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Serum amyloid A (SAA) in viral infection: rubella, measles and subacute sclerosing panencephalitis (SSPE)

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SUMMARY

Serum amyloid A (SAA) levels were determined in the serial serum samples of eight rubella, 10 measles and seven subacute sclerosing panencephalitis (SSPE) patients. An early rise in SAA levels was detected in the acute phase in rubella and measles, followed by a prompt decrease in the convalescent phase. In a number of measles and rubella patients from whom early serum samples were available, the rise of SAA levels could be demonstrated before specific viral antibodies could be detected by complement fixation (CF) (measles) and haemagglutination inhibition (HI) (rubella). In only one rubella and one measles patient was no rise of SAA level detected. In SSPE only a moderate increase in SAA levels was noted except in one patient during a temporary deterioration, at which time the SAA level was very high; it returned to close to normal shortly thereafter. The possibility that SAA levels might be of value in monitoring the severity of infections, the recovery process and effects of anti-viral agents is discussed.

INTRODUCTION

Serum amyloid A (SAA) is a normal serum protein (Rosenthal & Franklin, 1975) which associates with a high density lipoprotein (Benditt & Eriksen, 1977). Being an acute phase reactant its level increases markedly in many bacterial infections, malignant disease and in response to a variety of immunological stimuli (Rosenthal & Franklin, 1975; Rosenthal & Sullivan, 1979). SAA seems to be the circulating precursor of the main fibrillar protein in secondary amyloidosis (Levin, Pras & Franklin, 1973; Husby & Natvig, 1974; Benson *et al.*, 1975). It has been suggested (Lavie, Zucker-Franklin & Franklin, 1980) that amyloid A (AA) is derived from SAA by proteolytic cleavage. In familial Mediterranean fever (FMF) as in tropical splenomegaly syndrome (TSS), which has a high incidence rate in Papua New Guinea (McAdam, 1981), there are high levels of SAA associated with the recurrent acute episodes of inflammation, fever and leucocytosis and some patients go on to develop amyloidosis (Ignaczak *et al.*, 1977). Six different species of SAA have already been identified (Bausserman, Herbert & McAdam, 1980), and various tissues have been reported as the site of SAA origin, viz connective tissue (Linder *et al.*, 1977), spleen (Baumal *et al.*, 1978), neutrophils (Rosenthal & Sullivan, 1978) and liver (Sipe, McAdam & Uchino, 1978; Benson & Kleiner, 1980). It has been suggested that SAA may be involved in immunoregulation by

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suppressing the immune response (Benson & Aldo-Benson, 1979). The appearance of SAA in various inflammatory processes and the need for biochemical markers in the detection of viral infections led us to look at SAA levels in several viral infections.

MATERIALS AND METHODS

Human sera. Single serum samples were obtained from 30 healthy adults aged 25–40, and serial serum samples were obtained from 25 patients including eight with rubella, 10 with measles and seven with subacute sclerosing panencephalitis (SSPE). CSF samples were obtained from four SSPE patients.

SAA levels. SAA levels were determined by RIA as previously described (Rosenthal & Franklin, 1975) with modifications using purified AA from human amyloidogenic spleen of the FMF patient and rabbit antibody against human AA (Franklin & Pras, 1969). Iodination of AA was performed by the chloramine T method (Hunter & Greenwood, 1962). The assay was carried out in barbital saline buffer 0·05 M, pH 8·2, in the presence of 0·5% bovine γ globulin, by incubation at 37°C for 2 hr, then overnight at 4°C. Separation of bound SAA from free was performed by centrifugation after the addition of 2 ml of 30% polyethylene glycol (mol. wt 6,000) to 1 ml of reaction mixture. The results of SAA levels are expressed in μg/ml AA equivalent.

Determination of HI antibodies to rubella. HI antibody titre was measured according to Norrby & Gollmer (1972), as previously described (Kahane, Goldstein & Sarov, 1979; Shani, Haikin & Sarov, 1981).

CF assay for antibodies to measles virus. The CF test was performed as described by Sever (1962): 2 units of lyophilized guinea-pig complement (Behringwerke, AG, Marburg, FRG) and 3 units of haemolysin (DIFCO, Detroit, Michigan, USA) were used.

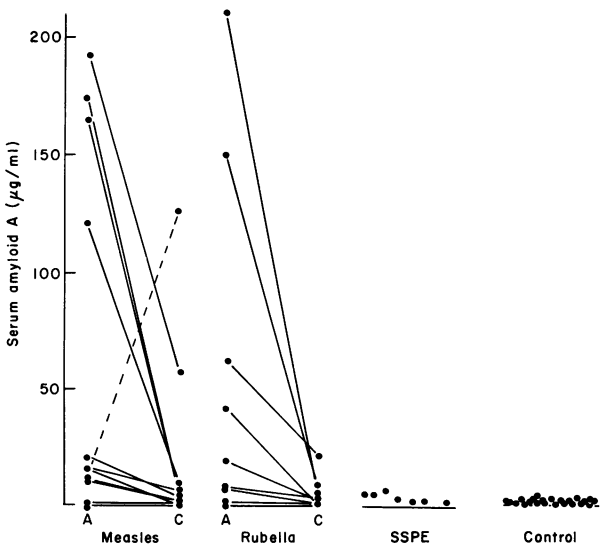

Fig. 1. SAA levels in patients with rubella, measles and SSPE compared to normal controls. Serum samples from infected patients and normal controls were tested for SAA level in acute and convalescent phase of rubella and measles; in the case of SSPE patients only maximal levels during the 1 year follow up are indicated. The high SAA level of one of these patients during the period of hospitalization is not included (see test). One patient with pneumonia following measles infection showed no decrease in SAA levels up to 29th day post-infection (dotted line). The results of SAA levels are expressed in μg/ml AA equivalent. A = acute; C = convalescent.

RESULTS

Determination of SAA levels in acute and convalescent sera of seven rubella patients (Fig. 1) indicates an early increase in SAA levels in the acute phase (range 7·3–210·0 μg/ml) followed by a decrease in the convalescent period. Thirty normal controls aged 20–40 had mean (m) SAA levels of 1·4 ± 0·2 μg/ml (mean ± standard deviation). In one patient elevated values of SAA were detected while the haemagglutination inhibition (HI) antibodies were still negative (titre < 8). In one rubella patient only no increase in the SAA level was detected. As can be seen in Fig. 1, the SAA level in nine measles patients was elevated in the acute phase (range 12·0–192·0 μg/ml), with a prompt decrease in the convalescent period. In five of nine measles patients elevated values of SAA could be detected while the CF antibodies were still negative (< 4). In one patient suffering from pnuemonia following measles infection (Fig. 1, dotted line) no decrease in SAA was detected up to the 29th day post-infection. No rise was noted in one of the measles patients but the first serum sample was taken 14 days after onset of illness.

A different pattern of SAA behaviour was noted in a group of seven SSPE patients who characteristically have persistent measles like virus infection and high titres of anti-measles antibody in serum and CSF. Four of these patients were followed up for 1 year. The SAA level was measured in sera and in a CSF sample from each. In all the SSPE patients a moderate increase in SAA level was noted (m = 3·1 ± 0·7 μg/ml in comparison to 1·4 ± 0·2 μg/ml in controls). One of the patients was hospitalized 1 year post-diagnosis with marked temporary deterioration. At this time, a high increase in SAA level was detected (630 μg/ml), which decreased to almost normal after 10 days. Of the four CSF samples available for testing, only one had a detectable amount of SAA (0·7 μg/ml).

DISCUSSION

The mechanism by which inflammatory stimuli induce synthesis of SAA has been a subject of active investigation (McAdam & Sipe, 1976; McAdam, 1981; Sipe *et al.*, 1979; Selinger *et al.*, 1980). McAdam & Sipe (1976) reported high levels of SAA in liver homogenates after lipopolysaccharide (LPS) treatment of mice. SAA was found to be synthesized *in vitro* by isolated hepatocytes cultured in the presence of a monokine derived from LPS stimulated macrophages, which has been termed SAA inducer (Selinger *et al.*, 1980). SAA inducer causes SAA synthesis when injected in LPS non-responder mice *in vivo* (Sipe *et al.*, 1979; Selinger *et al.*, 1980). Sztein *et al.*, (1981) have recently shown that highly purified preparations of murine lymphocyte activating factor (LAF), partially purified human LAF, otherwise known as interleukin 1 (IL1), and two species of partially purified rabbit endogenous pyrogen (EP) induce SAA synthesis in mice. Antiserum shown to block pyrogenic and thymocyte proliferating activities also blocked the SAA inducing activity suggesting the possibility that these are closely related if not identical molecules (Sztein *et al.*, 1981). It has been suggested that this monokine can thus account for several of the clinical features of diseases leading to secondary amyloidosis: fever, leucocytosis, lymphocyte activation with hyperglobulinaemia and elevated concentrations of SAA (McAdam, 1981).

The SAA response in mice to LPS begins with a latent period of 2–4 hr, followed by an exponential increase in SAA concentration over the subsequent 12–16 hr. Approximately 20–24 hr after LPS administration the SAA concentration declines and returns to base line values by 48 hr (Sipe, 1978).

The present study demonstrates an early rise in SAA levels in the acute phase of rubella and measles infections, followed by a decrease during the convalescent stage. In one patient suffering from pneumonia following measles infection no decrease in SAA levels was detected up to the 29th day post-infection (Fig. 1). In the case of subacute sclerosing panencephalitis (SSPE), a persistent infection, a slight increase of SAA was noted. Recently, Salonen & Vaheri (1981) reported elevated levels of C-reactive protein (CRP) in acute phase sera of persons with viral infections before virus specific serum antibody was detectable, and a decrease approaching normal values in most convalescent phase sera. The possibility that CRP and SAA might be of value in monitoring the

severity of infection, recovery process and possible effects of therapeutic agents needs to be examined. Since various species of SAA have already been identified (Bausserman *et al.*, 1980) determination of SAA levels may have more specific diagnostic value if it should be found that different SAA's are produced in response to different inducers.

We are currently investigating the mechanism of SAA induction by viral agents *in vitro*. Future studies are also intended to examine the possibility that a rise of SAA in FMF patients could be due to possible induction by periodic reactivation of a latent viral agent.

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