Serum amyloid A protein in acute viral infections

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Abstract

Concentrations of serum amyloid A protein (SAA) were measured in 254 children with viral diseases, including measles, varicella, rubella, mumps, echo-30 meningitis, chronic hepatitis B and C, and in eight with Kawasaki disease.

Latex agglutination nephelometric immunoassay was used for assaying SAA. In 191 out of 195 patients (98%), SAA concentrations became markedly raised in the acute phase of the viral disease: measles (97%), varicella (100%), mumps (95%), and echo-30 meningitis (99%) with mean titres of 82.4, 80.5, 60.2, 75.2, and 101.1 μg/ml respectively. This increase in SAA was followed by a rapid return to normal concentrations (<5 μg/ml) during convalescence. Remarkably higher concentrations of SAA (mean 1630 μg/ml) were detected in the acute phase of patients with Kawasaki disease, but in most of the children with chronic hepatitis B or C, the titres of SAA remained normal. There was no close correlation between SAA and serum concentrations for C1-α-acid glycoprotein, β2-microglobulin, transferrin, and IgG.

There was a clear correlation between SAA and C reactive protein concentrations, although SAA showed a greater incremental change than C reactive protein in the acute phase. In the acute phase of these viral diseases, 56% of the patients had raised SAA concentrations (>5 μg/ml) with normal C reactive protein concentrations (<5 μg/ml). These results indicate that SAA could be useful as an inflammatory marker in children with acute viral infections.

Patients and methods

PATIENTS

The study group consisted of 254 children with viral infections including measles (n=34), varicella (n=17), rubella (n=31), mumps (n=37), enterovirus infections (echo-30 meningitis, n=76), chronic hepatitis B (n=29), and chronic hepatitis C (n=30), and eight children with Kawasaki disease. The children were between 1 and 14 years old (mean 6.4 years). Measles, varicella, rubella, and mumps were diagnosed according to clinical findings and serological evaluations. Echo-30 meningitis was diagnosed based on clinical manifestations (fever, headache, vomiting, and stiff neck) and by lymphocytic pleocytosis of >30×10⁶ cells/l in the cerebrospinal fluid. Isolation of the echo-30 virus and/or serological examination by neutralising test were also conducted to confirm the diagnosis. Serum samples were collected during an epidemic in the Nagoya area in 1991.

Chronic hepatitis was defined as a continuous hepatic inflammatory process lasting one year or more. Patients with mild increases in serum aminotransferase activities (50–100 IU/l) were selected for study. Hepatitis C was diagnosed by detection of the C-100 antibody and by the presence of hepatitis C virus RNA using the polymerase chain reaction method. A diagnosis of hepatitis B was based on positive hepatitis B surface antigenemia and presence of anti-hepatitis B core antibody. The diagnosis of Kawasaki disease was based on clinical manifestations and laboratory data.

Sera were obtained from patients at designated intervals and stored at −70°C until analysis. Single serum samples were also obtained from 51 age matched healthy children.
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SAA ASSAY: LATEX AGGLUTINATION NEPHELOMETRIC IMMUNOASSAY
SAA was measured by latex agglutination nephelometric immunoassay as described previously. SAA was purified from the pooled sera of patients with rheumatic arthritis. Antiserum to SAA was raised in rabbits according to standardised procedures. Immunoglobulin G was isolated from the antiserum by diethylenetoaminoethanol cellulose ion exchange chromatography, adjusted to 1 mg/ml, and then conjugated to 10% polystyrene latex particles (mean diameter 0·1 μm) in 0·2 mol/l ammonium buffer at pH 8·2 for one hour at 37°C. After washing, latex particles were suspended at 0·1% in the same ammonium buffer containing 0·5% bovine serum albumin. For the assay, an automated immunochemistry analyser, LX-3000 (Eiken Chemical Co Ltd and AIC, Tokyo, Japan), was used. An aliquot of 20 ml of a test sample or assay standard, along with 240 μl of 0·05 mol/l hydroxyethylpiperazine—ethanesulphonic acid (HEPES) buffer at pH 7·4, and 80 μl of a latex solution were mixed and incubated in glass cells at 37°C. Light scattering was recorded at 45 seconds and 305 seconds after mixing, and any observed increases in this interval were used to calculate the SAA concentration. For the assay standard we used an SAA enriched high density lipoprotein.

ACUTE PHASE REACTANTS
For other acute phase reactants we also measured C reactive protein, β2-microglobulin (β2-M), α1-acid glycoprotein (α1-AG), transferrin, and IgG, in the same samples using the latex agglutination assay.

RESULTS
SAA CONCENTRATIONS IN PATIENTS
Fifty one normal controls aged 1–14 years (mean 5·8) had mean (SD) SAA concentrations of 1·72 (1·11) μg/ml. From these data an arbitrary upper limit for the normal SAA concentration was set at 5·0 μg/ml (mean + 3SD). In cases with acute viral diseases, SAA concentrations increased in the acute phase and declined thereafter throughout convalescence (see table 1). By contrast, however, in children with chronic hepatitis B and C the titre of SAA remained within normal at 3·0 (2·3) μg/ml in hepatitis B and 1·5 (1·1) μg/ml in hepatitis C (fig 1).

KINETICS OF SAA DURING THE COURSE OF ILLNESS
Changes in the SAA concentration were investigated at designated intervals. Figure 2 shows the kinetics for SAA in 12 cases of measles, 14

Table 1 Mean (SD) concentrations of SAA (μg/ml) in the acute and convalescent phase of illness

<table>
<thead>
<tr>
<th>Phase of illness</th>
<th>Viral infection</th>
<th>Measles (n=34)</th>
<th>Varicella (n=17)</th>
<th>Rubella (n=31)</th>
<th>Mumps (n=37)</th>
<th>Echo-30 meningitis (n=76)</th>
<th>Controls (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute</td>
<td></td>
<td>82·4 (113·3)</td>
<td>80·5 (148·9)</td>
<td>60·2 (71·9)</td>
<td>75·2 (160·2)</td>
<td>101·1 (157·0)</td>
<td>1·7 (1·1)</td>
</tr>
<tr>
<td>Convalescence</td>
<td></td>
<td>4·3 (3·3)</td>
<td>4·3 (2·1)</td>
<td>3·0 (2·1)</td>
<td>3·0 (2·0)</td>
<td>3·4 (1·6)</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 SAA concentrations in children with measles, rubella, varicella, mumps, echo-30 meningitis, hepatitis B, and hepatitis C compared with normal controls. SAA concentrations in serum samples from infected patients were obtained during the acute (A) and convalescent (C) phases of acute viral infections. The dotted line indicates the upper limit of the normal range.
of echo-30 meningitis, and eight of Kawasaki disease. Higher than normal SAA was detectable in all patients with measles on the day the rash appeared, but SAA titres sharply declined between days 4 and 7 in seven of these cases and remained above normal in the remaining five cases. SAA concentrations were raised at the onset of neurological symptoms in 13 of the 14 patients with echo-30 meningitis. In nine of these cases, the SAA titre decreased dramatically between days 4 and 8, but in the remaining five cases SAA concentrations continued to be high.

Remarkably higher titres in SAA were detectable in the acute phase of patients with Kawasaki disease, ranging from 250–3560 μg/ml (mean 1630 μg/ml), and the concentrations remained at higher concentrations (from 10–50 μg/ml) during convalescence.

**Figure 2** Kinetics for SAA concentrations in 12 children with measles, 14 with echo-30 meningitis, and eight with Kawasaki disease.

**Figure 3** Relation between SAA and other acute phase reactants (α₁-AG, β₂-M, transferrin and IgG) in sera during the acute phase of viral diseases.

**Figure 4** Relation between SAA and C reactive protein concentrations in children acute viral diseases. Dotted lines indicate the upper limit for the normal range.

**Table 2** SAA and C reactive protein in the acute and convalescent phase of illness. Figures are number (%)

<table>
<thead>
<tr>
<th>SAA (μg/ml)</th>
<th>C reactive protein (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute (n=195)</td>
<td>Convalescence (n=165)</td>
</tr>
<tr>
<td>SAA&lt;5</td>
<td>SAA&lt;5</td>
</tr>
<tr>
<td>≥5</td>
<td>81 (41.5)</td>
</tr>
<tr>
<td>&lt;5</td>
<td>110 (56.4)</td>
</tr>
<tr>
<td>Total</td>
<td>191 (97.9)</td>
</tr>
</tbody>
</table>

*Number of patients with measles, varicella, rubella, mumps, and echo-30 meningitis.

**RELATION BETWEEN SAA AND ACUTE PHASE REACTANTS**

A comparison between SAA and acute phase reactants was conducted after preliminary findings that showed increases in SAA during the acute phase of viral infections. No close relations, however, could be found between the SAA concentration and serum concentrations for α₁-AG, β₂-M, transferrin, or IgG (fig 3).

**RELATION BETWEEN SAA AND C REACTIVE PROTEIN**

A total of 195 children with acute viral infections were studied. In most of cases, acute phase proteins were measured on more than one occasion, resulting in 360 sets of measurements. A comparison of serum SAA and C reactive protein concentrations is shown in fig 4. A positive correlation (r=0.86) was seen over a wide range of concentrations, both within and above normal. SAA, however, showed a greater incremental change in the acute phase with maximum concentrations ranging from 2–900 μg/ml when compared with C reactive protein concentrations of 2–100 μg/ml. It is interesting to note that increased SAA concentrations were observed despite normal C reactive protein concentrations. Out of the 195 patients, 110 (56.4%) showed increases in SAA during the acute phase, although the C reactive protein concentrations remained normal (table 2). No correlation could be found for this increased SAA/normal C reactive protein condition and the type of disease. In 130 out of 165 patients (79%), SAA concentrations declined to within the normal range during convalescence, but remained above normal in the other 35 (21%) patients. These results indicate that SAA may be useful as an inflammatory marker in cases with acute viral diseases.

**Discussion**

SAA has been clinically evaluated as an acute phase reactant sensitive to serum in a number of inflammatory diseases. It has been shown to be especially useful in the close monitoring of disease activity in rheumatoid arthritis patients. Increased SAA concentrations have been seen
among serum samples taken from patients with viral diseases in the acute phase including cytomegalovirus, herpes simplex, rubella, measles, varicella, and herpes zoster.\textsuperscript{14} The significance of this phenomenon, however, has not yet been established, and SAA assaying has not been applied to a wide clinical use. One reason may be that the present methods for quantifying SAA, such as enzyme immunoassay,\textsuperscript{25-27} radioimmunoassay,\textsuperscript{28} and nephelometric immunoassay,\textsuperscript{29} are time consuming and unsuitable for application in clinical laboratories. For assaying SAA in this study we used the latex agglutination assay as it is simpler and faster than other methods that take more than an hour to complete. Accurate and rapid assessment of SAA values by this method could prove to be useful for evaluating acute viral diseases and thus should be included as a routine test in clinical applications.\textsuperscript{20}

To establish the usefulness of SAA in evaluating viral diseases we reviewed a large series of children with measles, varicella, rubella, mumps, and echo-30 meningitis. Findings revealed an early rise in SAA concentrations (98%) during the acute phase of viral diseases followed by a rapid return to normal concentrations (79%) in convalescence. However, SAA concentrations in 35 (21%) samples remained above normal even throughout convalescence. This may be due to the fact that most of these patients, especially those with measles and echo-30 meningitis, had not completely recovered from the disease by the time serum samples were obtained. These findings suggest that SAA could be useful as a marker of clinical recovery in patients with viral diseases. By contrast, we found that titres of SAA remained within normal ranges in patients with chronic hepatitis B and C.

Sera obtained from patients in the acute phase of viral disease showed no close correlation between SAA concentrations and serum concentrations for α1-AG, β2-M, transferrin, and IgG. These findings are of considerable interest as they illustrate the discrepancies among different acute phase proteins in patients with viral infections.

A comparison of SAA and C reactive protein concentrations revealed a correlation over a wide range both within and above normal. SAA, however, showed a greater incremental change in the acute phase of the disease when compared with C reactive protein. Moreover, raised SAA concentrations were observed despite normal C reactive protein concentrations, accounting for 56% of all acute phase samples. Unlike C reactive protein in bacterial infections, no reliable markers are available for monitoring viral diseases. Clinical signs and symptoms such as fever with no C reactive protein increase may suggest the presence of a viral infection. In our study more than half of the patients (56%) had increased SAA (>5 µg/ml) with no increases in C reactive protein (<5 µg/ml) during the acute phase. These results indicate that the assay of SAA in combination with C reactive protein could be useful to confirm a diagnosis of acute viral infection. Approximately 40% of our patients, however, demonstrated increases in both SAA and C reactive protein. This may be due to coexisting infections of a bacterial or other origin, although previous studies have suggested that C reactive protein concentrations increase in cases with severe viral infection alone.\textsuperscript{29}

The mechanism by which inflammatory stimuli induce the synthesis of SAA in hepatocytes and other acute phase proteins has been a subject of intense investigation in recent years. Many studies indicate that interleukin(IL)-1, IL-6, and the tumour necrosis factor are mediators for modulating of liver synthesis in these acute phase proteins.\textsuperscript{20} Our findings suggest that cytokines such as IL-1, IL-6, and TNF may be responsible for the increases in SAA, and that SAA synthesis in the hepatocyte may be more easily activated by IL-1 and IL-6 than C reactive protein or other acute phase proteins. During the silent stages of chronic hepatitis B and C, SAA concentrations do not show such increases probably due to the weak stimuli by cytokines.

As far as we know this is the first report of acute phase responses of SAA in Kawasaki disease, which is classified as a collagen vasculitis with unknown aetiology. In this study the concentration of SAA in Kawasaki disease was extremely high in the acute phase but declined thereafter within several days. The concentrations of SAA, however, remained at higher values (from 10-50 µg/ml) during convalescence. Monitoring SAA, therefore, may be useful as marker for disease activity and responses to treatment in Kawasaki disease.

In conclusion, an increase in SAA concentration may provide a useful marker of viral infection in cases where a differential diagnosis may be difficult clinically and where viral isolation may not be possible.

30 Yap SH, Moshaue HJ, Hazenberg BPC, et al. Tumor necrosis factor (TNF) inhibits interleukin (IL) 1 and/or IL-6 stimulated synthesis of C-reactive protein (CRP) and serum amyloid A (SAA) in primary cultures of human hepatocytes. Biochem Biophys Acta 1991;1091:405–8.