Monitoring Both Serum Amyloid Protein A and C-Reactive Protein as Inflammatory Markers in Infectious Diseases

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We examined serum amyloid protein A (SAA) and C-reactive protein (CRP) as inflammatory markers of viral and bacterial infections. Both acute-phase reactants increased in the acute stage and thereafter decreased in the convalescent stage. In viral infections, the mean serum concentrations of SAA during the acute stage were 141 mg/L in infections with adenovirus, 77 mg/L with measles virus, 63 mg/L with influenza virus, 55 mg/L with parainfluenza virus, 31 mg/L with respiratory syncytial virus, and 31 mg/L in aseptic meningitis. The mean serum concentration of CRP was 19 mg/L for adenovirus infection and <7 mg/L in all other viral infections. The SAA concentrations were 5- to 11-fold greater than the CRP concentrations. Both the SAA and the CRP concentrations were higher in bacterial infections than in viral infections. Changes in the concentrations of serum SAA paralleled those in serum CRP in bacterial infection; during the course of viral infection, however, serum SAA tended to disappear more quickly than CRP did. SAA appears to be a clinically useful marker of inflammation in acute viral infections, with or without significant changes in the CRP concentration.

Indexing Terms: acute-phase proteins · bacteria · viruses · infection · Kawasaki disease · latex agglutination nephelometric immunoassay

Serum amyloid protein A (SAA) is a putative serum precursor of the amyloid A protein, which consists of amyloid fibrils observed in secondary amyloidosis (1-3). Secondary amyloidosis occurs in patients with tuberculosis, leprosy, and rheumatoid arthritis. SAA has been detected in sera from patients with malignant tumors, autoimmune diseases, viral infections, and rejection of kidney transplants, and also in patients with secondary amyloidosis (4-14). Recently, SAA has been clinically evaluated as one of the sensitive acute-phase reactants in serum, together with C-reactive protein (CRP).

CRP concentration has been used as a sensitive inflammatory marker in bacterial infections. But in acute viral infections, CRP changes within a narrower range than in bacterial infections. Consequently, a more sensitive marker of inflammation is needed for viral infections. In this report, we compared the amounts of SAA and CRP in sera obtained from patients with viral infections, bacterial infections, and muco-cutaneous lymph node syndrome (MCLS; Kawasaki disease), which are representative of acute inflammatory diseases in children.

Materials and Methods

Patients

Patients were admitted to Saiseikai Central Hospital. Those with lower respiratory infection were diagnosed by chest roentgenographic findings and clinical examinations. On the day of hospitalization, nasopharyngeal secretions were obtained. Of these patients, 70 were diagnosed as having had respiratory syncytial virus (RSV) infection by the detection of RSV antigen with the RSV ELISA kit (Ortho Diagnostic Systems, Raritan, NJ) and (or) by serological response of complement-fixing antibodies. Other patients were diagnosed on the basis of their serological responses: 25 with influenza virus infection, 47 with parainfluenza virus infection, 29 with adenovirus infection, and 17 with measles virus infection. Fifty-three patients were diagnosed as having been infected with Mycoplasma pneumoniae by the isolation of M. pneumoniae and (or) serological responses. In 21 patients with aseptic meningitis, 14 patients were diagnosed as having had echo virus type 30 infection and 7 as having mumps meningitis by isolation of the respective viruses from their cerebrospinal fluids. For bacterial infections, we studied 8 patients with bacterial enterocolitis (5 Salmonella, 3 Campylobacter) and 10 patients with urinary tract infection (8 Escherichia coli, 1 Enterococcus faecalis, 1 Pseudomonas). Eight patients who fulfilled the MCLS diagnosis criteria (15) were also studied. All serum samples were stored at -70 °C.

SAA and CRP Assay

Concentrations of SAA were measured by latex agglutination nephelometric immunoassay (LA) with an automated immunochemistry analyzer (LX-3000; Eiken Chemical Co., Tokyo, Japan) (16). Briefly, 20 µL of serum was mixed with 240 µL of 0.1 mol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.4) and incubated at 37 °C for 130 s; then, 80 µL of latex reagent coated with antibodies to SAA, 1 g/L, was added. (The antibodies to SAA had been raised in rabbits immunized with purified human SAA emulsified with complete Freund's adjuvant.) Thereafter, the mixture was incubated at 37 °C, and the increase in the

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4 Nonstandard abbreviations: SAA, serum amyloid protein A; CRP, C-reactive protein; RSV, respiratory syncytial virus; MCLS, muco-cutaneous lymph node syndrome; LA, latex agglutination nephelometric immunoassay; and rIL, recombinant interleukin.

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intensity of the scattered light after the addition of latex reagent was measured. SAA-enriched high-density lipoprotein, in which SAA content was determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, was used as the assay standard as previously reported (17). Concentrations of CRP were also assayed by LA, with use of latex particles coated with antibodies to CRP (18). The detection limit was 1 mg/L for both proteins.

Results
Concentrations of SAA and CRP in Viral Infections
The changes in the concentrations of SAA and CRP during infection are shown in Figure 1. The acute-stage sera were obtained on the day of hospitalization; most of the convalescent stage sera were obtained 1 week later, when all symptoms had disappeared. In 29 patients infected with adenovirus, the mean concentration of SAA was 141 mg/L in the acute stage and decreased to 10 mg/L in the convalescent stage; the mean concentration of CRP was 19 mg/L in the acute stage and decreased to 2 mg/L in the convalescent stage. In the 25 patients infected with influenza virus, the concentrations of SAA and CRP were higher in the acute stage than in the convalescent stage. In 3 of the 47 patients infected with parainfluenza virus, the concentration of SAA increased in the convalescent stage; the CRP varied according to the change of SAA. In 68 of the 70 patients infected with RSV, the concentrations of SAA and CRP decreased in the convalescent stage. Essentially the same results were obtained in patients with aseptic meningitis. In most of the patients with virus infections, the concentration of SAA in the convalescent stage was <10 mg/L and that of CRP was <2 mg/L.

Concentrations of SAA and CRP in Bacterial Infections
The changes in the concentrations of SAA and CRP during infection are shown in Figure 2. In eight MCLS cases, the mean concentration of SAA was 513 mg/L, decreasing to 29 mg/L 1 week later. The mean concentration of CRP was 81 mg/L in the acute stage and decreased to 16 mg/L 1 week later. The concentrations of SAA and CRP decreased to normal range (SAA <10 mg/L, CRP <3 mg/L) by 2 or 3 weeks later (data not shown). In bacterial enterocolitis, urinary tract infection, and infection with M. pneumoniae, the concentrations of SAA and CRP returned to normal after 1 week.

In general, changes in the SAA concentration between the acute stage and the convalescent stage paralleled that of the CRP concentration in both viral and bacterial infections. We compared the disappearance of SAA and CRP in patients infected with RSV and those infected with M. pneumoniae, which involved a considerable number of patients. We defined as day 1 of illness the day when the patient had an axillary body temperature >38 °C. The changes in SAA and CRP concentrations are shown in Figure 3. In the patients infected with M. pneumoniae, SAA decreased in parallel with CRP. In the patients infected with RSV, however, SAA tended to disappear more quickly than CRP.

Fig. 1. Concentrations of SAA and CRP in sera during the acute stage (A, ○) and the convalescent stage (C, ○) in patients with viral infections When paired sera were available from the same patient, the concentrations of SAA (upper panels) or CRP (lower panels) in the two stages are connected with lines. Unconnected symbols represent sera obtained at only one stage. Bars represent the mean value ± SD.
The mean concentrations of SAA and CRP in the acute stage were highest overall in patients with MCLS. (Table 1). Among patients with viral infections, the highest concentrations of SAA and CRP were observed in patients with adenovirus infection. In patients with other viral infections, the SAA concentration ranged from 31 to 77 mg/L, whereas the CRP concentration varied within a narrower range (5-7 mg/L). The ratio of SAA to CRP was greatest in patients infected with measles virus and influenza virus. In the acute-stage sera overall, the concentration of SAA was 5- to 11-fold greater than that of CRP.

Discussion

In response to tissue injury or infection, a coordinated sequence of systemic and metabolic changes occurs, generally observed as increased serum concentrations of acute-phase proteins. McAdam et al. (19) reported that the SAA concentration began increasing within 12 h of etiocholanolone-induced inflammation in humans,
reaching a maximum value at ~48 h and returning to baseline values after 4 or 5 days. Moreover, the SAA time response was similar to that of CRP, a well-documented acute-phase protein. These proteins are synthesized in liver under the regulation of cytokines. Ramadori et al. (20) reported that intravenous injection of purified recombinant (r) murine interleukin (IL)-1 into endotoxin-resistant mice induced a dose-dependent increase in SAA-specific hepatic messenger RNA and an increase in plasma SAA concentration. Moshage et al. (21) reported that both rIL-1 and rIL-6 stimulated the liver synthesis of SAA and CRP and that anti-rIL-6 antibodies reduced the stimulatory effect of rIL-1 on the synthesis of these proteins in vitro in human hepatocyte cultures. They suggested that IL-6 played a key role in the stimulation of synthesis of SAA and CRP in human liver cells.

SAA is thought to be the precursor of the secondary amyloid fibril protein, but the significance of the increased concentration of SAA in inflammations is still unclear (1–3). Recently, Brinkerhoff et al. (22) reported that an SAA-like substance from rabbit synovial fibroblasts treated with phorbol myristate acetate, like human SAA, induced collagenase synthesis in rabbit or human fibroblasts. They suggested that SAA may thus provide a means to modulate connective tissue breakdown in inflammatory sites.

Increased concentrations of SAA have been reported in several acute virus infections: influenza, rhinovirus, measles, rubella, cytomegalovirus, varicella-zoster, and human immunodeficiency virus infections (10–13). Whicher et al. (12) observed a peak response of SAA and CRP on day 4 or 5 for experimentally induced infection with rhinovirus and on day 3 for influenza virus. Here, we compared the serum concentrations of SAA and CRP in patients with viral infections and bacterial infections. In patients with MCLS, for which the etiology and pathogen are still unknown, the SAA concentration increased the most, in accordance with the highest value of CRP. In bacterial enterocolitis and urinary tract infections, both SAA and CRP concentrations were increased in the acute stage. Most of the patients we examined had lower respiratory virus infections; their CRP concentrations increased only in adenovirus infection. In the other infections, CRP had normal values or only a little increase (<7 mg/L) in sera obtained during the acute stage. However, these patients' SAA concentrations increased in the acute stage and decreased more promptly during the course of viral illness than during bacterial illnesses (Figure 3). Thus, we suggest that the measurement of SAA is of value in monitoring the severity of inflammation and the recovery process in viral infections. In several sera from patients with acute viral lower respiratory infections, both SAA and CRP concentrations significantly increased (Figure 1). In these cases, we suspect the possibility of mixed infections with bacterial agents.

It is difficult to determine whether lower respiratory infections are caused simply by viral infection or complicated with bacterial infection. When both SAA and CRP concentrations increase, we advise initiating an appropriate antibiotic therapy on suspicion of complicated bacterial infection. SAA and CRP can be measured by LA, a simple, sensitive, and reliable method that does not require radioisotopes and yields results in ≤10 min. The determination of SAA concentration by the LA method appears to be more sensitive than CRP determinations for viral infections in clinical laboratory examination.

References
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Table 1. Serum Amyloid Protein A and C-Reactive Protein Concentrations in Acute-Phase Sera

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>SAA</th>
<th>CRP</th>
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<tr>
<td>SAA:</td>
<td></td>
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<tr>
<td>Adenovirus</td>
<td>29</td>
<td>2.15 ± 0.65 (141)</td>
<td>1.28 ± 0.61 (19)</td>
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<td>Measles</td>
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<td>1.89 ± 0.31 (77)</td>
<td>0.84 ± 0.51 (7)</td>
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<td>Influenza</td>
<td>25</td>
<td>1.80 ± 0.50 (63)</td>
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<tr>
<td>Parainfluenza</td>
<td>47</td>
<td>1.74 ± 0.73 (55)</td>
<td>0.78 ± 0.70 (6)</td>
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<tr>
<td>RSV</td>
<td>70</td>
<td>1.49 ± 0.76 (31)</td>
<td>0.76 ± 0.58 (6)</td>
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<tr>
<td>Aseptic meningitis</td>
<td>21</td>
<td>1.49 ± 0.73 (31)</td>
<td>0.71 ± 0.57 (5)</td>
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<td>MCLS</td>
<td>8</td>
<td>2.71 ± 0.36 (513)</td>
<td>1.91 ± 0.33 (81)</td>
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<td>Enterocolitis</td>
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<td>2.19 ± 1.03 (155)</td>
<td>1.32 ± 0.75 (21)</td>
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<td>Urin. tract infection</td>
<td>10</td>
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<td>1.39 ± 0.78 (25)</td>
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<td>M. pneumoniae</td>
<td>53</td>
<td>2.00 ± 0.62 (100)</td>
<td>1.14 ± 0.47 (14)</td>
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* Concentrations expressed as 10⁻⁶ (mean ± SD); numbers in parentheses are the mean arithmetic concentration.

Total Error Assessment of Five Methods for Cholesterol Screening

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We report the accuracy, imprecision, total analytical errors, and patient misclassification errors for cholesterol measured from capillary whole blood, venous whole blood, and venous plasma samples by five devices used in public cholesterol screening environments: Reflotron, Vision, Ektachem DT-60, QuickRead, and Liposcan. None of the methods met the National Cholesterol Education Program (NCEP) performance recommendations of 3% CV with 3% bias. The Vision and Reflotron methods used with venous samples gave individual results with total errors consistent with a combined CV and bias in the 4–5% range; capillary blood samples had total errors >5% (combined CV and bias criteria). The DT-60 performance was near the 5% total error criterion for capillary samples and was >5% for venous samples. Misclassification of individuals into desirable or referral groups for venous samples was as great as 5.1% for the DT-60, 5.7% for the Vision, and 7.1% for the Reflotron. Misclas-

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