Clin. exp. Immunol. (1986) **66**, 691–700

Circulating serum amyloid P component is the precursor of amyloid P component in tissue amyloid deposits

MARILYN L. BALTZ, D. CASPI, D. J. EVANS* I. F. ROWE, C. R. K. HIND & M. B. PEPYS *MRC Acute Phase Protein Research Group, Immunological Medicine Unit, Department of Medicine and *Department of Histopathology, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, United Kingdom*

(Accepted for publication 10 June 1986)

SUMMARY

Intravenous administration of ^{125}I-labelled isolated mouse serum amyloid P component (SAP) to mice with systemic amyloidosis was followed by specific deposition of the labelled protein in amyloidotic organs. Although only a small proportion of the total injected dose became localized in this way, the amount correlated with the quantity of amyloid present in different organs and was greatest in the spleen. No such localization was detected in the organs of control, untreated mice or animals which had received inflammatory stimuli but did not have amyloidosis. The labelled SAP was found by autoradiography to be present in the same distribution within the tissues as the Congophilic amyloid deposits. These observations establish directly, for the first time, that circulating SAP is the precursor of the amyloid P component (AP) in systemic amyloidosis. They were confirmed by the further finding that intravenous injection into amyloidotic mice of human SAP, either in whole human serum or in isolated pure form, was followed by appearance of the human SAP in the mouse amyloid deposits. In addition to elucidating one aspect of the pathogenesis of amyloid deposition and strengthening the homology of functional behaviour between SAP of different species, the present results suggest a means for selective targeting of diagnostic tracers and/or effector agents to amyloid deposits *in vivo*.

Keywords amyloidosis serum amyloid P component amyloid P component

INTRODUCTION

Systemic amyloidosis is a serious disorder which causes considerable morbidity and is usually fatal (Glenner, 1980a, b; Pepys, 1986). The deposits which accumulate and persist in the tissues consist predominantly of characteristic amyloid fibrils formed by particular proteins, notably immunoglobulin light chains in AL amyloid, amyloid A protein (AA) in reactive systemic amyloid and prealbumin in type I familial amyloid polyneuropathy. In addition, in all forms of systemic and localized amyloidosis, with the possible exception of the intracerebral plaques in Alzheimer's disease and senile dementia (Westermark *et al.*, 1982; Rowe *et al.*, 1984), there is also a minor non-fibrillar glycoprotein constituent known as amyloid P component (AP) (Pepys *et al.*, 1982; Pepys & Baltz, 1983). AP is apparently identical to a normal serum protein known as serum amyloid P

Correspondence: Professor M. B. Pepys, Immunological Medicine Unit, Department of Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, U.K.

component (SAP) by immunochemical testing, by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) and non-denatured gradient PAGE, by partial amino acid sequencing and in electron microscopic appearance (Pepys *et al.*, 1982; Pepys & Baltz, 1983). AP may be present in amounts of up to 15% of the mass of the amyloid fibrils in the deposits (Skinner *et al.*, 1980) and it has long been assumed that AP is derived from the circulating SAP although this has not previously been demonstrated.

We report here that both in the well-established murine model of AA amyloidosis induced by repeated injections of casein (Janigan, 1965) and in the accelerated mouse model of AA amyloidosis induced by so-called amyloid-enhancing factor (AEF) (Axelrad *et al.*, 1982), SAP from the circulation is indeed laid down as AP in the amyloid deposits. Furthermore we show for the first time that introduction into the plasma of SAP from another species, man, is followed by its deposition in the mouse amyloid. In addition to elucidating one aspect of the pathogenesis of amyloidosis, these findings may have implications for the diagnosis and management of amyloidosis in man.

MATERIALS AND METHODS

Mice. Male and female mice, 8–12 weeks old were used. CBA/ca mice were obtained from the National Institute of Medical Research (London, UK) and C57Bl/6 mice from OLAC Ltd (Bicester, Oxon, UK).

Induction of amyloid. Mice were injected subcutaneously (s.c.) daily five times per week for 4–6 weeks with 0·5 ml 10% w/v vitamin free casein (ICN Pharmaceuticals Inc., Cleveland, Ohio) in 0·05 M $NaHCO_3$ (Janigan, 1965). Amyloid enhancing factor (AEF) was prepared by the method of Axelrad *et al.* (1982) using the spleens of CBA/ca mice in which amyloidosis had been induced by repeated casein injections. For amyloid induction, mice were injected intravenously (i.v.) with 0·5 ml AEF followed immediately by a s.c. injection of 0·5 ml 2% w/v $AgNO_3$ in distilled water. Mice were killed at the times indicated in Results.

Histochemical staining. Immediately after death, spleens, and in some experiments liver and kidney, were snap frozen in Arcton 12 (ICI, Cheshire, UK) slushed over liquid N_2, and stored in liquid N_2. Serial sections 8 μm thick were cut on a cryostat. The presence of amyloid was confirmed by alkaline Congo red staining (Puchtler, Sweat & Levine, 1962), viewed in a Leitz Orthoplan microscope (E. Leitz (Instruments) Ltd, Luton, UK) equipped with polarizing filters. The presence of mouse SAP, human SAP or human C-reactive protein (CRP) in the deposits was sought by direct immunofluorescence staining using the fluoresceinated reagents detailed below and precisely the same method previously described (Baltz, Dyck & Pepys, 1980) with the single exception that the solution used for all washing procedures was 0·01 M Tris, 0·14 M NaCl, 0·002 M $CaCl_2$, 0·1% w/v NaN_3 at pH 8·0. The presence of human serum albumin (HSA) in sections was sought by indirect immunofluorescence using rabbit anti-HSA serum and fluoresceinated goat anti-rabbit IgG (Miles Scientific, Slough, UK). In all cases the specificity of positive staining was established by its complete abolition when each antiserum was absorbed before use with the relevant pure antigen. Sections of tissue from mice which had received ^{125}I-labelled SAP were subjected to autoradiography (Flitney, 1977) by the liquid emulsion technique (Ilford Liquid Emulsion K5 Size A, Ilford Ltd, Cheshire, UK) in order to evaluate the pattern of localization of the labelled material. After development of the autoradiographs the sections were stained with haematoxylin for morphological detail and Congo red to demonstrate the amyloid.

Electron microscopy. Tissue blocks of 1 mm^3 were fixed in phosphate buffered 3·4% v/v glutaraldehyde, postfixed in 1% w/v Millonigs osmium tetroxide, dehydrated and embedded in TAAB resin (TAAB Laboratories Ltd, Reading, UK). Thin sections were cut and stained with alcoholic uranyl acetate and Reynolds lead citrate. Photographs were taken on an AEI transmission electron microscope model 801 (Kratos Instruments, Manchester, UK).

Proteins. Mouse SAP, human SAP and human CRP were isolated and purified as previously reported (Pepys, 1979; de Beer & Pepys, 1982). HSA (crystallized and lyophilized) was obtained

from Sigma London Chemical Co Ltd (Poole, Dorset, UK). All proteins were dialysed into phosphate buffered saline, pH 7·4 (PBS) for injection *in vivo*.

Antisera. Monospecific rabbit and sheep antisera to SAP and CRP of man and mouse were raised by immunization with the isolated pure proteins. For immunofluorescence, the IgG_1 fraction of a sheep anti-human SAP (Dyck *et al.*, 1980), the $F(ab')_2$ fragment of a rabbit anti-mouse SAP (Baltz, Dyck & Pepys, 1980) and the IgG fraction of a rabbit anti-human CRP were conjugated with fluorescein isothiocyanate by standard methods.

Iodination of mouse SAP. Pure mouse SAP was radiolabelled with carrier-free Na-^{125}I (IMS.30, Amersham plc, Amersham, UK) using Iodogen (Pierce & Warriner UK Ltd, Chester, UK) (Salacinski *et al.*, 1981). Free ^{125}I was separated from protein bound ^{125}I by gel filtration in PBS on Sephadex G-25 (PD-10 column, Pharmacia GB Ltd, Milton Keynes, UK). The radiolabelled protein was sterilized by filtration through a 0·22 μm Millipore filter (Millipore UK Ltd, Harrow, UK). More than 95% of the radioactivity was precipitable with cold 20% trichloroacetic acid. When subjected to SDS-PAGE under reducing conditions the radiolabelled material migrated as a single band in the same position as unlabelled mouse SAP.

Plasma clearance studies. The rate of clearance of ^{125}I-mouse SAP from the circulation of amyloidotic and control mice was estimated as reported elsewhere (Baltz, Dyck & Pepys, 1985).

Localization of circulating mouse SAP in AEF-induced amyloidosis. Two groups of eight CBA/ca mice were injected s.c. with $AgNO_3$ alone or $AgNO_3$ preceded by an i.v. injection of AEF. On day 0, 1, 2 or 3 after $AgNO_3$ (with or without AEF) two mice in each group were injected i.v. with 3·3 μg of ^{125}I-mouse SAP (5×10^7 ct/min). All mice were killed on day 4, the spleens, livers and kidneys were removed, weighed, counted in a gamma counter and then snap frozen for histochemical processing. The counts were corrected for decay of the isotope.

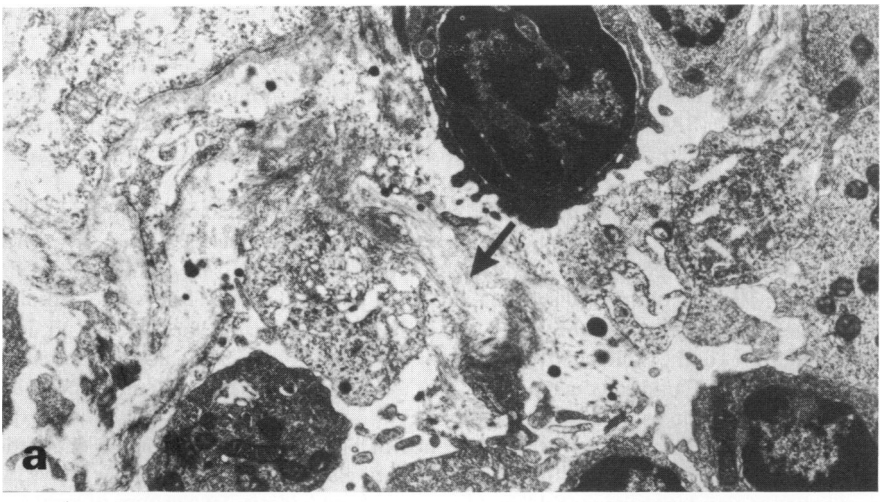
Persistence of ^{125}I-mouse SAP in AEF-induced amyloidosis. Mice in groups of the same size as above were treated identically throughout except that one group was killed on day 12 and the other group killed on day 29. The counts were corrected for decay of the isotope.

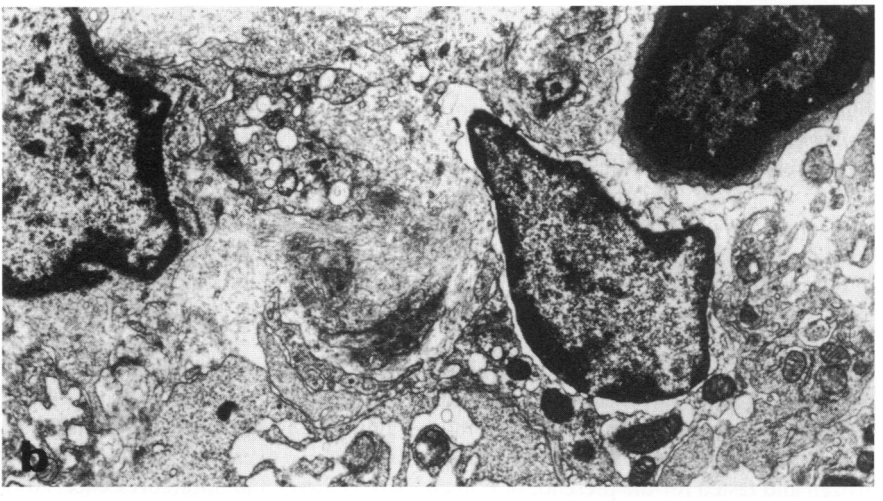
Localization and persistence of ^{125}I-mouse SAP in casein-induced amyloidosis. A group of eight C57Bl/6 mice with established casein-induced amyloid were given 3·3 μg each of ^{125}I-mouse SAP (5×10^7 ct/min) by i.v. injection on day 0. Two mice were killed at intervals thereafter, their spleens were removed, counted for radioactivity and then processed for histology. The counts were corrected for decay of the isotope.

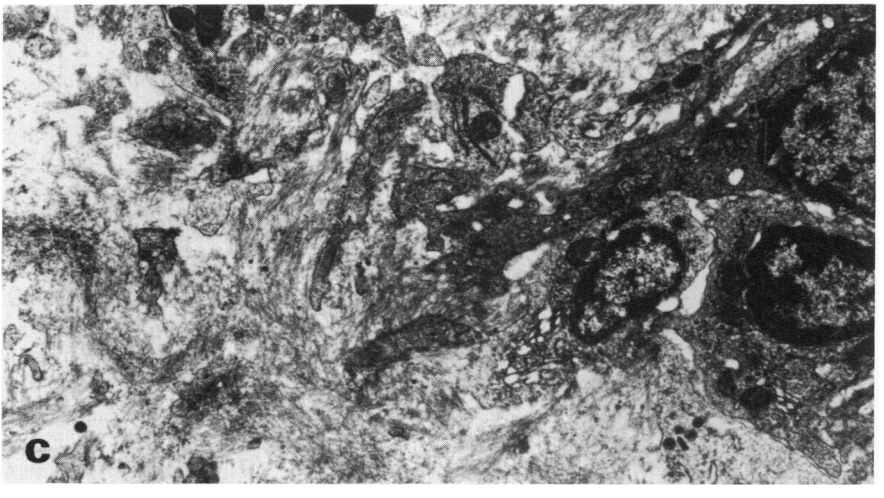
Localization of human SAP in deposits of mouse amyloid. Groups of CBA/ca mice were treated with AEF and $AgNO_3$, $AgNO_3$ alone or nothing. Isolated pure human SAP (100 μg–1·0 mg) or, in other experiments normal human serum (0·5 ml containing 25 μg of human SAP), was injected i.v. on day 3 and the animals were killed 1 day later. Their spleens, livers and kidneys were removed, snap-frozen and tested for the presence of human SAP by direct immunofluorescence. Additional controls were provided by the use in other groups of mice of isolated pure human CRP (300 μg) or HSA (300 μg). The presence of these proteins in the spleen was sought by immunofluorescence staining.

RESULTS

Kinetics of amyloid deposition in the AEF model. Four mice given a single i.v. injection of AEF followed by a single s.c. injection of $AgNO_3$ were killed exactly 24, 48, 72 and 96 h later. Although there was apparently some uptake of Congo red by sections of spleen at 24 h, no green bi-refringence was seen. At 48 h and subsequently, there was however, unequivocal Congophilic amyloid in a perifollicular distribution, increasing in amount up to 96 h. Electron microscopy was more sensitive in that extracellular fibrils with the typical morphology of amyloid were already demonstrable at 24 h and amounts increased rapidly thereafter (Fig. 1). Direct immunofluorescence with anti-mouse SAP antibodies produced only very weak perifollicular staining at 24 h, albeit definitely distinguishable from the complete lack of staining seen in normal spleen or spleen from mice which had received only $AgNO_3$ without AEF. Thereafter there was a rapid increase in the intensity of


a


b


c

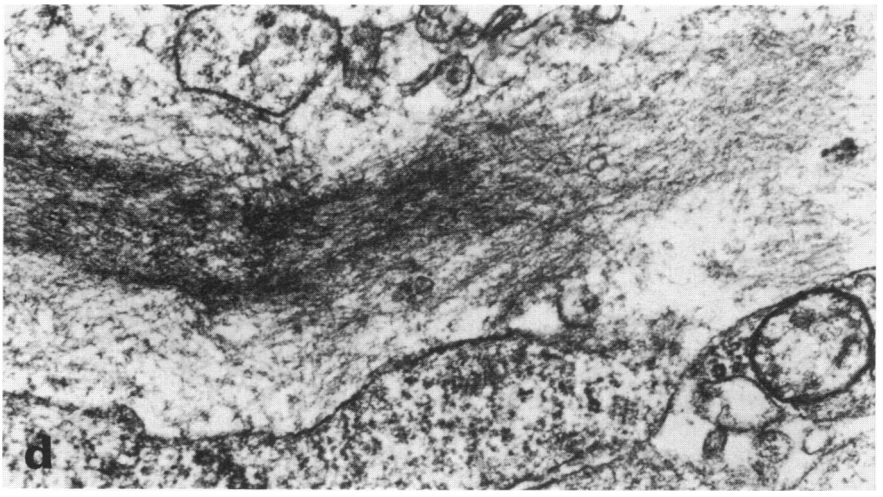


Fig. 1. Electron micrographs of spleen after AEF and $AgNO_3$ administration. (a) day 1, scanty deposit of amyloid seen as extracellular fibrils (arrowed) (× 9,450); (b) day 2, substantial increase in amyloid deposition (× 9,450); (c) day 3, extensive amyloid deposits present (× 9,450); (d) detail of amyloid fibrils (× 37,500) taken from a day 2 spleen; similar appearances were present at all times.

Table 1. Localization of circulating ^{125}I-mouse SAP in splenic amyloid deposits

Treatment	Amyloid present*	Day of ^{125}I-mouse SAP injection	ct/min/spleen†
AEF + $AgNO_3$	+	0	19,300
AEF + $AgNO_3$	+	0	13,978
$AgNO_3$	−	0	676
$AgNO_3$	−	0	6898
AEF + $AgNO_3$	+	1	36,988
AEF + $AgNO_3$	+	1	33,192
$AgNO_3$	−	1	2670
$AgNO_3$	−	1	1660
AEF + $AgNO_3$	+	2	80,670
AEF + $AgNO_3$	+	2	134,017
$AgNO_3$	−	2	3280
$AgNO_3$	−	2	3541
AEF + $AgNO_3$	+	3	159,925
AEF + $AgNO_3$	+	3	117,442
$AgNO_3$	−	3	6731
$AgNO_3$	−	3	1818

* Demonstrated by Congo red staining.

These values correspond to 0·028–0·32% of the injected dose for the amyloidotic mice and 0·001–0·013% for the control mice.

staining and by 96 h it was very marked, resembling the characteristic appearance of anti-SAP stained early amyloid deposits induced by casein (Baltz, Dyck & Pepys, 1980).

Localization of circulating ^{125}I-mouse SAP in AEF-induced amyloidosis. Much more of an injected dose of ^{125}I-mouse SAP became localized in the spleens of mice which had received AEF and then $AgNO_3$ than in spleens of mice which had received $AgNO_3$ only (Table 1). The spleens of these

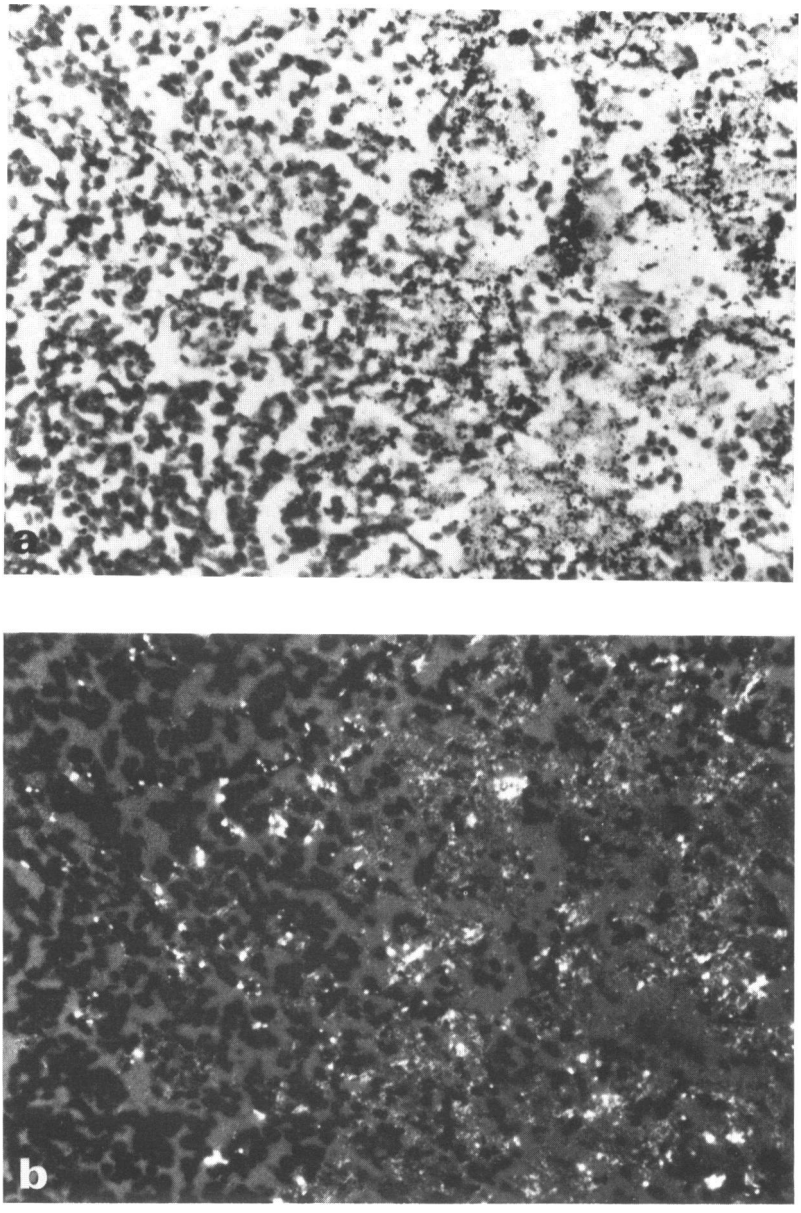
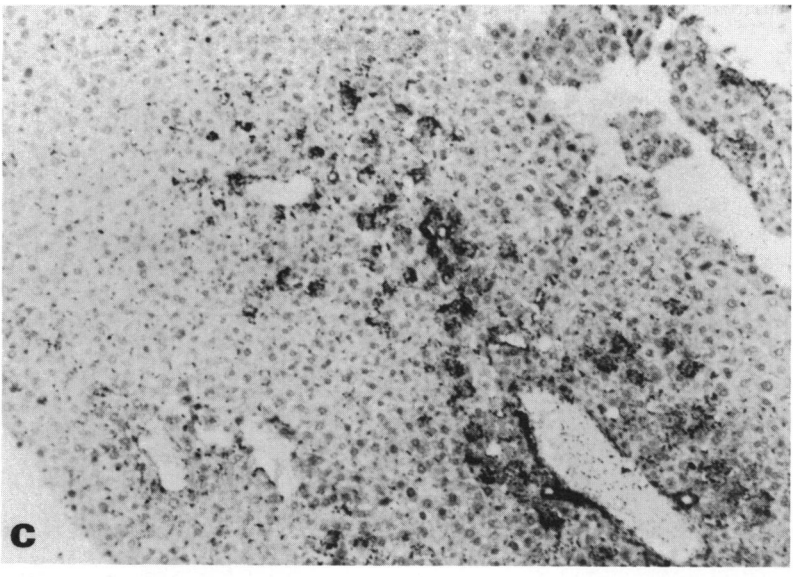
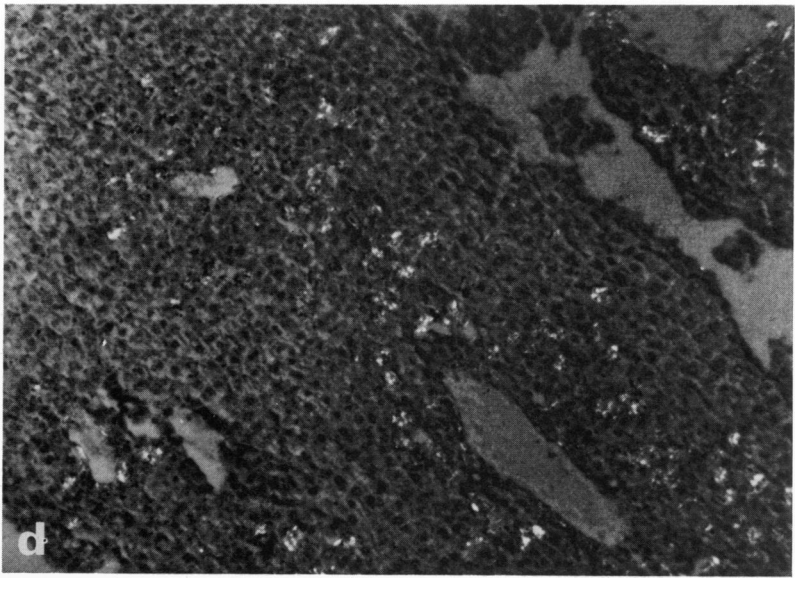


Fig. 2. Localization of ^{125}I-mouse SAP to amyloid deposits demonstrated by autoradiography. (a) periphery of splenic follicle showing black grains specifically located over amorphous Congo red stained amyloid deposits; (b) same field viewed in polarized light showing specific amyloid bi-refringence (magnification × 200); (c) liver showing black grains specifically located over scattered amyloid deposits; (d) same field viewed in polarized light showing specific amyloid bi-refringence (magnification × 100).

latter animals contained no amyloid demonstrable by any method, in contrast to the spleens of the AEF-treated group, as shown above. These observations suggest that the localization of ^{125}I-mouse SAP correlated with the deposition of amyloid and this was confirmed by autoradiography (Fig. 2) in which the grains corresponding to the presence of labelled SAP overlay precisely the perifollicular zones containing amyloid detected by Congo red and anti-SAP staining. The quantity of ^{125}I-mouse





SAP in the spleen increased *pari passu* with the increase in amyloid (Table 1) although it was never more than a small percentage of the total injected dose. In another experiment in which mice treated identically were maintained for longer periods, very little radioactivity (less than 10% of the values in Table 1 for each time point) was present in the spleen at 12 or 29 days after its injection although deposits of amyloid comparable to or greater than those at day 4 were still present.

The ^{125}I-mouse SAP was cleared from the plasma at the same rate in all mice regardless of prior treatment with AEF and of the presence or absence of amyloid. The half-time was 7·2–8·0 h, the same as has been reported previously for SAP in normal and acute phase mice and mice with casein-induced amyloid (Baltz, Dyck & Pepys, 1985). The failure to detect any acceleration of plasma clearance of SAP despite its unequivocal deposition in the amyloid is not surprising in view of the small proportion of the total intravascular pool which does localize (Table 1). Furthermore this

Table 2. Localization and persistence of ^{125}I-mouse SAP in casein-induced amyloidosis

Days after injection of ^{125}I-mouse SAP	ct/min/spleen
1	380,069*
	410,194*
5	100,148
	71,459
22	11,410
	7,890
29	10,266
	5,061

* The mean of these values represents 0.79% of the total injected dose.

observation indicates that deposition of SAP at extracellular sites in the tissues is not a major determinant of the rate of its passage from the intravascular compartment.

Localization of ^{125}I-mouse SAP in casein-induced amyloidosis. One day after injection of ^{125}I-mouse SAP into animals with established casein-induced amyloid there was highly significant localization of label in the spleen, the proportion of the injected dose being greater than that seen in the AEF model (Tables 1, 2). This difference correlated with the greater extent of the amyloid deposits after prolonged casein stimulation, extending between follicles and obliterating the follicles in some cases. The amount of label in the spleen decreased quite rapidly (Table 2) reaching 50% after 3 days and declining more slowly thereafter. There was, however, no change in the amount of amyloid present over this period. Entirely comparable correlations were observed between the

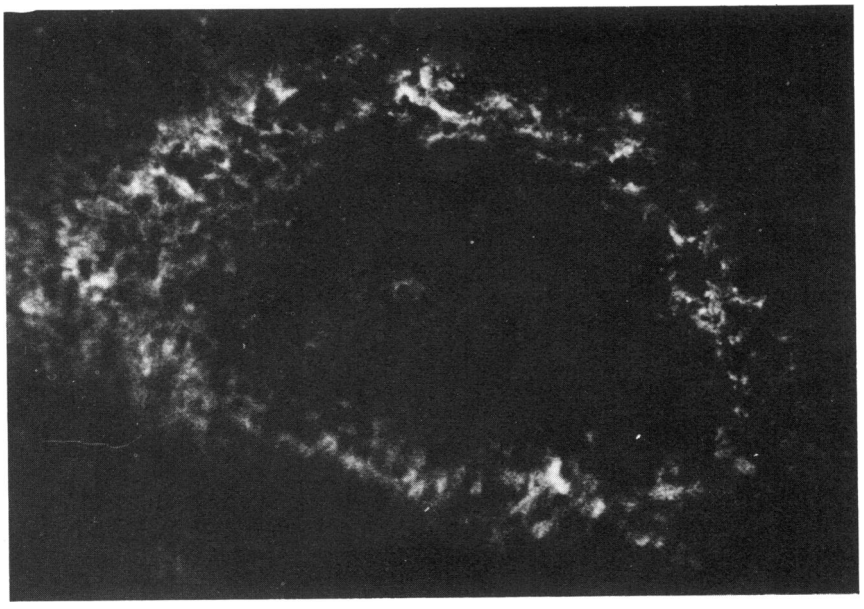


Fig. 3. Direct immunofluorescence staining with anti-human SAP of spleen from a mouse which had received pure human SAP by i.v. injection 3 days after AEF and $AgNO_3$ and 1 day before being killed. A single follicle with characteristic perifollicular staining is shown.

amount of ^{125}I-mouse SAP initially localizing to the liver and kidneys and the quantity of amyloid present. The amount of label localized in these organs also decreased at the same rate as in the spleen.

Localization of human SAP in deposits of murine amyloid. When isolated human SAP or whole human serum was administered to mice with either AEF or casein-induced amyloidosis, human SAP became demonstrable in the spleen, localized specifically to the amyloid deposits (Fig. 3). The intensity of staining with anti-human SAP corresponded to the quantity of Congo red positive amyloid present and was proportionately less in the liver and kidneys than in the spleen. No human SAP at all was detected in spleens of either normal or $AgNO_3$-treated mice without amyloid. Specificity of the deposition of SAP in amyloid deposits was established by the failure of the other human proteins, CRP and HSA, to localize in either amyloid-bearing (AEF-treated) or control mice.

In amyloidotic mice killed at various intervals after injection of human SAP the intensity of immunofluorescent staining of deposits with anti-human SAP decreased steadily from its peak at day 1 although some staining was still present after 30 days. Positive staining with anti-SAP persisted longer in the mice with casein-induced amyloid than in those with AEF induced amyloid, possibly corresponding to the greater amount of amyloid present. There was no appreciable change in the amount of amyloid present as the quantity of human SAP associated with it declined.

Interestingly there was no evidence of a significant inflammatory reaction in the organs containing human SAP, despite the fact that the protein is a good immunogen, nor was any circulating antibody response to human SAP detected by gel precipitation testing even at 30 days after injection (not shown).

DISCUSSION

The discovery of a protein component of amyloid deposits which is immunochemically identical to a normal, circulating plasma protein led to the conclusion that the latter is the precursor of the former (reviewed in Pepys *et al.*, 1982; Pepys & Baltz, 1983). Further work confirming the biochemical and structural similarities between SAP and AP reinforced this impression. The observation that SAP displays specific calcium-dependent binding properties for ligands including amyloid fibrils (Pepys *et al.*, 1979) provided for the first time a mechanism which could be invoked to explain the almost universal presence of AP in amyloid deposits.

The present results establish that SAP in the circulation is indeed deposited as AP in tissue amyloid. Furthermore they illustrate that whilst the initial deposition takes place rapidly, within hours, the persistence of AP is not prolonged. There is a fairly rapid turnover of AP in the deposits, with a half-time of days, presumably as a result of replacement of existing AP molecules by fresh SAP from the circulation. It is not clear whether such replacement reflects a metabolic change in the AP following its deposition or is merely a result of a dynamic equilibrium between free SAP in the plasma, free SAP in the tissue fluids and SAP molecules which have become attached as AP in the amyloid deposits. Regardless of the mechanism these observations demonstrate that amyloid deposits are not completely inert and this may encourage hopes of their therapeutic mobilization.

Our demonstration that human SAP, whether in isolated pure form or in its native state in whole normal human serum, is deposited in murine amyloid, extends the previously well recognized homology between these pentraxins of different species (Baltz *et al.*, 1982; Taylor *et al.*, 1982). We have also demonstrated that both react specifically with the newly synthesized monosaccharide ligand, methyl 4,6-O-(1-carboxyethylidene)-β-D-galactopyranoside (MOβDG) (Hind *et al.*, 1984a, b). The present finding confirms this in-vitro observation in terms of the only known in-vivo pathophysiological function of SAP; that is its propensity to deposit with amyloid fibrils.

In experiments (not shown) in which both labelled mouse SAP and human SAP were administered simultaneously to amyloidotic mice there was evidence of competition between the two for uptake in spleen. This finding taken together with the production of 'chimaeric' amyloid, that is xenogeneic AP with autologous AA fibrils, opens up the possibility of certain potentially important applications in the diagnosis and management of human amyloidosis. For example,

could suitably labelled autologous, isologous or heterologous SAP be used as a diagnostic probe for determining the presence, extent and distribution of amyloid deposits in man? In addition what effect may the sustained presence in amyloid deposits of xenogeneic (immunogenic) or some form of, for example, enzyme-conjugated AP, have on their persistence and effects? Although only a very small proportion of the total intravascular pool of SAP finds its way to amyloid deposits at any one time *in-vivo* these questions are certainly worth exploring and preliminary studies in the present mouse models are currently in progress. If promising results are obtained, their extension to man would not be far fetched considering the absence of any effective therapy for human systemic amyloidosis and its extremely poor prognosis in many cases.

Electron micrographs were taken by Andrew Westmuckett. This study was supported in part by MRC Programme Grant G979/51 to MPB.

REFERENCES

AXELRAD, M.A., KISILEVSKY, R., WILLMER, J., CHEN, S.J. & SKINNER, M. (1982) Further characterisation of amyloid-enhancing factor. *Lab. Invest.* **47,** 139.

BALTZ, M. L., DE BEER, F.C., FEINSTEIN, A., MUNN, E.A., MILSTEIN, C.P., FLETCHER, T.C., MARCH, J.F., TAYLOR, J., BRUTON, C., CLAMP, J.R., DAVIES, A.J.S. & PEPYS, M.B. (1982) Phylogenetic aspects of C-reactive protein and related proteins. *Ann. N.Y. Acad. Sci.* **389,** 49.

BALTZ, M.L., DYCK, R.F. & PEPYS, M.B. (1980) Amyloid P-component in mice injected with casein: identification in amyloid deposits and in the cytoplasm of hepatocytes. *Immunology* **41,** 59.

BALTZ, M.L., DYCK, R.F. & PEPYS, M.B. (1985) Studies of the *in-vivo* synthesis and catabolism of serum amyloid P component (SAP) in the mouse. *Clin. exp. Immunol.* **59,** 234.

DE BEER, F.C. & PEPYS, M.B. (1982) Isolation of human C-reactive protein and serum amyloid P component. *J. Immunol. Methods* **50,** 17.

DYCK, R.F., EVANS, D.J., LOCKWOOD, C.M., REES, A.J., TURNER, D., & PEPYS, M.B. (1980) Amyloid P-component in human glomerular basement membrane: abnormal patterns of immunofluorescent staining in glomerular disease. *Lancet* **ii,** 606.

FLITNEY, F.W. (1977) Autoradiography. In: *Theory and Practice of Histological Techniques.* (ed. by Bancroft, J.D. & Stevens, A.) p. 371. Churchill Livingstone, Edinburgh.

GLENNER, G.G. (1980a) Amyloid deposits and amyloidosis—the β-fibrilloses I. *New. Engl. J. Med.* **302,** 1283.

GLENNER, G.G. (1980b) Amyloid deposits and amyloidosis—the β-fibrilloses II. *New Engl. J. Med.* **302,** 1333.

HIND, C.R.K., COLLINS, P.M., CASPI, D., BALTZ, M.L. & PEPYS, M.B. (1984) Specific chemical dissociation of fibrillar and non-fibrillar components of amyloid deposits. *Lancet* **ii,** 376.

HIND, C.R.K., COLLINS, P.M., RENN, D., COOK, R.B., CASPI, D., BALTZ, M.L. & PEPYS, M.B. (1984) Binding specificity of serum amyloid P component for the pyruvate acetal of galactose. *J. exp. Med.* **59,** 1058.

JANIGAN, D.T. (1965) Experimental amyloidosis. Studies with a modified casein method, casein hydrolysate and gelatin. *Am. J. Pathol.* **47,** 159.

PEPYS, M.B. (1979) Isolation of serum amyloid P-component (protein SAP) in the mouse. *Immunology* **37,** 637.

PEPYS, M.B. (1986) Amyloidosis. In: *Immunologic diseases,* 4th edn. (ed. by M. Samter) Little, Brown & Co. Boston, in press.

PEPYS, M.B. & BALTZ, M.L. (1983) Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv. Immunol.* **34,** 141.

PEPYS, M.B., BALTZ, M., DE BEER, F.C., DYCK, R.F., HOLFORD, S., BREATHNACK, S.M., BLACK, M.M., TRIBE, C.R.F., EVANS, D.J. & FEINSTEIN, A. (1982) Biology of serum amyloid P component. *Ann. N.Y. Acad. Sci.* **389,** 286.

PEPYS, M.B., DYCK, R.F., DE BEER, F.C., SKINNER, M. & COHEN, A.S. (1979) Binding of serum amyloid P-component (SAP) by amyloid fibrils. *Clin. exp. Immunol.* **38,** 284.

PUCHTLER, H., SWEAT, F. & LEVINE, M. (1962) On the binding of Congo red by amyloid. *J. Histochem. Cytochem.* **10,** 355.

ROWE, I.F., JENSSON, O., LEWIS, P. D., CANDY, J., TENNENT, G.A. & PEPYS, M.B. (1984) Immunohistochemical demonstration of amyloid P component in cerebrovascular amyloidosis. *Neuropathol. appl. Neurobiol.* **10,** 53.

SALACINSKI, P.R.P., MCLEAN, C., SYKES, J.E.C., CLEMENT-JONES, V.V. & LOWRY, P.J. (1981) Iodination of proteins, glycoproteins, and peptides using a solid-phase oxidizing agent, 1, 3, 4, 6-tetrachloro-3 6-diphenyl glycoluril (Iodogen). *Analyt. Biochem.* **117,** 136.

SKINNER, M., PEPYS, M.B., COHEN, A.S., HELLER, L.M. & LIAN, J.B. (1980) Studies of amyloid protein AP. In: *Amyloid and Amyloidosis.* (ed. by G. G. Glenner, P. Pinho e Costa & F. Falcao de Freitas) p. 384. Excerpta Medica, Amsterdam.

TAYLOR, J.A., BRUTON, C.J., CLAMP, J.R., DE BEER, F.C., BALTZ, M.L. & PEPYS, M.B. (1982) Studies on the primary structure of mouse serum amyloid P component (SAP). *Ann. N.Y. Acad. Sci.* **389,** 471.

WESTERMARK, P., SHIRAHAMA, T., SKINNER, M., BRUN, A., CAMERON, R. & COHEN, A.S. (1982) Immunohistochemical evidence for the lack of amyloid P component in some intracerebral amyloids. *Lab. Invest.* **46,** 457.