M-12
HUMAN SURFACTANT PROTEIN A (SP-A) GENE-SPECIFIC AND ALLELE-SPECIFIC ANTIBODIES
Hepzhizah Rani S.Tagaram *, Guirong Wang *, Todd M. Umstead*, David S.Phelps*, Joanna
Floros *,**, Departments of Cellular and Molecular Physiology, Pediatrics, Obstetrics, and
Gynecology, Pennsylvania University State College of Medicine, Milton S. Hershey Medical
Center, Hershey, PA-17033.

Pulmonary surfactant, a lipoprotein complex, is essential for lung function. It lowers the
surface tension in the terminal airspaces or alveoli and thus prevents lung collapse at low lung
volumes. The surfactant protein A (SP-A) is involved in surfactant related functions and in
innate host defense functions. Two genes, SP-A1 and SP-A2, with several alleles identified for
each gene, encode human SP-A. Although the SP-A genes and their corresponding alleles share a
high level of sequence similarity, in vitro studies have identified functional, biochemical and
structural differences between the two genes and/or among alleles. Alterations in SP-A function
and/or levels have been associated with several pulmonary diseases. However, it is unknown
whether the differences in SP-A observed between healthy individuals and subjects with
pulmonary diseases reflect alterations in one or both SP-A genes. To address this issue, we began
developing gene and allele specific antibodies. For the gene-specific antibodies (Ab), we focused
our attention on the collagen-like domain of SP-A, because this region contains the “core” amino
acids (a. a.) that distinguish one gene from the other. Two 27 a. a. (Pro53-Gly39) peptides each
specific for each SP-A gene were synthesized. The peptides differed from one another at a. a.
positions 66, 73, 81, and 85. Affinity purified peptide antiserum was then checked for gene
specificity with in vitro expressed SP-A variants and with human SP-A purified from the
bronchoalveolar lavage (BAL) from alveolar proteinosis (AP) patients. The results showed that
the SP-A2 specific antibodies recognized in vitro expressed SP-A2 alleles but not SP-A1
variants, indicating that the SP-A2 specific Ab is indeed gene specific. However, this strategy
failed to generate SP-A1 specific antibodies. For allele-specific antibodies, we focused our
attention on the carbohydrate recognition domain (CRD) of SP-A and developed antibodies to a
12 a. a. (Ala217-β238) sequences. Amino acid Lys223 is unique for the SP-A 1A1/3 (alleles 1A1
and 1A3 are identical in the CRD), whereas the other SP-A alleles have a glutamine at this
position. The purified Ab was checked for allele specificity with in vitro expressed SP-A variants
and with the human SP-A purified from the bronchoalveolar lavage (BAL) from AP patients of
unknown genotype. The results showed that the SP-A 1A1/3 allele-specific Abs recognized the
invitro expressed SP-A 1A1/3 alleles, and SP-A samples from BAL with 1A1 genotype, indicating
the 1A1/3 Abs could recognize both in vitro expressed and native SP-A of 1A1 genotype.
However the allele specific abs exhibited low reactivity with non-IA1/3 alleles. Treatment with
endoglycosidase-H and β -mercaptoethanol enhanced the specificity of 1A1 allele-specific
antibodies. In Conclusion, we have been able to develop SP-A2 gene specific Abs and one SP-A
1A1/3 allele specific Abs. We plan to use these Abs to determine differences in protein between
the two genes and also among alleles. SUPPORTED BY: NIH HL 68947
T-1
SP-A, SP-D AND MBL BIND NUCLEIC ACID AND ENHANCE ITS CLEARANCE
Nades Palaniyar\textsuperscript{9}, Jeya Nadesalingam\textsuperscript{9}, Howard Clark\textsuperscript{9}, Michael J Shih\textsuperscript{7}, Alister W Dodds\textsuperscript{9},
and Kenneth BM Reid\textsuperscript{9} \textsuperscript{,} \textsuperscript{MRC} Immunochemistry Unit, Department of Biochemistry, The
University of Oxford, South, Parks Road, Oxford OX1 3QU, UK. \textsuperscript{2}Lung Biology Research Program, Hospital for Sick Children Research Institute, 555 University Avenue, Toronto,
Ontario M5G 1X8, Canada.

Collectins are a family of innate immune proteins that contain fibrillar collagen-like regions and globular carbohydrate recognition domains (CRDs). The CRDs of these proteins recognize various microbial surface-specific carbohydrate patterns, particularly hexoses. We hypothesized that collectins such as pulmonary surfactant proteins (SP-) A and D, and serum protein mannos-binding lectin (MBL), could recognize nucleic acids, pentose-based anionic phosphate polymers. Here, we show that collectins bind DNA from a variety of origins, including bacteria, mice and synthetic oligonucleotides. Pentoses such as arabinose, ribose and deoxyribose inhibit the interaction between SP-D and mannan, one of the well-studied hexose ligands for SP-D, and biologically relevant D-forms of the pentoses are better competitors than the L-forms. In addition, DNA and RNA polymer-related compounds, such as nucleotide di- and triphosphates, also inhibit the carbohydrate binding ability of SP-D, or ~60 kDa trimeric recombinant fragments of SP-D that are composed of the alpha-helical coiled-coil neck region and 3 CRDs (SP-D(n/CRD)), or SP-D(n/CRD) with 8 Gly-X-Y repeats (SP-D(GXY)8(n/CRD)). Direct binding and competition studies suggest that collectins bind nucleic acid via their CRDs as well as via their collagen-like regions, and that SP-D binds DNA more effectively than do SP-A and MBL at physiological salt conditions. Furthermore, the SP-D(GXY)8(n/CRD) fragment co-localize with DNA, and the protein competes the interaction between propidium iodide, a DNA-binding dye, and apoptotic cells. In conclusion, we show that collectins are a new class of proteins that bind free DNA and DNA present on apoptotic cells via both of their globular CRDs and collagen-like regions. Collectins may therefore play an important role in decreasing the inflammation caused by DNA in lungs and other tissues.

T-4
CHARACTERIZATION OF SP-A UPTAKE AND TRAFFICKING IN HUMAN MACROPHAGES
Joy Crowther and Larry Schlesinger \textsuperscript{,} \textsuperscript{Interdisciplinary Graduate Program in Immunology, University of Iowa, Iowa}
City, IA 52240; Departments of Medicine and Molecular Virology, Immunology, and Medical Genetics, and Center for Microbial Interface Biology, Ohio State
University, Columbus, OH 43210.

Recent work by several laboratories has shown that surfactant protein A (SP-A) can act as an anti-inflammatory molecule through its effects on macrophage function, including inhibition of cytokine and nitric oxide production. We have recently shown that SP-A reduces the macrophage oxidative burst in response to stimuli through inhibition of NADPH oxidase activity, by a mechanism which includes a decrease in p47\textsuperscript{phox} association with phagosomes [\textit{J. Immunol.} (2004) 172:6866]. The receptor(s) responsible for these effects of SP-A on primary human macrophages (M\textsuperscript{\text{\Phi}}) is not clear, although SP-A binding to several proteins, including SPAR, SIRP-1\textalpha, TLR2, and TLR4, has been described in cell lines or purified protein systems. In this study, we demonstrate high-affinity specific binding of SP-A to human M\textsuperscript{\text{\Phi}}. 83% of total SP-A binding to human M\textsuperscript{\text{\Phi}} was inhibited by treatment of cells with EDTA, indicating that this binding is calcium dependent. Although SP-A has been shown to be degraded by M\textsuperscript{\text{\Phi}}, the trafficking of SP-A through the macrophage following uptake has not been previously reported. We examined trafficking of SP-A in human M\textsuperscript{\text{\Phi}} by confocal microscopy, and found that SP-A is rapidly endocytosed by M\textsuperscript{\text{\Phi}} and co-localizes over time with the late endosomal marker LAMP-1 and the lysosomal marker cathepsin D. Based on our studies to date, we conclude that SP-A binds to receptor(s) on human M\textsuperscript{\text{\Phi}}, is endocytosed by a receptor-mediated process, and traffics through the endolysosomal pathway.