Pharmacoproteomics of Aspergillus fumigatus for identification of novel molecular targets having application immuno diagnosis and therapy

Rambir Singh

Abstract

Aspergillosis has emerged as threat to public health in recent past. Early stage diagnosis of aspergillosis has been difficult. There are limited options of effective drugs for treatment and invasive infections are always fatal. Importantly, clinical symptoms of aspergillosis overlap with those of TB. This often leads to misdiagnosis of aspergillosis as TB and wrong treatment. Hence, early diagnosis of aspergillosis is essentially needed. Currently, available late stage antigen based serological tests have limited diagnostic efficacy. Hence, there is urgent need for identification of novel molecules of diagnostic and therapeutic relevance. While working with Indian (ITCC-6604) and German (DAYA) strains of A. fumigatus, we were able to identify 111 cytosolic proteins spots from 2D gels. Out of these 111 protein spots, 66 proteins have been identified on comparison with available protein databases. Immuno-proteomics studies on these cytosolic proteins showed the presence of highly immunodominant IgG and IgE reactive proteins. Characterizations of these proteins have immense application in immuno-diagnosis and therapy of aspergillosis.

In recent decades, invasive aspergillosis (IA) has emerged as an important cause of morbidity and mortality in patients with prolonged neutropenia. However, several reports have recently described a rising incidence of IA in critically ill patients, even in the absence of an apparent predisposing immunodeficiency [1–6]. The incidence of IA in critically ill patients ranges from 0.3% to 5.8% [2, 3, 6], and carries an overall mortality rate > 80%, with an attributable mortality of approximately 20% [4, 5]. Critically ill patients are prone to develop immunologic derangement, which renders them more vulnerable for Aspergillus infections. The risk factors for IA include chronic obstructive pulmonary disease (COPD) and other chronic lung diseases , prolonged use of steroids , advanced liver disease chronic renal replacement therapy near-drowning, and diabetes mellitus.

The diagnosis of such IA is difficult because signs and symptoms are non-specific. The conventional diagnostic methods, such as tissue examination and microbial cultivation, may lack sensitivity in the first stages of infection in critically ill patients. As a result, the diagnosis of IA is often established after a long delay or following autopsy. Currently, the best-characterized circulating marker used in the diagnosis of IA is galactomannan (GM), which is present in the cell walls of most Aspergillus species. The commercial Platelia Aspergillus assay (BioRad™, Marnes-La-Coquette, France) has been included in the EORTC/MSG criteria for probable IA. However, a recent meta-analysis indicated that GM testing is more useful in patients with prolonged neutropenia (sensitivity, 72%-82%) than in non-neutropenic, critically ill patients (sensitivity, 40%-55%). Further studies suggested that the host immune status may influence GM release. It appears that GM production is proportional to the fungal load in tissues. Although neutropenic patients and non-neutropenic, critically ill patients are susceptible to IA, the pathology of the disease is quite different in these two groups of patients. In neutropenic patients and animal models, IA is characterized by thrombosis and hemorrhage from rapid and extensive hyphal growth. However, in non-neutropenic, critically ill patients and animal models, IA is characterized by limited angioinvasion, tissue necrosis, and excessive inflammation]. The limited angioinvasion and low fungal load result in a low level of GM released by the fungus. The use of the GM assay for the diagnosis of IA in non-neutropenic patients is very limited. Therefore, more prompt and accurate disease markers for early diagnosis are needed, which requires a thorough knowledge of fungal antigens detected in the serum or other body fluids of infected patients.

A. fumigatus is the most common opportunistic pathogen that causes life-threatening IA in human beings. The ability of A. fumigatus to acquire and process growth substrates from its host is dependent on factors released from the fungi. The extracellular proteins of A. fumigatus, which are released during the germination of conidia and growth of hyphae, consist of secreted enzymes, toxins, and other secondary metabolites which are pathogenic and responsible for invasion of the structural barrier of the host. Studies on the extracellular proteins of A. fumigatus and their immunogenic potential are therefore important for further understanding the pathogenesis of A. fumigatus and targets for the immunodiagnosis of the diseases. It is not surprising that some of the proteins may be major elicitors of specific immune responses, which could be brought into play to establish prognosis and develop new diagnostic procedures for IA.

We have recently observed that high levels of antibody against extracellular proteins of A. fumigatus are often present in the sera of critically ill patients with proven IA. This finding prompted us to discover the potential novel biomarkers for the diagnosis of IA in such patients. The investigation of specific

antigens is strongly supported by the combination of immunoproteomics and bioinformatics. The completion of the genomes of A. fumigatus and other Aspergillus species makes it possible to identify the antigens of Aspergillus species on a global scale. In this study we searched for the immunodominant antigens from the crude culture filtrate using an immunoproteomic approach. As a result, a total of 17 immunodominant antigens were identified. One of the antigens, thioredoxin reductase GliT (TR), which showed the best immunoactivity, was cloned and expressed in Escherichia coli.

Our results indicate that this protein could be useful for the early diagnosis of IA.

The immunoreactive proteins identified in this study may be helpful for the diagnosis of IA in critically ill patients. Our results indicate that TR and other immunodominant antigens have potential as biomarkers for the serologic diagnosis of invasive aspergillosis.

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Rambir Singh
University of Delhi, India E-mail: sehrawat_r@yahoo.com