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Chapter 1

General introduction and outline of the thesis

General introduction

Infectious diseases are a major cause of morbidity and mortality worldwide (1). Improved hygiene, the introduction of antibiotics and vaccination programs have successfully reduced the morbidity and mortality of many infectious diseases. However, the increasing incidence of antibiotic resistance and the enhanced risk of worldwide spreading due to increasing travelling stress the importance of improving treatment against infectious diseases. To achieve this, expanding our understanding of the host responses to microbial invasion is mandatory. This thesis is focussed on the role of CD44 and osteopontin (OPN) during primarily pulmonary bacterial infection and inflammation.

Pulmonary immune response

The alveolar membrane is the largest surface of the body in contact with the outside environment, and is continuously exposed to respiratory pathogens such as bacteria and viruses. In the upper respiratory tract physical mechanisms like coughing and sneezing are used as a first line of defense against potential pathogens. In case microbes enter the terminal airways, they can cause pneumonia, one of the most common infectious diseases and the most frequent source of sepsis (2). Innate immune cells like respiratory epithelial cells, in a normal alveolus covered with a surfactant layer, and resident alveolar macrophages (AM) are the first cells to encounter pathogens in the alveoli (Figure 1, reviewed in (3)). The innate immune system is able to detect pathogens via a limited number of pattern-recognition receptors (PRR) which recognise conserved motifs that are expressed by pathogens but are absent in higher eukaryotes (4). Among other receptor families such as scavenger receptors, complement receptors, Fc-receptors, C-type lectins and Nucleotide-binding oligodimerization domain proteins (NOD)-like receptors, Toll-like receptors (TLRs) have a central role as PRR in the initiation of cellular innate immune responses. Upon recognition of a pathogen, lung epithelial cells will secrete cytokines and chemokines, and antimicrobial peptides that have microcidal activity or inhibit bacterial growth. AM are able to recognize, bind and phagocytose pathogens and subsequently kill them intracellularly. These cells can also secrete several mediators including cytokines and chemokines that mediate recruitment and activation of neutrophils from the circulation. These cells are potent phagocytes and critical for the effective elimination of pathogens. In addition, AM can phagocytose apoptotic neutrophils and thereby contribute to the resolution of pneumonia. In a number

of murine pneumonia models depletion of AM or neutrophils resulted in increased lethality and worsened outcome of the disease indicating the significant role of these cells during pulmonary infection (reviewed in (5)). In addition to rapid initiation of the nonspecific inflammatory reaction, the innate immune response is thought to orchestrate the adaptive immune response that primarily consists of T-cell and B-cell responses that provide specific memory of infection (6).

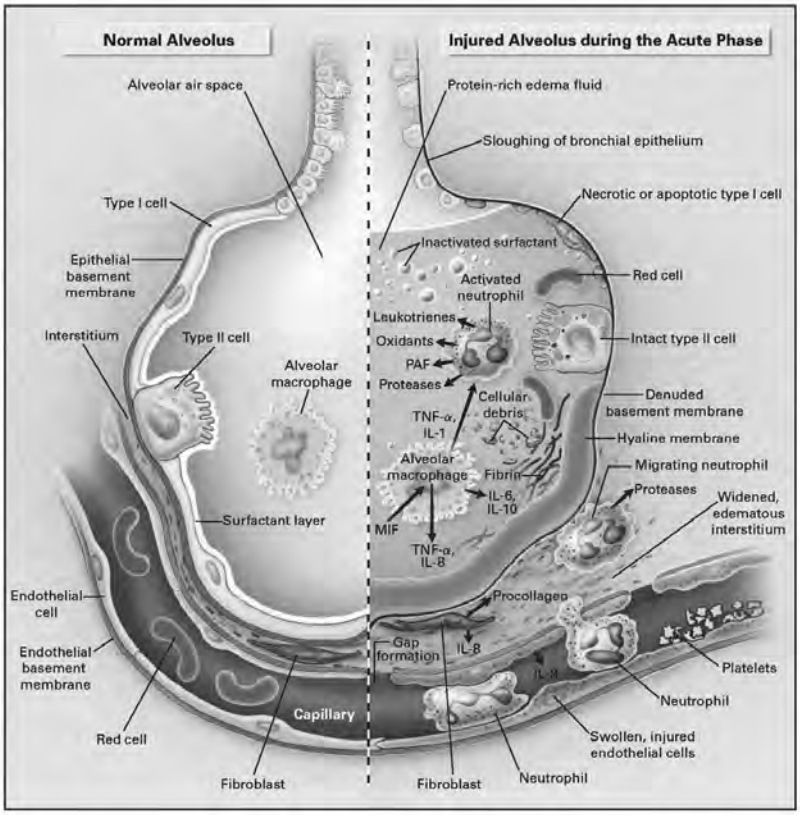

Figure 1. Depicted is a normal (left side) and inflamed (right side) alveolus. In the acute phase, there is sloughing of both the bronchial and alveolar epithelial cells, with the formation of protein-rich hyaline membranes on the denuded basement membrane. In the air space, an alveolar macrophage is secreting cytokines, including interleukin (IL)-1, IL-6, IL-8, and IL-10, and tumor necrosis factor (TNF)-α, which act locally to stimulate chemotaxis and activate neutrophils. Neutrophils are shown adhering to the injured capillary endothelium and marginating through the interstitium into the air space, which is filled with protein-rich edema fluid. Neutrophils can release oxidants, proteases, leukotrienes, and other proinflammatory molecules, such as platelet-activating factor (PAF). The influx of protein-rich edema fluid into the alveolus has led to the inactivation of surfactant. Adapted from (7).

CD44

The transmembrane glycoprotein CD44 was initially described in 1981 as a hyaluronic acid (HA) binding molecule (8) and as a homing receptor for lymphocytes (9). In the following decades, the diversity in structure and function of CD44 became clear. The single CD44 gene consists of 20 exons and encodes for a large variety of CD44 proteins. All isoforms share an extracellular N-terminal domain (coded by exon 1-5) which binds HA (10), and a C-terminal domain (coded by exon 16-20), which contains a transmembrane domain and an intracellular cytoplasmic domain (11). The cytoplasmic tail of CD44 interacts with proteins of the cytoskeleton including actin, ankyrin, ezrin, radixin and moesin (12-14), and connection of CD44 with the cytoskeleton was hypothesised to regulate HA binding to CD44. The standard form of CD44 is expressed by most tissues and consists of the structures encoded by exon 1-5 and 16-20 only (15). By alternative splicing of the 10 variable exons that are present in mice (exon v1-v10) many isoforms can be generated that differ in their extracellular domain (11). In addition to alternative splicing CD44 is altered by posttranslational modifications including N- and/or O-linked glycosylation and addition of glycosaminoglycan chains (Figure 2) (16, 17). Shedding of CD44 from cellular membranes results in soluble CD44 (sCD44). So far little is known about the function of sCD44 (18, 19). sCD44 is found in the circulation of normal humans (20) and mice (21), and the serum levels of sCD44 often correlate with inflammatory state and tumor progression (21-24).

CD44 functions and ligands

CD44 is widely expressed on a variety of cell types, including leukocytes and parenchymal cells (26, 27). This molecule has been implicated to mediate important physiological and pathological processes, such as cellular adhesion and migration by mediating cell-cell and cell-matrix interactions, cellular survival by affecting apoptosis and proliferation, and cytokine and chemokine responses (reviewed in (28, 29)).

The main ligand of CD44 is HA, a glycosaminoglycan that is a component of the extracellular matrix (Figure 3). Under physiologic conditions HA exists as a high molecular weight polymer of disaccharides that has a restricted tissue distribution and is mainly found in connective tissues such as cartilage and joints. However, during tissue injury and inflammation HA undergoes dynamic degradation resulting in accumulation of low molecular weight fragments (30). Binding of HA to CD44

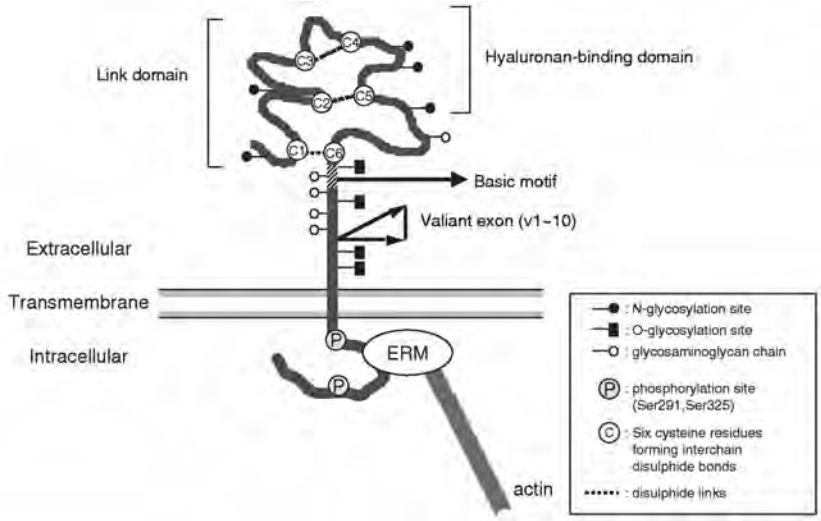

Figure 2. Structure of CD44 protein. CD44 type I transmembrane protein is composed of extracellular, transmembrane, and cytoplasmic domains. The globular region of CD44 in the extracellular amino-terminal domain has homology with the cartilage link protein, which is known as "link domain." Six cysteine residues that form interchain disulphide bonds confer stability on the link domain. The amino-terminal globular domain contains hyaluronan-binding motifs, which are in the link domain, and a basic motif that is outside the link domain. The extracellular domain is highly glycosylated. The stem structure of CD44 can be enlarged by the alternatively spliced variant exons (v1-v10) of CD44. The carboxy-terminal cytoplasmic domain interacts with ERM (ezrin, radixin, and moesin) proteins, which link to the actin cytoskeleton. From (25)

is strictly regulated and dependent on the cell type, state of cell activation and differentiation (31-33). HA may interact differentially with various cells and produce distinct biological effects depending on the molecular weight. HA fragments induce chemokine gene expression (34) and NF-κB activation (35) in AM, and promote angiogenesis (36), whereas high molecular weight HA does not. However, several HA induced effects appeared only partly CD44 dependent. In the past few years, it has become clear that TLRs may warn the host of danger in general by the ability to recognise endogenous mediators released during injurious processes (37). HA fragments are now known to additionally signal through TLR2 and TLR4, thereby affecting NF-κB activation and induction of inflammatory gene expression, cell survival and migration (38-41). Interestingly, recent studies have shown that CD44 positively regulates the induction of negative regulators of TLR signalling including IL-1R-associated kinase (IRAK)-M (42, 43). Moreover, CD44 is known to bind, internalise and degrade HA into inactive disaccharides (26, 44, 45), thereby clearing it from the extracellular compartment.

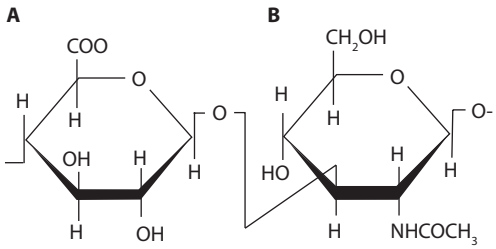

Figure 3. Hyaluronic acid is composed of repeating disaccharide units containing glucuronic acid (A) and *n*-acetylglucosamine (B). From (54).

OPN, the second major ligand of CD44, is a secreted phosphorylated glycoprotein (Figure 4) (46) that is expressed by a broad range of tissues and cells (47-49). Originally considered a bone matrix protein, OPN is now known to regulate inflammation, tissue remodeling and cell survival (49-51). It has been implicated as an important component of inflammation during both innate and adaptive immunity by mediating inflammatory cell differentiation, maturation and migration, and cytokine production (47, 49-53).

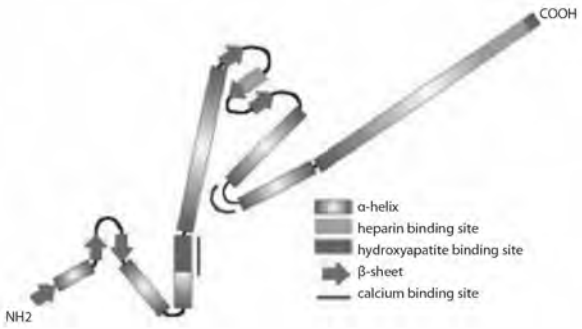

Figure 4. Osteopontin.

CD44 and osteopontin in lung disease

Under normal conditions CD44 is expressed by AM and pulmonary epithelial cells (55-57), and during inflammation CD44 expression in the lung can be upregulated (55, 58). Inflammatory cell recruitment is an important process during pulmonary inflammation (59), and CD44 is expressed on inflammatory, as well as endothelial and epithelial cells (60). Indeed, several studies suggest a central function for CD44 during sterile or infectious lung inflammation. CD44 on murine neutrophils is an

E-selectin ligand (61) and important for polarization and directed migration of those cells (62). Therefore, it can be anticipated that cell influx into the lung would be impaired in the absence of CD44; however, others suggested a negative regulation of epithelium-neutrophil interactions via CD44 (63). During ozone-induced airway hyperresposiveness inflammatory cell influx into the lungs was dependent on HA and CD44, and thus impaired in CD44 knockout (KO) mice (64). On the other hand, in a model of acute pulmonary lung inflammation induced by intratracheal lipopolysaccharide (LPS) treatment, neutrophils appeared earlier and remained enhanced in numbers in bronchoalveolar lavage fluid (BALF) from CD44 KO mice when compared to neutrophil numbers in BALF of wild-type (WT) mice (42), whereas during a low dose aerosol LPS challenge neutrophil numbers were lower in CD44 KO mice (65). Similarly, enhanced neutrophil recruitment in the absence of CD44 has been reported for *Mycobacterium* (*M*.) *tuberculosis* infection (66) and *Escherichia (E.) coli* induced pneumonia (67). In addition, CD44 has been shown to affect macrophage trafficking towards the lung. CD44 KO mice demonstrated increased macrophage numbers in their BALF during lung inflammation elicited by intrapulmonary delivery of LPS or bleomycin (42, 68), whereas decreased pulmonary macrophage numbers were found in CD44 KO mice after aerosol challenge with a low dose of LPS (65), during ozone-induced airway hyperresponsiveness (64) and tuberculosis (66). Moreover, cell migration and tissue damage are additionally affected by cytokine and chemokine release, a process that can also be influenced by CD44 (42). Taken together, the net effect of CD44 on pulmonary inflammation appears to be dependent on the stimulus, the severity of tissue damage and the predominant cell type involved in the inflammatory response. In line with the function of CD44 to take up and degrade HA (26), CD44 KO mice showed elevated levels of HA during sterile pulmonary inflammation (induced either by ozone (64), LPS (42), or bleomycin (68)), which was accompanied by sustained accumulation of inflammatory cells.

Patients suffering from diverse pulmonary diseases, including interstitial pneumonia, tuberculosis, silicosis and sarcoidosis, displayed enhanced OPN expression in their lungs as compared to healthy controls (69-71, 72, 73). In addition, patients with idiopathic pulmonary fibrosis demonstrated increased OPN levels in BALF (74). A possible protective role for OPN in mycobacterial infection was suggested by the observation that the extent of OPN protein expression in pathological lymph nodes from *M. bovis* BCG or *M. avium* infected patients was inversely correlated with disseminated infection and death (75). Furthermore, plasma OPN levels were

dramatically elevated in patients with interstitial pneumonia (76), in patients with sepsis of whom almost half suffered from pneumonia as the primary site of infection (77), and in patients with tuberculosis in which levels declined after successful therapy (78, 79). Moreover, a functional role for OPN has been described in several experimental models of non-infectious lung disease, including allergy and asthma (80, 81, 82), acute respiratory distress syndrome (73), granulomatous disease (83) and fibrosis (84, 85). However, knowledge on the functional role of OPN during pulmonary (bacterial) infection is limited.

Infection and inflammation models studied in this thesis

Respiratory tract infections

Infections of the respiratory tract are the 7th most prevalent cause of mortality in the United States (86, 87). According to the acquisition of pneumonia and the pathogens involved, community-acquired pneumonia (CAP) can be distinguished from nosocomial pneumonia. The pre-dominant pathogen responsible for CAP is *Streptococcus (S.) pneumoniae*, which is responsible for up to 60% of the cases. *S. pneumoniae* is a gram-positive diplococcus, of which the virulence is mainly determined by its capsule (88, 89). Nosocomial pneumonia usually occurs in patients with preexisting conditions and can be induced by various pathogens. *Klebsiella (K.) pneumoniae* is a gram-negative opportunistic rod-shaped bacterium that accounts for about 12% of hospital-acquired pneumonia cases (90, 91). Melioidosis is an important cause of severe sepsis in Southeast Asia and Northern Australia caused by the aerobic gram-negative soil-dwelling bacillus *Burkholderia* (*B.*) *pseudomallei* (92, 93). Infection is thought to occur by cutaneous inoculation or inhalation. More than half of patients with melioidosis presents with pneumonia associated with bacterial dissemination to distant organs (94), and all-cause mortality is as high as 50% in Northeast Thailand where the majority of reported cases occur (95). One of the most dramatic manifestations of chronic lung infection and inflammation is tuberculosis. This disease, caused by infection with the acid fast rod *M. tuberculosis*, is a serious threat to mankind. One-third of the world's population is infected with the tubercle bacillus and tuberculosis is responsible for two million deaths each year (96, 97). Upon infection with this pathogen healthy individuals develop a strong T-helper 1 (Th1) response which is able to contain the infection in granulomas and prevent active disease. However, *M. tuberculosis* bacilli are thereby not eradicated from the lungs and remain a potential danger to the infected individual (97).

Hyperoxia induced lung injury

Patients with acute lung injury (ALI) or acute respiratory distress syndrome (ARDS) almost invariably are given supplemental oxygen to maintain tissue oxygenation (98, 99). However, prolonged exposure to high oxygen concentrations can worsen or induce lung damage in already injured or previously healthy lungs (100). The mechanisms underlying hyperoxia induced lung injury are beginning to be understood. The inflammatory response to hyperoxia is dominated by the recruitment of neutrophils into the bronchoalveolar space and likely mediated by the generation of reactive oxygen species (98, 100). However it has become clear that neutrophils are not absolutely required for the induction of lung injury during hyperoxia (101-103).

Peritonitis

Peritonitis is most often caused by the presence of bacteria in the otherwise germ-free peritoneal cavity and is predominantly due to perforation of intestines. An immediate and adequate host defense is necessary to contain and kill the pathogen as due to the site of infection bacteria can quickly spread via the circulation, resulting in systemic inflammation and sepsis (2, 104). Like in the lung, upon bacterial appearance in the peritoneal cavity, resident peritoneal macrophages release chemoattractant factors to recruit circulating neutrophils and monocytes to kill the pathogen (105). *E. coli* is the most frequently isolated causative pathogen in peritonitis, which is responsible for 60% of the intraabdominal infections (106, 107).

Aim and outline of this thesis

The general aim of the research described in this thesis was to obtain more insight into the role of CD44 and OPN during infection and inflammation. The principal subject of investigation was pulmonary inflammation and (myco)bacterial respiratory tract infections. The first part of this thesis focuses on the role of CD44 during (pulmonary) inflammation and infection. **Chapter 2** describes the contribution of CD44 to hyperoxia induced lung injury. The function of CD44 during *E. coli* induced abdominal sepsis is reported in **chapter 3**. The role of CD44 in the acute and resolution phase during *S. pneumoniae* or *K. pneumoniae* induced pneumonia is described in **chapter 4** and **chapter 5**. TLRs are known to be important for pathogen recognition during pneumonia (108), and negative regulators of TLR-mediated immune responses are critical to attenuate TLR signaling and thereby prevent overwhelming inflammation (109). **Chapter 6** describes the role of the negative TLR regulator IRAK-M in the host

response during gram-negative and gram-positive pneumonia. In the next part of this thesis we report on the role of OPN during respiratory tract infections. The contribution of OPN to the host response during pneumonia caused by *K. pneumoniae* or *S. pneumoniae* infection is described in **chapter 7** and **chapter 8**. OPN levels in the circulation are enhanced in patients with sepsis, of whom almost half suffered from pneumonia as the primary site of infection (77), and in patients with tuberculosis (78, 79). Therefore, we investigated the role of OPN during severe gram-negative sepsis induced by *B. pseudomallei* infection and during chronic *M. tuberculosis* infection, which is described in **chapter 9** and **chapter 10**.

References

1. Organization WH. The World Health Report 2000, Health systems. *Improving Performance* 2000;Geneva, Switserland
2. Wheeler AP and Bernard GR. Treating patients with severe sepsis. *N Engl J Med* 1999;340:207-214
3. Martin TR and Frevert CW. Innate immunity in the lungs. *Proc Am Thorac Soc* 2005;2:403-411
4. Akira S, Uematsu S and Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124:783-801
5. Knapp S, Schultz MJ and van der Poll T. Pneumonia models and innate immunity to respiratory bacterial pathogens. *Shock* 2005;24 Suppl 1:12-18
6. Pasare C and Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. *Adv Exp Med Biol* 2005;560:11-18
7. Ware LB and Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000;342:1334-1349
8. Delpech B and Halavent C. Characterization and purification from human brain of a hyaluronic acid-binding glycoprotein, hyaluronectin. *J Neurochem* 1981;36:855-859
9. Jalkanen S, Bargatze RF, de los Toyos J and Butcher EC. Lymphocyte recognition of high endothelium: antibodies to distinct epitopes of an 85-95-kD glycoprotein antigen differentially inhibit lymphocyte binding to lymph node, mucosal, or synovial endothelial cells. *J Cell Biol* 1987;105:983-990
10. Lesley J, Hyman R and Kincade PW. CD44 and its interaction with extracellular matrix. *Adv Immunol* 1993;54:271-335
11. Screaton GR, Bell MV, Jackson DG, Cornelis FB, Gerth U and Bell JI. Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. *Proc Natl Acad Sci U S A* 1992;89:12160-12164
12. Lacy BE and Underhill CB. The hyaluronate receptor is associated with actin filaments. *J Cell Biol* 1987;105:1395-1404
13. Isacke CM. The role of the cytoplasmic domain in regulating CD44 function. *J Cell Sci* 1994;107 (Pt 9):2353-2359
14. Tsukita S, Oishi K, Sato N, Sagara J and Kawai A. ERM family members as molecular linkers between the cell surface glycoprotein CD44 and actin-based cytoskeletons. *J Cell Biol* 1994;126:391-401
15. Ponta H, Sherman L and Herrlich PA. CD44: from adhesion molecules to signalling regulators. *Nat Rev Mol Cell Biol* 2003;4:33-45
16. Brown TA, Bouchard T, St John T, Wayner E and Carter WG. Human keratinocytes express a new CD44 core protein (CD44E) as a heparan-sulfate intrinsic membrane proteoglycan with additional exons. *J Cell Biol* 1991;113:207-221
17. Bennett KL, Modrell B, Greenfield B, Bartolazzi A, Stamenkovic I, Peach R, Jackson DG, Spring F and Aruffo A. Regulation of CD44 binding to hyaluronan by glycosylation of variably spliced exons. *J Cell Biol* 1995;131:1623-1633
18. Cichy J, Bals R, Potempa J, Mani A and Pure E. Proteinase-mediated release of epithelial cell-associated CD44. Extracellular CD44 complexes with components of cellular matrices. *J Biol Chem* 2002;277:44440-44447
19. Cichy J and Pure E. The liberation of CD44. *J Cell Biol* 2003;161:839-843
20. Sjoberg S, Fogelstrand L, Hulthe J, Fagerberg B and Krettek A. Circulating soluble CD44 is higher among women than men and is not associated with cardiovascular risk factors or subclinical atherosclerosis. *Metabolism* 2005;54:139-141

21. Katoh S, McCarthy JB and Kincade PW. Characterization of soluble CD44 in the circulation of mice. Levels are affected by immune activity and tumor growth. *J Immunol* 1994;153:3440-3449
22. Skoumal M, Kolarz G, Haberhauer G, Feyertag J and Wottawa A. Is soluble CD44 variant isoform 5 useful as a predicting factor and a parameter for long term observation in rheumatoid arthritis? *Ann Rheum Dis* 2002;61:1036-1037
23. Niitsu N and Iijima K. High serum soluble CD44 is correlated with a poor outcome of aggressive non-Hodgkin's lymphoma. *Leuk Res* 2002;26:241-248
24. Kasuga I, Minemura K, Nasu H, Hibi N, Tago H, Nagate A, Kunisawa A, Ohyashiki JH and Ohyashiki K. Elevated serum soluble CD44 level in sarcoidosis. *Int J Mol Med* 2000;6:679-682
25. Nagano O and Saya H. Mechanism and biological significance of CD44 cleavage. *Cancer Sci* 2004;95:930-935
26. Culty M, Nguyen HA and Underhill CB. The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *J Cell Biol* 1992;116:1055-1062
27. Isacke CM and Yarwood H. The hyaluronan receptor, CD44. *Int J Biochem Cell Biol* 2002;34:718-721
28. Siegelman MH, DeGrendele HC and Estess P. Activation and interaction of CD44 and hyaluronan in immunological systems. *J Leukoc Biol* 1999;66:315-321
29. Ilangumaran S, Borisch B and Hoessli DC. Signal transduction via CD44: role of plasma membrane microdomains. *Leuk Lymphoma* 1999;35:455-469
30. Fraser JR, Laurent TC and Laurent UB. Hyaluronan: its nature, distribution, functions and turnover. *J Intern Med* 1997;242:27-33
31. Lesley J, English N, Charles C and Hyman R. The role of the CD44 cytoplasmic and transmembrane domains in constitutive and inducible hyaluronan binding. *Eur J Immunol* 2000;30:245-253
32. Lesley J, Hascall VC, Tammi M and Hyman R. Hyaluronan binding by cell surface CD44. *J Biol Chem* 2000;275:26967-26975
33. Underhill CB and Toole BP. Physical characteristics of hyaluronate binding to the surface of simian virus 40-transformed 3T3 cells. *J Biol Chem* 1980;255:4544-4549
34. McKee CM, Penno MB, Cowman M, Burdick MD, Strieter RM, Bao C and Noble PW. Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44. *J Clin Invest* 1996;98:2403-2413
35. Noble PW. Hyaluronan and its catabolic products in tissue injury and repair. *Matrix Biol* 2002;21:25-29
36. West DC and Kumar S. Hyaluronan and angiogenesis. *Ciba Found Symp* 1989;143:187-201; discussion 201-187, 281-185
37. Mollen KP, Anand RJ, Tsung A, Prince JM, Levy RM and Billiar TR. Emerging paradigm: toll-like receptor 4-sentinel for the detection of tissue damage. *Shock* 2006;26:430-437
38. Voelcker V, Gebhardt C, Averbeck M, Saalbach A, Wolf V, Weih F, Sleeman J, Anderegg U and Simon J. Hyaluronan fragments induce cytokine and metalloprotease upregulation in human melanoma cells in part by signalling via TLR4. *Exp Dermatol* 2008;17:100-107
39. Taylor KR, Trowbridge JM, Rudisill JA, Termeer CC, Simon JC and Gallo RL. Hyaluronan fragments stimulate endothelial recognition of injury through TLR4. *J Biol Chem* 2004;279:17079-17084
40. Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD and Horton MR. Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. *J Immunol* 2006;177:1272-1281
41. Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R and Noble PW. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005;11:1173-1179

42. Liang J, Jiang D, Griffith J, Yu S, Fan J, Zhao X, Bucala R and Noble PW. CD44 Is a Negative Regulator of Acute Pulmonary Inflammation and Lipopolysaccharide-TLR Signaling in Mouse Macrophages. *J Immunol* 2007;178:2469-2475
43. del Fresno C, Otero K, Gomez-Garcia L, Gonzalez-Leon MC, Soler-Ranger L, Fuentes-Prior P, Escoll P, Baos R, Caveda L, Garcia F, Arnalich F and Lopez-Collazo E. Tumor cells deactivate human monocytes by up-regulating IL-1 receptor associated kinase-M expression via CD44 and TLR4. *J Immunol* 2005;174:3032-3040
44. Knudson W, Chow G and Knudson CB. CD44-mediated uptake and degradation of hyaluronan. *Matrix Biol* 2002;21:15-23
45. Harada H and Takahashi M. CD44-dependent intracellular and extracellular catabolism of hyaluronic acid by hyaluronidase-1 and -2. *J Biol Chem* 2007;282:5597-5607
46. Weber GF, Ashkar S, Glimcher MJ and Cantor H. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science* 1996;271:509-512
47. Wang KX and Denhardt DT. Osteopontin: Role in immune regulation and stress responses. *Cytokine Growth Factor Rev* 2008;19:333-345
48. Sodek J, Ganss B and McKee MD. Osteopontin. *Crit Rev Oral Biol Med* 2000;11:279-303
49. Denhardt DT and Guo X. Osteopontin: a protein with diverse functions. *Faseb J* 1993;7:1475-1482
50. Scatena M, Liaw L and Giachelli CM. Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. *Arterioscler Thromb Vasc Biol* 2007;27:2302-2309
51. Denhardt DT, Noda M, O'Regan AW, Pavlin D and Berman JS. Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. *J Clin Invest* 2001;107:1055-1061
52. Nystrom T, Duner P and Hultgardh-Nilsson A. A constitutive endogenous osteopontin production is important for macrophage function and differentiation. *Exp Cell Res* 2007;313:1149-1160
53. Koh A, da Silva AP, Bansal AK, Bansal M, Sun C, Lee H, Glogauer M, Sodek J and Zohar R. Role of osteopontin in neutrophil function. *Immunology* 2007;122:466-475
54. Turino GM and Cantor JO. Hyaluronan in respiratory injury and repair. *Am J Respir Crit Care Med* 2003;167:1169-1175
55. Lackie PM, Baker JE, Gunthert U and Holgate ST. Expression of CD44 isoforms is increased in the airway epithelium of asthmatic subjects. *Am J Respir Cell Mol Biol* 1997;16:14-22
56. Svee K, White J, Vaillant P, Jessurun J, Roongta U, Krumwiede M, Johnson D and Henke C. Acute lung injury fibroblast migration and invasion of a fibrin matrix is mediated by CD44. *J Clin Invest* 1996;98:1713-1727
57. Givehchian M, Woerner SM, Lacroix J, Zoller M, Drings P, Becker H, Kayser K, Ridder R and von Knebel Doeberitz M. Expression of CD44 splice variants in normal respiratory epithelium and bronchial carcinomas: no evidence for altered CD44 splicing in metastasis. *Oncogene* 1996;12:1137-1144
58. Peroni DG, Djukanovic R, Bradding P, Feather IH, Montefort S, Howarth PH, Jones DB and Holgate ST. Expression of CD44 and integrins in bronchial mucosa of normal and mildly asthmatic subjects. *Eur Respir J* 1996;9:2236-2242
59. Craig A, Mai J, Cai S and Jeyaseelan S. Neutrophil recruitment to the lungs during bacterial pneumonia. *Infect Immun* 2009;77:568-575
60. Pure E and Cuff CA. A crucial role for CD44 in inflammation. *Trends Mol Med* 2001;7:213-221
61. Katayama Y, Hidalgo A, Chang J, Peired A and Frenette PS. CD44 is a physiological E-selectin ligand on neutrophils. *J Exp Med* 2005;201:1183-1189

62. Alstergren P, Zhu B, Glogauer M, Mak TW, Ellen RP and Sodek J. Polarization and directed migration of murine neutrophils is dependent on cell surface expression of CD44. *Cell Immunol* 2004;231:146-157

63. Si-Tahar M, Sitaraman S, Shibahara T and Madara JL. Negative regulation of epithelium-neutrophil interactions via activation of CD44. *Am J Physiol Cell Physiol* 2001;280:C423-432

64. Garantziotis S, Li Z, Potts EN, Kimata K, Zhuo L, Morgan DL, Savani RC, Noble PW, Foster WM, Schwartz DA and Hollingsworth JW. Hyaluronan mediates ozone-induced airway hyperresponsiveness in mice. *J Biol Chem* 2009;284:11309-11317

65. Hollingsworth JW, Li Z, Brass DM, Garantziotis S, Timberlake SH, Kim A, Hossain I, Savani RC and Schwartz DA. CD44 Regulates Macrophage Recruitment to the Lung in Lipopolysaccharide-induced Airway Disease. *Am J Respir Cell Mol Biol* 2007;37:248-253

66. Leemans JC, Florquin S, Heikens M, Pals ST, van der Neut R and Van Der Poll T. CD44 is a macrophage binding site for Mycobacterium tuberculosis that mediates macrophage recruitment and protective immunity against tuberculosis. *J Clin Invest* 2003;111:681-689

67. Wang Q, Teder P, Judd NP, Noble PW and Doerschuk CM. CD44 deficiency leads to enhanced neutrophil migration and lung injury in Escherichia coli pneumonia in mice. *Am J Pathol* 2002;161:2219-2228

68. Teder P, Vandivier RW, Jiang D, Liang J, Cohn L, Pure E, Henson PM and Noble PW. Resolution of lung inflammation by CD44. *Science* 2002;296:155-158

69. Kelly MM, Leigh R, Gilpin SE, Cheng E, Martin GE, Radford K, Cox G and Gauldie J. Cell-specific gene expression in patients with usual interstitial pneumonia. *Am J Respir Crit Care Med* 2006;174:557-565

70. Carlson I, Tognazzi K, Manseau EJ, Dvorak HF and Brown LF. Osteopontin is strongly expressed by histiocytes in granulomas of diverse etiology. *Lab Invest* 1997;77:103-108

71. O'Regan AW, Chupp GL, Lowry JA, Goetschkes M, Mulligan N and Berman JS. Osteopontin is associated with T cells in sarcoid granulomas and has T cell adhesive and cytokine-like properties in vitro. *J Immunol* 1999;162:1024-1031

72. Nau GJ, Guilfoile P, Chupp GL, Berman JS, Kim SJ, Kornfeld H and Young RA. A chemoattractant cytokine associated with granulomas in tuberculosis and silicosis. *Proc Natl Acad Sci U S A* 1997;94:6414-6419

73. Takahashi F, Takahashi K, Shimizu K, Cui R, Tada N, Takahashi H, Soma S, Yoshioka M and Fukuchi Y. Osteopontin is strongly expressed by alveolar macrophages in the lungs of acute respiratory distress syndrome. *Lung* 2004;182:173-185

74. Pardo A, Gibson K, Cisneros J, Richards TJ, Yang Y, Becerril C, Yousem S, Herrera I, Ruiz V, Selman M and Kaminski N. Up-regulation and profibrotic role of osteopontin in human idiopathic pulmonary fibrosis. *PLoS Med* 2005;2:e251

75. Nau GJ, Chupp GL, Emile JF, Jouanguy E, Berman JS, Casanova JL and Young RA. Osteopontin expression correlates with clinical outcome in patients with mycobacterial infection. *Am J Pathol* 2000;157:37-42

76. Kadota J, Mizunoe S, Mito K, Mukae H, Yoshioka S, Kawakami K, Koguchi Y, Fukushima K, Kon S, Kohno S, Saito A, Uede T and Nasu M. High plasma concentrations of osteopontin in patients with interstitial pneumonia. *Respir Med* 2005;99:111-117

77. Vaschetto R, Nicola S, Olivieri C, Boggio E, Piccolella F, Mesturini R, Damnotti F, Colombo D, Navalesi P, Della Corte F, Dianzani U and Chiocchetti A. Serum levels of osteopontin are increased in SIRS and sepsis. *Intensive Care Med* 2008;34:2176-2184

78. Inomata S, Shijubo N, Kon S, Maeda M, Yamada G, Sato N, Abe S and Uede T. Circulating interleukin-18 and osteopontin are useful to evaluate disease activity in patients with tuberculosis. *Cytokine* 2005;30:203-211

79. Koguchi Y, Kawakami K, Uezu K, Fukushima K, Kon S, Maeda M, Nakamoto A, Owan I, Kuba M, Kudeken N, Azuma M, Yara S, Shinzato T, Higa F, Tateyama M, Kadota J, Mukae H, Kohno S, Uede T and Saito A. High plasma osteopontin level and its relationship with interleukin-12-mediated type 1 T helper cell response in tuberculosis. *Am J Respir Crit Care Med* 2003;167:1355-1359

80. Simoes DC, Xanthou G, Petrochilou K, Panoutsakopoulou V, Roussos C and Gratziou C. Osteopontin deficiency protects against airway remodeling and hyperresponsiveness in chronic asthma. *Am J Respir Crit Care Med* 2009;179:894-902

81. Xanthou G, Alissafi T, Semitekolou M, Simoes DC, Economidou E, Gaga M, Lambrecht BN, Lloyd CM and Panoutsakopoulou V. Osteopontin has a crucial role in allergic airway disease through regulation of dendritic cell subsets. *Nat Med* 2007;13:570-578

82. Kohan M, Breuer R and Berkman N. Osteopontin induces airway remodeling and lung fibroblast activation in a murine model of asthma. *Am J Respir Cell Mol Biol* 2009;41:290-296

83. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, Rittling SR, Denhardt DT, Glimcher MJ and Cantor H. Eta-1 (osteopontin): an early component of type-1 (cell-mediated) immunity. *Science* 2000;287:860-864

84. Berman JS, Serlin D, Li X, Whitley G, Hayes J, Rishikof DC, Ricupero DA, Liaw L, Goetschkes M and O'Regan AW. Altered bleomycin-induced lung fibrosis in osteopontin-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2004;286:L1311-1318

85. Takahashi F, Takahashi K, Okazaki T, Maeda K, Ienaga H, Maeda M, Kon S, Uede T and Fukuchi Y. Role of osteopontin in the pathogenesis of bleomycin-induced pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2001;24:264-271

86. 2005. Deaths: Leading causes for 2002. *National Vital Statistics Report* 53:1-90

87. Laterre PF, Garber G, Levy H, Wunderink R, Kinasewitz GT, Sollet JP, Maki DG, Bates B, Yan SC and Dhainaut JF. Severe community-acquired pneumonia as a cause of severe sepsis: data from the PROWESS study. *Crit Care Med* 2005;33:952-961

88. Bartlett JG and Mundy LM. Community-acquired pneumonia. *N Engl J Med* 1995;333:1618-1624

89. van der Poll T and Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. *Lancet* 2009;374:1543-1556

90. Podschun R and Ullmann U. Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998;11:589-603

91. Kofteridis DP, Papadakis JA, Bouros D, Nikolaides P, Kioumis G, Levidiotou S, Maltezos E, Kastanakis S, Kartali S and Gikas A. Nosocomial lower respiratory tract infections: prevalence and risk factors in 14 Greek hospitals. *Eur J Clin Microbiol Infect Dis* 2004;23:888-891

92. Cheng AC and Currie BJ. Melioidosis: epidemiology, pathophysiology, and management. *Clin Microbiol Rev* 2005;18:383-416

93. Wiersinga WJ, van der Poll T, White NJ, Day NP and Peacock SJ. Melioidosis: insights into the pathogenicity of Burkholderia pseudomallei. *Nat Rev Microbiol* 2006;4:272-282

94. Currie BJ. Melioidosis: an important cause of pneumonia in residents of and travellers returned from endemic regions. *Eur Respir J* 2003;22:542-550

95. White NJ. Melioidosis. *Lancet* 2003;361:1715-1722

96. Dye C, Scheele S, Dolin P, Pathania V and Raviglione MC. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *Jama* 1999;282:677-686

97. Kaufmann SH. How can immunology contribute to the control of tuberculosis? *Nat Rev Immunol* 2001;1:20-30

98. Wheeler AP and Bernard GR. Acute lung injury and the acute respiratory distress syndrome: a clinical review. *Lancet* 2007;369:1553-1564

99. Leaver SK and Evans TW. Acute respiratory distress syndrome. *Bmj* 2007;335:389-394

100. Carvalho CR, de Paula Pinto Schettino G, Maranhao B and Bethlem EP. Hyperoxia and lung disease. *Curr Opin Pulm Med* 1998;4:300-304

101. Boyce NW, Campbell D and Holdsworth SR. Granulocyte independence of pulmonary oxygen toxicity in the rat. *Exp Lung Res* 1989;15:491-498

102. Shasby DM, Fox RB, Harada RN and Repine JE. Reduction of the edema of acute hyperoxic lung injury by granulocyte depletion. *J Appl Physiol* 1982;52:1237-1244

103. Perkowski S, Scherpereel A, Murciano JC, Arguiri E, Solomides CC, Albelda SM, Muzykantov V and Christofidou-Solomidou M. Dissociation between alveolar transmigration of neutrophils and lung injury in hyperoxia. *Am J Physiol Lung Cell Mol Physiol* 2006;291:L1050-1058

104. Holzheimer RG, Muhrer KH, L'Allemand N, Schmidt T and Henneking K. Intraabdominal infections: classification, mortality, scoring and pathophysiology. *Infection* 1991;19:447-452

105. Broche F and Tellado JM. Defense mechanisms of the peritoneal cavity. *Curr Opin Crit Care* 2001;7:105-116

106. Lorber B and Swenson RM. The bacteriology of intra-abdominal infections. *Surg Clin North Am* 1975;55:1349-1354

107. Nathens AB. Relevance and utility of peritoneal cultures in patients with peritonitis. *Surg Infect (Larchmt)* 2001;2:153-160; discussion 160-152

108. Tsai KS and Grayson MH. Pulmonary defense mechanisms against pneumonia and sepsis. *Curr Opin Pulm Med* 2008;14:260-265

109. Liew FY, Xu D, Brint EK and O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005;5:446-458