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Original article

Expression of the protein serum amyloid A in response to *Aspergillus fumigatus* in murine models of allergic airway inflammation

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ABSTRACT

Background: Serum amyloid A (SAA) is an acute phase protein that is elevated in blood during inflammation. The role of this protein in allergic diseases of airways remains unclear.
Aims: The objective of this study was to evaluate the SAA in blood, lung and bronchial cells in a murine model of bronchial hypersensitivity to *Aspergillus fumigatus*.
Methods: To achieve this purpose, different groups of 5-month-old mice were housed in cages containing hay bedding that was contaminated with *A. fumigatus* and were kept in an isolation room for 16 days to allow for the induction of allergic airway inflammation. Subsequently, the mice were then exposed once again to *Aspergillus* spores at 0, 2, 8, 24 and 72 h, and they were bled to acquire serum and sacrificed to obtain bronchoalveolar lavage fluid (BALF) or lung tissues for analysis. SAA levels were measured in lung, serum and BALF by dot blot assay and RT-PCR (reverse transcription polymerase chain reaction).
Results: The results indicated that SAA protein levels increased in both serum and lung within 2–24 h after mice were exposed to *Aspergillus* spores. Moreover, the SAA mRNA expression levels in the lungs and BALF cells demonstrated the same trend that was observed for the protein levels through the dot blot assay; in particular, SAA mRNA levels increased within the first hour after mice were exposed to *A. fumigatus*.
Conclusions: In this allergic airway model, we conclude that *A. fumigatus* can induce an acute inflammatory response in the airways through the stimulation of the SAA protein, increasing its levels in serum, lung tissue and BALF samples during the early hours of exposure of mice that have been sensitised for this fungus.

Expresión de la proteína amiloide A sérica como respuesta a *Aspergillus fumigatus* en modelos murinos de inflamación alérgica de las vías respiratorias

Palabras clave:
Proteína amiloide A sérica
Aspergillus fumigatus
Alergia
Inflamación de las vías respiratorias

RESUMEN

Antecedentes: La proteína amiloide A sérica (AAS) es un reactante de fase aguda cuyos valores sanguíneos aumentan durante los procesos inflamatorios agudos. Todavía no se ha dilucidado el papel que desempeña en las enfermedades alérgicas de las vías respiratorias.
Objetivos: El objetivo del presente estudio fue examinar los valores de AAS en sangre, tejido pulmonar y células bronquiales en un modelo murino de hipersensibilidad bronquial frente a *Aspergillus fumigatus*.
Métodos: Diferentes grupos de ratones de 5 meses de vida fueron alojados en jaulas cuyos lechos de paja estaban contaminados por *A. fumigatus* y se mantuvieron en una sala de aislamiento durante 16 días para permitir la inducción de inflamación alérgica de las vías respiratorias. Tras este período de inducción, a las 0, 2, 8, 24 y 72 h los animales se expusieron de nuevo a esporas de *Aspergillus*. En cada tiempo de reexposición se obtuvieron muestras sanguíneas de los animales y, acto seguido, fueron sacrificados para obtener líquido de lavado broncoalveolar y muestras de tejido pulmonar. La concentración de AAS se analizó mediante técnica de hibridación del ADN (Southern) y reacción en cadena de la polimerasa-retrotranscriptasa en muestras de suero, tejido pulmonar y células de líquido de lavado broncoalveolar.

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Resultados: Los resultados del presente estudio demuestran que, al cabo de 2-24 h de la exposición a *A. fumigatus* aumentaron los valores de proteína AAS en muestras de suero y tejido pulmonar. Además, en células de líquido de lavado broncoalveolar y muestras de tejido pulmonar los niveles de expresión de ARNm de AAS demostraron la misma tendencia, y, en particular, aumentaron al cabo de la primera hora de exposición a las esporas de *A. fumigatus*.
Conclusiones: En este modelo murino de alergia de las vías respiratorias, concluimos que *A. fumigatus* puede inducir una respuesta inflamatoria aguda de las vías respiratorias a través de la estimulación de la proteína AAS, aumentando su concentración sérica en muestras de tejido pulmonar y de líquido de lavado broncoalveolar durante las primeras horas de exposición de ratones sensibilizados frente a este hongo.

Allergic airway inflammation is one characteristic feature of asthma, with additional pathology including a reversible airway obstruction, airway hyperresponsiveness (AHR), the infiltration of eosinophils and T helper type 2 (Th2) cells into the airway submucosa, mucus hypersecretion, and airway remodelling.[2] Allergic airway diseases are inflammatory disorders in which aberrant immune regulation occurs and susceptible individuals display allergen-specific responses. In these responses, inflammatory cells are recruited to the asthmatic airways or are activated in situ. These inflammatory cells include mast cells, macrophages, eosinophils, T lymphocytes, dendritic cells, basophils, neutrophils, and platelets.[3]

Aspergillus fumigatus is a saprophytic fungus that can survive and grow on a wide variety of organic remains. Its most common ecological niche is the ground. Because of the ease of dispersion of its conidia, *A. fumigatus* is one of the most ubiquitous fungi in the world.[13] The small size of these conidia, which range from 2 to 3 μm, allows them to remain in suspension in the environment for a long period of time; thus, humans are constantly exposed to inhaling airborne conidia, which can allow these conidia to reach the pulmonary alveoli.[1] In immunocompetent patients, *A. fumigatus* can produce allergic bronchopulmonary aspergillosis (ABPA), allergic rhinosinusitis, and asthma.[11] *A. fumigatus* produces a significant number of allergenic molecules that react with IgE in asthmatic patients and in patients with ABPA.[10]

Serum amyloid A (SAA) is an acute phase protein that is elevated in the blood during infection, trauma, surgery, burn injury, tissue infarction, inflammation, neoplasia and stress.[7] SAA production is primarily induced by IL-6, IL-1 and tumour necrosis factor α (TNF-α), which are multifunctional cytokines that are produced by many different types of cells in the human body.[6,7] Traditionally, SAA was considered to be produced by hepatocytes and subsequently secreted into serum. However, one published study[22] has demonstrated that SAA mRNA is normally expressed in the epithelial components of a variety of human organs and tissues. SAA could be released locally in certain organ-specific diseases, as demonstrated by several different groups of researchers.[5,14,20] However, very few investigations have focused on the relationship between SAA and asthma. Several studies have indicated that a positive correlation exists between SAA and the prevalence of asthma and have concluded that bronchial asthma causes not only local inflammation but also systemic inflammation.[8] Moreover, other authors have demonstrated that blood SAA concentrations are greater than normal in patients with asthma and allergic rhinitis.[17]

The objective of the current study was to evaluate whether SAA levels increase in provoked lung, blood or bronchoalveolar lavage fluid (BALF) cells in the context of a murine model of allergy airway inflammation and to discover whether SAA could be used as an inflammatory marker of allergy airway inflammation. Our hypothesis is that within several hours, the components of *A. fumigatus* may stimulate innate immunity through increases in the levels of the acute phase protein SAA, a factor in the initial bronchial allergy inflammation response.

Materials and methods

Animals

For all of the experiments in this study, we used 5-month-old, sex- and age-matched Rockefeller (RK) mice. These animals were obtained from and maintained at the Animal Facility of the Universidad Austral de Chile. During the exposure of these animals to *A. fumigatus*, they were placed in an isolation room with appropriate ventilation and filtering systems. This study was approved by the Bioethics Committee for the Use of Animals in Biomedical Research of the Universidad Austral de Chile.

Exposure of mice to A. fumigatus *spores*

Different groups of 5-month-old mice (eight mice per group) were housed in cages containing hay bedding that was contaminated with *A. fumigatus* and were kept in an isolation room for 16 days to allow for the induction of allergic airway inflammation, as described by previously published procedures.[15] After 16 days of exposure to this mould, the mice were placed in a remission environment for 10 days with the purpose of having animals in basal inflammatory condition before starting the antigenic challenge. Subsequently, the mice were once again exposed to the *Aspergillus* spores. At 0, 2, 8, 24 and 72 h after this exposure, the mice were bled to acquire serum and sacrificed with an overdose of a sodium barbital anaesthetic (Serve, USA) to obtain BALF or lung tissues for analysis as described by previously published procedures.[15]

Determination of serum amyloid A levels in serum and lung by immunodetection

The determination of SAA levels in the lungs and sera of the mice was performed by the dot blot immunodetection technique, which was performed as described in previously published sources.[23] Briefly, 1 μl quantities of undiluted samples of serum and lung extract proteins were applied to a nitrocellulose membrane and allowed to dry completely. The membrane was then blocked for 1 hour (h) with the following blocking solution: 1× Tris Buffer Solution (TBS) (40 g NaCl, 12.1 g Tris, pH 7.6), 5% skim milk and 0.5% Tween-20. After the blocking was completed, the membrane was incubated overnight (with constant stirring) at room temperature with goat anti-mouse SAA1 antibody (R&D Systems, USA) that had been diluted by 1:1000 in blocking solution. Following this incubation, the membrane was washed three times with phosphate-buffered saline (PBS; pH 7.2) for 5 min per wash and incubated for 1 h with alkaline phosphatase-conjugated bovine anti-goat IgG (Jackson ImmunoResearch Laboratories, Inc., USA) that had been diluted by 1:2000 in blocking solution. The membrane was then washed again and equilibrated for 10 min with buffer for the alkaline phosphatase enzyme. Immunoreactivity