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RESEARCH ARTICLE

IN-VITRO INTERACTION OF SOLUBLE AND AMYLOID FORM OF SERUM AMYLOID A PROTEIN TO BC3H1CELLS

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ABSTRACT

The BC3H1 smooth muscle cells of mice brain, the study was carried out membrane binding. This is important in relation to the activity of membrane proteins, because losing the activity of such systems will ultimately lead to malfunction or death of the cell. The interactions of Serum Amyloid A (SAA) and Serum Amyloid A protofibrils with BC3H1 cells of the mouse are dealt with in detail to study the binding of SAA protofibrils in various conditions. The FACScan and MTT assay results have shown the SAA and SAA fibrils binding and cell toxicity with the BC3H1 cells with different concentrations of Serum amyloid P component and Amyloid enhancing factor. Specifically, cells were incubated with 1.25-6.25 μM SAA-FITC and SAA protofibrils-FITC assayed. The 50% viable BC3H1 cells at 4–6 μM with an LD50 of 3.5 μM. The interaction of serum amyloid A fibrils with a cell surface binding site/receptor might alter the local environment to cause cellular dysfunction and to be more favorable for amyloid formation. The RAGE (receptor for advanced glycation endproducts) a polyvalent receptor in the immunoglobulin super family has been implicated in binding with the isoform of SAA (SAA1.1) which has the highest fibirillogenic property. In the present study, concluding the SAA fibrils more binding and cell cytotoxicity than SAA protein.

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INTRODUCTION

The cell membrane is a dynamic entity, containing highly organized proteins, which are under constant thermal perturbation. The integrity of the membrane is maintained by the dynamic lipid matrix by acting as a thermal sink. Oxidative and ionic perturbations are other cellular insults. Various transporters nullify the ion fluctuation effects. The thermal fluctuations are transduced to the system through the cell membrane, and hyper thermal effects get attenuated through the expression of the heat shock proteins. The cells are protected from oxidative insults by the various anti oxidant mechanisms. The membrane is damaged when these intricate balances are lost. The interaction with toxic amyloid fibrils is another type of cellular insult for which the mechanism is not yet known. Explanations like oxidative and ionic imbalance are being given with a lot of experimental evidence. Recently, membrane-stabilized protofibrillar structures were implicated in amyloid-related toxicity to mammalian cellular system with membrane-amyloid interaction as the central point of focus in the understanding of amyloid chemistry. Yet another hypothesis predicts the specific denaturing ability of amyloid fibrils on interacting with native proteins.

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This is further supported by the research findings that amyloid have a very high structure breaking effect on vital proteins. Several groups have reported the loss of membrane-bound enzymatic activity due to amyloid interaction. Amyloid is one protein known to have anti chaperonic activity with very high membrane binding property. The toxic mechanism of any amyloid depends upon the specific and non-specific binding effect of amyloid assemblages in various forms.

The SAA proteins are highly conserved and ubiquitous in all vertebrate systems. They belong to the apolipoprteins (apoSAA) and are mainly associated with the HDL3 of serum HDL. Following infections or injuries, a set of systemic physiological responses is initiated, which are collectively referred to as the acute-phase response. Among others, these include fever, metabolic changes in several organ systems, high profile protein synthesis in the liver and production of proinflammatory cytokines. Together these provide optimal conditions for the restoration of homeostasis. The apoSAAs are one of the major classes of positive acute-phase reactants (A-apoSAA) for which plasma levels are elevated several hundred to several thousand folds during acute inflammation and injury (Hoffman and Benditt 1982; Kushner 1982). At present, four human SAA gene loci have been identified on chromosome 11 in this multigene family (Husbey *et al*., 1994). Two of them are acute-phase hyper inducible genes (SAA1

and SAA2, which encode A-apoSAAs), one is a pseudogene (SAA3) and one is expressed constitutively (SAA4, which encodes C-SAA) (Steel and Whitehead 1994). It has been shown that the synthesis of A-apoSAA is induced by proinflammatory cytokines (Betts *et al*., 1991; Raynes *et al*., 1991). In particular, the acute-phase genes coding for SAAs are regulated by IL-1 or TNF and reach the maximum stimulation in the presence of interleukin-6 (Grehan *et al*., 1997; Jiang *et al*., 1995; Lozanski *et al*., 1996; Rokita *et al*., 1994; Rygg 1996). While the primary source of circulating A-apoSAAs is the liver for the systemic acute-phase response (Kushner 1982), extrahepatic expression of these proteins has been demonstrated in different species and in various cell types, such as cultured smooth muscle cells, endothelial cells, monocytes, and macrophage cell lines (Ramadori *et al*., 1985; Meek and Benditt 1989; Webb *et al*., 1989; Meek *et al*., 1994). Therefore, it appears that these cells could provide a local source of SAA proteins. The local production of A-apoSAA raises the possibility that A-apoSAA may play a role related to the site of expression rather than to acute-phase systemic response.

This question is especially important in relation to the activity of membrane proteins, because losing the activity of such systems will ultimately lead to malfunction or death of the cell. In this study, the interactions of SAA and SAA proto fibrils with different cells of the mouse are dealt with in detail to study the binding of SAA proto fibrils in various conditions. Specifically, interaction of amyloid A fibrils with a cell surface binding site/receptor might alter the local environment to cause cellular dysfunction and to be more favorable for amyloid formation. Already RAGE (receptor for advanced glycation endproducts) a polyvalent receptor in the immuglulobulin superfamily has been implicated in binding with the isoform of SAA (SAA1.1) which has the highest fibirillogenic property.

MATERIALS AND METHODS

Isolation of SAA Protein

HDL was isolated from the pooled plasma by sequential centrifugation (Lindhorst *et al*., 1997). Briefly, the density of the plasma was adjusted to 1.063 g/l with solid KBr, followed by centrifugation at 10°C for 16 hrs at 75000g in a Beckman 50 Ti rotor. The top quarter, which contained VLDL and LDL, was aspirated and discarded. The pooled infranatants, adjusted to solvent density 1.21 g/l by addition of KBr, were re-centrifuged for 48 hrs at 106,000g in 50 Ti rotor. The top layers (HDL) were aspirated, pooled and concentrated with 0.15 M NaCl, 0.1% EDTA, pH 6.4 for 2 hrs at 40°C and then with 10% formic acid for 2 hrs using FILTRON. (GELMAN Sciences. India).

Gel Chromatography of HDL-SAA Protein

The concentrated HDL preparation was fractionated on a column 2.5 cm × 100 cm of Sephacryl S-200 (Pharmacia Biotech, Uppsala, Sweden) previously equilibrated with 10% (v/v) formic acid at a flow rate of 25 mL/hr. 5 mL fractions were collected using FPLC (Fast Protein Liquid Chromatography) Gradifrac™ system (Pharmacia Biotech, Uppsala, Sweden). Hiload pump p-1 fractions were monitored at 280 nm (Fig. 3a). Fractions were pooled and concentrated using FILTRON (GELMAN Sciences, India) and freeze-dried.

Anion Exchange Chromatography

The freeze dried sample obtained from the gel filtration step was dissolved in 10 mM tris (pH 8.2/8 M urea and was applied to a column (1 cm × 30 cm) of DEAE cellulose (Whatman DE-52) previously equilibrated with the same buffer. After loading, the column was washed further with 80 mL of initial buffer, using FPLC Gradifrac™ system (Pharmacia Biotech, Uppsala, Sweden). The column was then eluted with a linear gradient of 150 mL each at 10mM Tris (pH 8.2)/8 M urea and 150 mM Tris (pH 8.2)/8 M urea. The flow rate was 12 mL/hr and 3 mL fractions were collected (Fig. 3b). Fractions were pooled and concentrated using FILTRON (GELMAN Sciences, India). At the end of the gradient, the column was further washed with 7.5 mL of 1 M Tris (pH 8.2)/8 M urea. Protein was determined by the method of Lowry *et al* (Lowry *et al.,* 1951). Fractions were pooled and concentrated using FILTRON (GELMAN Sciences, India) and freeze-dried.

Characterization of SAA Protein

Column fractions were analyzed for their potential component on 15% (w/v) polyacrylamide gel in SDS system as described by Laemmli (1970) and 10-20% native-PAGE gradient gels system as described by Layfield *et al*. (1996).

RP-HPLC

The RP-HPLC equipment (Kaplan *et al.,* 1999) consisted of a Waters RP-HPLC pump 515 A and B (Waters, Milford, MA, USA) solvent delivery system with automated gradient controller coupled to a Shimadzu. SPD 10A UV-Visible detector (Shimadzu, Kyoto, Japan) with an 8 µl cell, a waters 746 data module. A Waters Spherisorb ODS 2 analytical column LC_{18} 25 cm × 4.6 mm (Waters, Milford, MA, USA) with 20 µl sample loop. The samples of amyloid protein were suspended in aqueous 0.1% TFA in water and filtered in 0.2 µ nylon membrane filter. RP-HPLC was performed over 45 min with a linear gradient at 77% acetonitrile in 0.1% TFA. The effluent was monitored at 220 nm.

Size Exclusion Chromatography (SEC)

Size exclusion HPLC columns were used to purify the amyloid protein, monitored with Shimadzu UV-Visible detector (Shimadzu, Kyoto, Japan) Ultrahydrogel 250™, Ultrahydrogel 500™ 7.8 × 300 mm column (Waters, Japan). Eluents consist of 20% acetonitrile 0.1% TFA solution and applied to the sample loop (20 µl per run). The elution was monitored at 220 nm; with isocratic flow rate at 0.8 mL/min. Signals were collected from Waters 746 data module (Waters, Milford, MA, USA).

Generation of SAA fibrils

Swiss white mice by producing a small sterile dorsal subcutaneously injecting 0.5 mL of 10% (w/v) Vitamin free Casein (ICN Pharmaceuticals, Cleveland, OH, USA) solution under the skin of the back (Botto *et al*., 1997; Benson *et al*.,

1977). After 24 hrs the mice were sacrificed and transferred to chilled plate.

Fluorescence Spectroscopy

Purified SAA and SAA protofibrils (400 µg/400 µl 0.001% NH_4OH, pH 9.0) were labeled with fluorescein isothiocyanate (FITC) (Fluka, USA) by dialysis through a 10000 Da cutoff membrane. FITC (0.4 mg) was dissolved in 40 µl DMSO and added to 40 mL of PBS adjusted to pH 9.0 with NH_4OH. After 12 hrs at 4°C the SAA-FITC and SAA protofibrils-FITC was dialyzed against milli-Q water, at 4°C (Linke *et al* 1991). 1.25-6.25 µM SAA-FITC and SAA protofibrils-FITC assayed for fluorescence intensity (FL, at λ_{max} 530±15 nm) (PERKIN-Elmer Chemiluminescence, USA).

Single Photon Counting

1.25-6.25 µM SAA-FITC and SAA protofibrils-FITC assayed for lifetime measurements (FL, at λ_{max} 530±15 nm) (Spectra physics, IBH Consultants, Scotland, UK).

Induced Circular Dichroic Spectroscopic Studies

1.25-6.25 µM SAA-FITC and SAA protofibrils-FITC assayed for induced CD measurements (Jasco J-715 Spectropolarimeter, Tokyo, Japan).

FACScan Analysis

BC3H1 cells were incubated with 1.25-6.25 µM SAA-FITC and SAA protofibrils-FITC assayed for 24 hrs at 4°C, washed in PBS, fixed immediately in 1% paraformaldehyde in PBS, and assayed for cellular fluorescence (FL, at λ_{max} 530±15 nm), forward scatter (FSC), and side scatter (SSC). Binding was analyzed on a FACScan flow cytometer using FACScan analysis software WINMDI (Becton Dickinson, San Jose, CA, USA).

MTT Assay

1.25-6.25 µM SAA-FITC and SAA protofibrils-FITC assayed incubation with BC3H1 cells for 24 hrs. Added of 200 µl fresh medium at the end of the growth period and add 50 µl of MTT to all wells in columns 1 to 6. Wrap plates in aluminum foil and incubate for 4 hrs in a humidified atmosphere at 37°C. This is a minimum incubation time and plates can be left for up to 8 hrs. Remove the medium and MTT from the wells and dissolve the remaining MTT-formazan crystals, adding 200 µl of DMSO to all wells in columns 1 to 6. Add glycine buffer (25 µl per well) to all wells containing the DMSO. Record absorbance at 570 nm immediately, since the product is unstable. The wells in column 1, which contain medium, MTT, but no cells, are used to blank the plate reader.

RESULTS

Deposition of amyloid A fibrils in tissues resulting in the displacement of normal structure and cellular dysfunction is the characteristic feature of systemic amyloidosis. This work was initiated to explore whether SAA monomeric and protofibrillar aggregates would bind to the different cell lines from mouse brain BC3H1 (smooth muscle cells). This was selected as model system for parenchymal cerebral cortex system where localized amyloid seems to be the maximum effect. The use of parenchymal cells for the study of SAA gains importance as the SAA expression is observed in the parenchymal cells in the inflammatory condition (Guo *et al*., 2002). It has shown in under the systemic amyloid condition the serum amyloid protofibrils pass the blood brain-barrier and bind widely in the parenchymal region (Asokan, 2014). It is also observed that the crossing of BBB is accompanied by the expression of Aβ peptides 20 times more than the control indicating the importance of the SAA interaction to the cells of parenchymal origin. Further, since SAA the value dramatically increased in the systemic circulation during the infection condition their interaction with the cell lines gains importance (Yamada 1999). Binding SAA of protein and AA protofibirls seemingly target the membranes (Yan *et al.,* 2000). Immediately after synthesis, the SAA is released from the cell and binds with HDL and is never found in the free form. In the circulation it is rapidly cleared: its half-life in mice is 30-50 min faster than apolipoprotein (Benson *et al*., 1995; Kluve-Beckerman *et al*., 1997). As the amyloid toxicity depends upon the assembled state of the protein in this study measured the cell-protein interaction in both monomer and assembled form. To determine the interaction in this study conjugated the proteins with FITC, which could be identified by fluorescence and absorption, distinctly.

Fluorescence spectroscopy

SAA-FITC binding to each other was studied using fluorescence intensity changes. The binding activity of protein was detected using fluorescence spectral change. The principle of the method is based on the reported phenomenon of fluorescence quenching in the protein abundantly labeled by fluorophores (this phenomenon is caused by an interaction of aromatic rings of different fluorophore molecules and interaction of disulfide bonds with fluorophores) and the fluorescence increases greatly after polypeptide chain breaks occur.

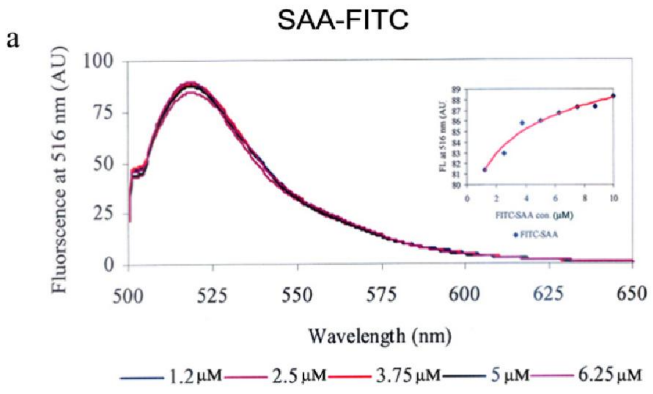

Fig. 1a. Fluorescence spectra of SAA-FITC (a) SAA-FITC with increasing concentrations of (1.25-6.25 µM) SAA-FITC and insert plot shows (1.2-6.25 µM) concentration Vs Fluorescence

The signal change in FITC fluorescence is applied here to study the nature of interaction of serum amyloid components with each other. The SAA is covalently linked to FITC. Typical increase in fluorescence spectra was observed on

increasing the SAA-FITC concentration. At higher SAA-FITC concentration, the self-quenching of fluorescence was observed (Fig. 1a). Linear increase in FITC fluorescence was observed during addition of SAA protofibril-FITC. This linear increase was diminished after addition of SAA-FITC protofibril at a concentration greater than 5 µM. The deviation of fluorescence curve may be associated with aggregation of SAA protofibril resulted in interaction of aromatic side chains with each other near the fluorophore (Fig. 1b).

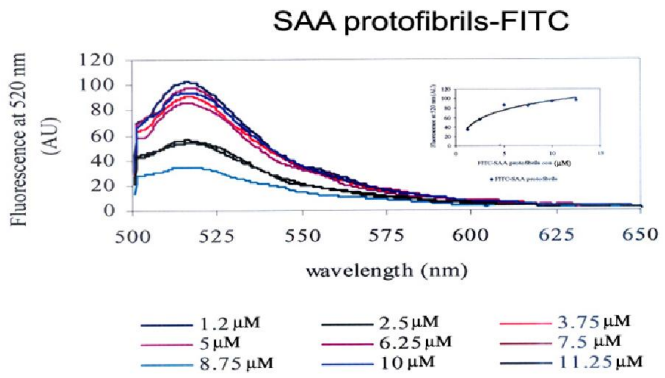

Fig. 1b. Fluorescence spectra of SAA protofibrils-FITC (b) SAA protofibrils-FITC with increasing concentrations of (1.25-11.25 µM) SAA protofibrils-FITC and insert plot shows (1.2-6.25 µM) concentration Vs Fluorescence

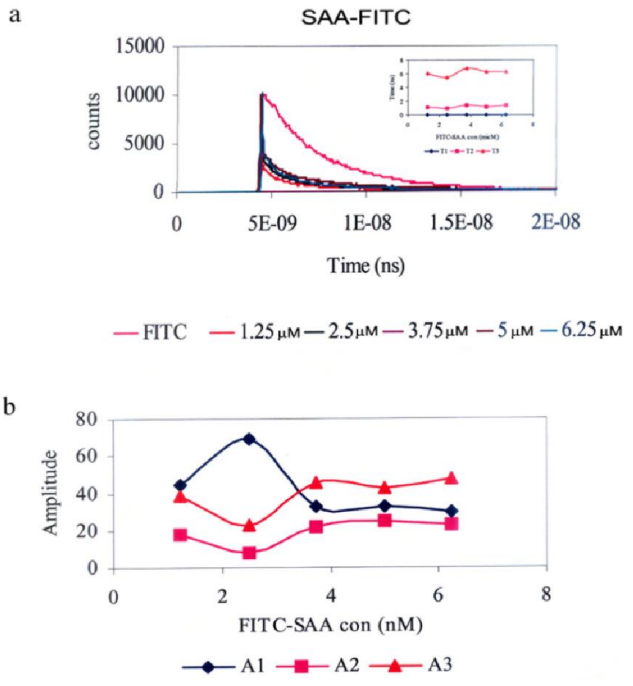

Fig. 2a. Life time measurements of fluorescence decay of SAA-FITC with increasing concentration of (1.25-6.25 µM) SAA-FITC, (b) increasing concentration of (1.25-6.25 µM) SAA-FITC Vs Amplitude and insert plot shows (1.2-6.25 µM) concentration Vs time

Fluorescence Lifetime Measurements

The fluorescence life of a fluorophore depends strongly on the properties of the molecules surrounding the probe. A decrease in fluorescence intensity followed by a decrease of the lifetime suggests an increase in excitation energy transfer, a photochemical reaction or other quenching processes. One of the most widely used fluorophores to label proteins for structural and conformational studies is fluorescein isothiocyante (FITC). Fluorescence dyes differ in their ability to quench. The more hydrophobic the dye the lower ratio of dye: protein where quenching will occur. The effect of SAA concentration on lifetime of FITC is indicated in Fig.2a. The average fluorescence lifetime increased from 2.5 to 3.3 ns indicating that the fluorophore is in highly polar environment than in the previous state. In high SAA concentration (6.25 µM), the association of SAA results in exposure of more FITC to the polar environment. The amplitude for short lifetime (τ_1) was decreased significantly from 45% to 30% during addition of SAA protein (Fig. 2b). However, long-lived species increased from 38 to 47% during further SAA incubation.

Induced CD Measurements

The CD spectrum of SAA-FITC, which was concentration-dependent, is shown in Fig. 3a.

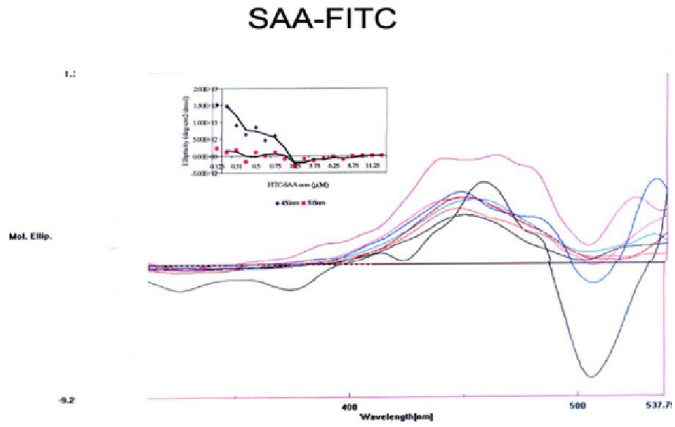

Fig. 3a. CD spectra of SAA-FITC (a) SAA-FITC with increasing concentration of (1.25-6.25 µM) SAA-FITC and insert plot shows (1.25-6.25 µM) concentration Vs elipticity at 450 and 505 nm

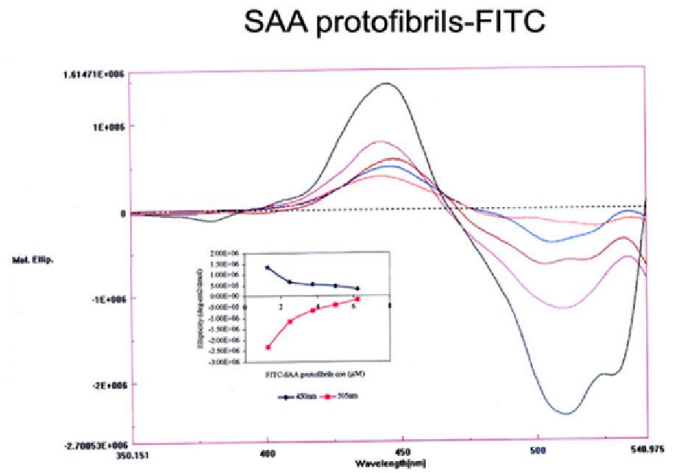

Fig. 3b. CD spectra of SAA protofibrils-FITC (b) SAA protofibrils-FITC with increasing concentration of (1.25-6.25 µM) SAA protofibrils-FITC and insert plot shows (1.25-6.25 µM) concentration Vs elipticity at 450 and 505 nm

The association SAA at different concentrations altered the CD band at 450 and 505nm and became maximal at 1.25nM of SAA. Further addition of SAA did not alter the Molar ellipticity. The SAA-FITC protofibrils addition decreased the

positive band at 450 nm and increased the negative band 505 nm (Fig. 3b). There was no significant change in the CD band at 450 nm indicated in plot

Binding Study of Monomeric and Protofibrils of SAA to BC3H1 Cells of Mouse

Viability test for the SAA protein (1.25-6.25 µM) (Fig. 4a) BC3H1 cells (Fig. 4b) Viability test for the SAA protofibrils (1.25-6.25 µM). SAA-FITC binding to BC3H1 cells was analyzed by flow cytometry and the respective histogram is shown in Fig. 5a and b. The shifting of cell population towards higher fluorescence side in the histogram reveals the binding of monomeric SAA-FITC to cell populations. The forward and side scatter is not changed significantly indicating that volume and granularity is not affected during serum amyloid binding to the respective cell lines. SAA protofibril-FITC also binds to BC3H1 cells similar to monomeric SAA as shown in cytometry histogram (Fig. 5b).

Table 1. Determination of affinity constant (Kd)

Cell type	Monomeric SAA (µM)	Protofibrillar SAA (µM)
BC3H1	0.55	0.65

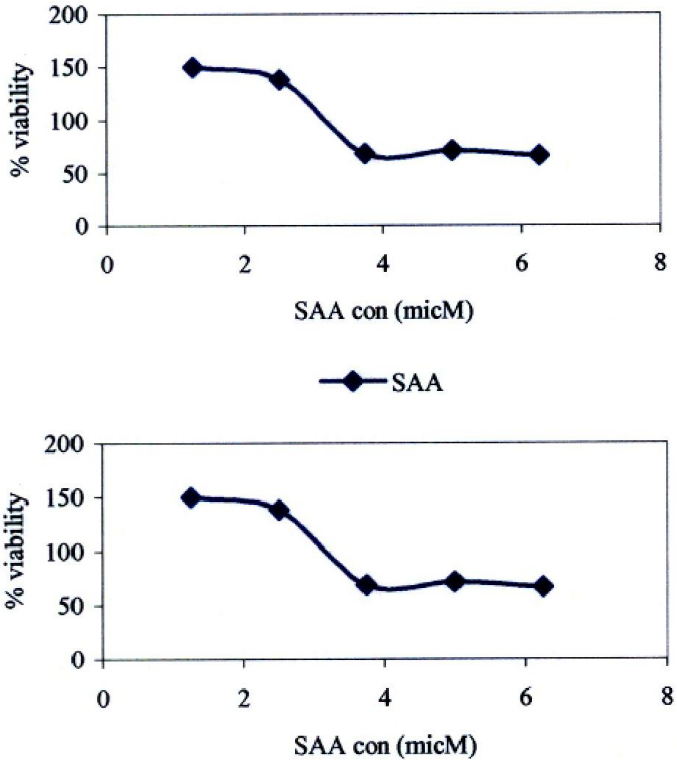

Fig. 4. Viability test for the SAA protein (1.25-6.25 µM) (a) BC3H1 cells
(b) Viability test for the SAA protofibrils (1.25-6.25 µM)

Affinity constants (Wang *et al*., 1996) for monomeric and fibrillar SAA-FITC were calculated from the flow cytometry experiments given in Table 1. The affinity constants for SAA-FITC in monomeric and fibrillar form remains same in all the cell lines indicating the presence of high affinity binding sites for serum amyloid in both the forms. Concentration dependent toxicity was observed in SAA binding. (Fig. 4a and 4b), show the changes in cell viability induced by exposure to 1.25-6.25 µM SAA for 24 hrs caused significant cell death. Incubation of varying concentrations of SAA resulted in BC3H1, only 50% viable BC3H1 cells at 4–6 µM with an LD_{50} of 3.5 µM.

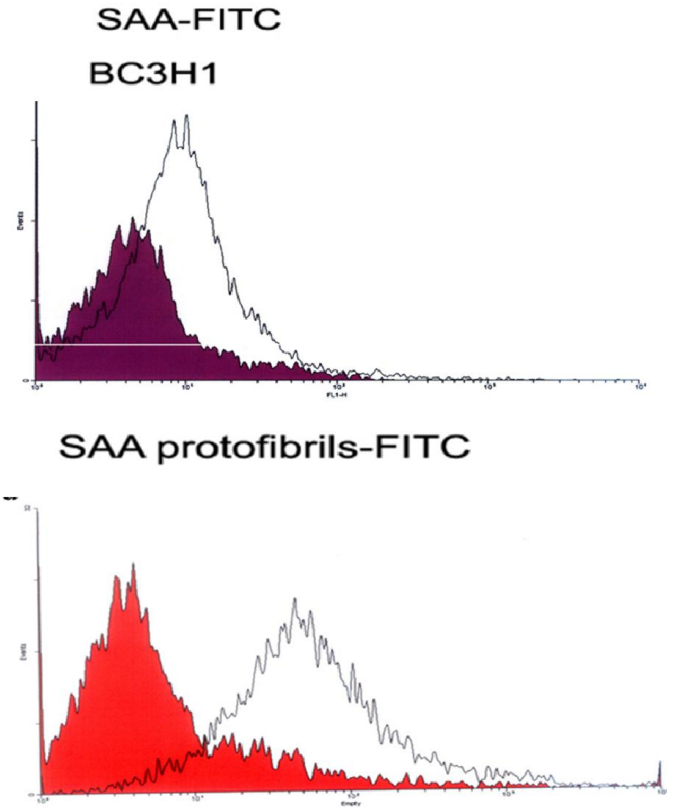

Fig. 5. Fluorescence histogram of SAA-FITC and SAA protofibrils-FITC in mouse BC3H1 cells (a) histogram shows filled curve control cells, black curve shows SAA-FITC (2.5 µM), violet curve shows increasing concentration of SAA-FITC (6.25 µM), (b) histogram shows filled mouse BC3H1 cells, black curve shows SAA protofibrils-FITC (2.5 µM), red curve shows increasing concentration of SAA protofibrils-FITC (6.25 µM)

DISCUSSION

The multiexponential decay of SAA-FITC is possibly due to heterogeneity of conformational isomers of the protein, time dependent relaxation around the excited state, or the intrinsic heterogeneity of the FITC around the SAA protein. SAA is showing heterogeneity because it contains three additional lysine molecules labeled FITC together with amino terminal region on the protein molecule. For many fluorophores, intermolecular interactions and energy transfer between molecules in close proximity to one another result in self-quenching. Molecular association can be assessed, if during this process, the molecular environment changes around the fluorescent probes used to label the desired protein. Using polarity changes near the probe, accessibility of the binding site of the protein by various molecules of interest can be evaluated. Conformational change of the fluorophore (FITC) induced by binding of proteins can cause a change in the fluorescence intensity. Time resolved fluorescence spectroscopy enables the independent observation of different populations of a fluorophore (free or protein bound) in contrast to the steady state fluorescence spectroscopy. Flow cytometric analysis suggested that interaction of SAA with its receptor on BC3H1 was stronger. Moreover, FITC labeled SAA was found

to bind, in a saturable manner, to all the cells, reaching a binding affinity of ~10^{-9} M. Although the chemical structure of SAA is defined, presence of its putative receptor on various target cells is unknown. Previous research work has shown that SAA acts as a chemo attractant and regulator of the migration of monocytes, polymorphonuclear cells, and lymphocytes through its avid interaction. The human SAA binds to T lymphocytes with a dissociation constant Kd=10^{-9} M.

The principal role of SAA during the acute-phase reaction appears to be the association with HDL-particles and subsequent changes of apolipoprotein composition and metabolic properties of its physiological carrier. Because SAA may displace apolipoprotein A-I, the major apolipoprotein of HDL, it is hypothesized that SAA could alter the protective function of HDL during 'reverse cholesterol transport (Artl 2000). SAA binding acute phase reaction was studied with macrophages (representative peripheral cells) revealed that the binding affinity for SAA is enhanced. Earlier findings suggest that extracellular matrix proteins appear to serve as a temporary anchorage sites for SAA and amyloid A. (Preciado-Patt *et al.,* 1996). Though SAA exists predominantly with HDL, its physiological function is unknown. From the side scatter and forward scatter values of SAA treated cells, it is clear that SAA binding does not alter the size and granularity of cells. Moreover, SAA had no effect on either protein synthesis or DNA synthesis, suggesting that SAA specifically altered lipid synthesis. Earlier findings suggest that acute-phase SAA down-regulates lipid biosynthesis in cultured aortic smooth muscle cells in a time and dose-dependent manner. For SAA, the situation is different since SAA, an acute phase reactant, rapidly integrates into HDL. Since HDL are potent LPS neutralizing entities by themselves. Studying the LPS-SAA interaction in acute phase sera will help to elucidate the exact role for SAA. In a clinical study the exact ratio of free and HDL-associated SAA will be determined in septic patients.

The SAA proteins comprise a family of molecules that are apolipoproteins being primarily associated with HDL. Triggered by inflammation, e.g. tissue damage or infection and after stimulation of hepatocytes by cytokine-mediated processes, the concentrations of SAA may increase during the acute-phase reaction to levels 1000-fold greater than those found in the noninflammatory state. The principal role of SAA during the acute-phase reaction appears to be the association with HDL-particles and subsequent changes of apolipoprotein composition and metabolic properties of its physiological carrier. Because SAA may displace apolipoprotein A-I, the major apolipoprotein of HDL, it is hypothesized that SAA could alter the protective function of HDL during 'reverse cholesterol transport. During this process native HDL is able to bind to peripheral cells and then to promote cholesterol efflux. Subsequently, acquired cholesterol is transferred either to lipoproteins other than HDL or directly transported to the liver.

The major findings of the present study are as follows: (1) Binding studies demonstrated that during the acute-phase reaction binding affinity and holoparticle uptake of HDL by J774 macrophages (a representative of peripheral cells) is enhanced, whereas HUH-7 Hepatoma cells (a representative of liver cells mediating uptake of HDL-associated cholesterol) have decreased affinity and HDL-holoparticle uptake (2) The presence of A-SAA on HDL specifically increases selective uptake of HDL-associated cholesterylester and holoparticle uptake by J774 macrophages. In contrast to these findings, the selective uptake of HDL-associated cholesterylester and holoparticle uptake by HUH-7 cells was decreased during the acute-phase reaction. (3) During cholesterol efflux experiments, it became apparent that the ability of acute-phase HDL with regard to cellular cholesterol removal from J774 macrophages was considerably lowers (30-35%) than for native HDL. In contrast, HDL-mediated cholesterol efflux from the plasma membrane of HUH-7 cells was slightly enhanced by acute-phase HDL. (4) Using ligand blotting experiments, two binding proteins for native and acute-phase HDL on J774 macrophages with molecular masses of 100 and 72 kDa could be observed. A single binding protein of 100 kDa could be observed on HUH-7 cells. Collectively, the present findings support the hypothesis that A-SAA may command HDL-particles during the acute-phase reaction to peripheral cells. Subsequently, the net transfer of cholesterol during the acute-phase reaction seems to be directed towards macrophages. It seems that changes in the apolipoprotein moiety of HDL appear to transform an originally antiatherogenic HDL-particle into a proatherogenic lipoprotein particle.

In the present study, we sought to define the region of acute phase apoSAA involved in cholesterol binding and to investigate the ability of constitutive apoSAA4 to bind cholesterol. Binding of [3H] cholesterol to apoSAAp was inhibited by unlabeled cholesterol (1-100 nM), but not significantly by vitamin D and estradiol. Direct binding of acute phase, but not constitutive, apoSAA to the surfaces of polystyrene microtiter wells was strongly diminished in the presence of cholesterol. The ability of apoSAA to bind cholesterol was inhibited by antibodies to human apoSAA1 and to peptide 1-18 of apoSAA1. There was only slight inhibition of cholesterol binding by antibodies to peptide 40-63, and no inhibition by antibodies to peptides spanning regions containing amino acid residues 14-44 and 59-104. [3H] Cholesterol uptake by neonatal rabbit aortic smooth muscle and HepG2 cells was enhanced by a synthetic peptide corresponding to amino acids 1-18 of hSAA1, but not by peptides corresponding to amino acids 1-18 of hSAA4. [3H] Cholesterol uptake by HepG2 cells was slightly increased by a peptide corresponding to amino acids 40-63 of hSAA1. These findings suggest that apoSAA modulates the local flux of cholesterol between cells and lipoproteins during inflammation and atherosclerosis (Liang and Sipe 1995) The this study data reported here demonstrate that SAA and amyloidotic AA bind with cholesterol rich region of brain cells, smooth muscle (BC3H1) cells of the brain. Previous workers have observed that acute-phase SAA binds and transports cholesterol into aortic smooth muscle and HepG2 cells (Liang and Sipe 1995; Liang *et al*., 1996) suggesting that SAA might have a role in cholesterol homeostasis during chronic inflammation in diseases including atherosclerosis.

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