Serum amyloid A has little effect on high density lipoprotein (HDL) binding to U937 monocytes but may influence HDL mediated cholesterol transfer.

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There are 4 serum amyloid A (SAA) genes in man [1,2]. SAA1 and SAA2, encode SAA whose concentration can increase up to 1000 fold during the acute phase response [API]. SAA associates with the high density lipoprotein (HDL) fraction HDL3 on which it is normally a minor apolipoprotein [3]. During the APR HDL is SAA enriched and apolipoprotein A1 (apoA1) depleted [4]. HDL is thought to be central in reverse cholesterol transport (RCT), the transport of cholesterol from peripheral tissues to the liver for excretion in bile [4]. The APR is a host response to infection or injury and it has been suggested that the increased SAA content of HDL may alter the balance of HDL mediated cholesterol transport so that cholesterol is directed towards sites of tissue damage where it would be used for repair [5].

apoA1 containing lipoproteins are considered to be anti-atherogenic [6]. The decrease of HDL apoA1 content as the particle is SAA enriched may be detrimental if maintained for a period of time as in chronic or recurring inflammation. The continued recycling of cholesterol may lead to accumulation in various cell types, contributing to the development of fatty streaks as seen in atherosclerosis. Patients with chronic inflammatory conditions such as rheumatoid arthritis have increased incidence of atherosclerosis [7].

We decided to investigate the hypothesis that SAA affects HDL metabolism. As macrophages are a major cell type in atherosclerotic plaques and precursors to foam cells which develop due to intracellular cholesterol accumulation, the cell line chosen was the human monocye cell line U937.

HDL was purified from plasma (1mM EDTA by ultracentrifugation at 15,000g/ml and then 3 times at 1% g/ml) purified HDL was dialysed into 1mM EDTA/BS and stored at 4°C. SAA was purified from plasma containing more than 100mg/ml SAA as measured by a competitive SAA [8]. SAA of >95% purity was isolated using affinity chromatography, gel filtration and ionic exchange [9]. The purified protein was freeze dried and stored at -20°C. HDL was incubated at 37°C for 24hr and HDL/SAA was resolated by ultracentrifugation at 1.21g/ml. For the binding experiments [EDL] and HDL/SAA was radiolabelled with [3H] by the iodine monochloride method [10].

Binding experiments: U937 were washed and suspended in RPMI 2% FCS and placed on ice for 1hr. 100uL cell suspension was added to microtitre plates containing [3H]HDL/SAA or [3H]HDL with SAA so that final volume was 500uL. After a 2hr incubation at 4°C bound [3H]HDL was separated from free [3H]HDL by centrifugation and the cells thoroughly washed with PBS and lysed in 1mL 0.5M NaOH. For influx experiments (EDL) was loaded with [3H]cholesterol by exchange with phosphatidylcholine liposomes. Cells were added to wells containing [3H]cholesterol without or with SAA and incubated for 24hr at 37°C. The supernatant was removed and washed and lysed as above.

There was no significant change in [3H]HDL binding with the addition of SAA (Fig. 1). It made no difference whether SAA was incorporated into HDL prior to radiolabelling or added to the cells separately. [3H]cholesterol preparations from several donors have shown the same results.

Effect of [3H]cholesterol was increased as HDL concentration in the medium increased (Table 1). There was a small increase in efflux on addition of SAA which was greatest at the lowest HDL concentration used. There was loss of an SAA effect on the HDL influx into U937. The largest change on SAA addition was seen at 1ug/ml HDL wherein there was a decrease in influx. It is thought that cholesterol transfer between cells and HDL has two components, a specific interaction involving binding to a receptor, and a non-specific interaction that is non-receptor dependent [4]. SAA did not alter HDL binding to U937's but there appeared to be a limited influence on cholesterol efflux which suggests that, at least here, SAA may be involved in non-receptor dependent component of cholesterol transfer. The increase in efflux has been reported in a number of separate experiments conducted with different batches of HDL and SAA. The greatest increase in efflux was demonstrated at 1ug/ml HDL. In other experiments the changes have varied from non- significant to highly significant (2 fold increase) and have been observed over 0-100ug/ml HDL. In the initial cellular cholesterol acceptor is thought to be pre-

Table 1. Cholesterol transfer of [3H]cholesterol between U937 and HDL. SAA: ug/ml. Data are shown as supernatant cpm SAA for the efflux experiment and cell lysate cpm SAA for the influx experiment. *p<0.1, **p<0.01, ***p<0.001.

<table>
<thead>
<tr>
<th>HDL pg/ml</th>
<th>[3H]cholesterol efflux cpm cell protein</th>
<th>[3H]cholesterol influx cpm cell protein</th>
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<td>[3H]HDL</td>
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Fig. 1. The effect of SAA on [3H]HDL binding affinity to U937. Fig. 2. [3H]HDL, [3H]cholesterol cpm SAA, [3H]HDL/SAA (SAA = 1 ug/ml total protein).

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