A new tool to investigate differences between human SP-A1 and SP-A2

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SURFACANT PROTEIN A (SP-A), a ~35,000-molecular weight glycoprotein characterized by collagen and lectin domains, is the most abundant surfactant-associated protein in the lung (25). SP-A is a member of the collectin family of innate immune proteins, i.e., a calcium-dependent, pattern recognition molecule that binds to carbohydrate modifications on pathogens (1). SP-A aggregates and facilitates the clearance of lung pathogens by alveolar macrophages and also regulates the inflammatory state of the lung via a variety of mechanisms (3, 9, 18, 19).

SP-A is an evolutionarily conserved molecule that first appeared in the swim bladder of fish as long as 300 million years ago (20). Most mammals have one copy of the conserved SP-A gene. However, in primates, the SP-A gene has duplicated, and therefore two SP-A proteins are expressed, SP-A1 and SP-A2 (4, 7). The genes encoding the two SP-A molecules are very similar, and the proteins themselves differ by only 8–10 amino acids (7). However, the two SP-A genes are differentially regulated by glucocorticoids, insulin, and cAMP (10, 14).

In humans, SP-A protein is most abundant in lung, but it has also been identified in submucosal glands of the conducting airways (2, 17) as well as in the gastrointestinal tract (11), female reproductive tract (13), and in the nasopharyngeal epithelium and associated glands (24). Interestingly, the mRNAs for the two human SP-A molecules do not colocalize completely. For example, SP-A2 mRNA is the predominant molecule expressed in submucosal glands of the conducting airways (2, 17) and in the nasal epithelium (8). In contrast, both genes are expressed in the distal lung, gastrointestinal system, and in the female reproductive system (7, 11, 13).

The study of recombinant SP-A1 and SP-A2 protein molecules has led to insights suggestive that they may have different functions. SP-A2 is able to bind to more carbohydrates and with greater affinity than SP-A1 (16). SP-A2 stimulates the phagocytosis of Pseudomonas aeruginosa by alveolar macrophages more than SP-A1 (15). SP-A2 protein also stimulates higher levels of TNF-α and IL-8 release from macrophages than SP-A1 (23).

Evidence from genetic association studies is also suggestive of different functions for the two human SP-A proteins. A polymorphism in the carbohydrate binding domain of the SP-A2 (but not SP-A1) gene has been linked to susceptibility to meningococcal disease in children (6). Likewise, a SP-A2 allele was found to be overrepresented in respiratory syncytial virus-infected infants compared with matched controls (12). The observation that SP-A2 alleles are associated with susceptibility to infections that initially target the pharynx and airways may be related to the differential expression of SP-A2 in submucosal glands (2, 17).

SP-A1 and SP-A2 are both expressed in lung alveolar type II cells (25). The native SP-A protein secreted by type II cells is thought to be a multimer of SP-A heterotrimers that consist of two SP-A1 molecules and one SP-A2 molecule (22). However, to date, no one has been able to measure the actual SP-A1 or SP-A2 content in biological samples because there have been no antibodies available that specifically and uniquely recognize either SP-A1 or SP-A2. Thus, the contribution of SP-A1 vs. SP-A2 to the total SP-A content in any biological system is currently unknown. Because the two human SP-A molecules are expressed in distinctive cell types throughout the body, differentially regulated and functionally distinct, such antibodies would be a valuable resource for further studies of SP-A1 vs. SP-A2. In the study by Tagaram et al., the current article in focus (Ref. 21, see article in this issue), the authors report the creation and characterization of an antibody directed specifically against SP-A1 that was used to create an ELISA for measuring SP-A1 content (21).

The SP-A1-specific primary antibody was produced in chickens, using a 21-amino acid peptide corresponding to the collagen-like region of the protein as the antigen. The antigenic peptide included three amino acids that differ between the SP-A1 and SP-A2 molecule, as well as three prolines, which were randomly either hydroxylated or non-hydroxylated in the antigen. The authors extensively characterized the affinity-purified antibodies and demonstrated binding specificity to several different alleles of SP-A1 recombinant protein and an absence of recognition of several alleles of SP-A2 recombinant protein using Western blot analysis, ELISAs, and immunofluorescence staining of CHO cells stably transfected to express either the SP-A1 or SP-A2 protein. This is the first demonstration of an antibody that specifically recognizes only one of the human SP-A proteins.

An ELISA, created using the chicken antibody, was used to specifically measure SP-A1 in bronchoalveolar lavage fluid (BALF) from healthy subjects and patients. The authors hypothesized that the SP-A1 content of bronchoalveolar lavage would differ in patient groups, i.e., healthy subjects, alveolar proteinosis patients, and cystic fibrosis (CF) patients. The percent of BALF SP-A consisting of the SP-A1 protein was ~30% in young individuals (~20 years), and this proportion declined significantly with age. Even though total SP-A content was significantly decreased in the pediatric CF patients, the proportion of SP-A1 was significantly elevated in their BALF compared with either healthy subjects or with age-matched non-CF patients. Higher proportions of SP-A1 were detected in culture-positive CF-BALFs than in culture-negative CF-BALFs. These are important new findings supporting the concept that the two human SP-A proteins have distinctive functions, are
regulated differentially, and may be independently associated with certain disease processes.

The availability of the SP-A1-specific antibody described in this paper should allow studies of the individual and unique roles of these two proteins to proceed with increased power. It is tempting to speculate that the increased proportion of SP-A1 in the total SP-A of CF patients reflects a decreased content of SP-A2. SP-A2 is the predominant SP-A produced in submucosal glands of the conducting airways, and secretions from these glands are components of BALF. The presumed decreased SP-A2 content in CF-BALF may reflect a generalized impairment of secretion from submucosal glands in CF patients (5). The fact that within the CF patient population the lowest levels of SP-A2 are predicted to be present in the culture-positive BALF samples is suggestive that SP-A2 may play an important role in innate immunity in the conducting airways of the human lung. An antibody specific for human SP-A2 would be another important advance in the field of human SP-A biology.

REFERENCES