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Antibodies to human serum amyloid P component eliminate visceral amyloid deposits

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Abstract

Accumulation of amyloid fibrils in the viscera and connective tissues causes systemic amyloidosis, which is responsible for about one per thousand deaths in developed countries[1]. Localised amyloid can also be very serious, for example cerebral amyloid angiopathy is an important cause of haemorrhagic stroke. The clinical presentations of amyloidosis are extremely diverse and the diagnosis is rarely made before significant organ damage is present[1]. There is therefore a major unmet medical need for therapy which safely promotes the clearance of established amyloid deposits. Over 20 different amyloid fibril proteins are responsible for different forms of clinically significant amyloidosis and treatments that substantially reduce the abundance

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Author contributions The study was conceived, designed and supervised by M.B.P. K.B., M.C.K. and S.E. performed all the experimental animal work. G.A.T., A.L., J.A.G., S.M. and A.P.D. performed or contributed to the histological studies. Amyloid scoring was performed by K.B., M.C.K., S.E., J.D.G. and M.B.P. W.L.H., P.P.M., J.R.G., D.J.M., G.W.T. and V.B. conducted the immunochemical, radiochemical and immunoassay studies. A.P. undertook the statistical analyses. A.R.B. produced the sheep anti-human SAP and control antisera. M.B. supplied the complement knockout mice. J.D.G. and P.N.H. contributed to experimental design. The paper was written by M.B.P. and reviewed and approved by all co-authors.

Author information Reprints and permissions information is available at www.nature.com/reprints. M.B.P. is the inventor on patents covering SAP as a therapeutic target in amyloidosis and amyloid-associated diseases, and the use of CPHPC for SAP depletion, owned by Pentraxin Therapeutics Ltd, a University College London spinout company in which he and P.N.H. own shares. Pentraxin Therapeutics Ltd owns the patents on CPHPC itself and has licensed the intellectual property relevant to the present work to GlaxoSmithKline.

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of the respective amyloid fibril precursor protein can arrest amyloid accumulation[1]. Unfortunately control of fibril protein production is not possible in some forms of amyloidosis and in others is often slow and hazardous[1]. There is no therapy that directly targets amyloid deposits for enhanced clearance. However, all amyloid deposits contain the normal, non-fibrillar, plasma glycoprotein, serum amyloid P component (SAP)[2, 3]. Here we show that administration of anti-human SAP antibodies to mice with amyloid deposits containing human SAP, triggers a potent, complement dependent, macrophage-derived giant cell reaction which swiftly removes massive visceral amyloid deposits without adverse effects. Anti-SAP antibody treatment is clinically feasible because circulating human SAP can be depleted in patients by the bis-D-proline compound, CPHPC[4], thereby enabling injected anti-SAP antibodies to reach residual SAP in the amyloid deposits. The unprecedented capacity of this novel combined therapy to eliminate amyloid deposits should be applicable to all forms of systemic and local amyloidosis.

Serum amyloid P component (SAP) is selectively concentrated in amyloid deposits by its avid binding to all amyloid fibril types[2,3]. SAP binding stabilises amyloid fibrils, protects them from proteolysis *in vitro*[5] and contributes to pathogenesis of systemic amyloidosis *in vivo*[6]. We therefore developed a novel bis-D-proline compound, (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid, (CPHPC), which is bound with high affinity by human SAP and triggers its rapid clearance by the liver, thereby depleting circulating SAP by more than 90% for as long as the drug is administered[4,7]. However, some SAP remains bound to amyloid even after months of CPHPC treatment[7]. Here we have targeted this residual SAP with IgG antibodies, triggering the body's potent phagocytic clearance mechanisms (Supplementary 1).

Splenic and hepatic AA amyloid deposition, closely resembling human systemic AA amyloidosis, was induced by chronic inflammation in C57BL/6 mice deficient in mouse SAP but transgenic for human SAP[4]. Human SAP is present in their circulation, in normal extracellular matrix[8,9] (Supplementary 2), and in the amyloid deposits (Supplementary 3), just as in humans. Amyloid was quantified in each mouse by whole body retention of ^{125}I-SAP (ref. [10]) and the mice were allocated to three groups closely matched for age, sex and amyloid load. The model closely reflects clinical amyloidosis because human SAP binds much more avidly to amyloid than does mouse SAP[10], and CPHPC depletes circulating human but not mouse SAP *in vivo*[4]. Two groups of mice then received CPHPC at 1 mg/ml in their drinking water for the rest of the experiment. Circulating human SAP was depleted but, as in humans treated with CPHPC, significant amounts of SAP remained in the amyloid deposits (Supplementary 4). Five days after starting on CPHPC, one group received a single intraperitoneal injection of 50 mg of the IgG fraction of monospecific polyclonal sheep anti-human SAP antiserum, containing 7 mg of anti-SAP antibody. A control group received 50 mg of unrelated sheep IgG (Supplementary 4). The third group received no treatment and thus controlled for spontaneous regression of AA amyloid[11]. Twenty eight days after the antibody or control IgG injection, the visceral amyloid load was scored histologically and human SAP was quantified in the individual sera and organs (Supplementary 4).

There was dramatically less amyloid after treatment with CPHPC plus anti-SAP antibody than in the other two groups but there was no difference between CPHPC alone and no treatment (Fig. 1, Supplementary 4). Apart from the amyloid deposits there were no other significant histological abnormalities in any animal. Anti-SAP antibody thus produced remarkable regression of amyloid with no disruption to the normal parenchymal or connective tissue structure of the liver, spleen or other organs. Furthermore there were no clinical or biochemical adverse effects, no mice died during the experiment and body weights remained constant (Supplementary 5).

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Systemic amyloid deposits are characteristically acellular with no surrounding inflammatory reaction (Figs. 2l, 2p, 3a). However by 24 h after injection of anti-SAP antibody all the deposits were densely infiltrated with mononuclear inflammatory cells and some granulocytes (Figs. 2b, 2m, 2q). Most infiltrating cells stained strongly with antibody to F4/80, a global macrophage marker (Fig. 2b). No such staining was present in amyloid deposits in mice not receiving anti-SAP. On day 2 macrophages surrounding the amyloid were fusing to form multinucleate giant cells and stained strongly for CD68, a marker of phagocyte endocytotic activity, which co-localised with staining for the amyloid fibril AA protein and mouse complement component C3 (Figs. 2e-2k, 3b, 3c, Supplementary 6). By day 4 the deposits were less abundant and were fragmented by numerous multinucleate giant cells surrounding and engulfing islands of amyloid (Figs. 2c, 2d, 2e, 2n, 2r). At day 7 residual amyloid was mostly being degraded within the cytoplasm of decreasing numbers of giant cells. Amyloid clearance was largely complete by about day 16 with remarkable restoration of normal tissue architecture and absence of any residual cellular infiltrate (Figs. 2o, 2s).

Human SAP binds avidly to mouse AA deposits *in vivo* and persists there with a half life of 3-4 days, while circulating human SAP is cleared in mice with a half life of 3-4 hours and is undetectable in the plasma after 3 days[4,10]. Amyloid deposits in non-transgenic AA amyloidotic C57BL/6 mice were thus loaded with human SAP by a single intraperitoneal injection of 10 mg of the isolated pure protein and anti-human SAP antibody was injected 3 days later without the need for CPHPC. The same highly reproducible amyloid elimination occurred as in the human SAP transgenic mice and this approach facilitated analysis of the mechanisms responsible.

In contrast to the clearance of amyloid deposits in wild type mice, significantly more amyloid remained after anti-SAP treatment of complement deficient animals lacking either C1q[12] or C3[13] (Supplementary 7), demonstrating that the antibody effect is largely complement dependent. IgG antibody alone could potentially engage phagocytic cells via their Fc(γ) receptors and, although amyloid clearance was much reduced in the absence of complement, the persistent deposits in complement deficient mice were more fragmented than in untreated controls, suggesting some direct antibody effect. There was more complete amyloid elimination in some C1q deficient mice than in C3 deficient animals (Supplementary 7) suggesting that complement activation may occur in the absence of C1q but that C3 is critical. Consistent with this observation, $F(ab)_2$ anti-SAP antibody treatment reduced amyloid load but was significantly less effective than intact IgG antibody (Supplementary 8). $F(ab)_2$ antibodies activate the alternative pathway, independently of C1q, and it is likely that the high dose of $F(ab)_2$ which was used (Supplementary 8) triggered some complement activation. Full efficacy of anti-SAP antibody thus requires the Fc region but cellular recognition by Fc(γ) receptors is not a major factor since $F(ab)_2$ was more effective in complement sufficient mice than IgG antibody in complement deficient animals.

When macrophage activity was ablated using liposomal clodronate[14], anti-SAP antibody produced no reduction of amyloid load (Supplementary 9), demonstrating that macrophages were the essential final effectors of amyloid clearance. Macrophages are largely responsible for the normal, clinically silent, resolution of non-infective tissue injury and for remodelling of non-cellular matrix. The failure to spontaneously clear amyloid deposits, which are composed only of autologous constituents, is therefore remarkable especially as, despite their inherent stability, amyloid fibrils can be digested by proteinases and phagocytic cells *in vitro*[5], especially when opsonised by antibody[15]. *In vivo* macrophage responses to different types of amyloid have been reported occasionally[16-19], and amyloid deposits sometimes regress when fibril precursor protein abundance is sufficiently reduced[20, 21]. However

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amyloid usually accumulates with little or no local cellular or systemic inflammatory response. The serendipitous effect of CPHPC in depleting circulating SAP but leaving some SAP in amyloid deposits enabled the present use of anti-SAP antibodies to trigger unprecedented, clinically silent, elimination of visceral amyloid deposits by macrophages.

The same therapeutic approach should be effective in human amyloidosis, using human or humanised monoclonal antibodies or other antibody constructs. We therefore investigated two of our mouse monoclonal IgG2a anti-SAP antibodies, designated SAP-5 and Abp1, which bound to human SAP with similar affinities, on rates and off rates (Supplementary 10), which activated mouse complement *in vitro* producing C3 cleavage comparable to that produced by the sheep polyclonal anti-human SAP, and which had similar plasma half lives of ~4 days in wild type C57BL/6 mice. IgG2a antibodies were selected because mouse IgG1 activates mouse complement poorly if at all[22]. SAP-5 and Abp1 recognised different epitopes on human SAP (Supplementary 10) but were each as potent as the polyclonal sheep anti-SAP in eliminating amyloid *in vivo* (Supplementary 11 and 12).

Anti-SAP antibody could potentially elicit tissue damaging inflammation in amyloidotic tissues. However the present notable absence of any adverse effects presumably reflects the physiological nature of the macrophage reaction and is encouraging for clinical use of CPHPC and anti-SAP. Nevertheless, appropriate caution will be essential because systemic amyloidosis patients have widespread amyloid deposits in sensitive tissues, including the heart, blood vessel walls and nerves, which are not involved in the mouse AA model. Also, the trace amount of human SAP in normal glomerular basement membrane[8] and elastic fibre microfibrils[9] is a potential undesirable target for anti-SAP antibodies. It is therefore reassuring that there was no change in plasma biochemistry or any histological abnormality in human SAP transgenic mice treated with CPHPC followed by anti-human SAP antibodies (Supplementary 13).

Anti-Aβ antibodies are under intense investigation for treatment of Alzheimer's disease and an *in vivo* imaging study[23] has shown binding to some human systemic AL amyloid deposits by a monoclonal anti-light chain antibody which produces clearance of artefactual local human AL amyloidomas in mice[24],[25]. However therapeutic anti-fibril antibodies will have to be reactive with each different type of amyloid whereas anti-SAP antibody treatment is applicable to all forms of amyloidosis and all human amyloid deposits. Since the SAP which is universal in amyloid is derived from the circulation, anti-SAP antibodies and complement proteins will also be able to reach the deposits, and macrophages are present in, or can access, all tissues. Management of systemic amyloidosis will always require maximum efforts to reduce amyloid fibril precursor protein production, if that is feasible, but the capacity to eliminate existing amyloid deposits would be a major therapeutic advance. A research and development collaboration between UCL and GlaxoSmithKline is now working towards clinical evaluation of this approach, and a candidate monoclonal anti-SAP antibody has been fully humanised for exploration of safety, efficacy and optimal clinical dosing.

METHODS SUMMARY

Induction of murine AA amyloidosis using amyloid enhancing factor and repeated casein injections, estimation of amyloid load *in vivo* and *in vitro*, and quantification of human SAP in serum and tissue extracts, were conducted as previously reported [6],[4],[10]. Sheep and mouse anti-human SAP antibodies were raised by immunisation with isolated pure human SAP[26] and mouse anti-human SAP hybridomas were cloned by standard methods.

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Supplementary Material

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Acknowledgments

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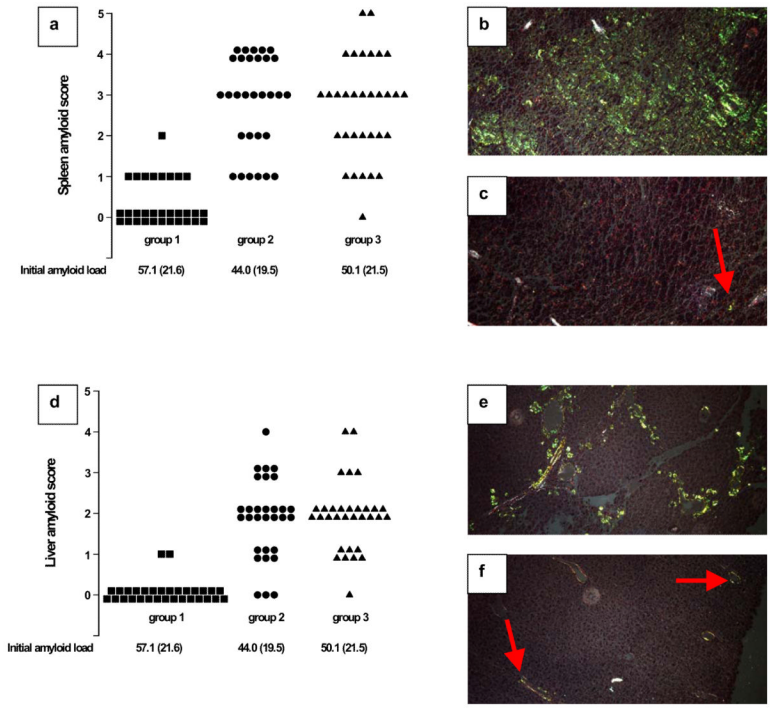

Figure 1. Elimination of visceral amyloid in AA amyloidotic mice after treatment with anti-SAP antibody

Systemic AA amyloidosis was established in C57BL/6 SAP deficient mice transgenically expressing human SAP, which were allocated to 3 groups closely matched for age, sex and whole body amyloid load, the latter shown as mean (SD) per cent whole body retention of ^{125}I-human SAP tracer (one way ANOVA comparing the 3 group means, P=0.054). The mice were treated with CPHPC and a single dose of sheep anti-human SAP antibody (group 1, n=31), with CPHPC and the same dose of an irrelevant sheep anti-human antibody (group 2, n=30), or left untreated (group 3, n=32). Amyloid load was determined histologically 28 days later. **a**, Each point is an individual spleen amyloid score. 0, no amyloid detected; 1, one or more trace specks; 2, marginal zone traces; 3, general marginal zone deposits; 4, heavy marginal zone deposits; 5, heavy marginal zone and extensive interfollicular deposits. Kruskal-Wallis test comparing the 3 groups, P<0.0001. Dunn's multiple comparison test: 1 vs 2 P<0.001; 1 vs 3 P<0.001; 2 vs 3 not significant P>0.05. **b**, Congo red stained spleen section showing the pathognomonic amyloid green dichroism, score = 5. **c**, As in **b** but with amyloid score = 1; single amyloid speck is arrowed. **d**, Individual liver amyloid scores. 0, no amyloid detected; 1, trace specks; 2, traces in/around most portal tracts; 3, significant deposits in/around all portal tracts; 4, extensive portal and parenchymal deposits. Kruskal-Wallis test, P<0.0001. Dunn's multiple comparison test: 1 vs 2 P<0.001; 1 vs 3 P<0.001; 2 vs 3 not significant P>0.05. There were no significant differences in the average spleen or liver amyloid scores between males and females within any of the groups (not shown). **e**, Congo red stain of liver amyloid, score = 4. **f**, Liver amyloid score = 1; arrows indicate amyloid specks.

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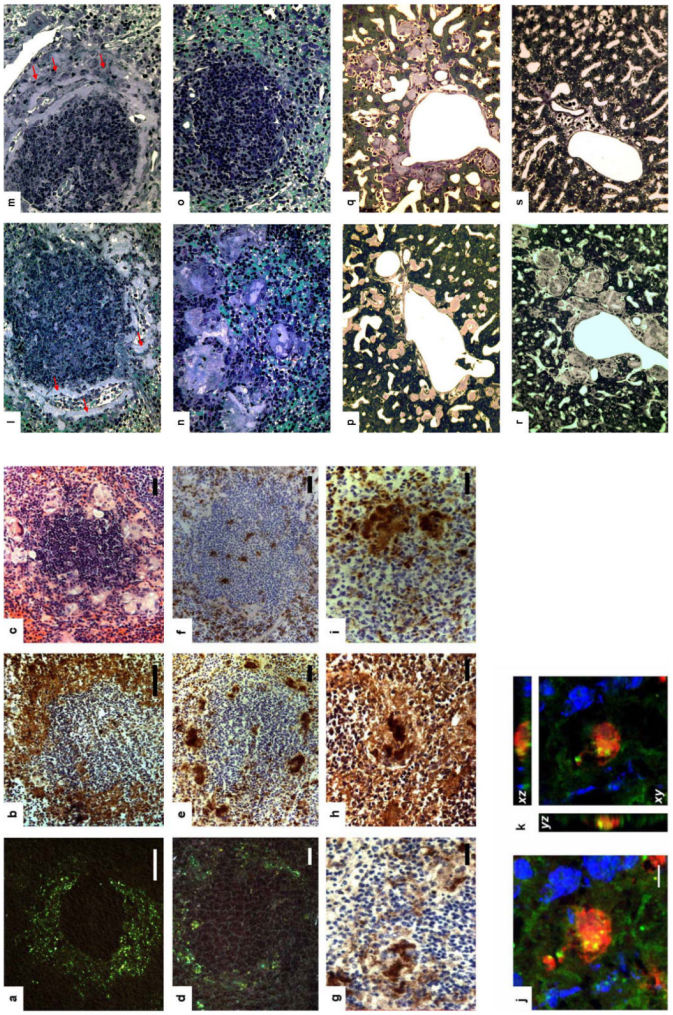

Figure 2. Cellular infiltration and amyloid destruction after administration of anti-SAP antibody

a, Congo red stained spleen section one day after anti-SAP antibody treatment showing typical heavy marginal zone amyloid. **b**, Anti-F4/80 stained adjacent section showing intense infiltration of the amyloid deposits with phagocytic cells. **c**, Hematoxylin and eosin stained spleen section 4 days after anti-SAP antibody treatment, showing multiple multinucleate giant cells surrounding and engulfing amyloid in the marginal zone. **d**, Congo red stained adjacent section showing marked fragmentation and reduction of marginal zone amyloid. **e**, Anti-CD68 stained adjacent section, showing massive infiltration of the marginal zone amyloid by phagocytically active macrophages and giant cells. Scale bars in a–f are 100 μm. **f**, Anti-CD68 stained spleen section from a control, untreated, amyloidotic mouse, showing no positive cells in the pale amorphous marginal zone amyloid deposits. **g,** Splenic marginal zone amyloid deposit from mouse 4 days after treatment with anti-SAP antibody, stained with antibodies to mouse AA, the amyloid fibril protein. **h,** Adjacent section stained with anti-mouse complement component C3. **i,** Adjacent section stained with anti-mouse CD68. Scale bars in g-i are 20 μm. **j,** Extended focus confocal view (z-projection) of the same spleen immunostained for CD68 (red), SAA (green) and counterstained with Hoechst 33342 (blue), confirming the close co-localisation of amyloid and active phagolysosome fusion in macrophages and giant cells as they ingest and destroy the amyloid deposits that have been opsonised by anti-SAP antibody and complement. **k,** Orthogonal views of same stain as **j**, showing ingested amyloid within a macrophage. Scale

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bar 5 μm. **l**-**o**, Spleen, and **p**-**s**, liver, thin sections from mice in this experiment stained with toluidine blue. Control mice, not treated with anti-SAP antibody, show abundant amorphous amyloid deposits, pale blue in spleen (red arrows, **l**) and pink in liver (**p**) with the characteristic absence of any surrounding inflammatory reaction or cellular infiltrate. **m** and **q**, One day after anti-SAP antibody treatment showing intense, predominantly mononuclear, cell infiltration (**m**, red arrows) in and around the amyloid. **n** and **r**, Five days after anti-SAP antibody treatment showing fusion of macrophages to form multinucleate giant cells surrounding and infiltrating the deposits and containing large masses of ingested amyloid undergoing degradation. **o** and **s**, Sixteen days after anti-SAP antibody administration showing complete elimination of amyloid deposits with no residual cellular infiltrate and restoration of normal tissue architecture.

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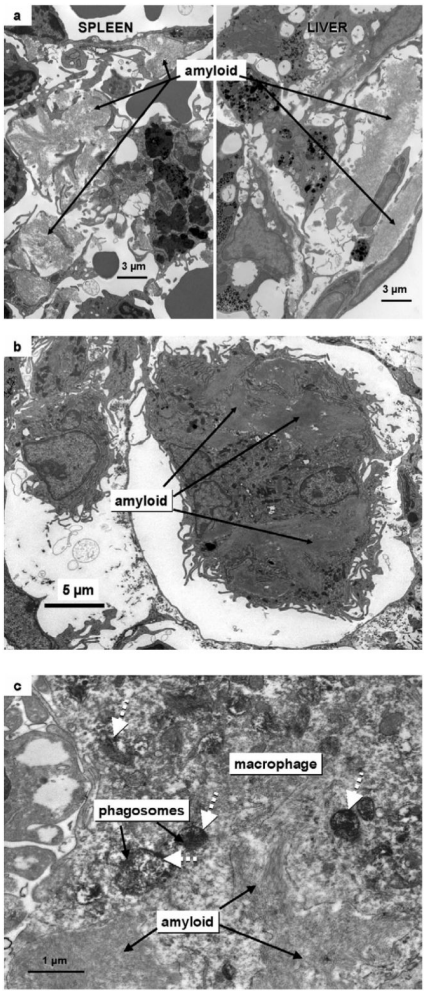

Figure 3. Electron micrographs of amyloid destruction after anti-SAP antibody treatment

a, Spleen and liver from a control AA amyloidotic mouse loaded with human SAP, which did not receive anti-SAP antibody, showing extracellular masses of fibrillar amyloid with the characteristic absence of any inflammatory cells or cellular reaction. **b**, Liver from an AA amyloidotic mouse loaded with human SAP, examined 5 days after administration of anti-SAP antibody, showing a multinucleate giant cell surrounding, internalising and digesting large masses of amyloid. **c**, Anti-CD68 immunostain of spleen removed one day after administration of anti-SAP antibody to an AA amyloidotic mouse loaded with human SAP, showing a macrophage surrounding, infiltrating and ingesting amyloid. The dark peroxidase reaction product (white arrows) identifies phagolysosome fusion around internalised fragments of amyloid.