PROSTACYCLIN AND THROMBOXANE PRODUCTION FROM MACROPHAGES OF AMYLOID RESISTANT AND SENSITIVE MICE*

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ABSTRACT

Peritoneal macrophages from amyloid resistant A/J mice produced more prostacyclin (PGI$_2$) and prostaglandin E$_2$ (PGE$_2$) and less thromboxane A$_2$ (TXA$_2$) into the incubation medium than similarly prepared macrophages from CBA/J mice. When CBA/J mice were given injections of azocasein sufficient to produce amyloid in the spleen, the amount of PGI$_2$, but not PGE$_2$ or TXA$_2$ found in the medium from incubated macrophages was significantly decreased. This inhibition of PGI$_2$ synthetase (and/or augmentation of PGI$_2$ catabolism) was also found in the macrophages from azocasein treated A/J mice and to a lesser extent in water injected CBA/J and A/J mice. However, this inhibition of PGI$_2$ synthetase in response to azocasein injections occurred much more slowly in the A/J macrophages than in the CBA/J macrophage. It is suggested that in the presence of elevated serum AA an altered arachidonic acid metabolism by cells of the mononuclear phagocytitic system may contribute to a susceptibility to amyloidosis.

INTRODUCTION

Amyloidosis is characterized by the deposition of a variety of extracellular proteins with specific physical properties. One of these proteins known as AA is thought to be a catabolic product of a structurally related serum protein with similar antigenic properties but higher molecular weight than AA. SAA which is a normal constituent of serum, increases dramatically in response to an inflammatory or immune stimulus [1]. While the consensus is that this is an appropriate homeostatic response to injury, the deposition of AA is clearly a pathologic one.

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Recent evidence implicating the macrophage in this pathologic process suggests that either the altered release of a factor necessary for the degradation of SAA or inappropriate SAA uptake and degradation within the macrophage, may be responsible. First there is the historical observation that amyloid is often laid down in close association with the reticuloendothelial system [2]. Second, enzymes of monocytic origin have been shown to degrade serum amyloid [3] and thirdly, it is well established that the in vivo environment of the macrophage is the main determinant of its activation and secretory products [4].

In general, the arachidonate products, i.e., prostaglandins (PG's), and thromboxanes (TX's) are potent regulators of cellular metabolism and are proposed as intercellular mediators of the immune and inflammatory response [5]. They appear to act primarily in the cells in which they are synthesized often by changing intracellular cyclic nucleotide levels [6]. Since monocytes and macrophages, of all the cells in the immune response, appear to be a major source of these products [7], we set out to determine if macrophages of amyloid sensitive and resistant strains of mice showed differences in their arachidonate metabolism either before, during, or after production of amyloid.

METHODS

Animals

Six to eight week old, female CBA/J (amyloid sensitive) and age, sex matched A/J (amyloid resistant) mice obtained from Jackson Labs, Maine, were used. One group from each strain was injected daily with 0.3 ml of sterile distilled water and the other with the same volume of 10% azocasein (the amyloidogenic agent). In some experiments a group of mice were left untreated. Four to five animals were used in each group.

Macrophages

At the end of the injection period (13 days for Experiment 1, 10 days for Experiment 2 and various times for Experiment 3) mice were sacrificed by cervical dislocation, the abdominal wall exposed and the peritoneal cavity injected with 6 ml of HBSS buffer. After 2 min the exudate was removed and spun at 400 g for 5 min. The cells were then washed twice with 2 ml volumes of HBSS. The cells were resuspended in RPMI with 10% fetal calf serum at a concentration of 1.5 × 10^6 (Expts. 1 and 3) or 0.5 × 10^6 (Expt. 2). After adhering cells to polystyrene flasks or microtiter wells for 2 h at 37°C in a humidified atmosphere the non-adherent cell population was removed. In Experiment 2 only, this 2 h incubation was done in the absence and presence of 100 µg/ml of azocasein. The adherent cells were washed with two further 2 ml volumes of buffer. The total non-adherent cell population which was washed off, was counted and the remaining adherent cells incubated in a further volume of RPMI with 2.5% fetal calf serum for 20 to 48 h. The supernatants were stored at -20°C for subsequent PG or TX analysis. Results were expressed as total amount of PG or TX products per unit time per 10^6 adhered cells (Expts. 1 and 3) or per unit time per ml (Expt. 2).

Kidney Slices

Kidneys were removed from azocasein injected, water injected, or untreated mice (Experiment 2 only) and freed from surrounding fat and blood vessels. Very thin slices were prepared using a Stadie Riggs micrometer. Two slices from the same kidney area of each mouse were incubated under 5% CO₂ 95% O₂ for 1 h in Krebs Ringer bicarbonate buffer. The incubation medium was assayed for PG's and TX and results expressed as ng/PG or TX/100 mg wet wt/hr.