

# Serum Amyloid P Component and Systemic Fungal Infection: Does It Protect the Host or Is It a Trojan Horse?

Stephen A. Klotz,<sup>1</sup> Richard E. Sobonya,<sup>2</sup> Peter N. Lipke,<sup>3</sup> and Melissa C. Garcia-Sherman<sup>3</sup>

<sup>1</sup>Division of Infectious Diseases, and <sup>2</sup>Department of Pathology, University of Arizona, Tucson; and <sup>3</sup>Department of Biology, City University of New York at Brooklyn

It is a striking observation that tissue of patients invaded by the deep mycoses often lacks evidence of an inflammatory response. This lack of host response is often attributed to neutropenia secondary to chemotherapy. However, systematic studies do not support this simplistic explanation. However, invasive fungal lesions are characterized by abundant fungal functional amyloid, which in turn is bound by serum amyloid P component (SAP). We postulate that SAP is important in the local immune response in invasive fungal infections. The interaction between fungal functional amyloid, SAP, and the immune response in deep mycoses is discussed.

**Keywords.** amyloid; candidiasis; functional amyloid; fungi; serum amyloid P component.

We recently discovered an interaction between serum amyloid P component (SAP) and systemic fungal infections. Before outlining the importance of this interaction, we briefly discuss and define some of the important terminology and relationships between amyloid and fungal infections.

## AMYLOIDS

Amyloids are aggregates of misfolded proteins forming characteristic fibrils (and protofibrils) that disrupt tissues and cells and lead to clinical disease. Amyloid fibrils are formed from crossed  $\beta$ -sheets of many copies of misfolded protein. They have a strong affinity for one another as well as for amyloidophilic dyes such as Congo red and thioflavin T. There are many amyloidoses, which are systemic diseases characterized by deposits of amyloid fibrils; each disease with its unique misfolded protein precursor [1]. The most

common is AL amyloidosis where overproduction of the lamda light chain of immunoglobulins forms deposits in many organs, particularly the kidneys, leading to renal failure. Predominately intracellular amyloid is seen in the prion diseases where amyloid fibrils form from the prion protein or Alzheimer's where the A $\beta$  peptide is found intracellularly. These diseases feature amyloid, but they are not amyloidoses because the latter comprises extracellular amyloid deposits [1].

## FUNCTIONAL AMYLOIDS

Functional amyloids are proteins forming amyloid fibrils that serve a physiologic purpose. They are even found in man, for example, Pmel 17 protein found in melanosomes [2]. Functional amyloids are ubiquitous in bacteria and fungi where amyloid fibrils often serve an attachment purpose [3]. One such amyloid is found within a *Candida albicans* glycoprotein adhesin found on the fungal cell surface [4]. After attachment to a peptide, stretching the molecule causes it to spontaneously form amyloid near the N-terminal immunoglobulin-like domain [5]. This process will be discussed further later in the article.

## SERUM AMYLOID P COMPONENT

Serum amyloid P component is a glycoprotein found in serum and is a component of the innate human immune

response. No human has been described as lacking SAP, and therefore it is undoubtedly a critical serum protein. Serum amyloid P component and C-reactive protein (CRP) are both proteins that respond to inflammation. C-reactive protein increases in concentration many hundredfold in response to inflammation, whereas SAP in humans maintains a constant concentration in plasma. Therefore, CRP is defined as an acute-phase reactant, whereas SAP is not. Serum amyloid P component is a pentavalent glycoprotein that circulates in the plasma at a concentration of 30–40  $\mu\text{g}/\text{mL}$  [6]. Serum amyloid P component is an evolutionarily conserved component of the innate immune response that binds to amyloid, chromatin, nucleoli [7], and apoptotic and necrotic cells as well as various microbes in a  $\text{Ca}^{2+}$ -dependent manner. It is a member of the pentraxin superfamily of proteins that share calcium-dependent ligand binding and a similar pentavalent structure. Each protomer of SAP is a 25 kDa domain with a  $\beta$ -jelly roll fold, and the pentamer has a total  $M_r$  of 127 310 [8]. Serum amyloid P component and CRP are short pentraxins and share 51% homology. Other pentraxins structurally similar to SAP and CRP have quite different sources and roles within the host. For example, pentraxin 3 is released by macrophages, endothelial cells, fibroblasts,

Received 9 June 2016; accepted 28 July 2016.

Correspondence: S. A. Klotz, Division of Infectious Diseases, 1501 N. Campbell, University of Arizona, Tucson, AZ 85724 (sklotz@u.arizona.edu).

Open Forum Infectious Diseases®

© The Author 2016. Published by Oxford University Press on behalf of the Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com. DOI: 10.1093/ofid/ofw166

and dendritic cells in response to presence of the cytokines tumor necrosis factor- $\alpha$  and interleukin (IL)-1 $\beta$  [9].

Amyloid deposits, whether extracellular or intracellular, also contain SAP. For example, SAP may constitute up to 15% of the mass of extracellular amyloid deposits such as occur in the various amyloidoses [1]. Serum amyloid P component is also found intracellularly on the amyloid particles of Alzheimer's disease [10]. Consequently, SAP is an integral part of diseases caused by amyloid by binding to and stabilizing intra- and extracellular amyloid deposits. One effective treatment of amyloidoses is to deplete SAP in plasma, which leads to dissociation of SAP from amyloid deposits, lessening the burden of disease. Even after this procedure, some SAP remains on the amyloid deposits, and this can be scavenged by use of anti-SAP antibodies that target the remnant fraction of bound SAP, bind complement, and promote removal of the glycoprotein by macrophages [11].

## DISCUSSION

We became interested in SAP after the serendipitous discovery of SAP binding to the cell surface of *Candida* invading the human gastrointestinal tract [12]. In previous studies, we had demonstrated the spontaneous formation of amyloid within glycoprotein adhesins on the cell surface of *C. albicans* in vitro. We characterized the critical role fungal amyloid played in the aggregation of one fungus to another as well as attaching fungi to broad range of human proteins [5]. To study this process in human disease, we stained for the presence of fungal amyloid in autopsy tissue of patients dying with invasive gastrointestinal candidiasis. Many of these patients, but not all, were neutropenic. Congo red and thioflavin T staining demonstrated an abundance of amyloid within the fungal structures. We also showed that a peptide probe for a specific amyloid sequence of *C. albicans* adhesins labeled the fungus both in autopsy sections and in vitro [13]. As part

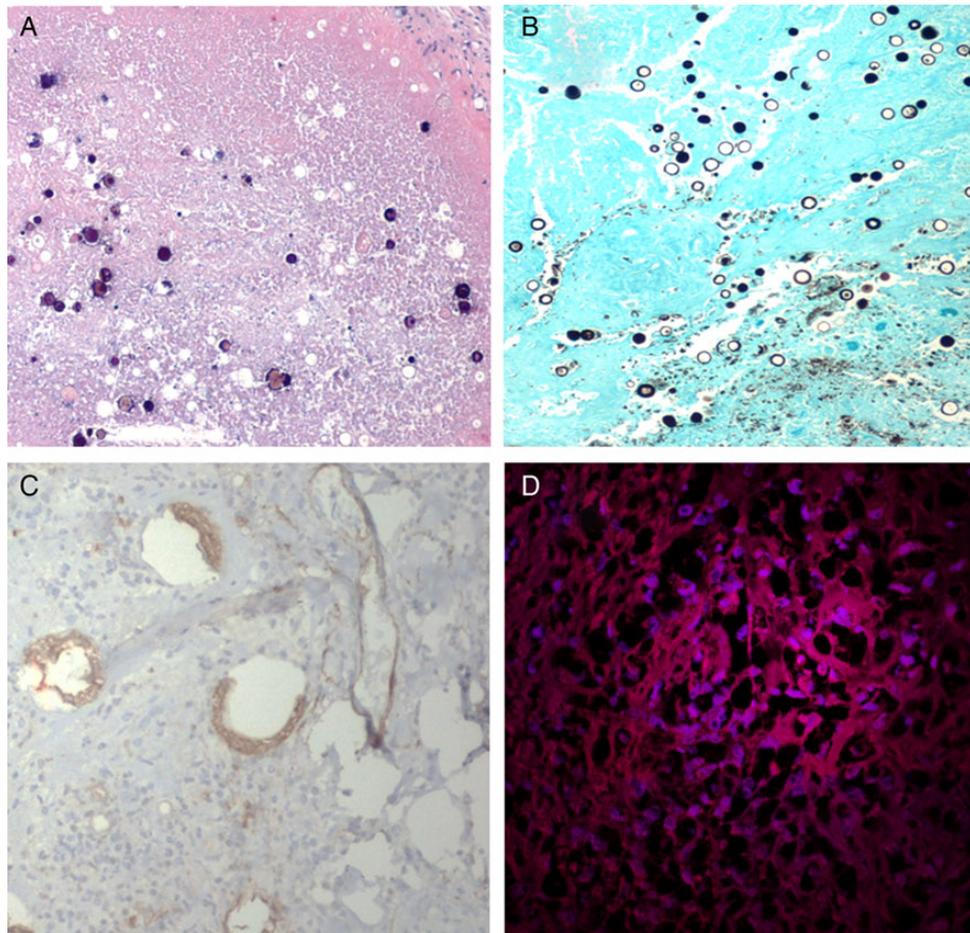
of the routine laboratory workup for amyloid in tissue, antibody to serum amyloid A and SAP were applied. Although there was no evidence of deposition of serum amyloid A, SAP was present in abundance on the surface of the fungi from 23 autopsies with invasive gastrointestinal candidiasis. We were caught off guard because we were ignorant of SAP and its role in the human body. It suddenly became a top research interest for us. Returning to in vitro experimentation, we found that the interaction of *C. albicans* and SAP was specific and occurred after the spontaneous formation of amyloid on the fungal cell surface. This was demonstrated by expressing *C. albicans* glycoprotein adhesins in *Saccharomyces cerevisiae* strains that did not bind SAP. These experiments involved incubating fungi in human serum as well as reconstituted SAP [12]. Serum amyloid P component bound to *S. cerevisiae* when the yeast was expressing an amyloid-forming version of the *C. albicans* adhesin, Als5p (an attachment protein on the cell wall of the fungus that binds to peptides), but did not bind to yeast without the adhesin or yeast expressing a nonamyloid version of Als5p. Serum amyloid P component also bound abundantly to wild-type *C. albicans* [12].

We also observed that there were few neutrophils in the infected gastrointestinal tissue, and their absence did not correspond with the circulating white blood cell count [12]. Because amyloid deposits characteristically lack an inflammatory response even when the abnormal protein is present in massive quantities, great enough to compromise organ function [1], we wondered whether the lack of tissue neutrophils might be due to the presence of fungal amyloid and the SAP that coated the cell wall. Although the role of SAP in infectious disease processes is not understood, Pepys and coworkers in 2000 [14] questioned whether SAP protected the host or masked the bacterial pathogen from the immune response. In their experiments, transgenic mice expressing human SAP and control mice

not expressing human SAP were challenged intravenously with *Streptococcus pyogenes* and *Escherichia coli*. They observed that SAP bound to both bacteria, but survival was worse in mice expressing human SAP. This finding suggested the title of their paper: "Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen" [14]. Thus, although SAP binds to bacteria, viruses, and fungi, the consequences of such binding to fungal pathogens is currently unknown.

A recent review discussed the immunomodulatory properties of SAP, including reduction of neutrophil adhesion and increases in macrophage secretion of the anti-inflammatory cytokine IL-10 by macrophages bound by SAP [15]. Similar modulations of the host response may occur at the local level when SAP binds to the invading fungi, perhaps leading to the poor inflammatory response to invading fungi.

More recent work extends our findings with fungal amyloid to include other pathogenic fungi that bind SAP [16]. We studied autopsy tissue from individuals with invasive aspergillosis, mucormycosis, and coccidioidomycosis. A frequent finding in cases of invasive aspergillosis and mucormycosis is the absence of an inflammatory response to the invading fungi, similar to what was observed in invasive candidiasis. Local tissue inflammation did not correspond to peripheral white blood cell counts, and the lack of inflammation did not always correlate with peripheral neutropenia. Because invasive candidiasis, aspergillosis, and mucormycosis occur in neutropenic patients, it is generally assumed that the neutropenia in tissue is due to the chemotherapy-mediated repression of bone marrow. However, systematic pathologic investigation of tissue from patients dying of these diseases demonstrates that patients with neutropenia may have some element of inflammation (however slight), and patients with normal or elevated white blood cell counts may have no inflammation in the area of tissue invasion [17, 18]. In



**Figure 1.** (A) Hematoxylin and eosin stain of lung tissue showing a large caseating granuloma with multiple spherules of *Coccidioides* species. In the caseous debris, there are no host cellular elements. Spherules appear as circles without pigment; there are many calcium deposits as well, which are bluish-red (magnification,  $\times 200$ ). (B) Grocott's methenamine silver stain showing empty spherules with refractile double walls lacking central pigmentation (magnification,  $\times 200$ ). (C) Lung tissue stained for serum amyloid P component (SAP). Brown stain indicates presence of SAP, especially on endospores lining the inside of spherules (magnification,  $\times 200$ ). (D) Combined Congo red and thioflavin T stain of lung tissue showing endospores within lung (fluorescent magenta particles amongst the red lung tissue).

addition, allogeneic hematopoietic stem cell transplant recipients after recovery from neutropenia had an acellular response to invading *Aspergillus* that appeared similar to that seen in neutropenic patients [19]. Looking at multiple tissues including lung, heart, brain, kidney, and spleen from autopsy, we found that once again there was abundant fungal amyloid stained with thioflavin T and Congo red present in lesions caused by *Aspergillus*, *Mucorales*, and *Coccidioides*. Furthermore, SAP coated the cell surface of the fungal structures. Figure 1 shows the typical findings of coccidioidal lesions of the lungs. On hematoxylin and eosin stain, many *Coccidioides* spherules are present and there are no

acute inflammatory cells (Figure 1A). The spherules are identified more readily in the silver stain (Figure 1B), and Figure 1C demonstrates the staining for SAP. It is present on the spherule walls and especially prominent where endospores are seen. Figure 1D shows the results of staining infected lung tissue with Congo red and thioflavin T demonstrating fluorescent amyloid fibrils within the structures. Rarely were neutrophils seen in the adjacent tissue, and they may have been physically excluded from infiltrating the fungi where hyphae were in a “starburst” configuration in aspergillosis and in columns of closely apposed bundles of hyphae in mucormycosis.

## CONCLUSIONS

Our work suggests that cell surface amyloid is extremely common amongst pathogenic fungi and likely contributes significantly to their morphology, histology, and even persistence in tissue [16]. This persistence is due in part to the protease-resistant nature of amyloid and colocalized SAP [20]. The close apposition of fungal cells in fungal abscesses, especially *Aspergillus* and *Mucorales*, leaves little space for host cells to intercalate among the hyphae. Perhaps even more important is that human SAP binds to the fungal amyloid and would therefore provide a local milieu where natural

host immunity may be dampened, ie, host response would be damped because neutrophils would not adhere to SAP-coated fungi. Furthermore, macrophages respond to SAP by upregulating anti-inflammatory IL-10 [15]. Serum amyloid P component is protease-resistant [20] and therefore persists in tissue masking fungi from the host response. Thus, it appears SAP functions like a Trojan horse in its interaction with pathogenic fungi allowing fungi to escape the normal host inflammatory response. It appears to coat the fungal cell surface, which may protect the fungi from cellular attack. It has been proposed that competing SAP off fungi could be a potential therapeutic target [21]. In conclusion, it is clear that SAP binds to fungi in human disease, and the significance of this interaction will be determined by further experimental work.

#### Acknowledgments

**Financial support.** Funding for this manuscript was intramural as well as from RO1 GM 098616 awarded to P. N. L.

**Potential conflicts of interest.** All authors: No potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

#### References

1. Pepys MB. Amyloidosis. *Annu Rev Med* **2006**; 57:223–41.
2. Fowler DM, Koulov AV, Alory-Jost C, et al. Functional amyloid formation within mammalian tissue. *PLoS Biol* **2006**; 4:e6.
3. Otzen D. Functional amyloid: turning swords into plowshares. *Prion* **2010**; 4:256–64.
4. Ramsook CB, Tan C, Garcia MC, et al. Yeast cell adhesion molecules have functional amyloid-forming sequences. *Eukaryot Cell* **2010**; 9:393–404.
5. Lipke PN, Ramsook CB, Garcia-Sherman MC, et al. Between amyloids and aggregation lies a connection with strength and adhesion. *New J Sci* **2014**; 2014:815102.
6. Pepys MB, Dash AC, Markham R, et al. Comparative clinical study of protein SAP (amyloid P component) and C-reactive protein in serum. *Clin Exp Immunol* **1978**; 32:119–24.
7. Pepys MB, Booth S, Tennent GA, et al. Binding of pentraxins to different nuclear structures: C-reactive protein binds to small nuclear ribonucleoprotein particles, serum amyloid P component binds to chromatin and nucleoli. *Clin Exp Immunol* **1994**; 97:152–7.
8. Hutchinson WL, Hohenester E, Pepys MB. Human serum amyloid P component is a single uncomplexed pentamer in whole serum. *Mol Med* **2000**; 6:482–93.
9. Vilahur G, Badimon L. Biological actions of pentraxins. *Vascul Pharmacol* **2015**; 73:38–44.
10. Al-Shawi R, Tennent GA, Millar DJ, et al. Pharmacological removal of serum amyloid P component from intracerebral plaques and cerebrovascular A $\beta$  amyloid deposits in vivo. *Open Biol* **2016**; 6:150202.
11. Richards DB, Cookson LM, Berges AC, et al. Therapeutic clearance of amyloid by antibodies to serum amyloid P component. *N Engl J Med* **2015** 17; 373:1106–14.
12. Gilchrist KB, Garcia MC, Sobonya RE, et al. New features of invasive candidiasis in humans: amyloid formation by the fungi and deposition of serum amyloid P component by the host. *J Infect Dis* **2012**; 206:1473–8.
13. Garcia-Sherman MC, Lysak N, Filonenko A, et al. Peptide detection of fungal functional amyloids in infected tissue. *PLoS One* **2014**; 9:e86067.
14. Noursadeghi M, Bickerstaff MC, Gallimore JR, et al. Role of serum amyloid P component in bacterial infection: protection of the host or protection of the pathogen. *Proc Natl Acad Sci U S A* **2000**; 97:14584–89.
15. Cox N, Pilling D, Gomer R. Serum amyloid P: a systemic regulator of the innate immune response. *J Leukoc Biol* **2014**; 96:739–43.
16. Garcia-Sherman MC, Lundberg T, Sobonya RE, et al. A unique biofilm in human deep mycoses: fungal amyloid is bound by host serum amyloid P component. *NPJ Biofilms Microbiomes* **2015**; 1: pii: 15009.
17. Stergiopoulou T, Meletiadis J, Roilides E, et al. Host-dependent patterns of tissue injury in invasive pulmonary aspergillosis. *Am J Clin Pathol* **2007**; 127:349–55.
18. Ben-Ami R, Luna M, Lewis R, et al. A clinicopathological study of pulmonary mucormycosis in cancer patients: extensive angioinvasion but limited inflammatory response. *J Infect* **2009**; 59:134–8.
19. Shaikat A, Bakri F, Young P, et al. Invasive filamentous fungal infection in allogeneic hematopoietic stem cell transplant recipients after recovery from neutropenia: clinical, radiologic, and pathologic characteristics. *Mycopathologica* **2005**; 159:181–8.
20. Tennent GA, Lovat L, Pepys MB. Serum amyloid P component prevents proteolysis of amyloid fibers. *Proc Natl Acad Sci U S A* **1995**; 92:4299–303.
21. Pepys MB. Invasive candidiasis: new insights pre-saging new therapeutic approaches? *J Infect Dis* **2012**; 206:1339–41.