because samples containing an excess of immune complex are automatically rediluted and rerun.

Last but not least, the presence of HARAs against rabbit IgG was clearly established in the two patients by using a semiquantitative ELISA method from the supplier of Thymoglobuline. The heterophilic antibodies were of the G and M classes; however, we assume that only IgG interfered in the CRP assay because the interference was completely removed by preincubation of serum with protein G (which does not recognize IgM). Protein A binds to all human immunoglobulins (except IgG3 and IgD), whereas Protein G recognizes all IgG subclasses but not the other human Ig classes (23).

Thus, the most likely explanation for the falsely increased CRP values is cross-reaction of the patients’ heterophilic antibodies (HARAs) with the rabbit antibodies from the suppliers. Interference in immunoassays by such heterophilic antibodies has often been reported, mainly after exposure of the patient to murine immunoglobulins (16) and in two-site sandwich immunoassays (24). There have been few reports on interference by HARAs: one case involved a patient treated with rabbit ALG who had an inappropriately high serum erythropoietin concentration in a radioimmunoassay (20). In a Japanese report (25), a false-positive CRP result in a radial immunodiffusion method was found in a patient with leukemia. Other pathological situations sometimes associated with falsely increased CRP values may be ruled out in both patients on the basis of clinical and biological findings (no dysglobulinemia or increased rheumatoid factor).

This report is the first description of false-positive sCRP values in patients with HARAs, using a rapid and frequently used immunoturbidimetric assay method. The presence of heterophilic antibodies should be suspected in any graft recipient receiving antibody-mediated immunosuppression. Because these ALGs may be obtained from rabbits, horses, or goats, precautions should be taken concerning the animal species producing the anti-CRP used in the assay. Although not observed with IgG or albumin, such cross-reactions must be suspected with all serum protein immunoassays according to the protein concentration, the sample dilution, and the heterophilic antibody titer of the patient’s serum.

In conclusion, serum CRP and other protein assay results should be critically interpreted by physicians and biologists, especially after day 11 of antilymphocyte immunosuppressive treatment. Two possibilities concerning the implications of a false-positive CRP result in patient management should be considered. In the first possibility, if only the sCRP is abnormal without other biochemical changes, the clinician should investigate background events (e.g., infection, blood transfusion, or venous thrombotic events). The second possibility associates a false-positive sCRP with other biochemical signs of renal dysfunction (increased creatininemia and microalbuminuria), suggesting a rejection episode pointing to renal biopsy and high-dose corticosteroids. When a clinically unexplained increase in sCRP occurs, we recommend repeat analysis of a more dilute sample and/or immunoassay using antisera from a different animal species.

References
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