Invasive Candidiasis: New Insights Presaging New Therapeutic Approaches?

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(See the major article by Gilchrist et al, on pages 1473–8.)

The expanding universe of amyloid has revealed an intriguing new galaxy, invasive candidiasis, with the publication by Gilchrist et al in this issue of the Journal. Invasive infection of the gut by Candida albicans is a serious, intractable condition that occurs most commonly but not exclusively in neutropenic patients and for which there is very poorly effective treatment. Gilchrist et al now report that amyloid fibrils are produced on the surface of the invading fungal cells in vivo, just as they are in vitro, and that human serum amyloid P component binds to the fibrils both in vitro and in vivo. What does this mean?

Amyloid, used in the strict sense, which is essential for correct diagnosis and management of sick patients, is a pathological extracellular deposit composed predominantly of characteristic protein fibrils [1]. About 30 different, unrelated human proteins are now known to form amyloid fibrils in different clinical conditions. Despite widely different structures among their precursor molecules, all amyloid fibrils share the same cross-β core structure and all bind alkaline alcoholic Congo red to produce unique red-green birefringence when observed in strong cross-polarized light. This pathognomonic tincctorial property is the gold standard for histopathological diagnosis of amyloid. The fibrillar cross-β aggregate is a very stable protein assembly, and almost any polypeptide can form amyloid-like fibrils in vitro if subjected to suitable denaturing conditions that enable refolding. In vivo most types of amyloid fibrils are formed by misfolding of native globular proteins, often associated with limited proteolytic cleavage, and some are derived from natively unfolded precursors, but it is not known why only approximately 30 among all human proteins form amyloid in vivo.

Regardless of their different protein compositions, all amyloid fibrils are rigid, non–branching, of indeterminate length, about 10 nm in diameter, and composed of 2–6 twisted protofibrils. In addition to the fibrils, amyloid deposits in vivo also always contain abundant heparan and dermatan sulfate proteoglycans and free glycosaminoglycan chains, some of which are tightly associated with the fibrils. Amyloid deposits also always contain serum amyloid P component (SAP), a normal plasma protein of the pentraxin family that shows avid calcium dependent binding to amyloid fibrils of all types [2]. Indeed, such binding by SAP is an essential feature, along with birefringence after Congo red staining, for identification of protein fibrils as amyloid.

Amyloid deposits disrupt the structure and function of tissues and organs, leading to disease, known as amyloidosis. In systemic amyloidosis, the deposits can occur very widely in connective tissue, including blood vessel walls, and in the parenchymal tissue of any organ except in the brain. Systemic amyloidosis is a very serious condition and is usually fatal, causing about 1 of 1000 deaths in developed countries. There are several different acquired forms of systemic amyloidosis that complicate various primary diseases, by far the most common being AL type caused by monoclonal immunoglobulin light chains in the various plasma cell dyscrasias. There are also many very rare hereditary forms caused by mutations that destabilize the native fold of the respective amyloidogenic precursor protein. In local amyloidosis, the deposits are confined to a single anatomical site, tissue, or organ, for example, focal AL type amyloidomas can occur anywhere; another example is cerebral amyloid angiopathy, a very common and important cause of cerebral hemorrhage. In addition to systemic and local amyloidosis, in which the amyloid deposits are definitely the cause of disease, microscopic amyloid deposits are also always present in the brain in Alzheimer’s disease and in...
the pancreatic islets in type-2 diabetes, but in neither case is it known whether these deposits have direct pathogenic significance. These 2 very important diseases should not therefore be considered as forms of amyloidosis. Similarly, intracerebral amyloid deposits are present only in some types of transmissible spongiform encephalopathy, and experimental models show clearly that amyloid deposition is not required for full expression of prion disease. Finally, the pathogenesis of many other diseases involves misfolding of autologous proteins and deposition of abnormal aggregates, often with cross–β structure. Biophysicists and other non-clinical scientists have persistently conflated these deposits with amyloid and the diseases with amyloidosis. From the perspective of correct diagnosis and appropriate therapy, this confusion is dangerously misleading: Parkinson’s disease, with α-synuclein aggregates in the cytoplasm, and Huntington’s disease, with intranuclear huntingtin aggregates, are definitely not forms of amyloidosis.

The discovery that various microorganisms normally produce cross–β, amyloid–like fibrils opened a whole new vista. The functional participation of such fibrils in biofilm formation is clearly relevant to infectivity and potentially also to pathogenesis and virulence. The new findings of Gilchrist et al take this further. Their demonstration that both yeast and filamentous forms of invasive C. albicans produce surface amyloid fibrils in vivo and that these fibrils are always coated with SAP starts to unravel molecular mechanisms of pathogenesis and suggest a novel therapeutic approach. A key observation is that, even in patients with normal or elevated neutrophil counts, these pivotal host defense cells ignore the invading fungus. Long-standing knowledge of amyloidosis and previous work on SAP indicate why this might be.

Despite being abnormal deposits of autologous proteins and glycans, amyloid deposits are almost entirely ignored by neutrophils and macrophages. This is a notable contrast with the normal function of the phagocytes in silently and effectively clearing tissue debris, for example after injury and hemorrhage. The lack of phagocytic cell reaction to amyloid is not well understood, but it is clearly not consistent with claims that SAP is an opsonin that interacts with cell surface Fc receptors [3], because all amyloid deposits in humans in vivo are richly coated with SAP but are ignored by phagocytes. Indeed, there is compelling experimental evidence that SAP bound to the surface of bacteria is in fact a potent anti–opsonin both in vitro and in vivo [4]. Bacteria that are phagocytosed and killed by neutrophils in the absence of SAP are protected from phagocytosis and killing when coated with SAP, and crucially, in vivo depletion of SAP in mice abrogates this protection [4]. In contrast, for bacteria to which SAP does not bind, SAP contributes, by hitherto unknown mechanisms, to host defense [4]. Together with the binding of DNA and chromatin by SAP [5, 6], this is probably the normal role of human SAP.

Amyloid fibrils are stabilized in vitro by binding of human SAP and protected from cleavage by proteases or phagocytic cells [7], and SAP knockout mice show delayed and reduced experimental amyloid deposition [8], identifying SAP as a therapeutic target in amyloidosis. The novel bis(D-proline) drug, (R)-1-[(R)-2-carboxy-pyrrrolidin-1-yl]-6-oxo-hexanoyl] pyrrolidine-2-carboxylic acid (CPHPC) was therefore developed as a specific inhibitor of SAP binding to amyloid fibrils [9]. CPHPC was then serendipitously found to produce sustained almost complete depletion of circulating SAP for as long as the drug is administered [9]. Neither CPHPC nor persistent SAP depletion have had any adverse effects in adults [10], and the drug is now in development by GlaxoSmithKline, in combination with humanized monoclonal antibodies against SAP, for treatment of systemic amyloidosis [11].

The Gilchrist findings are entirely consistent with the apparent amyloid protective role of SAP and with the compelling evidence from experimental bacterial infection models that SAP shields pathogens from phagocytosis and enhances their pathogenicity. Potential availability of CPHPC, a safe drug that very effectively depletes human SAP in vivo, thus offers the attractive possibility of a completely novel approach to treatment. Preventing SAP from coating the surface of invading Candida should expose the pathogen to recognition and destruction by neutrophils and macrophages and thereby, perhaps, enable normal host defense mechanisms to be effective without the need for toxic antifungal drugs with limited efficacy. The same approach may also be applicable to other pathogens that have harnessed the amyloid structural signature to deploy this human protein against the host.

Notes

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