Brief Definitive Report

EXPRESSION OF THE THIRD MEMBER OF THE SERUM AMYLOID A GENE FAMILY IN MOUSE ADIPOCYTES

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Serum amyloid A (SAA) is a family of proteins found circulating mainly associated with high density lipoproteins (HDL) (1–4). They behave as acute phase reactants. Only trace amounts are found normally but, in response to inflammatory conditions or injury, the levels are elevated several hundred fold (1, 5–7). The dramatic elevation of SAA levels under these conditions suggests an important functional role for SAA. In the mouse, SAA is encoded by a family of three active genes (8, 9) (SAA_1, SAA_2, and SAA_3). SAA_1 and SAA_2 are HDL apoproteins but SAA_3 protein has not been identified in serum. The major site of SAA_1, SAA_2, and SAA_3 synthesis is the liver, where, after LPS stimulation, mRNA for each of the three genes is elevated ~1,000-fold (10). SAA genes are also expressed in extrahepatic tissues in response to LPS injection (11, 12), however, this is almost exclusively limited to expression of the SAA_3 mRNA (11). To determine the source of SAA_3 mRNA in extrahepatic sites, an SAA_3-specific cRNA probe was hybridized in situ to histological sections of tissues obtained after injection of mice with LPS. The observations indicate that extrahepatic SAA_3 expression is accomplished largely by a dispersed cell system, adipocytes. In testis and spleen certain other nonadipocytes also express SAA_3 mRNA.

Materials and Methods

In Situ Hybridization and cRNA Probes. Tissue samples from unstimulated mice (BALB/c) and from mice 18 h after LPS injection were fixed in a solution of 4% paraformaldehyde, 0.1 M PBS. Preparation of fixed tissues, ^3H-labeled cRNA probes, and subsequent in situ hybridization was carried out as described elsewhere (12a).

One cRNA probe designed to detect only the SAA_3 mRNA and another specific for SAA_1 and SAA_2 mRNA were prepared as described elsewhere (13), except that the SAA_1/SAA_2 cRNA was transcribed from 81 bp of 3′ noncoding sequence after cleavage of plasmid p275 with Hae III restriction enzyme.

Results

Results of hybridization of liver sections with [^3H]SAA_3 cRNA are shown in Fig. 1. Liver sections from treated mice (Fig. 1 *a*) show many silver grains overlying all hepatocytes with some variation in signal intensity. Only occasional silver grains are observed over liver sections from untreated mice (Fig. 1 *b*). Hybridization to cells other than hepatocytes is also observed. Labeled cells within blood vessels ap-

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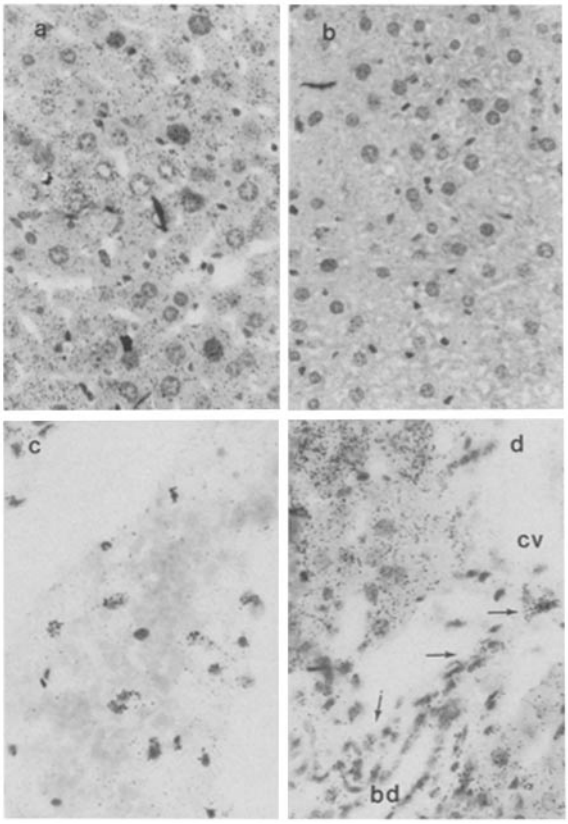

FIGURE 1. Autoradiographs of liver tissue sections from LPS-injected (*a*, *c*, and *d*) and control uninjected (*b*) mice after hybridization in situ with SAA_3 [^{3}H]cRNA. *a* and *b* show hepatocytes, *c* shows plasma and fixed cells in a central vein, and *d* is a section through a bile duct (*bd*) and central vein (*CV*). Arrow in *d* points out labeled nonhepatocyte cells.

pear to be monocytes/macrophages (Fig. 1 *c*); nonhepatocyte cells adjacent to bile duct epithelium (Fig. 1 *d*) and occasional cells among those lining the central veins and sinuses are labeled (not shown).

Several nonhepatic tissues were also examined. The SAA_3 cRNA hybridized to adipocytes of fat tissue adherent to the adrenal gland (Fig. 2, *a* and *b*) and aorta (Fig. 2, *c* and *d*) of treated mice but not to tissues of control mice. Adipocytes within the connective tissues adherent to the intestinal tract and fat tissue within lung also specifically hybridized with the SAA_3 cRNA (not shown).

In testis and spleen, SAA_3 mRNA is expressed by cells other than adipocytes. Leydig cells of testes from LPS-treated mice show specific hybridization with the SAA_3 cRNA (Fig. 3 *a*) but not with the SAA_1/SAA_2 cRNA (Fig. 3 *b*). In situ hybridization of spleens from treated mice revealed that some mononuclear cells, located only in the parafollicular zones, express SAA_3 mRNA (Fig. 3 *c*). Hybridization of [^{3}H]SAA_3 cRNA is not observed in spleen sections from control mice (Fig. 3 *d*).

Discussion

In a previous study (11), we found by blot analysis of extracted RNA that SAA_3 mRNA was expressed in all tissues examined after mice were treated with LPS. Esti-

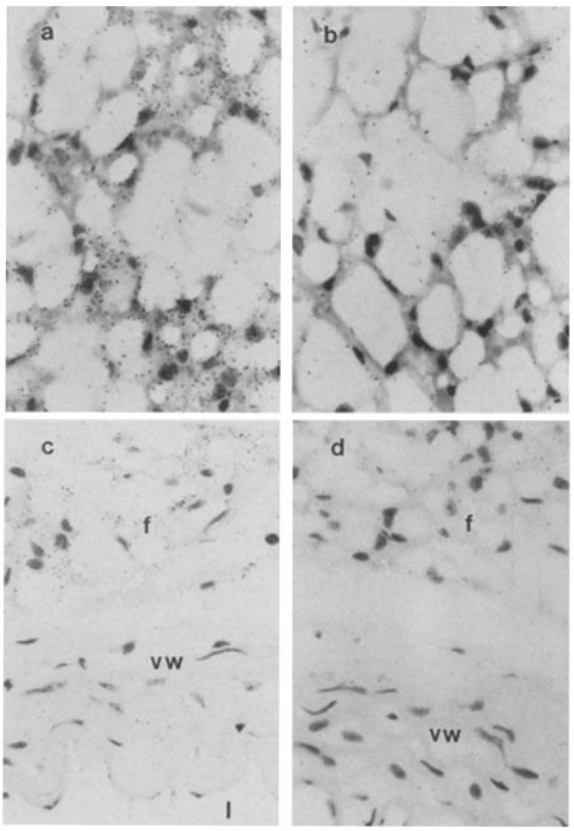

FIGURE 2. Autoradiographs of tissue section of peri-adrenal (*a* and *b*) and peri-aortic (*c* and *d*) fat tissue. Tissue sections from mice injected with LPS were hybridized in situ with SAA_3 [^{3}H]cRNA (*a*) and SAA_1/SAA_2 [^{3}H]-cRNA (*b*). Aorta from LPS-injected (*c*) and control uninjected mice (*d*) were hybridized with SAA_3 [^{3}H]cRNA. The lumen (*l*), vessel wall (*vw*), and fat (*f*) of the aortas are indicated.

mates showed the highest level of SAA_3 mRNA in nonhepatic cells was in peritoneal macrophages and was equivalent to the amount found in liver RNA of LPS-injected mice. Such macrophage-related cells are resident within many tissues and monocytes/macrophages accumulate in tissues that are sites of injury (14). Because LPS-activated macrophages express high levels of SAA_3 mRNA, it seemed reasonable to ask whether a single dispersed cell system, such as the reticuloendothelial system, might be the source of extrahepatic SAA_3 mRNA. To pursue this notion we used in situ hybridization to identify the cells expressing SAA_3 mRNA. The specificity of in situ hybridization was established as follows. (*a*) Two probes were used, one that detected SAA_1 and/or SAA_2 mRNAs, and the other specific for SAA_3 mRNA (13). In almost all extrahepatic tissues (excepting kidney and intestine), LPS induces only expression of SAA_3 mRNA (11). The SAA_1/SAA_2–specific cRNA probe, when hybridized to sections of tissues from mice injected with LPS was negative. (*b*) As a further control in the hybridization procedure, we used tissues from unstimulated mice. These were hybridized with the SAA_3 cRNA simultaneously with tissue sections from LPS-injected mice. The SAA_3 probe did not hybridize to any control tissues. As indicated above, in liver sections from LPS-injected mice, all hepatocytes expressed SAA_3 mRNA (Fig. 1). However, some cells other than hepatocytes also expressed the SAA_3 mRNA, these included cells lining sinusoids and

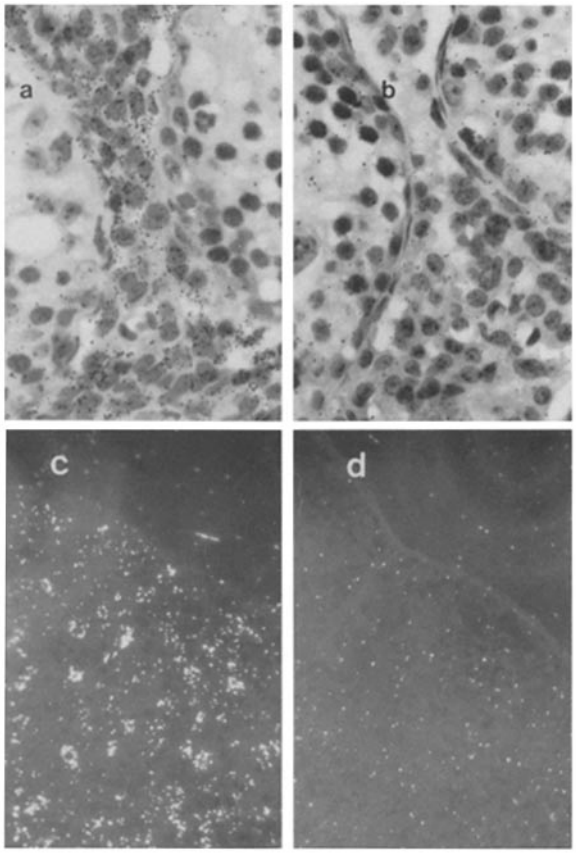

FIGURE 3. Autoradiographs of tissue sections of testis (*a* and *b*) and spleen (*c* and *d*) from a mouse injected with LPS and hybridized with SAA_3 [^{3}H]-cRNA (*a* and *c*) and SAA_1/SAA_2 [^{3}H]cRNA (*b* and *d*). Photomicrographs of spleen sections were produced with dark field illumination. The photomicrographs show only the parafollicular regions adjacent to the spleen capsule and a blank, nontissue area in the upper right corner.

central veins (data not shown) and cells adjacent to bile duct epithelium (Fig. 1 *d*). Some of these SAA_3 mRNA-containing cells in liver appear to be Kupffer cells. This finding in liver differs from the expression of SAA_1 and SAA_2 mRNA in liver, which is restricted to hepatocytes (12a).

It is clear in mice injected with LPS that extrahepatic SAA_3 mRNA is expressed by adipocytes of fat tissues adherent to (or an integral part of) organs such as adrenal gland, aorta (Fig. 2), lung, ileum, and large intestine. The observations indicate that while one dispersed system of cells is responsible for much of the SAA_3 mRNA found in the extrahepatic sites, other specialized cells of some tissues also do so. This population of cells includes Leydig cells of testis and other as yet undefined cells in the spleen (Fig. 3). Since monocytes/macrophages (11) (Fig. 1 *c*) express high levels of SAA_3 mRNA, SAA_3 expression in the spleen may be due to resident-free and/or resident-fixed macrophages populating the parafollicular zones. While the adipocytes are prominent producers of SAA_3 mRNA, it seems that macrophage-related reticuloendothelial cells, including Kupffer cells, alveolar macrophages, and connective tissue histiocytes, may express the SAA_3 gene. Additionally, preadipocytes may also express the gene and be indistinguishable from fibroblasts in certain tissues.

Our finding that expression of SAA_3 mRNA in all tissues after LPS injection is due largely to adipocytes raises new possibilities regarding functions of SAA_3. The role of SAA_3 in host response to injury, trauma, and inflammation must be distinct from that of apoSAA_1 and apoSAA_2. The differences are emphasized by the fact that SAA_3 mRNA is not expressed in response to all of the inflammatory stimuli that can induce SAA_1 and SAA_2 mRNA expression (11). Moreover, SAA_3 protein has not been found to be an HDL apoprotein, as are SAA_1 and SAA_2 (1, 15). Furthermore, except for hepatocytes, the cells that express SAA_3 mRNA after LPS treatment of mice are distinct from those expressing SAA_1 and SAA_2 mRNA. By analogy with the cell type-specific expression of other genes (16), this suggests that there is a difference in the enhancer-promoter elements of the SAA_3 compared with SAA_1 and SAA_2 genes. This is consistent with the marked difference in structure of the SAA_3 gene when compared with SAA_1 and SAA_2 genes (8). The high levels of SAA_3 mRNA expression in adipocytes and Leydig cells suggests a role for SAA_3 in lipid and steroid metabolism and/or transport in response to injury, reminiscent of the role of other apolipoproteins in cholesterol and lipid transport (17).

Summary

Three homologous genes that code for three related proteins comprise the serum amyloid A (SAA) family in the mouse. Endotoxin induces equally vigorous expression of mRNAs for the three SAA genes in liver. In extrahepatic tissues SAA_1 and/or SAA_2 mRNAs have been found only in kidney and intestine, however, SAA_3 is expressed in all extrahepatic tissues thus far examined. This observation raised the question: is SAA_3 mRNA expressed by a single cell system dispersed throughout all tissues, or by differentiated cells of each tissue? This question was explored in various tissues by in situ hybridization with a single-stranded cRNA probe specific for SAA_3 mRNA. We found expression in the liver of SAA_3 mRNA by other cells as well as by hepatocytes. A common feature among extrahepatic tissues was SAA_3 mRNA expression in adipocytes. SAA_3 mRNA was also found in two nonadipose cells, Leydig cells of the testis, and some of the cells located in parafollicular zones of the spleen.

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