

## Acute phase response of serum amyloid A protein and C reactive protein to the common cold and influenza

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**SUMMARY** C reactive protein (CRP) and serum amyloid A protein (SAA) are sensitive and rapid acute phase reactants, and their measurement for monitoring inflammatory disease and assessing the prognosis in secondary amyloidosis is gaining widespread acceptance. The changes in these proteins in eight subjects suffering from natural colds, 15 subjects with experimentally induced colds (rhinoviruses E1, 3, 9, 14, or 31), and eight with experimentally induced influenza (A/Eng/40/83) were studied. SAA concentration increased in 21 of the 23 subjects with natural or experimental rhinovirus colds (mean increase 95 mg/l); CRP concentration increased in 11 (mean increase 11 mg/l). All subjects with influenza showed pronounced increases in SAA concentrations (mean increase 642 mg/l) while six showed increases in CRP concentration (mean increase 22 mg/l). All these increases were highly significant ( $p < 0.001$ ). Asymptomatic excretors of both rhinovirus and influenza virus showed significant increases in SAA concentration ( $p = 0.015$  for rhinovirus and  $p < 0.001$  for influenza virus) but not in CRP concentration. No changes in SAA or CRP values were seen in 12 volunteers after challenge with saline.

These observations suggest that caution is required in the interpretation of estimations of SAA concentration and that it may be too sensitive an acute phase protein for clinical use as its concentration may be raised in both trivial and asymptomatic viral infections.

The acute phase response comprises an increase in hepatic synthesis and plasma concentration of a group of proteins probably involved in the inflammatory process as mediators, inhibitors, scavengers, and immune regulators.<sup>1</sup> It results from the action of the cytokines interleukin-1,<sup>2</sup> released by activated macrophages, and epidermal T cell activating factor, released by damaged epidermal cells.<sup>3</sup> Macrophages release interleukin-1 in response to bacterial lipopolysaccharide, muramyl dipeptide, lymphokines, and possibly to any form of membrane perturbation, including phagocytosis,<sup>2</sup> thus providing an explanation for the acute phase response of most forms of inflammation. It is not known whether other inducers of the acute phase response such as prostaglandins<sup>4</sup> act via macrophage interleukin-1 production or directly on the liver.

The various acute phase proteins differ in their

time course and incremental responses to inflammatory stimuli.<sup>5</sup> A number of recent observations suggest that serum amyloid A protein (SAA) is the most sensitive acute phase protein, its serum concentration increasing when others, such as C reactive protein (CRP), remain unaffected.<sup>6,7</sup> Viral infections have been reported to cause little acute phase response and raised CRP concentrations have been used to distinguish illness of bacterial origin from those of viral aetiology.<sup>8,9</sup> More recently, however, certain viral infections have been shown to cause substantial increases in both CRP and SAA concentrations.<sup>10-12</sup>

In view of the increasing use of acute phase protein measurements to detect and monitor inflammation<sup>13</sup> and in particular the recent interest in the diagnostic potential of SAA estimations,<sup>14-19</sup> we thought it pertinent to establish the response of this protein to the "common cold," which might interfere with the interpretation of such measurements.

## Subjects and methods

The study population consisted of 61 volunteers housed in isolation at the Common Cold Unit and eight subjects with wild colds, who were members of the pathology department at the Bristol Royal Infirmary. The volunteers were examined before entry into the study and were free from clinical evidence of infection or inflammation. Eight subjects were subsequently excluded from the data owing to the presence of increased SAA concentrations at the outset. In all except one of these concentrations returned to normal over the next 10 days and probably represented mild or subclinical infections. The one remaining volunteer probably has early rheumatoid arthritis.

Virus or saline was instilled into the nose after a quarantine period of three days and samples of blood for acute phase protein measurement were taken by venepuncture; the serum obtained was stored at  $-20^{\circ}\text{C}$  until analysis. In addition to the preinoculation sample daily samples were obtained from some subjects in order to establish the time course of the acute phase response. Thereafter samples were obtained on days 3 (influenza study) and 4 or 5 (rhinovirus study and controls). The severity of the illness was assessed by the standard methods of the unit.<sup>20</sup> Nasal secretions were cultured for viruses and antibody titres were measured in serum collected before and two to three weeks after virus challenge. Thirty six subjects were challenged with bacteriologically sterile nasal washings containing an untyped human rhinovirus (HRV), E1, HRV9, HRVs 9 and 14, HRVs 3 and 9, or HRVs 3 and 31. Thirteen subjects were challenged with diluted allantoic fluid containing influenza virus A/Eng/40/83. Twelve subjects (controls) had saline but no virus instilled into their noses. The subjects with wild colds provided blood samples, as described, on day 3 after the onset of symptoms and in health.

SAA was measured by a modified radial immunodiffusion method<sup>21</sup> using antiserum from Atlantic Antibodies, Westbrook, Maine, USA. The lower limit of sensitivity of the assay is  $0.002\text{ g/l}$  (normal range  $<0.002\text{--}0.030\text{ g/l}$ ). CRP was measured by simple radial immunodiffusion<sup>22</sup> with antiserum from Seward Laboratories, London. The lower limit of sensitivity of the assay is  $0.005\text{ g/l}$  (normal range  $<0.005\text{--}0.011\text{ g/l}$ ). The within batch precision (CV) is 6% for SAA and 7.5% for CRP. Sequential samples from individual subjects were analysed within the same analytical batch. The significances of the increases in SAA and CRP concentrations were assessed by Mann-Whitney tests as the data were not normally distributed. In the case of CRP the probabilities are, at best, estimates of

the true significance owing to the inability of our assay to measure values in the lower part of the normal range.

This study was approved by the ethical committee at Northwick Park Hospital.

## Results

### WILD COLDS AND EXPERIMENTAL RHINOVIRUS INFECTION

Preliminary studies indicated that the peak SAA and CRP response occurred in parallel on days 4 or 5 after viral challenge. Fig. 1 shows an example of the time course of the changes in serum SAA concentration in one subject. Symptoms generally appeared about 36–48 h after challenge.

The results are summarised in the Table. A total of twenty three subjects suffered symptomatic colds (eight wild colds and 15 in volunteers); 21 of these showed increases in SAA concentration, which in 20 subjects rose above the upper limit of the normal range. Eleven showed increases in CRP concentration, which in nine rose above normal. The increases in both proteins were significant ( $p < 0.001$ ). The size of the acute phase response showed no relation to the type of virus administered, the severity of the cold, or the increase in serum antibody concentration.

Thirteen subjects did not develop significant colds. Ten of these, however excreted virus, of whom nine showed increases in SAA ( $p = 0.05$ ) but not CRP concentrations. Of the three who did not excrete virus two showed slight increases in SAA concentration. The SAA results are shown in Fig. 2 and the CRP results in Fig. 3.

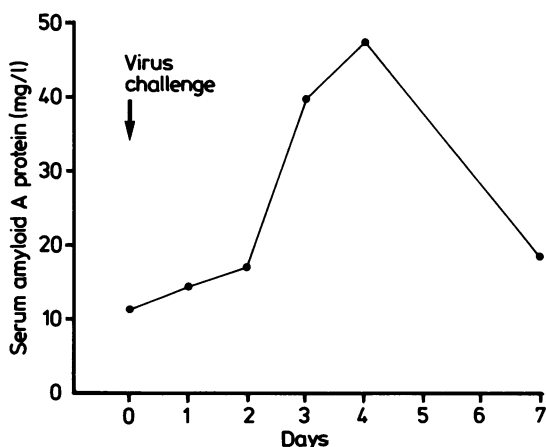


Fig. 1 Time course of changes in serum amyloid A protein concentration after challenge with human rhinovirus HRV9 in one subject who developed symptoms.

*Virus excretion and acute phase protein changes in subjects*

Group	No subjects	Virus isolated*	Increased serum concentrations	
			SAA	CRP
Rejects	8		8	—
Colds				
Symptomatic volunteers	15	+ 15	21	11
Wild colds	8		(20 > normal)	(9 > normal)
Asymptomatic volunteers	13	+ 10 - 3	9 2	—
Influenza				
Symptomatic	8	+ 8	8	6
Asymptomatic	5	+ 3 - 2	3 —	—
Controls	12	—	—	—

\*+ = virus isolated; — = virus not isolated.  
SAA = serum amyloid A protein.  
CRP = C reactive protein.

EXPERIMENTAL INFLUENZA A VIRUS INFECTIONS

Thirteen subjects were challenged with influenza A virus. Eight developed an influenza like illness which started about 24 h after challenge, and all showed

pronounced increases in SAA concentration (Table). Of these, six showed increases in CRP concentration. The overall increase in both cases was significant ( $p = 0.001$ ) (Figs. 2 and 3). Sequential samples were obtained from five of those who developed influenza and the time course of the

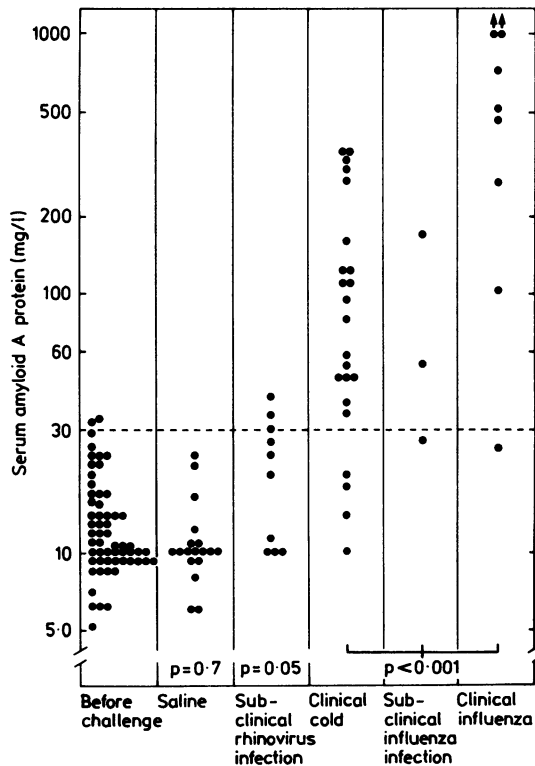


Fig. 2 Serum amyloid A protein concentrations in the different groups of subjects after challenge. The significance of the increase in each group over prechallenge concentrations ( $p$ ) is shown.

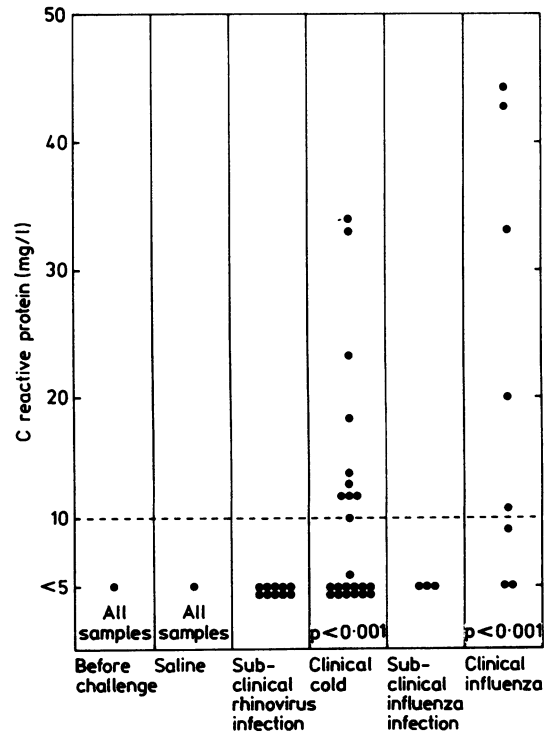


Fig. 3 Serum C reactive protein concentrations in the different groups of subjects after challenge. The significance of the increase in each group over the prechallenge concentrations ( $p$ ) is shown.

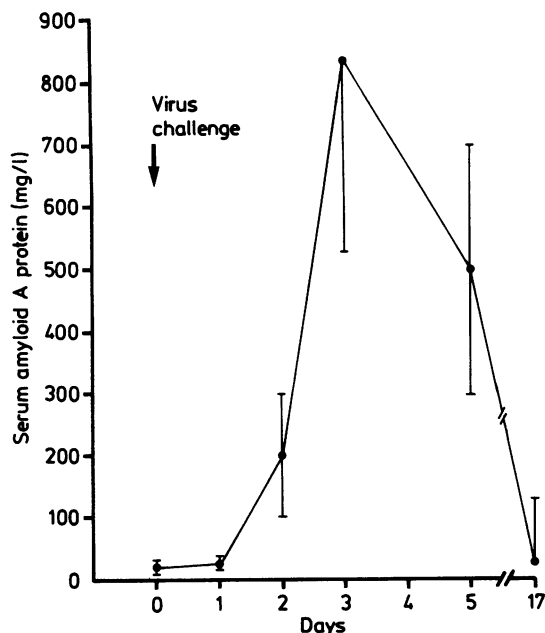


Fig. 4 Time course of changes in serum amyloid A protein following challenge with influenza virus A/Eng/40/83 in five subjects who developed symptoms. Mean and SEM are shown.

response showed that both SAA and CRP reached their maximum concentrations on day 3 after viral challenge. SAA changes are shown in Fig. 4.

Three subjects did not develop a clinical illness but did excrete virus and all showed increases in SAA concentrations (overall  $p < 0.001$ ) but not in CRP concentrations. Two subjects who neither developed symptoms nor excreted virus showed no increase in either acute phase protein.

#### CONTROLS

In the group of 12 volunteers inoculated with saline there was no significant change in the serum concentration of SAA or CRP on days 4 or 5 after the instillation of sterile saline into the nose ( $p = 0.70$  for SAA).

#### Discussion

As far as we are aware acute phase responses have not been previously described in infection with human rhinoviruses. Increased amounts of CRP have been found in the serum of patients with rubella, herpes simplex, cytomegalovirus, influenza A and B, and enterovirus.<sup>10</sup> Raised SAA concentrations have been found in acute serum samples from patients with cytomegalovirus, herpes simplex, rubella, measles, varicella, and zoster, though the

time course of the response was not studied.<sup>11, 12</sup> In this study almost all subjects with a natural cold or developing a clinical illness after infection with a HRV or influenza A virus showed increased concentrations of SAA. Increases in CRP were less striking and present in only about half of the volunteers developing colds.

The time course of the changes in SAA and CRP concentration showed a peak response on day 4 or 5 for rhinovirus infections and on day 3 for influenza. In both cases, therefore, the interval is similar after the onset of symptoms and we are thus sampling at the same time relative to the clinical illness. At this time we would expect to obtain peak values for both conditions (Figs. 1 and 4).

It is of considerable interest that increases in SAA concentration were also found in most asymptomatic subjects from whom a rhinovirus or influenza virus was isolated. This may have resulted from a local inflammatory response associated with macrophage interleukin-1 release or from interferon release by virus infected cells. Interferon preparations administered systemically have been shown to potentiate the endotoxin induced production of interleukin-1 by man.<sup>23, 24</sup> Our findings suggest that an increase in SAA concentration may provide a useful marker of viral infection in cases where viral isolation may not be possible. It may also be a useful way of identifying the host response to a virus infection even when there are no signs or symptoms.

This study shows again that SAA is a more sensitive acute phase protein than CRP since its concentration increases when that of CRP does not and it does so to a much greater extent. This sensitivity to common and trivial viral illness and even subclinical infections must be borne in mind when it is used as a marker of inflammation in other clinical contexts, such as monitoring the activity of chronic inflammatory diseases.

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