

AMYLOIDOGENESIS  
One Serum Amyloid A Isotype is Selectively Removed  
from the Circulation

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Serum amyloid A (SAA)<sup>1</sup> is presumed to be the precursor for amyloid A protein, the main protein constituent found in the amyloid fibrils of reactive amyloidosis (1-3). In the mouse SAA is encoded by a family of three genes (Morrow, John F., personal communication). SAA<sub>1</sub> and SAA<sub>2</sub> are synthesized in the liver by hepatocytes (4-10) and are found circulating in nearly equal quantities associated with high density lipoprotein (HDL) (11). SAA<sub>3</sub> mRNA is also expressed in the liver; however, the corresponding polypeptide has not been identified. We have found that of the three possible SAA gene products, SAA<sub>2</sub> appears to be the sole precursor of murine amyloid fibril protein AA (12). We wish to determine by what mechanism this monotypic fibril formation occurs. One can conceive of three ways in which this could come about: (a) the gene for SAA<sub>2</sub> could be active in tissues in which the deposits occur; (b) amyloid A fibril protein could be derived from a circulating SAA<sub>2</sub> precursor whose concentration is selectively enhanced relative to the other SAA proteins during the induction of the disease process; and (c) there could be selective SAA<sub>2</sub> removal from a circulating pool of possible precursors and deposition of SAA<sub>2</sub> as amyloid A protein.

We designed the experiments described here to decide among these three alternatives. To differentiate among the several possible mechanisms of amyloidosis, we searched for evidence of local SAA synthesis and examined SAA metabolism during amyloid induction at the level of SAA gene expression, synthesis, secretion, and circulatory levels. We conclude that amyloid A protein monotypy is not due to local SAA<sub>2</sub> production, nor is it a reflection of selective increase of SAA<sub>2</sub> isotype levels in the circulation. However, it does involve the selective and rapid removal of SAA<sub>2</sub> from the circulating pool of both SAA<sub>1</sub> and SAA<sub>2</sub>. Our experimental analysis clearly indicates that a combination of some feature of SAA<sub>2</sub> interacting with itself and/or local tissue constituents is responsible for the appearance of amyloid fibrils with AA<sub>2</sub> as the major constituent peptide.

Materials and Methods

*Amyloid Induction and Histological Evaluation of Tissue Amyloid Deposits.* Male CBA mice 2-3 mo old were used throughout the study. Amyloidosis was induced by daily

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<sup>1</sup> *Abbreviations used in this paper:* HDL, high density lipoproteins; SAA, serum amyloid A protein.

subcutaneous injection of casein as described by Benson (13). At 0, 1, 5, 10, 15, and 20 d during casein-injection, animals were anesthetized, bled, and the spleens and livers were removed. 5- $\mu$ m histological sections of spleens were examined by polarized light after staining with Congo red (14). The areas of specific Congo red dichroic birefringence were found to correlate well with the area of the hyaline material observed in adjacent tissue sections stained with hematoxylin and eosin (data not shown); hence, for ease of measurements, tissues stained with the latter were used for morphometry (15) to estimate splenic amyloid content. At each time point four randomly chosen fields from each of four spleens were quantitated. Relative areas were estimated with a 400-point grid on projected sections enlarged  $\sim$ 400 times, and amyloid content was computed as the percent of spleen occupied by the amyloid deposits.

**RNA Isolation and Cell-free Translation.** Liver RNA was isolated after homogenization of tissue in SDS-proteinase K buffer (16). RNA was obtained from spleens after homogenization in guanidium isothiocyanate (17).

Cell-free translation of total RNA (200  $\mu$ g/ml) or poly A<sup>+</sup> RNA (50  $\mu$ g/ml) was carried out in a rabbit reticulocyte translation system (18) in the presence of L-[<sup>35</sup>S]methionine (New England Nuclear, Boston, MA; 1 mCi/ml,  $>1,000$  Ci/mM). SAA translation products (preproteins) were immunoprecipitated with rabbit anti-apoSAA antibody, affinity-purified as previously described (6). Immune complexes were recovered by absorption to pansorbin (Calbiochem-Behring, La Jolla, CA). The washed precipitates were solubilized by boiling for 2 min in SDS sample buffer (19) and analyzed by SDS-urea-PAGE and fluorography. The microsome preparation used in the cell-free translations was a gift from Dr. Mark Lively (20).

**Hepatocyte Isolation and Culture.** Hepatocytes were harvested as described previously (6) from mice 1 or 20 d after initiation of casein treatment. Routine yields were 20–40  $\times$  10<sup>6</sup> hepatocytes per liver with  $\sim$ 90% viability as judged by trypan blue exclusion. Washed hepatocytes were resuspended in media containing 10% FCS and 3  $\times$  10<sup>6</sup> cells were placed into 25 cm<sup>2</sup> flasks (Corning Glassworks, Corning, NY). After 6 h, the medium was replaced with RPMI 1640 without FCS or methionine. After a 30-min incubation this medium was replaced with the same medium containing L-[<sup>35</sup>S]methionine (200  $\mu$ Ci/ml) and aprotinin (100  $\mu$ g/ml; Sigma Chemical Co., St. Louis, MO) for 4 h. The medium was then removed and an aliquot of each medium was prepared for SDS-urea-PAGE.

**Radioimmunoassay, IEF, and SDS-Urea-PAGE and Densitometry.** Blood was collected by cardiac puncture of ether-anesthetized mice. Serum SAA concentrations were determined by RIA as previously described (11). HDL was then prepared from serum of individual mice by sequential ultracentrifugation and the delipidated apoproteins were analyzed by equilibrium IEF in pH 3.5–10.0 ampholine gradients (LKB Instruments, Inc., Gaithersburg, MD) (11). SDS-urea polyacrylamide gels were prepared and used as described (6). After electrophoresis slab gels were fixed and prepared for fluorography with EN<sup>3</sup>HANCE (New England Nuclear) and exposed to X-omat AR film (Eastman Kodak Company, Rochester, NY). Fluorographs and stained IEF gels were quantified using a microdensitometer (Joyce Loebel and Co., Malden, MA) equipped with an integrator.

**Recombinant SAA cDNA.** A recombinant SAA cDNA clone was isolated using the procedures described by Maniatis et al. (21). Briefly, poly A<sup>+</sup> RNA was isolated from the liver of a male Balb/c mouse 20 h after an intraperitoneal injection of LPS (11). Double stranded cDNA was synthesized and inserted into the plasmid pBR322 by the G-C tailing method. The chimeric plasmids were used to transform *E. coli* strain RRI. After differential colony screening (22) of tetracycline resistant clones with <sup>32</sup>P-labeled cDNA derived from poly A<sup>+</sup> liver RNA of normal and LPS-stimulated Balb/c mice, potential SAA cDNA clones were rescreened by hybrid selection of SAA mRNA. SAA cDNA clones were identified by cell-free translation of hybrid selected mRNA (21) and identification of SAA translation products on SDS-urea polyacrylamide gels. One clone (pSAA<sub>1</sub>) with an SAA cDNA insert of  $\sim$ 400 bp, hybrid-selected SAA<sub>1</sub> and SAA<sub>2</sub> mRNA equally (data not shown) and was confirmed to code for SAA<sub>1</sub> (23) by its nucleotide sequence (24). The 5' end of this clone (unpublished results) covers the highly conserved region spanning amino acid residues 32 to 44 found in the three SAAs and the amyloid A protein (25).

**Northern Blot Analysis.** RNA was denatured and electrophoresed as described by Lehrach et al. (26). Total RNA (0.5 mg/ml) was denatured at 60°C for 15 min. 5 µg of RNA was then electrophoresed through 1.5% agarose gels buffered with 10 mM phosphate, 5 mM NaOAc, 1 mM EDTA, and containing 3% formaldehyde. Fractionated RNA was transferred to nitrocellulose in 20× SSC (3 M NaCl, 0.3 M sodium citrate, pH 7.0) and baked at 80°C. Hybridization was carried out using buffers essentially described by Wahl et al. (27). Prehybridization buffer contained 50% formamide, 5× SSC, 4× Denharts, 150 µg/ml herring sperm DNA, 100 mM NaH<sub>2</sub>PO<sub>4</sub>, 1% glycine, and 50 µg/ml polyadenylic acid (poly A). Prehybridization was for 2 h at 45°C. Hybridization buffer contained four parts prehybridization buffer and one part 50% (wt/vol) dextran sulfate. Hybridization was performed at 45°C for 16–18 h with 10<sup>6</sup> cpm/ml denatured, nick-translated (28), SAA-specific cDNA (10<sup>8</sup> cpm/µg) isolated from pSAA<sub>1</sub>. RNA blots were washed three times at room temperature with 2× SSC, 0.1% SDS for 20 min each, followed by three washes in 0.1× SSC, 0.1% SDS at 55°C for 15 min each. Comparison of SAA<sub>1,2,3</sub> mRNA sequences (John F. Morrow, personal communication; 23) revealed that SAA<sub>1</sub> and SAA<sub>2</sub> are highly conserved with 96% homology. SAA<sub>3</sub> mRNA is 73% homologous to SAA<sub>1</sub> mRNA, but contains a 150-nucleotide region of 90% homology to our pSAA<sub>1</sub> cDNA. Using these standard blot washing conditions, regions of ≥90% homology would form stable hybrids. 28S and 18S RNA were visualized on the nitrocellulose after autoradiography of the blot by staining in 0.04% methylene blue in 0.5 M sodium acetate, pH 5.5.

## Results

**Time Course of Amyloid Deposition.** The changes with time of the relative volume of splenic tissue occupied by amyloid substance is shown in Fig. 1. With either Congo red or H & E staining, no amyloid material was detected until day 5, at which time ~2% of splenic volume was occupied. The quantity of amyloid substance increased rapidly thereafter, and by day 20 represented ~30% of the total spleen volume.

**There is No Detectable Synthesis of SAA by the Spleen.** In order to determine whether or not spleen cells contribute to amyloid substance accumulation via SAA synthesis and secretion, a recombinant SAA cDNA, which hybridizes to all three SAA sequences, was used to detect SAA mRNA. Fig. 2 shows the result of hybridizing the <sup>32</sup>P-labeled SAA cDNA to liver RNA, which synthesizes large amounts of SAA in response to inflammatory stimuli, and spleen RNA after electrophoresis through agarose and transfer to nitrocellulose. Liver SAA mRNA was elevated to a high level 1 d after casein injection (lanes 1 and 2). No SAA

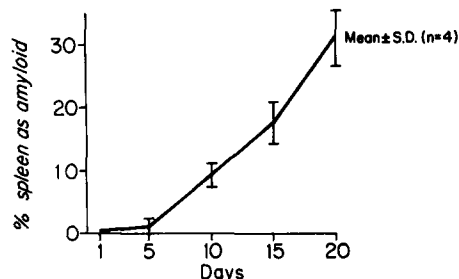


FIGURE 1. Time course of amyloid deposition. At the indicated times spleens were removed and prepared for histological examination. As described in Materials and Methods, amyloid cross-sectional area was computed by morphometry. Time points represent mean percentages from four fields of each of four spleens.

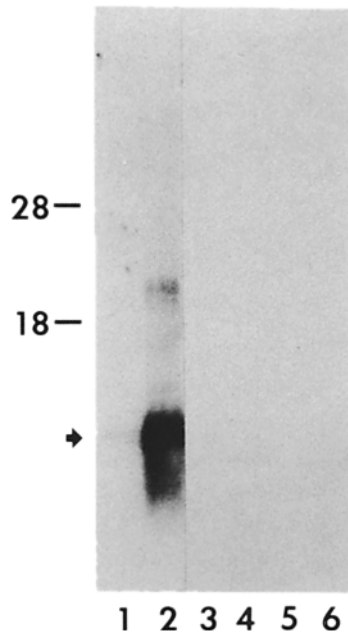


FIGURE 2. Northern gel analysis of liver and spleen RNA. Total RNA from liver and spleen was isolated at various times during amyloid induction. 5  $\mu$ g of RNA were then separated on agarose (1.5%) formaldehyde denaturing gels, transferred to nitrocellulose, and the nitrocellulose filter was hybridized to nick translated SAA cDNA as described in Materials and Methods. The RNA examined was extracted from the following tissues: Lane 1 and 2 are liver from either uninjected animals (lane 1) or 1 day after casein injection (lane 2). Lanes 3–6 were from spleens from uninjected animals (lane 3) or from animals after 7, 14 and 21 d of casein injections, respectively. The position of the 28s and 18s ribosomal RNA is indicated.

mRNA was detected in spleen of control animals or during amyloid substance deposition (lanes 3–6). Quantitative Northern blot analysis at a sensitivity capable of detecting  $\geq 1\%$  of normal liver SAA mRNA did not reveal splenic SAA mRNA (data not shown). In addition, no SAA products could be detected from the cell-free translation of splenic RNA (data not shown). Furthermore, no SAA mRNA was detected after casein injection in a variety of other tissues examined (unpublished observation).

*Circulating Levels of SAAs.* Serum SAA levels 1 d after the first casein injection (Fig. 3), were elevated  $>50$ -fold above baseline ( $280 \pm 58 \mu\text{g/ml}$ ). Thereafter, SAA levels remained elevated but slowly declined to a low of  $\sim 110 \pm 35 \mu\text{g/ml}$  by day 15 and remained at this level despite continued casein injections.

Analysis of SAA<sub>1</sub> and SAA<sub>2</sub> levels revealed that on day 1 SAA<sub>1</sub> was  $120 \mu\text{g/ml}$  while SAA<sub>2</sub> was  $160 \mu\text{g/ml}$  (SAA<sub>2</sub>/SAA<sub>1</sub> ratio, 1.28). Over the next 19 d SAA<sub>1</sub> dropped slightly to  $100 \mu\text{g/ml}$  while the SAA<sub>2</sub> level dropped dramatically to  $15 \mu\text{g/ml}$  (SAA<sub>2</sub>/SAA<sub>1</sub> ratio, 0.15). A second similar experiment (data not shown) yielded essentially the same result, although total serum SAA rose to only  $190 \mu\text{g/ml}$ , the SAA<sub>2</sub>/SAA<sub>1</sub> ratio declined with similar kinetics from 1.4 on day 1 to 0.12 on day 20.

*Changes in Hepatic SAA<sub>1</sub> and SAA<sub>2</sub> mRNA Assayed by Cell-free Translation.* In order to measure hepatic SAA<sub>1</sub> and SAA<sub>2</sub> mRNA levels, we first determined

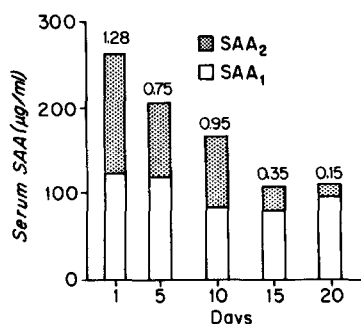


FIGURE 3. Serum SAA levels during amyloid induction (amyloidogenesis). Serum was collected from mice as described under Materials and Methods and SAA concentration determined by RIA. The concentration of circulating SAA<sub>1</sub> and SAA<sub>2</sub> during amyloid induction was determined as follows: animals were bled and HDL isolated as described under Materials and Methods. The HDL apoproteins from four animals at each time point were separated on equilibrium IEF gels, and SAA<sub>2</sub> to SAA<sub>1</sub> ratios quantitated to be  $1.28 \pm 0.13$ ,  $0.75 \pm 0.25$ ,  $0.95 \pm 0.12$ ,  $0.35 \pm 0.14$ ,  $0.15 \pm 0.01$  for day 1 through day 20 consecutively. The ratio data was then used to estimate the concentration of SAA<sub>1</sub> and SAA<sub>2</sub> from the total SAA levels determined by RIA. The number at the top of each bar is the SAA<sub>2</sub>/SAA<sub>1</sub> ratio.

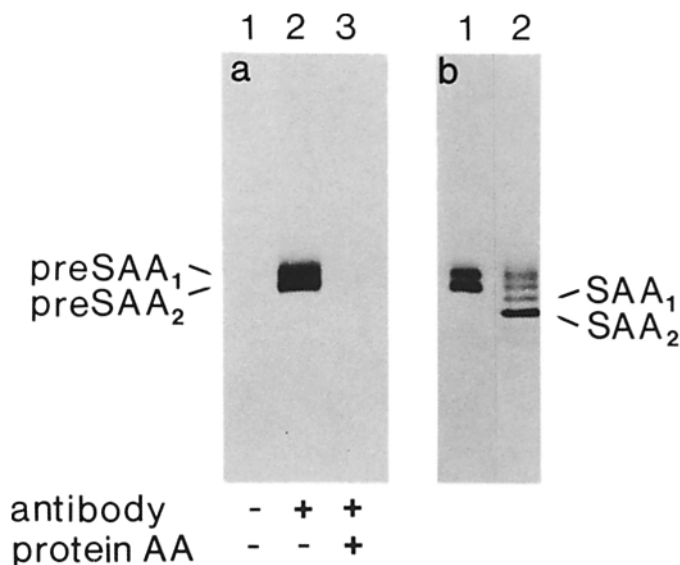


FIGURE 4. Identification of SAA<sub>1</sub> and SAA<sub>2</sub> cell-free translation products. Poly A<sup>+</sup> RNA isolated from murine liver 24 h after casein was translated in the presence of [<sup>35</sup>S]methionine, antibody precipitated, and separated on an SDS-urea-polyacrylamide gels as described under Materials and Methods. (a) In lane 1 antibody precipitation with hyperimmune rabbit IgG; lane 2, antibody precipitation with affinity-purified anti-SAA antibody; and lane 3, as in lane 2, but in the presence of murine protein AA (100 μg/ml). (b) Cell-free translation of total RNA isolated from mouse liver 24 h after casein injection and antibody precipitated as in Fig. 4a, lane 1, translation without microsomes; and lane 2, translation in the presence of microsomes. The position of SAA<sub>1</sub> and SAA<sub>2</sub> HDL apoproteins is indicated in the right margin.

that SAA<sub>1</sub> and SAA<sub>2</sub> preproteins synthesized in a cell-free translation system could be identified. Poly A<sup>+</sup> RNA isolated from murine liver 20 h after casein injection was translated in the presence of L-[<sup>35</sup>S]methionine. Two polypeptides

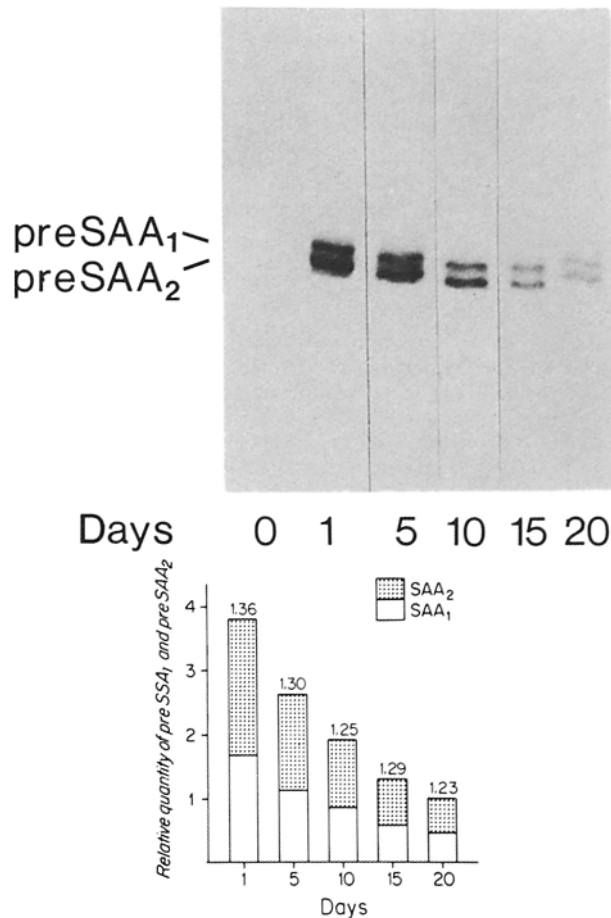


FIGURE 5. (*top*) Time course of SAA<sub>1</sub> and SAA<sub>2</sub> mRNA level during amyloid induction. Total RNA was isolated from murine liver, translated in the presence of L-[<sup>35</sup>S]methionine, and antibody precipitated as described. Total <sup>35</sup>S counts per minute was determined by TCA precipitation. The antibody precipitate from 200,000 cpm of total TCA precipitated translated proteins was separated on SDS-urea-polyacrylamide gels. A representative fluorograph is shown in this figure. (*bottom*) Ratio of SAA<sub>1</sub> and SAA<sub>2</sub> mRNA during amyloid induction. L-[<sup>35</sup>S]methionine, SAA antibody-precipitated translation products were separated on SDS-urea-polyacrylamide gels and radiofluorographs prepared (as in top). SAA<sub>1</sub> and SAA<sub>2</sub> preproteins were quantitated by microdensitometry of appropriately exposed fluorographs. The number at the top of the bars is the SAA<sub>2</sub>/SAA<sub>1</sub> preprotein ratio. RNA from two livers at each time point was analyzed.

were specifically immunoprecipitated in approximately equal amounts (Fig. 4*a*, lanes 1 and 2), and as expected of preproteins, migrated with mobilities of peptides appropriately larger than SAA<sub>1</sub> and SAA<sub>2</sub>. Pretreatment of the antibody with murine protein AA completely blocked antibody precipitation of the translated proteins (lane 3). Fig. 4*b* depicts results of cell-free translations of total RNA in the absence (lane 1) or presence (lane 2) of microsomes (which remove the NH<sub>2</sub> terminal signal peptide from secretory proteins) (29). As in Fig. 4*a*, two SAA preproteins are immunoprecipitated from the reaction. Translations carried

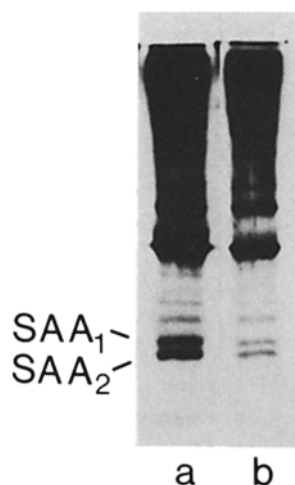


FIGURE 6. Secretion of SAA by hepatocytes. Hepatocytes were isolated and labeled as described under Materials and Methods from mice (a) 1 d or (b) 20 d after the start of casein injections. Equal volumes of media from a 25-cm<sup>2</sup> flask containing  $3 \times 10^6$  cells were analyzed from each sample. Identification of SAA<sub>1</sub> and SAA<sub>2</sub> HDL apoproteins is in the left margin.

out in the presence of microsomes result in processed SAA proteins with the same mobility as authentic SAA<sub>1</sub> and SAA<sub>2</sub> isolated from HDL.

The relative abundance of hepatic SAA<sub>1</sub> and SAA<sub>2</sub> mRNA was determined by quantitation of [<sup>35</sup>S]methionine-labeled SAA<sub>1</sub> and SAA<sub>2</sub> preproteins, identified after cell-free translation of liver mRNA obtained from animals over the time course of amyloid induction. Fig. 5(*top*) shows the following: no specific radioactivity was precipitated in translation products from uninduced animals (lane 1). One day after casein injection, SAA translation products were easily detected and represented ~3.4% of total TCA precipitable counts translated. The level of SAA translation products showed a gradual decline and by day 20 was ~30% of that at day 1. As shown in Fig. 5(*bottom*), the relative SAA<sub>1</sub> and SAA<sub>2</sub> preprotein levels did not change significantly over the course of the amyloid deposition (day 1, 1.36; day 20, 1.23). This result is in contrast to the ratios of circulating SAA<sub>2</sub>/SAA<sub>1</sub> shown in Fig. 2, and clearly shows that the SAA<sub>1</sub> and SAA<sub>2</sub> mRNA ratio is not altered during amyloidogenesis.

*Synthesis and Secretion of SAA by Hepatocytes In Vitro.* Although hepatic SAA<sub>1</sub> and SAA<sub>2</sub> mRNA ratios remained unchanged during amyloid deposition, the relative rates of mRNA translation or relative efficiency of secretion may be changed and result in altered SAA<sub>2</sub> to SAA<sub>1</sub> ratio in the circulation. We obtained information on the relative rates of secretion of each SAA isotype by intact hepatocytes from freshly isolated hepatocytes 1 and 20 d after initiation of casein injections (Fig. 6). Proteins secreted into the medium during a 4-h labeling period with [<sup>35</sup>S]methionine were separated by SDS-urea-PAGE and the quantity of SAA<sub>1</sub> and SAA<sub>2</sub> estimated by microdensitometry of appropriately exposed autoradiographs (data not shown). Although the amount of each SAA isotype secreted by hepatocytes after 20 d of casein injection was reduced to ~25% of that secreted after day 1, the ratio of SAA<sub>2</sub>/SAA<sub>1</sub> on day 1 and day 20 was the

same (1.27). This result is in good agreement with the relative levels of SAA<sub>1</sub> and SAA<sub>2</sub> mRNA (Fig. 4), and taken together these data show that SAA<sub>1</sub> and SAA<sub>2</sub> mRNA levels and their synthesis and secretion by the liver decline in a parallel manner during induction of amyloidosis by multiple casein injections.

### Discussion

Amyloidosis of the reactive variety may complicate chronic inflammatory diseases such as rheumatoid arthritis and tuberculosis (2). Extracellular amyloid deposits composed of fibrils accumulate in extracellular locations in a variety of tissues, resulting in organ dysfunction (1-3, 12, 30-37). The fibrils are composed mainly of protein AA, an 8.5-kD peptide homologous with the NH<sub>2</sub>-terminal portion of the acute-phase HDL-associated apoprotein, serum amyloid A protein.

What is the underlying cause of amyloid formation? The commonly accepted hypothesis of amyloid deposition is that amyloid fibril protein A is derived from circulating SAA because of its sustained high levels during chronic inflammatory conditions. However, no certain evidence has been produced to sustain this supposition. Although an elevated SAA level is a predisposing condition for amyloid formation, high SAA levels alone are not sufficient. Of patients with chronic inflammatory diseases, only some individuals develop amyloidosis (2). Mouse model systems do not show a 1:1 correlation between SAA level and susceptibility to amyloid deposition (13, 38). Therefore, it appears that factors other than high SAA levels are involved in the pathogenesis of amyloidosis.

Our recent evidence that murine protein AA is derived from only one SAA isotype (12) indicates that an important feature of murine amyloidosis involves a mechanism specific for SAA<sub>2</sub> deposition. SAA<sub>2</sub> overproduction could result in ineffective degradation during normal catabolic mechanisms, or SAA<sub>1</sub> synthesis could cease during the long inflammatory period of amyloid deposition, leaving only SAA<sub>2</sub> as a possible circulating precursor to AA protein. Another possibility is that structural features of the SAA<sub>2</sub> protein alone may confer amyloidogenic potential without the need for altered metabolic pathways, or that SAA<sub>2</sub> could be synthesized and deposited locally rather than derived from the circulation as suggested by other investigators (39-45), and as is the case in endocrine and cutaneous amyloidosis (46). In order to differentiate among the several possible mechanisms of amyloidosis, we searched for evidence of local SAA synthesis and examined SAA metabolism during amyloid induction at the level of SAA gene expression, synthesis, and secretion and circulatory levels.

Having used a probe capable of detecting all three expressed SAA genes (Fig. 2), our findings that at no time during amyloid formation does splenic tissue contain detectable levels of SAA mRNA leave no doubt that the source of AA protein in the mouse is not local, but must be sequestered from the circulation. In addition, to determine if tissues other than liver synthesized SAA, we searched for SAA mRNA in RNA extracted from lung, heart, kidney, brain, skeletal muscle, and testis. We did not detect SAA mRNA in normal controls or at any time during amyloid induction in these tissues.

Our analysis of circulating SAA showed that monotypic amyloid formation is not a reflection of circulating SAA<sub>2</sub> levels, either by having a large preponderance of SAA<sub>2</sub> compared with SAA<sub>1</sub>, or by the lack of circulating SAA<sub>1</sub>. In fact, SAA<sub>2</sub>



serum levels are substantially reduced during the course of amyloid formation. This could be due to differential isotype synthesis and secretion or altered clearance from the circulation. However, the relative levels of SAA<sub>1</sub> and SAA<sub>2</sub> mRNA remained constant (Fig. 5), as did the amounts of SAA<sub>1</sub> and SAA<sub>2</sub> synthesized and secreted by hepatocytes (Fig. 6). The tenable explanation, therefore, is that SAA<sub>2</sub> is removed from the circulation faster than SAA<sub>1</sub>. This differential clearance of SAA isotypes is in marked contrast to equal clearance rates of SAA<sub>1</sub> and SAA<sub>2</sub> observed in normal mice (47) and mice during an acute phase response (Hoffman, Jeffrey S., unpublished observation). Thus protein AA monotypy in murine amyloid deposits must be the result of events that favor the rapid local removal of SAA<sub>2</sub> from a circulating pool containing both SAA<sub>1</sub> and SAA<sub>2</sub>.

Several factors may make SAA<sub>2</sub> amyloidogenic. The structure of the SAA<sub>2</sub> polypeptide may be important for amyloid deposition. Comparison of mouse SAA<sub>1</sub> and SAA<sub>2</sub> sequences deduced from cloning data (Morrow, John F., personal communication) (23) reveals nine relatively conservative amino acid residue differences. Among them is a third methionine residue at position 76 in SAA<sub>2</sub>. Our amino acid analysis data (34) indicate that this third methionine is included in the murine protein AA polypeptide (the NH<sub>2</sub> terminal portion of SAA<sub>2</sub>, ~8.5 kD). It is of interest that in an autosomal dominant form of genetically determined amyloidosis, familial amyloidotic polyneuropathy (FAP), a variant transthyretin is synthesized with a methionine substitution for a valine. Both the normal and variant proteins are found in the circulation, but only the variant transthyretin accumulates as amyloid fibrils (48, 49). This suggests that in the local environment where amyloid deposits occur, an additional methionine residue may confer increased protein-cell and/or protein-protein affinities. However, the significance of an additional methionine residue or other amino acid differences between SAA<sub>2</sub> and SAA<sub>1</sub> awaits further investigation. In addition, further studies are necessary to elaborate the details surrounding the nature of the interaction of SAA<sub>2</sub>, leading to its rapid and selective removal from the circulation and resulting in local amyloid deposition.

### Summary

The deposits of fibrils found in amyloidosis of the A type are derived from only one of the three serum amyloid A (SAA) gene products, namely SAA<sub>2</sub>. In order to explore the mechanism of SAA isotype-specific amyloid protein AA deposition, the molecular kinetics of the serum amyloid proteins were examined in CBA mice during casein induction of amyloidosis. The presence of SAA mRNA in spleen was searched for; hepatic SAA<sub>1</sub> and SAA<sub>2</sub> mRNA levels, rates of specific protein synthesis and secretion by hepatocytes, and serum levels were measured during a 20-d period of amyloid induction. We observed the following: small amounts of amyloid substance appeared in the spleen by day 5 and increased steadily over the ensuing 15 d to occupy nearly 30% of splenic volume by day 20. No SAA mRNA was detected in spleen at any time during induction of amyloid formation. Total serum SAA levels peaked 1 d after we began casein treatment, and thereafter declined. This decline was accounted for entirely by a dramatic fall in SAA<sub>2</sub>, while SAA<sub>1</sub> levels remained nearly constant throughout.

The ratios of hepatic SAA<sub>2</sub>:SAA<sub>1</sub> mRNA, as determined by *in vitro* translation, remained constant during the 20-d period, as did amounts of SAA<sub>1</sub> and SAA<sub>2</sub> synthesized and secreted by freshly isolated hepatocytes. These data indicate that the deposition of amyloid A protein derived from SAA<sub>2</sub> is not due to local SAA production in spleen, nor excessive SAA<sub>2</sub> production compared with SAA<sub>1</sub>, but involves the selective and accelerated removal of SAA<sub>2</sub> from the circulating pool of both SAA<sub>1</sub> and SAA<sub>2</sub>.

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