

2009

Moraxella catarrhalis-induced innate immune responses in human pulmonary epithelial cells and monocytes

Miao Chen

Medical University of Ohio

Follow this and additional works at: <http://utdr.utoledo.edu/theses-dissertations>

Recommended Citation

Chen, Miao, "Moraxella catarrhalis-induced innate immune responses in human pulmonary epithelial cells and monocytes" (2009). *Theses and Dissertations*. 1051.

<http://utdr.utoledo.edu/theses-dissertations/1051>

This Dissertation is brought to you for free and open access by The University of Toledo Digital Repository. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of The University of Toledo Digital Repository. For more information, please see the repository's [About page](#).



COLLEGE OF GRADUATE STUDIES

THE UNIVERSITY OF TOLEDO

***Moraxella catarrhalis*-induced innate immune responses in human
pulmonary epithelial cells and monocytes**

Doctor of Philosophy

Miao Chen

University of Toledo, Health Science Campus

2009

Examination Committee

Major Advisor: Kevin Z. Pan, M.D., Ph.D.

Academic Advisory Committee: Robert M. Blumenthal, Ph.D.

Linyu Chen, M.D., Ph.D.

James P. Trempe, Ph.D.

Randall G. Worth, Ph.D.

DEDICATION

I would like to dedicate this work to my grandfather, Zhiwen Chen. I will always miss you. May you rest in peace.

ACKNOWLEDGEMENTS

I would like to thank Dr. Kevin Z. Pan, my major advisor, for his constant encouragement, guidance and support during the last four years of my graduate study.

I would like to thank my committee members: Dr. Robert M. Blumenthal, Dr. Linyu Chen, Dr. James P. Trempe, and Dr. Randall G. Worth for their valuable suggestions on my project. A special thanks to Dr. Stanley G. Sawicki and Dr. Dorothea L. Sawicki, you are more like my friends and family.

I would like to thank all the members of Dr. Pan's lab: Dr. Xuesong Huang, Dr. Wei Liu, Dr. Sumanta Mukherjee, Dr. Pablo Serrano, and Dr. Byung Cheol Lee, Kuladeep Sudini, Sara Campbell, Sushovita Mukherjee, Yiqing Pan, Christopher Fisher, and Laura Smith. We do create a great research environment in the lab and always help each other out.

I would like to thank everyone from the Department of Medical Microbiology and Immunology at the University of Toledo. I really appreciate everything you have done for me.

Finally, I deeply thank my father Yanfeng Chen and my mother Guangxian Wang, I can not make it without your support.

TABLE OF CONTENTS

| | |
|----------------------------|-----|
| DEDICATION..... | ii |
| ACKNOWLEDGEMENTS..... | iii |
| TABLE OF CONTENTS..... | iv |
| INTRODUCTION..... | 1 |
| LITERATURE REVIEW..... | 6 |
| MATERIALS AND METHODS..... | 44 |
| RESULTS..... | 49 |
| DISCUSSION..... | 72 |
| CONCLUSIONS..... | 79 |
| REFERENCES..... | 80 |
| ABSTRACT..... | 113 |

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is defined as “*a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases.*” (Pauwels et al., 2001; Rabe et al., 2007). Such inflammatory responses can lead to mucus hypersecretion and scarred bronchial tubes, creating an ideal breeding place for bacterial infections (Hogg et al., 2004). The predominant bacteria isolated from patients with COPD are *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* (Monso et al., 1995; Ball et al., 1995; Murphy et al., 2005). Although many studies have already confirmed the important roles of *H. influenzae* and *S. pneumoniae* in the pathogenesis of COPD, it was only until recently that *M. catarrhalis* was estimated to colonize up to 32% of patients with COPD and caused about 10% of acute exacerbations and disease progression of COPD (Sethi et al., 2002; Murphy et al., 2005). Most of the research has focused on identifying outer membrane virulence factors of *M. catarrhalis* for vaccine development, with some hopeful candidates including the adhesins: UspA1 (Lafontaine et al., 2000; Hill and Virji, 2003), Hag (Pearson et al., 2002; Holm et al., 2003), and McaP (Timpe et al., 2003); the serum resistance factor UspA2 (Cope et al., 1999; Meier et al., 2002). However, little is known about how colonized *M.*

catarrhalis interacts with the host, especially the inflammatory cells such as pulmonary epithelial cells and monocytes, and thus initiates innate immune response in the lung.

Exacerbation of COPD is characterized by acutely worsening symptoms, especially increased dyspnea, and is the major cause of increased mortality and diminished quality of life (Seemungal et al., 1998; Donaldson et al., 2006). Clinical data has shown that bacterial infections cause over half of acute exacerbations of COPD (Sethi et al., 2008). As a primary interface between the internal body and the external environment, the respiratory epithelium is the first place where local immune activation happens (Diamond et al., 2000). Thus epithelial cells lining the airways and the alveolar surface area are a crucial site for host defense. In a healthy human body, the lung is normally populated with commensal microbes, which produce a ‘tolerance’ response and homeostasis is maintained (Hippenstiel et al., 2006). In contrast, in patients with COPD, the lung epithelium has usually lost its integrity due to long term exposure to cigarette smoke or air pollutants, resulting in increased susceptibility to potential pathogens (Wilson, 1998; Soler et al., 1999). Pulmonary epithelial cells directly increase the release of antimicrobial peptides into the lumen of the airways and initiate non-specific inflammatory responses, producing chemokines and cytokines into the submucosa (Barnes, 2008). Along the chemokine gradients, inflammatory cells such as monocytes, dendritic cells and neutrophils are recruited to the site of infection, helping to kill the invasive bacteria and mounting exaggerated inflammatory responses (Mosser et al., 2008; Hammad et al., 2008; Zemans et al.,

2009). The acute exacerbation phase is always associated with increased airway inflammation, compared with stable COPD state. It is therefore important to study the interaction between bacteria and cells of innate immune system, which might provide hints regarding new therapeutic targets of COPD.

Like other cells of the innate immune system, pulmonary epithelial cells depend on the germline-encoded pattern recognition receptors (PRRs) to recognize specific pathogen associated molecular patterns (PAMPs) on potential pathogens and then initiate the host defense mechanisms (Janeway and Medzhitov, 2002). Cellular PRRs belong to different functional and structural groups, which include the Toll-like receptors (TLRs), NOD-like receptors (NODs), scavenger receptors, and lectin receptors (Gordon, 2002). Among these PRRs, TLRs constitute the family of most important and intensely studied PRRs (Akira et al., 2004). For most Gram-negative bacteria such as *M. catarrhalis*, TLR2 recognizes lipopeptides, lipoprotein, and peptidoglycan (PGN); while TLR4, in association with MD2, recognizes the lipid A moiety of lipopolysaccharide (LPS) or lipooligosaccharide (LOS) (Janeway et al., 2002; Akira et al., 2006). MD2 is a small secreted glycoprotein, which forms a stable complex with TLR4 on the cell surface; it plays an essential role in TLR4 signaling and intracellular trafficking (Shimazu et al., 1999; Nagai et al., 2002).

The key early event of these recognitions is mediated by CD14, which concentrates ligands including lipopeptides, LTA, PGN and LPS, and then transports them to specific TLRs (Van Amersfoort et al., 2003). CD14 is mainly expressed on myeloid cells (monocytes, macrophages and neutrophils) in two forms: a

membrane-bound glycosyl phosphatidyl inositol (GPI)-anchored protein (mCD14) and a soluble proteolytic fragment lacking the GPI anchor (sCD14) (Haziot et al., 1988; Simmons et al., 1989; Frey et al., 1992). When CD14 was first identified as receptor for LPS and PGN (Wright et al., 1990; Gupta et al., 1996), people did not know how it could initiate intracellular signaling events because CD14 lacks a transmembrane domain. TLR2 and TLR4/MD2 were subsequently shown to form co-receptor complexes with CD14 for the signaling entity (Medzhitov et al., 1997; Kirschning et al., 1998; Chow et al., 1999). CD14 contains 10 leucine-rich repeat (LRR) motifs, which shares the similar structure with 21 LRRs on the extracellular domains of TLRs (Kim et al. 2005). This region may be the possible binding site for both PAMPs and co-receptors. In endothelial or epithelial cells that do not express mCD14, sCD14 released from CD14 bearing cells could increase the response to endotoxin (Pugin et al., 1993; von Asmuth et al., 1993). However, the precise role of CD14 in pathogenesis of COPD is not yet clear.

Recently, the new chemokine CCL20/MIP-3 α was found to be highly increased in total lung and sputum samples of patients with COPD, compared with non-smokers and smokers without COPD (Demedts et al., 2007). CCL20/MIP-3 α acts on its specific receptor, CCR6, strongly stimulating chemotaxis for dendritic cells (DCs) and lymphocytes and has weakly effect on monocytes and neutrophils (Hieshima et al., 1997; Schutyser et al., 2003). There are also significantly elevated numbers of DCs infiltrated into the epithelium and adventitia of small airways of patients with COPD, such increase is correlated with the severity of the disease (Demedts et al.,

2007). Moreover, cigarette smoke exposure-induced accumulation of DCs, granulocytes and T lymphocytes was significantly attenuated in the CCR6 knockout mice, compared with their wild-type littermates (Bracke et al., 2006). All these data suggest that CCL20/MIP-3 α might be an important mediator in the development of COPD. However, the major producer of CCL20/MIP-3 α and the specific signaling pathway that regulates its expression are not fully understood.

We found that *M. catarrhalis* could induce inflammatory responses by human pulmonary epithelial cells, including CCL20/MIP-3 α and CXCL8/IL-8 expression, and NF- κ B activation. *M. catarrhalis*-induced CCL20/MIP-3 α secretion is mainly through the TLR2-MyD88-TRAF6-NF- κ B signaling pathway. The TLR4 signaling pathway might not be activated on pulmonary epithelial cells, because they lack two important co-receptors, CD14 and MD2. Our hypothesis is that CD14 might mediate crosstalk between monocytes and pulmonary epithelial cells through TLRs signaling. In work presented here, we report that both mCD14 and sCD14 expression levels are very low in naïve monocytic THP1 cells, but are greatly increased by 1 α , 25-dihydroxy VD₃ and ligands of TLRs. Elevated CD14 stimulated TLR2- and TLR4-mediated inflammatory responses in THP1 cells. Also sCD14 activated the TLR4 signaling pathway and enhanced TLR2-induced responses on pulmonary epithelial cells, mounting an amplified inflammatory responses upon stimulation of *M. catarrhalis*. Moreover, antibody specific for CD14 inhibited such effects on both THP1 cells and pulmonary epithelial cells. Thus upon bacterial infection, CD14 plays an important role in the pathogenesis and exacerbation of COPD, and could be a new

therapeutic target for inhibiting abnormal inflammation during the development of COPD.

LITERATURE REVIEW

Chronic Obstructive Pulmonary Disease

Definition

According to Global Initiative for Chronic Obstructive Lung Disease (GOLD), chronic obstructive pulmonary disease (COPD) is defined as “*a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases.*” (Pauwels et al., 2001; Rabe et al., 2007). COPD is now ranked as the fourth leading cause of chronic morbidity and mortality in the United States, accounting for 122,000 deaths, 661,000 hospitalizations, 2 million emergency department visits and more than 17 million physician or hospital outpatient visits annually (CDC, 2006). It also represents an enormous economic burden on the health care system, with direct costs of \$18 billion and indirect costs of \$14.1 billion in the United States in 2002 (Rabe et al., 2007). Due to increasing environmental pollution and cigarette smoking rate, the World Health Organization (WHO) predicted that COPD will be the fifth most prevalent disease and third leading cause of death by 2020 (Lopez and Murray, 1998).

Pathogenesis and Pathology

It is still not clear that how cigarette smoke and noxious particles induce the

pathogenesis of COPD, because a quarter of COPD patients are non-smokers (Salvi and Barnes, 2009). It is highly possible that these irritants contain a high concentration of free radicals, which cause oxidative stress and thus initiate chronic inflammation in the lung (Bowler et al. 2004). Such chronic inflammation in bronchi usually develops into obstructive bronchiolitis, one of the main pathologic conditions of COPD. It is defined as “*the persistence of a productive cough for more than three months in two successive years*” (Ball, 1995). Another condition that occurs deeper in distal tissue to the terminal bronchioles is emphysema, which is characterized by “*enlargement of air spaces and destruction of lung parenchyma, loss of elasticity and closure of small airways, but without obvious fibrosis*” (Hogg and Senior, 2002). Both symptoms are caused by abnormal inflammatory responses at particular airways, and contribute to the airflow limitation in COPD to a variable extent (Barnes, 2000).

COPD and Asthma

Asthma is also an inflammatory pulmonary disease, sharing some common symptoms with COPD such as narrowed airways and increased inflammation. In contrast to COPD, asthma is usually developed in early childhood due to environmental and genetic factors, especially inhalation of allergens. The typical symptom is intermittent wheezing and shortness of breath, and the inflammation is mainly located in the larger airways, but not in the lung parenchyma, which is usually reversible (Barnes, 2008a). COPD generally starts in one’s middle age, distinctively causing shortness of breath on exertion, and is persistent and slowly progressive over time. In addition, the inflammation of COPD is not fully reversible in most cases.

Most importantly, the pattern of inflammation is also a significant difference between COPD and asthma. Infiltrated inflammatory cells in COPD mainly include monocytes and neutrophils, recruited by chemokines such as CXC-chemokine ligand 8 (CXCL8)/interleukin 8 (IL-8) and CC-chemokine ligand 2 (CCL2)/monocyte chemoattractant protein 1 (MCP-1). CD8⁺ T cells/cytotoxic T (T_C) cells and T helper 1 (T_{H1}) cells, recruited by some CXC-chemokine ligands, are also involved. Together with local activated epithelial cells and macrophages, these cells release large amounts of chemokines and cytokines, as well as some proteases and elastases, causing mucus hypersecretion and tissue damage, such as elastin degradation and fibrosis (Barnes, 2008a,b). In asthma, inhaled allergens activate mainly mast cells and dendritic cells (DCs), which release CCL17 and CCL22 to chemoattract T_{H2} cells. T_{H2} cytokines, including IL-4, IL-5 and IL-13, are highly induced, which stimulate B cells to produce immunoglobulin E (IgE) and recruit eosinophils (Barnes, 2008a,b). However, it needs to be noticed that there is no absolute standard to distinguish these inflammatory cells and mediators between the two diseases. Both macrophages and DCs are increased in the lungs of patients with COPD and asthma. T_{H2} cells are also found increased in bronchoalveolar lavage (BAL) fluid of patients with severe COPD (Barczyk et al., 2006; Kurashima et al., 2006).

Diagnosis and Treatment

To diagnose COPD, the patient should be first examined for the “*symptoms of cough, sputum production, or dyspnea, and/or a history of exposure to risk factors for the disease*” (Pauwels RA et al., 2001; Rabe KF et al., 2007). Then diagnosis should

be confirmed by spirometry, which should be undertaken in all patients who may have COPD. The volume of air forcibly exhaled from the point of maximal inspiration (FVC), and the volume of air exhaled during the first second of this maneuver (FEV_1), need to be measured. Compared to the reference value, according to sex, age, height and race, decreased FEV_1 and FVC should be observed. A ratio of FEV_1/FVC less than 0.7 would indicate airflow limitation that is not fully reversible (Pellegrino et al., 2005). Current treatments for COPD depend on the severity of disease. For stable COPD, the patient should first reduce the exposure to risk factors. Smoking cessation effectively reduces the symptoms. To manage the airflow limitation, bronchodilator medications, such as β_2 -agonists, anticholinergics or theophylline, could be used separately or in combination (Pauwels RA et al., 2001; Rabe KF et al., 2007).

Exacerbation of COPD

Definition

As COPD progresses, acutely worsening symptoms, also called exacerbations, tend to become more frequent. Exacerbation is defined as “*an event in the natural course of the disease characterized by a change in the patient’s baseline dyspnea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD*” (Burge and Wedzicha, 2003). Exacerbation is now the leading concern in the natural course of COPD, because it accounts for not only the major portion of health care spending on COPD but also most of the morbidity and mortality. It has been

shown that acute exacerbation is the main cause of hospitalization among COPD patients, and such costs represent up to 63% of patients with exacerbation, far exceeding the costs of patients with stable COPD (Miravittles et al., 2002). Moreover, treatment failures happen more frequently for patients with severe COPD than early stage patients (Donaldson and Wedzicha, 2006). Such failures could further increase the frequency of acute exacerbation and accelerate the progression of COPD. Exacerbation significantly affects the patients' life quality, reducing exercise capacity and health status (Seemungal et al., 1998). Some independent studies have established that in-hospital mortality rates of acute exacerbation range from 8% to 11%, and one year mortality rates range from 23% to 43% (Connors et al., 1996; Groenewegen et al., 2003).

Causes of Exacerbation of COPD

Clinical data suggests that respiratory bacteria and virus infections cause about 30% and 23% of exacerbations, respectively. Mixed infections were found in a quarter of the total cases, and the remaining 22% was due to environmental pollution or other unknown etiology (Papi et al., 2006). Several studies have estimated that over half of exacerbations are associated with increased colonization of bacteria, including *Haemophilus influenzae* (11% of all exacerbating patients), *Streptococcus pneumoniae* (10%) and *Moraxella catarrhalis* (10%) (Sapey and Stockley, 2006). In some cases, specific immune responses to the isolated bacteria were found (Sethi et al., 2001), supporting a direct link between bacterial infection and exacerbation of COPD. Most importantly, these bacteria may amplify the pulmonary inflammation to

a higher extent than stable COPD, though the precise mechanism is still poorly understood. Viral infection of the respiratory tract also plays an important role in exacerbation of COPD. Rhinovirus, respiratory syncytial virus (RSV) and influenza are most common viruses have been associated with the disease (Wedzicha, 2004). Serological studies have shown that rhinovirus, RSV and influenza were detected in 23%, 11.4% and 28%, respectively in the patients with exacerbation; whereas in stable COPD patients, the rates were all between 1% and 6% (Greenberg et al., 2000; Sapey and Stockley, 2006). Interestingly, exacerbations happen more frequently in winter, the same period that viral infections are more common in the community. Also, 25% of the patients with exacerbation had co-infection of both bacteria and virus. Such concomitant infections usually cause more severe symptoms and longer recovery time (Celli and Barnes, 2007). However, controversy still remains about whether bacterial or viral colonizations cause exacerbation or whether colonizations are just outcomes of the impaired mucociliary clearance as COPD develops. So future study need to focus on the cellular and molecular mechanisms of exacerbations, especially how bacteria and viruses trigger the amplification loop of inflammation in the lung.

Diagnosis and Treatment

According to the definition, the clinical diagnosis of exacerbation of COPD should first focus on three major symptoms: increased shortness of breath, increased sputum volume, and increased sputum purulence (Rodríguez-Roisin, 2006). More importantly, some similar pulmonary diseases, such as pneumonia, pneumothorax,

lung cancer or upper airway obstruction should be examined to determine their roles as the causes for the symptoms or just as the complications. Spirometry is less useful in diagnosis, but should be measured during the recovery period to monitor the improvement. Finally sputum samples or protected brush specimens should be screened by the bacteria and virus culture to identify the possible prevalence pathogens (Rodríguez-Roisin, 2006). Pharmacological treatment for the exacerbation of COPD is often called “ABC approach”, referring to the three commonly used medicines (antibiotics, bronchodilators and corticosteroids) (Currie and Wedzicha, 2006). Antibiotics are most effective in severe exacerbations with increased sputum volume and purulence, due to a highly possibility of bacteria infections. Other advantages are less risk of treatment of failure, oral delivery and economically affordable. Bronchodilators are the most essential drug in managing both stable COPD and exacerbation. Easy administered by inhalation, bronchodilators could induce smooth muscle relaxation and bronchodilation through β_2 adrenoceptors. In most cases of exacerbation of COPD, oral corticosteroids are recommended because significant improved lung function, shortened length of hospital stay and fewer relapses were observed following administration. However, long term usage of corticosteroids could cause more side effects than benefits.

Inflammation in COPD

Inflammation plays a central role in pathogenesis of COPD at different stages. During the initial development of COPD and the following stable state, the chronic inflammatory responses activate pulmonary epithelial cells and local macrophages. Different chemokines are released to recruit the immune cells, including monocytes, neutrophils, DCs, T_C and T_{H1} cells, to the bronchi (Pauwels et al., 2001; Rabe et al., 2007). Highly induced cytokines, oxidative stress and proteases contribute to the tissue damage in small airways and lung parenchyma, causing bronchiolitis and emphysema. While during the acute episode of exacerbations, the mild inflammations are amplified to excessive extent. However, the mechanisms of such amplification are not fully understood.

Inflammatory Cells

Pulmonary Epithelial cells. As a primary interface between the internal body and the external environment, the respiratory epithelium is the first place at which local inflammatory responses start. Thus epithelial cells lining the airways and the alveolar surface area are a crucial site for host defense. In healthy human lung, the ‘tolerance’ response and homeostasis is maintained between normal commensal microbes and the epithelium (Hippenstiel et al., 2006). In the patients with COPD, lung epithelium has often lost its integrity due to overexposure to cigarette smoke or air pollutants; and airway epithelial cells are more readily activated by the increased contact with the potential pathogens. It has been proved that airway epithelial cells not only provide a passive barrier function, but also actively contribute to the innate immunity (Wilson,

1998; Soler et al., 1999). Just like other cells of the innate immune system, pulmonary epithelial cells depend on the germline-encoded pattern recognition receptors (PRRs) to recognize specific pathogen associated molecular patterns (PAMPs) on pathogens (Janeway and Medzhitov, 2002).

Activated lung epithelial cells can induce two complementary parts of the innate immune response. One is the increased release of antimicrobial factors into the lumen of the airways, such as β -defensin and cathelicidin, LL-37/hCAP-18 (Bals, 2000). Antimicrobial peptides have a broad spectrum activity against Gram-positive and Gram-negative bacteria, as well as some fungi and viruses. Their antimicrobial activities are due to the amphipathic and cationic properties, such that they readily bind to membrane bilayers and form pores to kill target organisms (Brogden, 2005). The complementary part of the inflammatory caused by activated lung epithelial cells is secretion of cytokines and chemokines into the submucosa (Diamond et al., 2000; Bals and Hiemstra, 2004). Such inflammatory factors recruit phagocytes including monocytes and neutrophils that serve to remove invading pathogens that are not cleared by the epithelium itself. If the infection is still out of control, DCs and lymphocytes will be recruited to mount adaptive immunity (Diamond et al., 2000; Bals and Hiemstra, 2004).

Monocytes and Macrophages. Alveolar macrophages play a critical role in innate and adaptive immunity, as well as the pathophysiology of COPD. The numbers of macrophages are increased about 10 to 25 fold in airways, lung parenchyma, BAL fluid and sputum samples of patients with COPD, compared with healthy people

(Barnes, 2004a). Such increases are also correlated to the severity of COPD (Di Stefano et al., 1998). Two mechanisms are generally considered to contribute to the increased numbers of macrophages in the lungs of COPD patients: one is the prolonged survival of local alveolar macrophages, and another is the increased recruitment of peripheral naïve monocytes (Shapiro, 1999; Tetley, 2000; Barnes, 2004a). Local alveolar macrophages are normally quiescent, producing little inflammatory mediators and displaying no phagocytic activity (Lambrecht, 2006). Once activated, they can reacquire the suppressive phenotype after a few days or be cleared by mucociliary clearance and lymphatic drainage (Lambrecht, 2006; Barnes, 2004a). These effects prevent over reaction of macrophages that might easily damage the alveoli. In contrast, in the COPD patients, the impaired physical clearance system allows pathogens to activate the macrophages over a longer period and prolong their survival time. On the other hand, activated pulmonary epithelial cells and local macrophages would release large amounts of chemokines, such as CXCL8/IL-8 and CCL2/MCP-1. As a result, these chemokines bind to G protein coupled receptors (GPCR) CXCR1 and CXCR2 on naïve monocytes, chemoattracting them from peripheral blood into the alveolar space. These freshly recruited monocytes then differentiate into tissue resident macrophages or DCs, depending on different stimuli. Activated macrophages can directly engulf the infectious microbes by phagocytosis and secrete different inflammatory mediators to help to clear the remaining pathogens. These mediators include lipid mediators, chemokines, cytokines, growth factors, reactive oxygen and nitrogen species, which are all important in maintaining the

homeostasis, airway remodeling and host defense.

Dendritic Cells (DCs). DCs are considered to be the most important antigen-presenting cells (APCs) in immune system, linking innate and adaptive immunity. Precursor DCs circulate in the blood and are found in smaller quantities near mucosal surfaces. They are immature and serve to sample the potential invading material. During inflammation, their numbers are enriched by migration of DCs from peripheral blood or differentiation of infiltrated monocytes (Hammad and Lambrecht, 2008; Tsoumakidou et al. 2008). CCL20/macrophage inflammatory protein 3 α (MIP-3 α) is the most important chemokine, mediating the chemotaxis through binding to its receptor CCR6 on DCs. Allergens, PAMPs and other cytokines, including thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF- α), IL-1 β , IL-25, and IL-33, can activate DCs through different receptors (Banchereau et al. 2000). Once activated, DCs increase cell surface costimulatory molecule expression and the production of various inflammatory mediators. More importantly, they can internalize antigens and then migrate to lymph nodes, modulating the proliferation of lymphocytes.

Neutrophils. Neutrophils are members of the polymorphonuclear leukocytes (PMNs) (or granulocytes). They originate in bone marrow and circulate in the blood. Like monocytes, they can also be recruited to the site of inflammation by attaching to vascular endothelium and then passing through the blood vessels. The most efficient chemoattractants for neutrophils are CXCL8/IL-8, interferon- γ (IFN- γ) and

complement component 5a (C5a) (Cowburn et al, 2008). In patients with COPD, increased numbers of neutrophils could be found in sputum, BAL and lung parenchyma, more in the small airways than the large airways (Quint and Wedzicha, 2007). Neutrophils are also phagocytes, so they can ingest microbes and particles. They are also the major producers of reactive lipid mediators, cytokines, chemokines, oxygen metabolites, antibacterial peptides, and tissue damaging enzymes (Dockrell and Whyte, 2006; Quint and Wedzicha, 2007).

Lymphocytes. Two types of lymphocytes dominate in the airways of patients with COPD: T_H1 and T_C cells. Both lymphocytes express the chemokine receptor CXCR3, thus can be recruited by its ligands such as CXCL9/monokine induced by IFN- γ (MIG), CXCL10/IFN- γ -induced protein (IP-10) and CXCL12/stromal cell-derived factor-1 (SDF-1) (Barnes, 2008a; Cosio et al., 2009). These ligands can all be induced by IFN- γ and are highly expressed in the airways of COPD patients. T_H1 cells play an important role in T_H1/T_H2 balance by producing typical T_H1 cytokines, including IFN- γ , TNF- α and IL-2. They are mainly involved in cellular immunity, enhancing the killing capacity of the macrophages, helping the proliferation of T_C cells and secreting opsonizing antibodies. Unlike T_H1 cells that bind major histocompatibility complex (MHC) class II, T_C cells recognize MHC class I and have a major function following viral infection. They can release perforins and granulysin, which form pores in the virus infected cells. The target cells are directly cytolysed or go through apoptosis induced by the granzymes (Baraldo et al., 2007).

Inflammatory Mediators

Cytokines. Cytokines are defined as a number of soluble substances secreted by immune cells, that have a variety of immunoregulating effects on the other cells. The action of cytokines may be autocrine or paracrine, but not endocrine. Cytokines play essential roles in initiating and amplifying chronic inflammation, and contribute to the structural remodeling of the respiratory tract in patients with COPD. The most important cytokines involved in pathogenesis of COPD are proinflammatory cytokines, such as TNF- α , IL-1 β , and IL-6 (Barnes, 2004b and 2008b). TNF- α is highly elevated in the sputum samples of COPD patients, especially during exacerbations (Keatings et al., 1996; Aaron et al., 2001). It has been proved that monocytes, macrophages and CD8⁺ T cells are the main sources of TNF- α . TNF- α exhibits broad inflammatory effects, activating NF- κ B and p38 mitogen-activated protein kinases (MAPK) signaling pathways, and inducing the transcription of genes for inflammatory mediators in pulmonary epithelial cells, monocytes, macrophages and neutrophils. *In vitro*, IL-1 β significantly activates alveolar macrophages from patients with COPD, inducing expression of inflammatory cytokines, chemokines, and especially matrix metalloproteinases 9 (MMP9) (Russell et al., 2002; Culpitt et al., 2003). *In vivo*, though some studies have reported that IL-1 β has increased levels in patients with COPD (Casadevall et al., 2007; Doz et al., 2008), the significance of this correlation is still unclear. Moreover, Mahajan et al. (2008) found that serum IL-1 β levels were higher in patients with asthma than COPD, suggesting that it could be used as a biomarker to differentiate asthmatics from patients with COPD. Similar to TNF- α , IL-6 is also increased in patients with COPD and exacerbations (Bhowmik et

al., 2000; Barnes, 2004b and 2008b), and is mainly released by monocytes (Aldonyte et al., 2003). However, in addition to proinflammatory effects, IL-6 also exhibits anti-inflammatory functions: inhibiting TNF- α and IL-1 β and activating IL-10 expression. Thus the precise role of IL-6 in COPD should be examined by its overall effects on other inflammatory mediators. Another important cytokine is IFN- γ , which is mainly produced by T_H1 and T_C cells. IFN- γ activates STAT1/T-bet signaling pathway, inducing T_H1 cytokine expression and decreasing that of T_H2 cytokines (Szabo et al., 2002). IFN- γ also increases the expression of CXCR3 by T_H1 and T_C cells, boosting the infiltration of both lymphocytes into the airways (Barnes, 2004b).

Chemokines. Chemokines are a family of cytokines with low molecular weight that exhibit a broad spectrum of chemotactic effects. According to the differences in the spacing of their critical cysteine residues, chemokines are categorized into four groups, C, CC, CXC and CX3C chemokines. Some chemokines bind to single receptors, and others bind to more than one receptor on target cells (Gutierrez-Ramos et al., 2000). All of these chemokine receptors belong to GPCRs. During the pathogenesis of COPD, chemokines initiate the infiltration of inflammatory cells, such as monocytes, neutrophils, DCs and lymphocytes, and regulate their proliferation, differentiation and lifespan.

Among these chemokines, CCL2/MCP-1 and CXCL8/IL-8 are most intensely studied in the pathogenesis of COPD (de Boer et al., 2000). CCL2/MCP-1 levels are elevated in sputum, BAL and lung parenchyma of patients with COPD (Capelli et al., 1999; Traves et al., 2002). Alveolar macrophages, epithelial cells and T lymphocytes

are believed to be the main producers. CCL2/MCP-1 specifically binds to CCR2 and recruits monocytes to the site of inflammation. CXCL8/IL-8 is also found highly elevated in sputum samples of patients with COPD, especially during exacerbations (Yamamoto et al., 1997; Gompertz et al., 2001). CXCL8/IL-8 is synthesized and released by pulmonary epithelial cells, macrophages and neutrophils through different signaling pathways. A variety of stimuli, including TNF- α , IL-1 β , TLR ligands and oxidative stress, can all induce its expression (Barnes, 2004b). In contrast to CCL2/MCP-1, CXCL8/IL-8 can bind to both CXCR1 and CXCR2. CXCR2 has a higher affinity for CXCL8/IL-8 than CXCR1 and can be shared with some other CXC chemokines (Barnes, 2004b). Since CXCL8/IL-8 mainly induces the chemotaxis of monocytes and neutrophils, there is a correlation with CXCL8/IL-8 level and neutrophilic inflammation in the airways of patients with COPD (Hill et al., 2000; Patel et al., 2002). CXCL8/IL-8 activates both phosphoinositide 3 kinase (PI3K)/anti-apoptotic kinase (Akt) and Ras GTPases/MAPK signaling pathways, thus inducing expression of inflammatory mediators and proteases.

Another new chemokine that might be important in development of COPD is CCL20/ macrophage inflammatory protein 3 α (MIP-3 α). CCL20/MIP-3 α is a relatively new chemokines, first cloned by Hieshima et al. (1997). Interestingly, CCL20/MIP-3 α is located at chromosome 2q35-36, whereas other CC-chemokines usually map to chromosome 17q11-22. By analyzing the promoter region, several transcriptional binding sites are identified, including κ B, activator proteins (AP-1, AP-2), C-EBP, Sp1 and ESE-1 (Nelson et al., 2001; Schutyser et al., 2003).

Expression study indicates that CCL20/MIP-3 α is predominantly expressed in lymph nodes, appendix, PBL, fetal liver, and fetal lung; and in several cell lines, such as promyelocytic leukemia HL-60, HeLa cell S3, chronic myelogenous leukemia K-562, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW480, lung alveolar type II epithelial cell A549 and melanoma G361 cell lines (Rossi et al., 1997). It can be induced by lipopolysaccharide (LPS), TNF- α and IFN- γ , and down-regulated by IL-10. However, the signaling pathway that regulates its expression is not fully understood. CCL20/MIP-3 α is strongly chemotactic for DCs and lymphocytes, and has weaker effects on monocytes and neutrophils. Recently, Demedts et al. (2007) reported that CCL20/MIP-3 α mRNA levels in total lung and protein levels in sputum were significantly higher in patients with COPD, compared with non-smokers and smokers without COPD. Moreover, there are also significantly elevated numbers of DCs infiltrated into the epithelium and adventitia of small airways of patients with COPD; such increases correlate with the severity of the disease. CCR6, the only specific receptor for CCL20/MIP-3 α , is expressed on DCs isolated from human lung, as revealed by RT-PCR and flow cytometry (FCM) (Demedts et al., 2007). These data suggest that CCL20/MIP-3 α can bind to CCR6 on the DCs, mediating the migration of DCs to the lung during development of COPD. Bracke et al. (2006) demonstrated that cigarette smoke exposure induced an infiltration of innate and adaptive immune cells in the BAL of both CCR6 knockout mice and wild-type mice. However, the accumulation of DCs, granulocytes and T lymphocytes was significantly attenuated in the CCR6 KO mice,

compared with their wild-type littermates. The inflammatory responses and some important inflammatory mediators, such as CCL20/MIP-3 α , CCL2/MCP-1 and MMP-12 were also significantly attenuated in CCR6 knockout mice. Moreover, there is no effect on the development of airway remodeling and emphysema in CCR6 deficient mice upon chronic cigarette smoke exposure. In our study, we also found CCL20/MIP-3 α could be highly elevated in both pulmonary epithelial cells and monocytes through TLR2 or TLR4 signaling pathways. Thus future studies should focus on the interaction between CCL20/MIP-3 α and CCR6 in development of COPD, and its potential to be a therapeutic target.

Lipid mediators. Two essential lipid mediators involved in the pathogenesis of COPD are prostaglandin E2 (PGE₂) and leukotriene B4 (LTB₄). PGE₂ belongs to family of prostanoids and is mainly induced by cyclooxygenase (COX) during the inflammation. It has potential activities of both bronchoconstriction and bronchodilatation by binding to different receptors, thus is a critical regulator of local airway inflammation (Barnes, 2004b). When bound to prostaglandin receptor E2 (EP₂), PGE₂ inhibits the release of TNF- α from monocytes (Meja et al., 1997) and enhances the anti-inflammatory effects (Au et al., 1998). When bound to EP₄, it stimulates gastric mucus secretion (Takahashi et al., 1999). Although Pavord and Tattersfield (1995) claimed that PGE₂ had bronchoprotective effect and clinical data showed that there is an increase in the concentration of PGE₂ in exhaled breath of COPD patients (Montuschi et al., 2003), its role in pathogenesis of COPD needs to be updated. LTB₄ is a chemoattractant for neutrophils and lymphocytes. LTB₄ is elevated

in sputum and exhaled breath condensate of patients with stable COPD, and further elevated during exacerbations (Montuschi et al., 2003; Biernacki et al., 2003; Barnes, 2004b). LTB₄ binds to two receptors, BLT1 on neutrophils and BLT2 on T lymphocytes (Yokomizo et al., 1997 and 2000). Lee et al. (1999) found that blocking the LTB₄ receptor could inhibit LPS, GM-CSF, dexamethasone (DEX) and LTB₄-induced neutrophil survival, suggesting LTB₄ signaling pathway to be a potential therapeutic target for COPD.

Reactive oxygen species. Reactive oxygen species (ROS) are free radicals that contain the oxygen atom, and are found at high concentration in cigarette smoke and air pollution. Endogenous ROS can be produced from macrophages, neutrophils and epithelial cells (MacNee, 2001). Excessive ROS usually accumulate from long term exposure to cigarette smoke or air pollution and activated inflammatory cells, damaging the antioxidant defense system and causing oxidative stress. Such oxidative stress has multiple effects that contribute to the development of COPD. ROS can activate macrophages, neutrophils, pulmonary epithelial cells and lymphocytes through ERK and p38 MAPK signaling pathway, inducing the activation of transcriptional factors such as NF- κ B and AP1 (MacNee, 2001; Rahman and Adcock, 2006). Thus expression of several inflammation mediators, including CXCL8/IL-8, TNF- α , MMP-9 and elastase, would be increased. ROS directly damage DNA, RNA and proteins, inducing apoptosis in target cells (Haddad, 2004). Oxidative stress can also activate histone acetyltransferase (HAT), which opens up the chromatin structure and increases the transcription of multiple inflammatory genes (Rahman, 2003).

Several antioxidants have been used in clinical trials to study their therapeutic effects in treating COPD.

Proteases. The interaction between proteases and elastin has been identified for decades as a critical mechanism for the development of emphysema. Various proteases could break down elastin, the elastic protein in connective tissues, thus contributing to the loss of elasticity in the lung parenchyma and causing emphysema. Recently, more and more proteases have been found to degrade elastin, among which neutrophil elastase (NE) is the most important. NE is a serine protease secreted mainly by neutrophils during inflammation, which can be inhibited by α 1-antitrypsin (α 1-AT) (Ioachimescu and Stoller, 2005). Patients with inherited α 1-AT deficiency were shown to develop early-onset emphysema and NE/ α 1-AT complexes were found increased in BAL of COPD patients who are smokers (Yoshioka et al., 1995). NE can also induce the expression of some mucins (MUC5AC) and cytokines (CXCL8/IL-8) in airway epithelial cells (Barnes, 2004b). NE knockout mice showed tolerance against the cigarette smoke-induced emphysema and resulted in reduced neutrophil infiltration in the lungs (Shapiro et al., 2003).

Another important family of proteases is matrix metalloproteinases (MMPs). MMPs are zinc-dependent endopeptidases that aim to degrade extracellular matrix (ECM), regulate expression of inflammatory mediators, and cleave cell surface receptors to release some apoptotic ligands (Shapiro and Senior, 1999). MMP-1, MMP-9 and MMP-12 have more critical roles in the pathogenesis of COPD than other MMPs. Their levels are increased in the BAL and lung parenchyma of patients

with emphysema or COPD (Imai et al., 2001; Vernooij et al., 2004; Babusyte et al., 2007). Moreover, cigarette smoke-induced emphysema is prevented in MMP-12 knockout mice and correlated with decreased IL-13 and IFN- γ expression, as well as reduced monocyte infiltration (Zheng et al., 2000; Lanone et al., 2002). However, MMP-9 knockout mice only show protection against development of small airway fibrosis, but not against cigarette smoke-induced emphysema (Lanone et al., 2002). Overall, all these data suggest that MMP inhibitors, such as tissue inhibitor of metalloproteinases (TIMPs), and NE inhibitors might have clinical benefit for the emphysema and COPD treatments.

Growth factors. Growth factors, including transforming growth factor (TGF), epidermal growth factor (EGF), vascular-endothelial growth factor (VEGF) and fibroblast growth factors (FGF), mainly cause the structural remodeling in small airways and lung parenchyma to induce fibrosis. TGF- β is one of the most important growth factors excessively expressed during the development of COPD (de Boer et al., 1998; Takizawa et al., 2001). It is induced by oxidative stress or MMP-9, which would then activates the SMAD (vertebrate homologues of *Sma* and *Mad*) signaling pathway (Heldin et al., 1997). Transcriptional factors, such as NF- κ B, AP1 and PI3K/Akt are then activated, leading to the production of various ECM proteins and decreased degradation (Moore et al., 2008). However, TGF- β also has immunoregulatory functions that mediate the apoptosis of T lymphocytes or regulatory T cells (Tregs), leading to an anti-inflammatory effect (Hodge et al., 2003; Wan and Flavell, 2007).

Moraxella catarrhalis

Moraxella catarrhalis is a Gram-negative aerobic diplococcus, which was first identified in 1896 as *Mikrokokkus catarrhalis* (Karalus and Campagnari, 2000). However, its essential role as a pathogen in otitis media (OM) and lower respiratory tract infections was not seriously considered almost until this century. Because *M. catarrhalis* can be isolated from the oropharynx or nasopharynx of up to 5% of healthy adults, and such colonization does not cause any disease, it was originally thought to be a nonpathogenic commensal organism (Sethi and Murphy, 2001). Thus clinical studies have ignored potential involvement of *M. catarrhalis* in bacteria infections caused diseases for quite a long time. It was only two decades ago that the importance of *M. catarrhalis* as a major cause of the middle ear and respiratory tract infection was first confirmed (Murphy, 1996 and 1998). Now, it has been reported that *M. catarrhalis* is the third leading cause of COPD, falling behind *Streptococcus pneumoniae* and *Haemophilus influenzae* (Sethi and Murphy, 2001), and causes approximately 2 to 4 million exacerbations of COPD annually in the United States (Murphy et al., 2005).

***Moraxella catarrhalis* in COPD**

Since *M. catarrhalis* is a human-specific pathogen that does not cause any persistent infection in rats, mice, SCID mice, gerbils, chinchillas or adenovirus-compromised chinchillas, there is no successful animal model to examine its role in OM or COPD (Verduin et al., 2002). Among the few studies that were

conducted *in vivo*, the mouse pulmonary clearance model was used to test the efficacy of clearance (Unhanand et al., 1992; Chen et al., 1996; Murphy et al., 1998). But this instillation model involves surgically exposing the trachea and injecting *M. catarrhalis* through the tracheal wall, directly into the localized segment of the lung; this is difficult to do accurately and is time-consuming. To overcome these difficulties, Hu et al. (1999) introduced a new aerosol challenge mouse model, which generates an aerosol of *M. catarrhalis* suspension in a nebulizer and inhalation exposure system. However, in this model, *M. catarrhalis* is cleared by host within 48 hours post-inoculation, and does not cause any persistent infection or initiate disease that could mimic respiratory infection in human lung (Hu et al., 1999 and 2000; Jiao et al., 2002). Thus better animal models are still needed to study the role of *M. catarrhalis* in infectious diseases.

Although it has been estimated that *M. catarrhalis* colonizes 5 to 32% of patients with COPD at any time, significantly higher than the fraction in healthy adults (1 to 5%) (Pollard et al., 1986; Vaneechoutte et al., 1990a), little is known about its relationship to the pathogenesis of COPD or exacerbations. By following 81 COPD patients monthly and during exacerbations for totally 57 months, Sethi et al. (2002) have first demonstrated that acquisition of a new strain of *H. influenzae*, *S. pneumoniae*, or *M. catarrhalis* is associated with the occurrence of an exacerbation. During the same year, Bakri et al. reported that from the sputum and serum samples from 21 patients with exacerbations associated with *M. catarrhalis*, they successfully detected serum IgG and sputum IgA specific to antigens on the bacterial surface

(Bakri et al., 2002). Based on these studies, Murphy et al. (2005) measured the frequency and duration of *M. catarrhalis* carriage and the systemic and mucosal antibody responses in serum and sputum samples to homologous infecting strains. Furthermore, they excluded the possibility of alternative causes of other bacterial pathogens. By comparing the colonization patterns with the clinical symptoms of COPD, they estimated that about 10% of 560 exacerbations were likely caused by *M. catarrhalis* (Murphy et al., 2005). Interestingly, they found that all patients with COPD can clear *M. catarrhalis* from the respiratory tract efficiently (median, 42 days); while other pathogens, such as *H. influenzae*, usually colonize the host for much longer. Thus, more efforts should be made to understand which host defensive mechanism is involved in clearing *M. catarrhalis* and prevent its reacquisition.

Currently, influenza and pneumococcal vaccinations are recommended by the Advisory Committee on Immunization Practices for persons with COPD. Such vaccinations can yield substantial health benefits to persons with chronic lung disease, associated with reduction in the rate of outpatient visits, hospitalization, and death, while also saving money. However, due to the lack of animal model, little is known about the potential protective antigens of *M. catarrhalis* and no vaccine is developed yet to prevent its infection. Moreover, 90% of *M. catarrhalis* strains express β -lactamases, making them resistant to penicillin-based antibiotics (Karalus and Campagnari, 2000). Inappropriate antibiotic treatment contributes to the worldwide emergence of antibiotic-resistant strains of *M. catarrhalis* over the past decades. Therefore our study, based on an understanding of the molecular pathogenesis of *M.*

catarrhalis infections will be helpful for the development of alternative therapeutic strategies.

Potential Virulence Factors

As an extracellular pathogen of the respiratory tract, virulence of *M. catarrhalis* is determined by its ability to adhere and colonize to the host mucosal surface (Verduin et al., 2002). Some molecules such as adhesins and serum resistance that contribute to virulence of the pathogen are called virulence factors. They are usually expressed on the outer membrane of the pathogen, or might be conserved among different strains, and can be recognized by the host immune systems to induce strong immune responses. Thus, these virulence factors would be the potential antigens for vaccine development. Several virulence factors of *M. catarrhalis* have been identified, such as lipooligosaccharide (LOS), UspA1 (Lafontaine et al., 2000; Hill and Virji, 2003), UspA2 (Cope et al., 1999; Meier et al., 2002), McaP (Timpe et al., 2003) and Hag (Pearson et al., 2002; Holm et al., 2003). Different with most other Gram-negative bacteria, which express lipopolysaccharide (LPS), LOS is the most abundant molecule on the outer membrane of *M. catarrhalis*. LOS also consists of core oligosaccharide and lipid A domains, but lacks saccharide side chains. 95% of all *M. catarrhalis* strains contain LOS in three serotypes: A (61%), B (29%), and C (5%) (Vaneechoutte et al., 1990b). The *lpxA* gene was found essential for the biosynthesis of lipid A in *M. catarrhalis*. The *lpxA* mutant strain showed complete loss of LOS, exhibiting reduced adherence to epithelial cells, decreased toxicity and increased clearance in mice (Peng et al., 2005). Conjugated detoxified LOS triggered immune

responses in both mice and rabbits (Gu et al., 1998; Hu et al., 2000; Jiao et al., 2002). While specific antibodies to LOS of *M. catarrhalis* could be detected in patients with COPD (Sethi and Murphy, 2001), such antibodies only modulate weakly responses against heterologous serotypes (Jiao et al., 2002; Yu and Gu, 2005).

Toll-like Receptors

Bacterial attachment to the respiratory mucosa is a crucial first step towards colonization of the human epithelium and therefore the development of COPD or exacerbations (Hippenstiel et al., 2006). As a result, it is important to study the molecular mechanisms that allow pulmonary epithelial cells to recognize the potential pathogens and modulate protective responses of innate immunity. As a primary interface between the internal body and the external environment, the respiratory epithelium not only provides a passive barrier function, but also actively contributes to the innate immune system (Bals et al., 2004). The key early event in local immune activation is the loss of epithelial barrier integrity, leading to increased exposure of resident cells to both pathogenic and non-pathogenic microbes. Most of pathogens have distinctive biological macromolecules present on their surface called pathogen associated molecular patterns (PAMPs). Cells of the innate immune system, including macrophages, DCs and epithelial cells, depend on germline-encoded pattern recognition receptors (PRRs) to bind to PAMPs (Janeway and Medzhitov, 2002). Cellular PRRs belong to different functional and structural groups, which include the Toll-like receptors (TLRs), NOD-like receptors (NODs), scavenger receptors, and

lectin receptors (Gordon, 2002).

Introduction

During the last decade, TLRs are becoming the most intensely studied PRRs. *Toll* gene was first described in *Drosophila*, which encodes a receptor critical for dorsoventral polarization and antimicrobial resistance (Anderson et al., 1985; Hashimoto et al., 1988). As the vertebrate counterparts of Toll in *Drosophila*, thirteen TLRs (TLR1 to TLR13) have been identified in mammalian species, and TLR11 to TLR13 are expressed in mice but not in humans (Takeda et al., 2003). The TLRs are type I integral membrane glycoproteins, which contain a single spanning transmembrane domain with an intracellular C-terminal portion and an extracellular N-terminal portion. TLRs and interleukin-1 receptors (IL-1Rs) belong to TLR/IL-1R superfamily because they share a conserved cytoplasmic Toll/IL-1R (TIR) domain, which is characterized by three highly homologous regions (known as boxes 1, 2 and 3) (Slack et al., 2000). However, TLRs have tandem repeats of leucine-rich repeats (LRRs) in their extracellular domain, whereas IL-1Rs have three immunoglobulin-like domains (Akira and Takeda, 2004).

Of ten members (TLR1 to TLR10) expressed in human, TLRs are divided into five subfamilies: TLR2 (TLR1, 2, 6, and 10), TLR3, TLR4, TLR5, and TLR9 (TLR7, 8, and 9) (Medzhitov, 2001; Takeda et al., 2003). The specific ligands for each of these TLRs are listed in Table 1. Depending on molecular patterns of their ligands, TLRs have different subcellular localizations. TLR2, TLR4 and TLR5 mainly express on the cell surface, whereas TLR3, TLR7 and TLR9 express intracellularly, requiring

Table 1. Toll-like receptors and their ligands.

| Receptor | Ligand | Origin of ligand |
|----------|---|---|
| TLR1 | Triacyl lipopeptides Soluble factors | Bacteria and mycobacteria <i>Neisseria meningitidis</i> |
| TLR2 | Lipoprotein/lipopeptides Peptidoglycan Lipoteichoic acid Lipoarabinomannan Phenol-soluble modulins Glycoinositolphospholipids Glycolipids Porins Atypical lipopolysaccharide Atypical lipopolysaccharide Zymosan Heat-shock protein 70 | Various pathogens Gram-positive bacteria Gram-positive bacteria Mycobacteria <i>Staphylococcus epidermidis</i> <i>Trypanosoma cruzi</i> <i>Treponema maltophilum</i> <i>Neisseria</i> <i>Leptospira interrogans</i> <i>Porphyromonas gingivalis</i> Fungi Host |
| TLR3 | Double-stranded RNA | Viruses |
| TLR4 | Lipopolysaccharide Taxol Fusion protein Envelope protein Heat-shock protein 60 Heat-shock protein 70 Type III domain A of fibronectin Oligosaccharides of hyaluronic acid Polysaccharide of heparan sulphate Fibrinogen | Gram-negative bacteria Plants Respiratory syncytial virus Mouse mammary-tumour virus <i>Chlamydia pneumoniae</i> Host Host Host Host Host |
| TLR5 | Flagellin | Bacteria |
| TLR6 | Diacyl lipopeptides Lipoteichoic acid Zymosan | <i>Mycoplasma</i> Gram-positive bacteria Fungi |
| TLR7 | Imidazoquinoline Loxoribine Bropirimine Single-stranded RNA | Synthetic compounds Synthetic compounds Synthetic compounds Viruses |
| TLR8 | Imidazoquinoline Single-stranded RNA | Synthetic compounds Viruses |
| TLR9 | CpG-containing DNA | Bacteria and viruses |
| TLR10 | N.D. | N.D. |

(Adapted from Akira and Takeda, 2004 and modified)

endosomal maturation in the signaling process. For most of the Gram-negative bacteria, TLR2 and TLR4 are the most important PRRs.

TLR2

In the TLR2 subfamily, TLR2 dimerizes or associates with TLR1 or TLR6 to recognize a wide variety of PAMPs, including lipopeptides or lipopeptides, peptidoglycan (PDG) and lipoteichoic acids (LTA) (Takeda et al., 2003). TLR2-deficient mice have higher susceptibility to bacteria infections (Takeuchi et al., 1999a and 2000). TLR1 and TLR6 are 69.3% identical in overall amino acid sequence; especially their TIR domains are highly conserved, with over 90% identity (Takeda et al., 2003). *Tlr1* and *Tlr6* locate very closely on chromosome 4p14, both consisting of one exon (Takeuchi et al., 1999b); therefore they might be the products of an evolutionary duplication. As shown by co-immunoprecipitation, both TLR1 and TLR6 physically interact with TLR2 in the cells (Ozinsky et al., 2000). Takeuchi et al. (2001) demonstrated that macrophages from TLR6-deficient mice did not show any inflammatory response to mycoplasmal macrophage-activating lipopeptide 2 (MALP-2), but responded normally to other bacterial lipopeptides. Macrophages from TLR2-deficient mice showed no response to either type of lipopeptide (Takeuchi et al., 2001). The same group also revealed the role of TLR1, showing that macrophages from TLR1 knockout mice had impaired response to a native mycobacterial 19-kDa lipoprotein and a synthetic triacylated lipopeptides but responded normally to diacylated lipopeptides (Takeuchi et al., 2002). Moreover, co-expression of TLR1 and TLR2 can enhance the synthetic lipopeptides-induced NF- κ B activation. These data

suggest that TLR1 or TLR6 might be necessary in recognition of distinctive ligands, but such function could be fulfilled only in the presence of TLR2. On the other hand, TLR1 or TLR6 may also compensate for each other in some cases, such as in the recognition of OspA of *Borrelia burgdorferi* (Alexopoulou et al., 2002). However, it is unknown whether such dimerization of TLR2 with TLR1 or TLR6 is constitutive or induced by stimulation.

TLR4

The involvement of TLR4 in LPS recognition has first been proved as *Tlr4* mutation was identified in two LPS hyporesponsive mice. Both mouse strains, C3H/HeJ and C57BL10/ScCr, are highly resistant to LPS and more susceptible to Gram-negative infections. It was originally thought to be a mutation of the *Lps* allele (*Lpsd/d*) that confers such effects, which was later confined to be interval spanning in three transcription units including *Tlr4* (Qureshi et al., 1999). The C3H/HeJ mouse strain has a point mutation in the third exon of the *Tlr4*, predicted to replace a highly conserved proline at position 712 with histidine (Poltorak et al., 1998; Qureshi et al., 1999). C57BL/10ScCr mice were identified to be homozygous for a null mutation of *Tlr4* (Poltorak et al., 1998; Qureshi et al., 1999). In addition, TLR4 knockout mice also exhibit defects in response to LPS (Hoshino et al., 1999). All these data suggest that TLR4 is an essential PRR for the recognition of LPS.

MD2. Overexpressing TLR4 in human embryonic kidney (HEK293) cells, that do not normally express TLR4, did not confer LPS-induced activation (Kirschning et al., 1998). This result indicated that some factors or subunits are required for LPS

signaling through TLR4. A year later, MD2 was first identified to be the key molecule that associates with the extracellular domain of TLR4 and modulates the LPS induced responses. Miyake et al. (1998) first cloned RP105, a LRR molecule expressed on lymphocytes and physically associated with MD1. Because the extracellular LRR of RP105 is very similar to *Drosophila* Toll, they hypothesized that there might be a homolog of MD1 that could associate with human TLRs (Miyake et al., 1998; Miura et al., 1998). By comparing the MD1 amino acid sequence to human cDNA clones, they finally located a new accessory protein, which shares 23% identity with MD1, and estimated that MD2 physically forms a complex with TLR4 on cell surface (Shimazu et al., 1999). Such TLR4/MD2 complexes efficiently mediated bacterial endotoxin induced responses. Loss of function assays further confirmed that a point mutation of MD2 in a highly conserved region (C95Y) cause hyporesponse to LPS stimulation, which can be complemented by transfecting wild type MD2 (Schromm et al., 2001). Different cells isolated from MD2-deficient mice exhibited impaired responses to LPS. Also, MD2-deficient mice are resistant to LPS-induced endotoxin shock, similar to TLR4-deficient mice (Nagai et al., 2002). Individual MD2 mainly consists of two forms: a 10 to 15kDa monomer with seven Cys residues and two N-linked glycosylation sites; and a 20 to 25kDa multimers with differentially glycosylated forms (Ohnishi et al., 2001; da Silva Correia and Ulevitch, 2002). MD2 was shown to bind to TLR4 in the endoplasmic reticulum (ER)/cis-Golgi, and then be transported to the cell surface, where soluble MD2 is secreted from TLR4/MD2 complex (Visintin et al., 2000). More importantly, secreted monomeric MD2 exists as

a heterogeneous collection of disulfide linked oligomers, which confers LPS responsiveness on those cells with only TLR4 expression on the cell surface (Mullen et al., 2003). Thus MD2 is an accessory mediator required for LPS-induced responses in TLR4 signaling pathway. Secreted MD2 could serve as a soluble receptor to activate TLR4-mediated responses on cells that do not express MD2.

CD14. LTA, PGN or LPS binding to CD14 is the key early event of TLR2 or TLR4/MD2 signaling. CD14 was first described in late 1980s as a differentiation antigen of monocytes (Goyert et al., 1988). Since Wright et al. (1989 and 1990a) found that CD18 deficient cells could still respond to LPS, they identified a new receptor as CD14, which binds with LPS and LPS binding protein (LBP) complex (Wright et al., 1990b). LBP is a trace plasma protein that binds to the lipid A moiety of LPS in an acute phase reactant, increasing the sensitivity of cells to LPS (Tobias et al., 1986; Schumann et al., 1990). LBP binds with the individual LPS molecules and then transfer the ligands to CD14 on the cell surface. CD14 is a glycoprotein with 356 amino acids, encoded on chromosome 5q 23-31 (Goyert et al., 1988). It is mainly expressed on myeloid cells (monocytes, macrophages and neutrophils) in two forms: a membrane-bound glycosylphosphatidylinositol (GPI)-anchored protein (mCD14) and a soluble proteolytic fragment (sCD14) lacking the GPI anchor (Haziot et al. 1988; Simmons et al. 1989; Frey et al. 1992).

Two soluble forms of CD14 are constitutively generated: the 55kDa form is directly secreted without GPI anchor or shredded by phospholipase from mCD14; the 49kDa form is cleaved from cell membrane by proteolytic cleavage through a serine

protease (Bazil et al., 1989; Bufler et al., 1995; Kirkland and Viriyakosol, 1998). To confirm its critical role in mediating LPS-induced responses, transgenic mice overexpressed human CD14 exhibited hypersensitive to LPS (Ferrero et al., 1993); whereas CD14 deficient mice are highly resistant to LPS challenge (Haziot et al., 1996). Also, other bacterial membrane components, such as PGN and LTA, were later identified to be the ligands of CD14 (Pugin et al., 1994; Kusunoki et al., 1995; Gupta et al., 1996). It was believed that these ligands have different binding sites for LPS, and do not need to be aided by LBP (Landmann et al., 2000). Moreover, sCD14 could mediate the endotoxin-induced responses on the cells that do not express CD14, such as endothelial and epithelial cells (Frey et al., 1992; Haziot et al., 1993; Pugin et al., 1993a,b). However, during that time, people did not know how CD14 could initiate an intracellular signaling event because it lacks a transmembrane domain. With the discovery of TLRs, TLR2 and TLR4/MD2 have been proved to form co-receptor complex with CD14 for the signaling entity (Landmann et al., 2000). CD14 has a high leucine content (17.7% in human CD14 and 15.5% in murine CD14), which contains 10 LRRs that shares the similar structure with 21 LRRs on extracellular domain of TLRs (Ferrero et al., 1990; Kim et al. 2005). This region is the potential binding site of CD14 and TLR2 or TLR4/MD2, thus would activate the intracellular signaling. CD14 enhances TLR2 or TLR4/MD2 mediated inflammatory responses, characterized by release of the inflammation mediators and activation of transcriptional factors (Yang et al., 1998; Kirschning et al., 1998; Jiang et al., 2000).

TLR Signaling

After binding with their ligands, TLRs undergo dimerization and conformational change that recruit the downstream signaling molecules to associate with the TIR domain. These TIR-domain-containing adaptors include myeloid differentiation factor 88 (MyD88), TIR domain-containing adaptor protein (TIRAP; also known as MyD88-adaptor-like protein, MAL), TLR-associated activator of interferon (TRIF), TRIF-related adaptor molecule (TRAM; also known as TIR-domain-containing molecule 2, TICAM2) (Barton and Medzhitov, 2003; Akira, 2003). Depending on the involvement of MyD88, these different downstream signaling pathways are categorized into two major pathways: MyD88-dependent and MyD88-independent/TRIF-dependent pathway (McGettrick and O'Neill, 2004; Akira and Takeda, 2004). MyD88-dependent pathway is shared by TLR2, 4, 5 and 9 subfamilies, mainly activating IL-1R-associated kinases (IRAKs) and TNF-receptor-associated factor 6 (TRAF6). By contrast, MyD88-independent/TRIF-dependent pathway is only shared by TLR3 and TLR4, leading to the activation of IFN-regulatory factor 3 (IRF3) through TRIF or TRAM (Akira and Takeda, 2004).

MyD88-dependent signaling pathway. MyD88 was first identified as a novel myeloid differentiation primary response gene that is activated in IL-6-stimulated myeloid precursor cells and induce the terminal differentiation into macrophages (Lord et al., 1990). MyD88 contains an amino (N)-terminal death domain (DD) and a carboxy (C)-terminal TIR domain, which are linked by an intermediary domain (ID). It was later found to be an adaptor protein, which recruits IRAKs to the TLRs or IL-1R complex following stimulation (Muzio et al., 1997; Dunne et al., 2003).

MyD88 forms a homodimer when recruited to the receptor complex, and associates with IRAKs through a DD-DD interaction (Dunne et al., 2003). Four IRAKs (IRAK1, IRAK2, IRAK4 and IRAK-M) have been identified in mammals, each containing an N-terminal DD and a central serine/threonine-kinase domain (Janssens and Beyaert, 2003). IRAK4 seems to be first recruited to MyD88 and subsequently mediates phosphorylation of IRAK1 (Li et al., 2002). It has been shown that IRAK1-deficient mice showed only decreased responses to IL-1 β or other TLRs ligands (Swantek et al., 2000), whereas IRAK4-deficient mice showed no response (Suzuki et al., 2002). Thus IRAK4 functions upstream of IRAK1 and both are required for TLR signaling. TRAF6 is also recruited to the receptor complex, by associating with phosphorylated IRAK1. TRAF6 was identified as a new member of TRAF family, which involved in mediating IL-1 signaling (Cao et al., 1996). TRAF family proteins are constituted with an N-terminal zinc-binding domain (containing a RING finger followed by several zinc fingers), a coiled-coil domain (TRAF-N) and a highly conserved C-terminal domain (TRAF-C) (Bradley and Pober, 2001). The zinc-binding domain is essential for activation of downstream signalings, whereas the TRAF domains (both TRAF-N and TRAF-C) mediate self-association and interactions with upstream receptors and other signaling molecules. The consensus sequence Pro-X-Glu-X-X-(aromatic/acidic residue) has been identified as the TRAF6-binding motif, which is also found in IRAKs (Ye et al., 2002). Phosphorylated IRAK1 and TRAF6 then dissociate from the receptor and form a complex with TGF- β activated kinase 1 (TAK1) and two TAK1-binding proteins (TAB1 and 2) at the plasma

membrane (Shibuya et al., 1996; Wang et al., 2001; Takaesu et al., 2000). TAK1 is a MAPK kinase kinase (M3K), which has been shown to be essential for TLR/IL-1R-induced NF- κ B activation (Takaesu et al., 2003). TAB2 plays an important role in facilitating the binding of TAK1 and TRAF6, whereas TAB1 enhances the kinase activity of TAK1 (Wang et al., 2001; Takaesu et al., 2000). After the degradation of IRAK1 at the plasma membrane, the remaining complex translocates to the cytosol and recruits two ubiquitin ligases: ubiquitin conjugating enzyme 13 (UBC13) and ubiquitin-conjugating enzyme E2 variant 1 (UEV1A) (Deng et al., 2000). Two enzymes induce the ubiquitylation of TRAF6, thus leading to the phosphorylation and of TAK1. Activated TAK1 phosphorylates MKK3/6 or MKK4/7, inducing the activation of MAPK p38 or c-Jun N-terminal kinase (JNK), respectively (Wang et al., 2001). In addition, TAK1 phosphorylates the inhibitor of NF- κ B (I κ B) kinase (IKK) complex, which consists of IKK- α , IKK- β and IKK- γ . Activated IKK complex induces phosphorylation of I κ B α , following by ubiquitylation and subsequent degradation. This allows the release of κ B subunit p50/p65(RelA) to migrate to the nucleus and induce target genes expression (Karin and Ben-Neriah, 2000). In MyD88-dependent pathway, a second TIR-domain-containing adaptor protein TIRAP was found involved in TLR2 and TLR4 signaling pathway (Horng et al., 2001; Fitzgerald et al., 2001). Unlike MyD88, TIRAP does not have a DD and may act upstream of MyD88 (Horng et al., 2002).

MyD88-independent signaling pathway. It was found that MyD88 knockout mice lack the ability to respond to LPS and cytokines produced by macrophages were

diminished. However, both NF- κ B and MAPK can still be activated, only with delayed kinetics (Kawai et al., 1999). Moreover, LPS-induced TNF- α , IL-6 and IL-1 β expressions were abolished in MyD88-deficient macrophages; while some IFN-regulated factors, such as CXCL10/IP10, was induced by LPS, but not TLR2 ligands (Kawai et al., 2001). This evidence suggests the existence of a MyD88-independent signaling pathway. Later, it was proved that TRIF is the essential adaptor in the TLR3- and TLR4-mediated MyD88-independent pathway (Yamamoto et al., 2003a; Hoebe et al., 2003). TRIF is another TIR domain-containing adapter, which mainly binds with TLR3 or TLR4 and regulates the expression of IFN- β (Yamamoto et al., 2002; Oshiumi et al., 2003). TRIF recruits and activates TRAF6, thus inducing the activation of NF- κ B pathway. Also, TRIF activates two kinases, IKK- ϵ and TBK1 (TRAF-family-member associated NF- κ B activator (TANK)-binding kinase 1), which phosphorylate IFN-regulatory factor 3 (IRF3) (Sharma et al., 2003; Fitzgerald et al., 2003a). Activated IRF3 forms homodimers and translocates to the nucleus, recruiting the co-activators p300 and CBP (cAMP responsive element binding protein (CREB)-binding protein) to initiate the transcription of the type I IFN. Then released type I IFNs (mainly IFN- β) activate several IFN-inducible genes through JAK (Janus activated kinase)-STAT (signal transducer and activator of transcription) signaling pathway (Akira and Takeda, 2004). It seems that this TRIF-dependent signaling pathway is dominant in TLR3-mediated responses, whereas TLR4 depend on both MyD88-dependent and –independent pathways. Also, TLR4 employs a specific TIR domain-containing adaptor, TRAM, to

facilitate the association of TRIF (Yamamoto et al., 2003b; Fitzgerald et al., 2003b).

Figure 1. Toll-like receptor signaling.

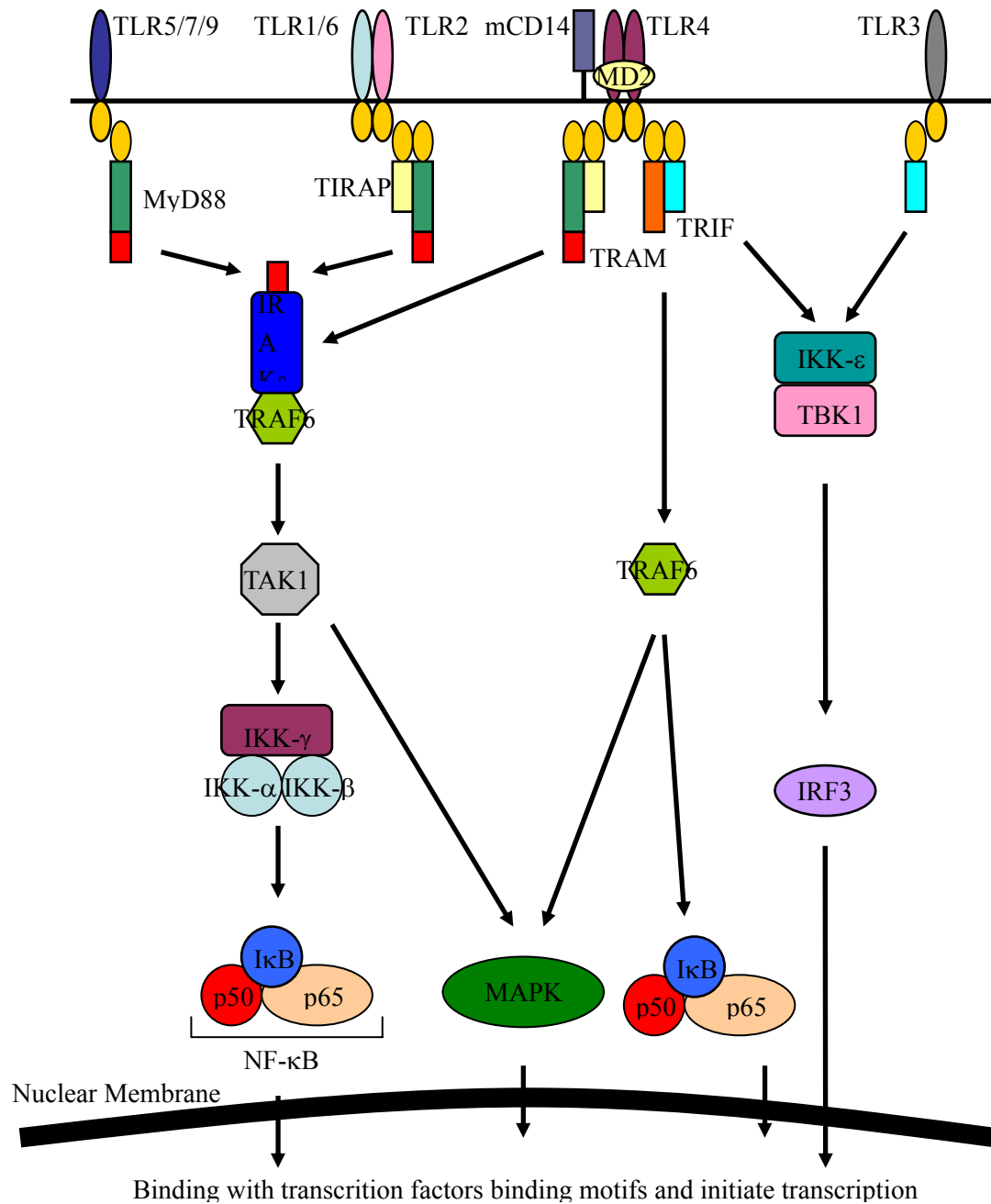


Figure 1. TLR2, 4, 5 and 9 subfamilies share the MyD88-dependent pathway, which mainly through MyD88 and TRAF6 to induce the activation of NF- κ B and MAPK. By contrast, TLR3 and TLR4 share the MyD88-independent/TRIF-dependent pathway, which activate IRF3 to induce the type I IFN expression. (Adapted from Akira and Takeda, 2004 and modified).

MATERIALS AND METHODS

Reagents and Antibodies

E. coli (0111:B4) ultra pure LPS and Pam₃CSK₄ were purchased from InvivoGen (San Diego, CA). Phospho-IκBα (Ser32/36) (5A5) antibody and total IκBα antibody were purchased from Cell Signaling Technology (Beverly, MA). Goat anti-mouse IgG-HRP and goat anti-rabbit IgG-HRP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Transfection reagent FuGENE 6 was purchased from Roche (Indianapolis, IN). Inhibitors MG-132, BAY11-7082, SP600125, SB203580, U0126 and LY294002 were purchased from Calbiochem (San Diego, CA). MD2 antibody and FITC conjugated antibody: TLR2-FITC and TLR4-FITC were purchased from (Imgenex, San Diego, CA). CD14-FITC antibody was purchased from (BD Sciences, San Jose, CA). TLR2, TLR4, CD14 and isotype control antibodies were purchased from eBioscience (San Diego, CA). 1α, 25-dihydroxy VD₃ and β-actin antibody was from Sigma (Saint Louis, MO).

Cell Culture and Bacterial Strain

The human lung epithelial A549 cells (ATCC CCL-185) were maintained in Ham's F12 (Cellgrow, Mediatech Inc, Hemdon, VA); human bronchial epithelial BEAS-2B cells (ATCC CRL-9609) were maintained in Keratinocyte-SFM (Gibco-BRL, Invitrogen, Carlsbad, CA); and the naïve monocytic THP1 cells (ATCC TIB-202) were maintained in RPMI 1640 (Gibco-BRL, Invitrogen, Carlsbad, CA). All

media were supplemented with 10% FBS and 100 U/ml penicillin, and 100 µg/ml streptomycin. HEK 293 cells stable transfected with plasmid encoding TLR2, TLR4 was selected and maintained in DMEM with G418, empty vector cDNA3.1 was used as control. HEK293 cells stable transfected with TLR4/MD2/CD14 was selected and maintained in DMEM with blasticidin and hygromycin.

M. catarrhalis wild-type strain O35E was kindly provided by Dr. Eric Lafontaine (University of Georgia, Athens, GA). For infection experiments, *M. catarrhalis* strain was grown overnight at 37°C on Todd Hewitt agar (BD Sciences, San Jose, CA). Bacteria were suspended in PBS and adjusted to an optical density (OD) at 600nm of 0.750 [$\cong 10^9$ colony-forming units (cfu)/ml]. The tissue culture medium was replaced with medium lacking antibiotics, and the indicated multiplicity of infection (MOI) of *M. catarrhalis* was added to cultured cells. The microplates were centrifuged at 165g for 5min to ensure bacteria contact cells.

Real-time PCR

A549 or BEAS-2B cells were plated at a density of 1.5×10^5 per well of a 24 well plate overnight. After stimulation with indicated time, total RNA was extracted from the cells with TRIzol reagent (Invitrogen, Carlsbad, CA) and removed the contaminant DNA by DNA-free kit (Ambion, Austin, TX). cDNA was then synthesized by Omniscript RT kit (Qiagen, Hantsworth, CA), according to the manufacturer's instructions. Real-time PCR was carried out using SYBR Green 1, (Molecular Probes, Eugene, Oregon) master mix containing hot start Taq and

dNTPs (Denville Scientific Inc., Metuchen, NJ), as well as gene-specific primers in the ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA). The primer sequences were as follows: GAPDH: forward 5'-GGGAAGGTGAAGGTCGGAGT-3', reverse 5'-TCCACTTTACCAGAGTTAAAAGCAG-3'; CCL20/MIP3 α : forward 5'-TCCACTTTACCAGAGTTAAAAGCAG-3', reverse: 5'AAGTTGCTTGCTGCTTCTGATTCG-3' (IDT, Coralville, IA). At the end of each reaction, melting curve analysis was performed to confirm the amplified product was specific. The cycle threshold (Ct) value was calculated from amplification plots, and gene expression was normalized using the Ct of the housekeeping gene GAPDH. Relative quantity (fold induction) of the target gene mRNA was then calculated using the comparative Ct method ($2^{-\Delta\Delta Ct}$).

ELISA

After stimulation, culture media at indicated time points were collected and centrifuged at 3,000xg for 5 min. Supernatants were then processed to measure the secreted CCL20/MIP-3 α , IL-8 and soluble CD14 levels by ELISA (R&D systems, Minneapolis, MN), according to the manufacturer's instructions.

Western Blotting

The cells were washed twice with PBS and then treated with 100 μ L of lysis buffer for 15 min on ice. After centrifuging the cell lysate at 12,000xg for 15 min, discarded the cell pellet and added 5x SDS buffer to the supernatant. Equal amounts

of cell lysate were loaded in each lane and separated by SDS-polyacrylamide gel (10%) electrophoresis. Fractionated proteins were blotted onto a nitrocellulose membrane. Blots were probed with specific primary antibodies overnight followed by respective horseradish peroxidase-labeled secondary antibody, and then visualized by SuperSignal West Pico kit (Pierce, Rockford, IL). For the total I κ B α detection, the probed membrane was stripped and re-probed with anti-I κ B α or anti- β -actin antibody to detect each expression.

Plasmids and Dual Luciferase Activity Assay

The reporter construct pNF- κ B-Luc was kindly gifted by Dr. JD Li. It contains three copies of NF- κ B binding site present in the promoter linked to the firefly luciferase gene. pRL-TK was purchased from Promega (Madison WI). The cDNAs encoding the dominant negative mutants of MyD88 or TRAF-6 were cloned into the mammalian expression vector pcDNA3.1. The cells were co-transfected with pNF- κ B-Luc and pRL-TK for 24 h. In some experiments, pDN-MyD88 or pDN-TRAF-6 was also transfected, empty vector pcDNA3.1 was used as control. The cells were stimulated for 4 h, then collected and dual luciferase activities were determined by Dual Luciferase Assay kit (Promega) on a Monolight 3010 luminometer (Analytical Luminescence). All luciferase activity was normalized to *Renilla* luciferase activity and relative fold induction was calculated.

Flow Cytometry

TLR2-FITC, TLR4-FITC and CD14-FITC antibody was used to detect TLR2, TLR4 or membrane-bound CD14 on THP1 cells and both pulmonary epithelial cells. FITC conjugated isotype antibody was used as control. Samples were measured in a FACScan instrument (BD Biosciences, San Diego, CA) and the data were analyzed using CellQuest software. Dead cells were identified by propidium iodide (PI) staining and excluded from the determinations.

Statistical analysis

Data are shown as mean and the standard error of the mean of at least three independent experiments or as indicated. Statistical analyses were performed with Student's *t*-test; statistical significance was accepted at a $P < 0.05$ indicated by asterisks.

RESULTS

***Moraxella catarrhalis* induces CCL20/MIP-3 α expression in human lung epithelial cells**

Following bacteria infection, pulmonary epithelial cells can initiate inflammatory responses, by secreting chemokines and cytokines into the submucosa. This plays an important role in the pathogenesis of COPD (Diamond et al., 2000). As a highly induced chemokine in human lung responding to PAMPs, CCL20/MIP-3 α is an important chemoattractant of lymphocytes and DCs, and induces further adaptive immunity (Schutyser et al., 2003). To study host-pathogen interactions between *M. catarrhalis* and human airway epithelial cells *in vitro*, A549 and BEAS-2B cell lines were used as the cell culture model. Incubating the A549 and BEAS-2B cells with *M. catarrhalis* induced CCL20/MIP-3 α mRNA expression in a time- and dose-dependent manner (Fig. 2A and 2B). One hour post-infection, there were significantly increased mRNA levels in both cell lines, reaching the peak level at 3 h and decreasing thereafter. These data confirmed that *ccl20* is an early response gene in bacterially induced inflammatory responses (Sugita et al., 2002) and that *M. catarrhalis* induces such responses. The expression of CCL20/MIP-3 α was also measured at the protein level by ELISA. Similar expression pattern was found between A549 and BEAS-2B cells (Fig. 2C and 2D): release of CCL20/MIP-3 α reached the peak level around 12 h post infection and decreased at 24 hours.

Figure 2. *M. catarrhalis*-induced CCL20/MIP-3 α expression in A549 and BEAS-2B cells.

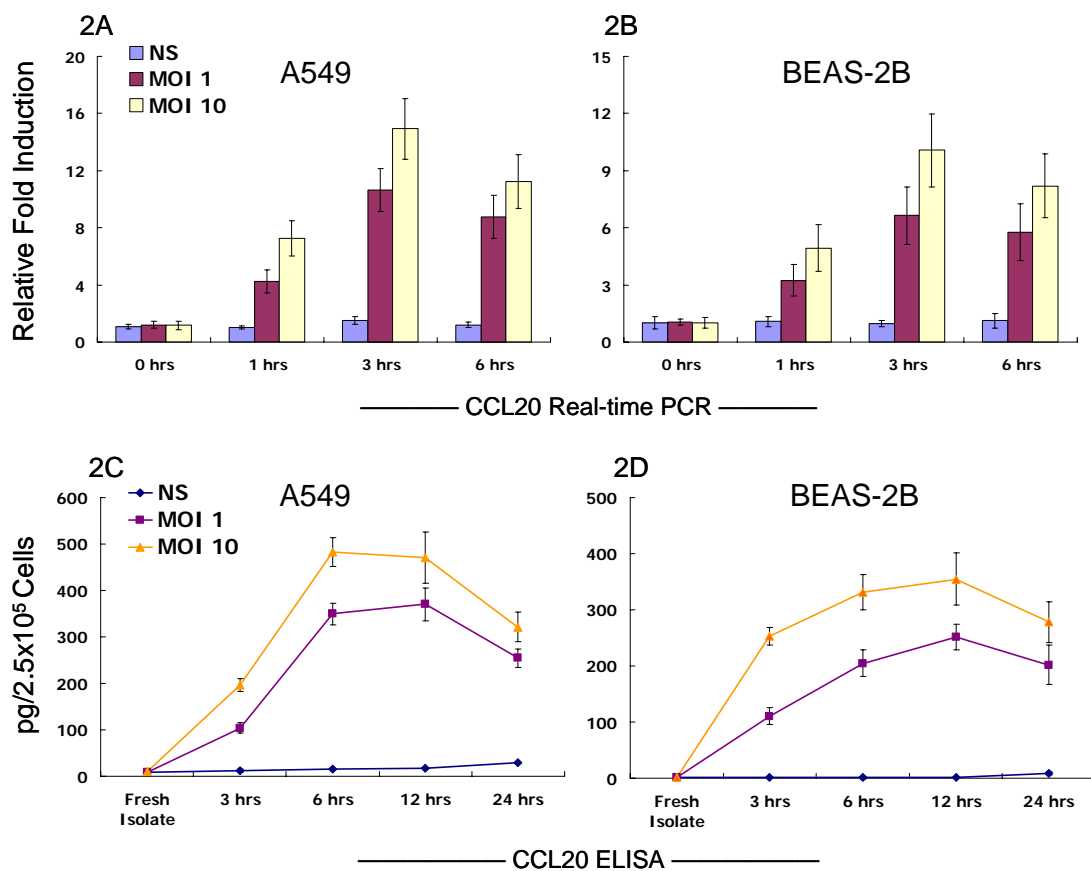


Figure 2. A549 (2A) or BEAS-2B (2B) cells were incubated with different MOI (0, 1, 10) of *M. catarrhalis* strain O35E at the indicated times. Total RNA was extracted and CCL20/MIP-3 α mRNA levels were detected by Real-time PCR. Supernatants of A549 (2C) and BEAS-2B cells (2D) were collected and CCL20/MIP-3 α release were measured by ELISA. Data presented are the mean and the standard error of the mean of four independent experiments.

***Moraxella catarrhalis* induces NF- κ B activation in human pulmonary epithelial cells**

In inflammatory responses, NF- κ B is a key transcription factor, regulating the expression of most of inflammation-related genes. Activation of NF- κ B is initiated by phosphorylation of I κ B α , which is an important member of the I κ B inhibitor protein

Figure 3. *M. catarrhalis*-induced NF- κ B activation in A549 and BEAS-2B cells.

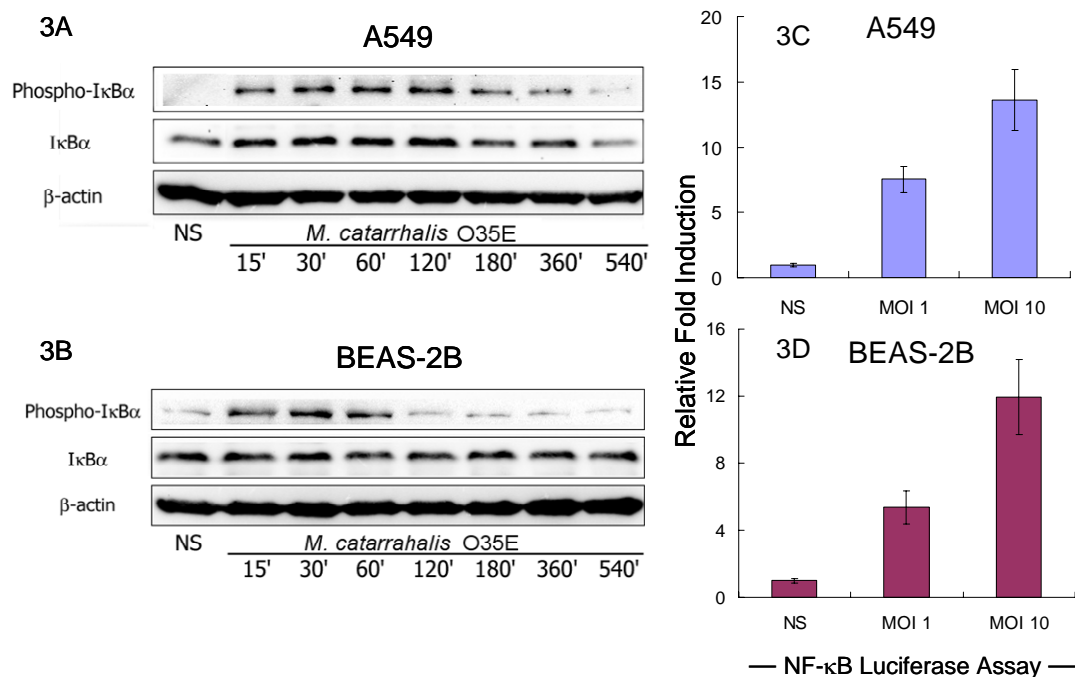


Figure 3. A549 (3A) or BEAS-2B cells (3B) were stimulated with MOI 10 of *M. Catarrhalis* strain O35E for indicated time. The cells were lysed in lysis buffer and Western blotting was used to detect the phosphorylated I κ B α , total I κ B α and β -actin. A549 (3C) or BEAS-2B cells (3D) were transfected with pNF- κ B-Luc and pRL-TK for 24 h. Then stimulated cells with indicated MOI of *M. catarrhalis* strain O35E for 4hr and the dual luciferase assay was conducted in duplicate and luciferase activity was notmalized with *Renilla* luciferase activity. Data presented are the mean and the standard error of the mean of three independent experiments.

family. Phosphor-I κ B α is ubiquitinated and then degraded in the proteasome, releasing κ B subunits to translocate to the nucleus (Perkins, 2007). To study whether *M. catarrhalis* induces NF- κ B activation, we first examined the time courses of *M. catarrhalis* induced-phosphorylation and degradation of I κ B α by western blotting. For A549 cells (Fig 3A), I κ B α phosphorylation became evident within 15 min, peaked at 60 min, and declined thereafter. BEAS-2B cells peaked earlier, at 30 min post-infection, and returned to baseline level within 2 h (Fig 3B). However no

significant degradation was observed for total I κ B α in both cell lines, which may be because phosphorylated I κ B α is only a small portion of total I κ B α . We next measured NF- κ B-dependent activation by dual luciferase assay. After 4 h stimulation with *M. catarrhalis* at different MOIs (0, 1, and 10), the activity of a NF- κ B-dependent promoter was greatly increased within 4 h in a dose-dependent manner in both A549 and BEAS-2B cells (Fig. 3C and 3D). All these data suggest that *M. catarrhalis* induces NF- κ B activation, through phosphorylation of I κ B α , in pulmonary epithelial cells.

TLRs, CD14 and MD2 expression on human pulmonary epithelial cells

Among multiple PRRs that are involved in recognizing Gram-negative bacteria, TLR2 and TLR4 are the most important. TLR2 mainly recognizes lipopeptides or lipoproteins, PDG and LTA; whereas TLR4 recognizes LPS (Takeda et al., 2003). MD2 is an essential co-receptor that associates with the extracellular domain of TLR4 and modulates the LPS induced responses (Shimazu et al., 1999). CD14 is a soluble PRR that binds to PAMPs, such as lipopeptides, PDG and LPS, and transports these ligands to TLR2 or TLR4 to initiate the intracellular signaling (Landmann et al., 2000). To study the expression of these PRRs, Flow cytometry (FCM) and western blotting were used to detect TLR2 and TLR4 expression on both A549 and BEAS-2B cells. Compared to isotype control, TLR2 and TLR4 receptor were detectable on both cells by FCM (Fig. 4A and 4B). Similarly, western blotting data confirmed their expression (Fig. 4E). However, CD14 (Fig. 4C and 4D) and MD2 (Fig. 4F), do not

express on A549 and BEAS-2B cells, as shown by both FCM and western blotting.

Figure 4. Expression of TLR2, TLR4, CD14 and MD2 on A549 and BEAS-2B cells.

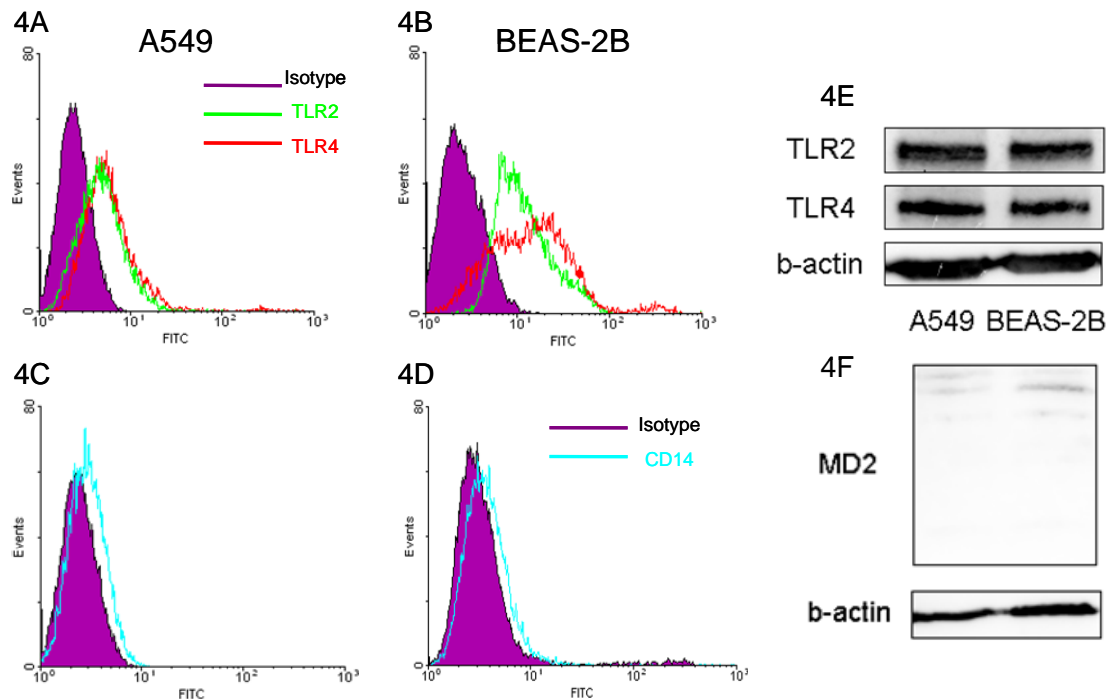


Figure 4. Expression of TLR2 and TLR4 on A549 and BEAS-2B cells were measured by FCM (4A and 4B) and western blotting (4E). Expression of CD14 and MD2 on A549 and BEAS-2B cells were measured by FCM (4C and 4D) and western blotting (4F), respectively. The filled distribution is that of the isotype controls (Fig 4A-4D).

***Moraxella catarrhalis*–induced inflammatory signaling is mainly through TLR2, not TLR4**

To study the involvement of TLRs in *M. catarrhalis*-induced inflammatory responses by human pulmonary epithelial cells, a loss of function assay was used. Functional antibody specific for TLR2 or TLR4 was incubated with A549 or BEAS-2B cells 1 h before infection. After incubation with *M. catarrhalis* for 9 h, TLR2 antibody significantly inhibited CCL20/MIP-3 α secretion in both cell

Figure 5. Antibody of TLR2, but not TLR4, inhibits *M. catarrhalis*-induced inflammatory responses in A549 and BEAS-2B cells.

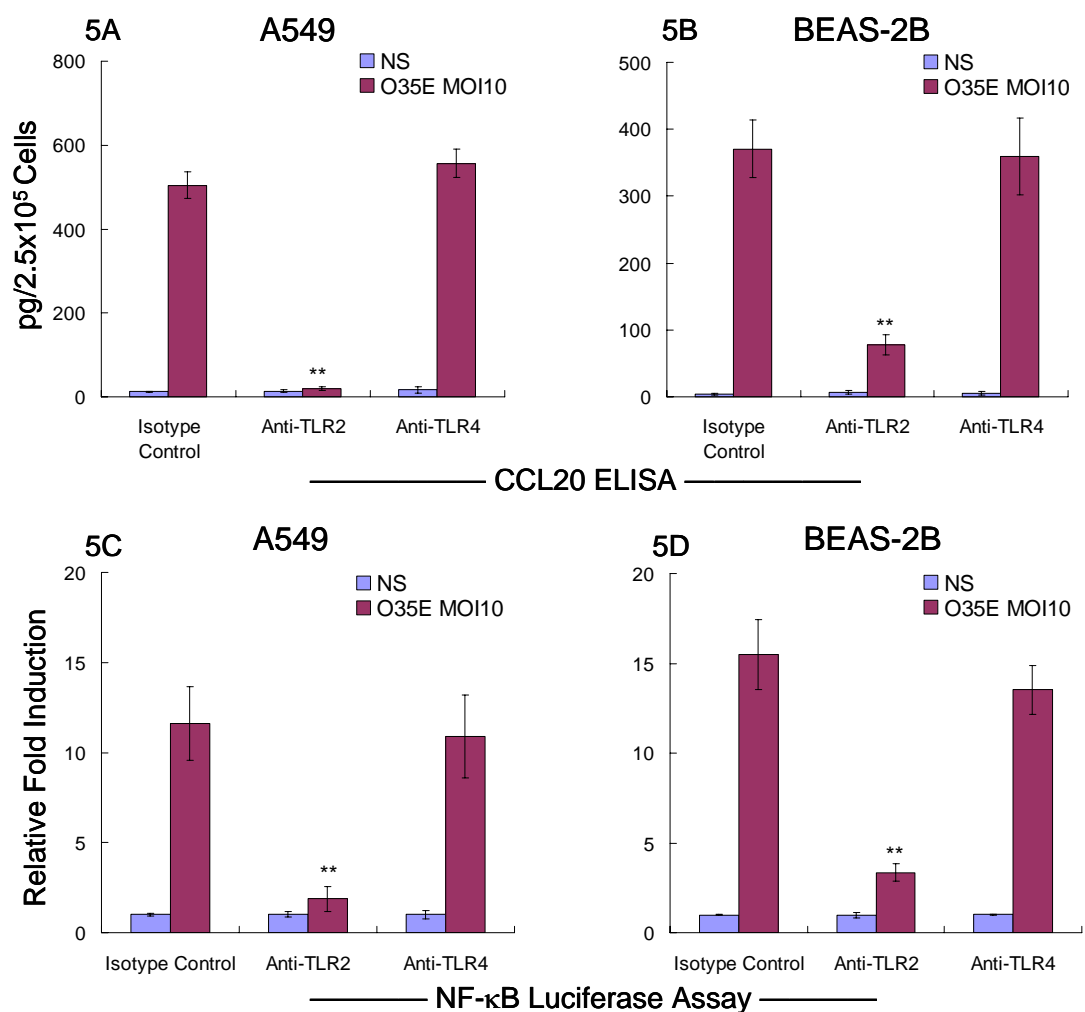


Figure 5. A549 or BEAS-2B cells were pretreated with anti-TLR2 or anti-TLR4 antibody (20 $\mu\text{g}/\text{mL}$) for 1h, and isotype antibody was used as control. The cells were then incubated with MOI 10 of *M. catarrhalis* strain O35E. At 9 h post infection, the supernatant was collected and ELISA was used to detect the level of CCL20/MIP-3 α (5A and 5B). To examine NF- κ B activation, A549 or BEAS-2B cells were transfected with pNF- κ B-Luc and pRL-TK for 24 h. The cells were pretreated with anti-TLR2 or anti-TLR4 antibody for 1 h and then infected with *M. catarrhalis* strain O35E (MOI 10) for 4 h and the dual luciferase assay was conducted in duplicate (5C and 5D). Luciferase activity was normalized with *Renilla* luciferase activity. Data presented are the mean and the standard error of the mean of four independent experiments. ** $P < 0.01$ by Student's t -test.

lines (Fig. 5A and 5B). In contrast, TLR4 antibody had no significant effect on *M.*

catarrhalis-induced CCL20/MIP-3 α expression. These antibody effects were confirmed by dual luciferase assay: only TLR2 antibody, but not TLR4 antibody, abrogated the *M. catarrhalis*-induced NF- κ B activation in both A549 and BEAS-2B cells (Fig. 5C and 5D). Thus, TLR2 seems to play a major role in regulating the *M. catarrhalis*-induced inflammatory responses in pulmonary epithelial cells.

MyD88 and TRAF6 are required for *M. catarrhalis*-induced CCL20/MIP-3 α expression

MyD88 and TRAF6 are two important adaptors in the TLR-mediated NF- κ B activation signaling pathways. MyD88 is constituted of a TIR domain, intermediary domain (ID) and death domain (DD). MyD88 interacts with TLRs through their TIR domains and initiates downstream signaling through a DD-DD interaction with IRAKs (Dunne et al., 2003). TRAF6 is characterized by an N-terminal zinc-binding domain (containing a RING finger followed by several zinc fingers), a coiled-coil domain (TRAF-N) and a highly conserved C-terminal domain (TRAF-C) (Bradley and Pober, 2001). The TRAF-N domain is essential for downstream signaling events, whereas the TRAF-C domain mediates self association and interactions with upstream receptors and signaling proteins. Dominant-negative (DN) MyD88 and DN TRAF6 alleles were generated by deleting the N-terminus, which can still interact with upstream receptors or signaling molecules, but can not initiate downstream signaling. Dual luciferase assay showed that A549 or BEAS-2B cells transfected with plasmids encoding DN MyD88 or DN TRAF6 showed impaired NF- κ B activation after *M.*

catarrhalis stimulation (Fig 6A and 6B). Empty vector pcDNA3.1 was used as control. The data indicate that both MyD88 and TRAF6 are involved in *M. catarrhalis*-induced NF- κ B activation in human pulmonary epithelial cells.

Figure 6. MyD88 and TRAF6 are involved in *M. catarrhalis*-induced NF- κ B activation in A549 and BEAS-2B cells.

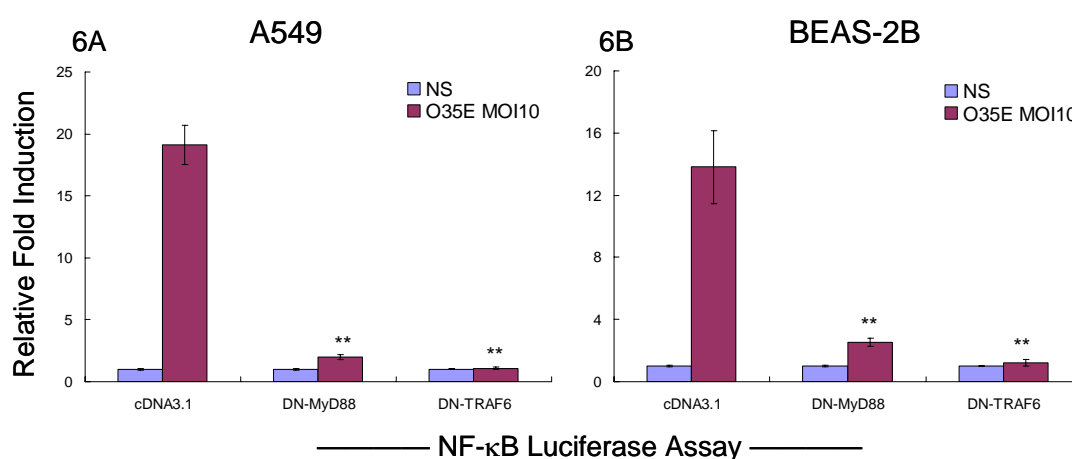


Figure 6. A549 and BEAS-2B cells were transfected with pNF- κ B-Luc and pRL-TK, as well as pDN-MyD88 or pDN-TRAF-6 for 24 h. Empty vector pcDNA3.1 was used as control. The cells were stimulated with MOI 10 of *M. catarrhalis* strain O35E for 4 h, then dual luciferase assay was conducted in duplicate and luciferase activity was normalized with *Renilla* luciferase activity (6A and 6B). Data presented are the mean and the standard error of the mean of three independent experiments. ** $P < 0.01$ by Student's *t*-test.

Involvement of transcription factors in *M. catarrhalis*-induced CCL20/MIP-3 α expression

NF- κ B, MAPKs and PI3K are all essential transcription factors in regulating the expression of inflammatory-related genes (Martin and Frevert, 2005). Pretreated A549 or BEAS-2B cells with MG132, a specific proteasome inhibitor, and BAY11-7082, an irreversible inhibitor of I κ B- α phosphorylation, could significantly inhibit *M.*

Figure 7. NF- κ B, p38 and MEK1/2 inhibitors blocked *M. catarrhalis*-induced CCL20/MIP-3 α expression.

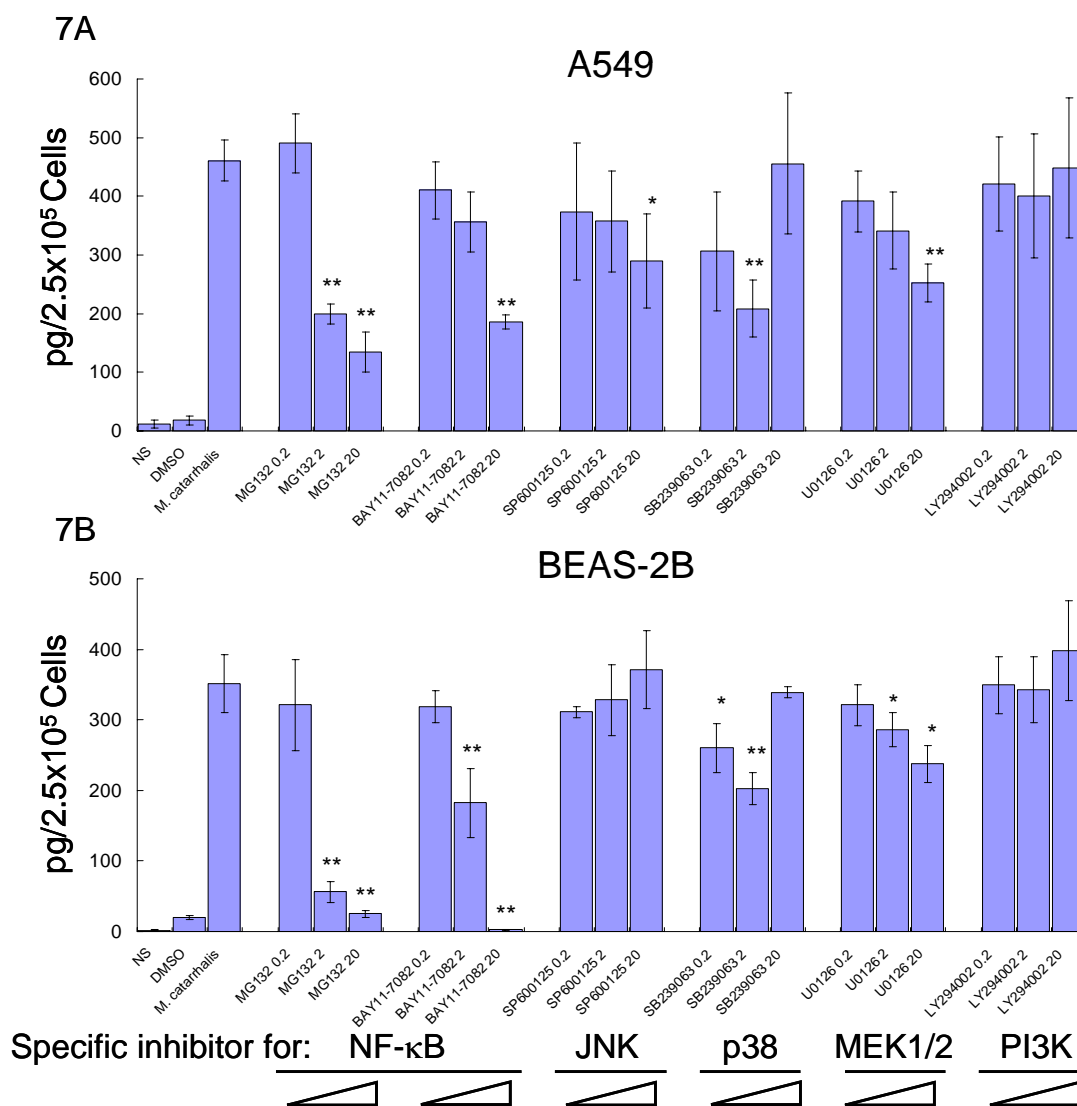


Figure 7. A549 (7A) and BEAS-2B (7B) cells were pretreated with different concentrations (0.2, 2, 20 μ M) of inhibitors for 1 h then incubated with MOI 10 of *M. catarrhalis* strain O35E for 9hr. The supernatant was collected and ELISA was used to detect the level of CCL20/MIP-3 α for each cell line. Data presented are the mean and the standard error of the mean of four independent experiments. * $P < 0.05$. ** $P < 0.01$ by Student's t -test.

catarrhalis-induced CCL20/MIP-3 α expression. SP600125 is an inhibitor of phosphorylation of c-Jun N-terminal kinase (JNK); SB203580 is a pyridinyl imidazole inhibitor of p38; and U0126 is a selective inhibitor of MEK1/2. We found

SP600125 only inhibited *M. catarrhalis*-induced CCL20/MIP-3 α secretion in A549 cells at highest dose (Fig. 7A). SB203580 and U0126 could significantly abrogate CCL20/MIP-3 α release; while for SB203580, the highest dose increased chemokine expression, possibly because of cell toxicity (Fig. 7A and 7B). However, PI3K inhibitor, LY294002, had no effect on *M. catarrhalis*-induced CCL20/MIP-3 α expression. Therefore, these data suggest that NF- κ B, p38 and MEK1/2 are the most important transcription factors, regulating the *M. catarrhalis*-induced CCL20/MIP-3 α production in lung pulmonary epithelial cells.

TLR2-, but not TLR4-mediated signaling can be activated in human pulmonary epithelial cells

Our previous data have suggested that *M. catarrhalis* activated human airway epithelial cells mainly through a TLR2-mediated signaling pathway. MyD88 and TRAF6 are two critical downstream adaptors, which mediate the activation of NF- κ B and MAPKs. To confirm the activation of TLR2 and TLR4 on A549 and BEAS-2B cells, we first stimulated both cells with either Pam₃CSK₄ or LPS, specific ligands for TLR2 and TLR4, respectively. We found that only Pam₃CSK₄ can induce NF- κ B activation and release of proinflammatory factors, such as CCL20/MIP-3 α and CXCL8/IL-8, in both cell lines; while there was no significant difference between LPS treated and untreated cells (Fig. 8A-8C). These data confirmed that TLR4-signaling can not be activated *in vitro* on A549 and BEAS-2B cells. This is consistent with the facts that TLR2 and TLR4 expression levels are similar in both

airway epithelial cell lines, whereas no CD14 and MD2 expression was detected. Thus we hypothesize that absence of these two mediators, might explain the lack of TLR4 signaling in pulmonary epithelial cells.

Figure 8. TLR2, but not TLR4 signaling can be activated in A549 and BEAS-2B cells.

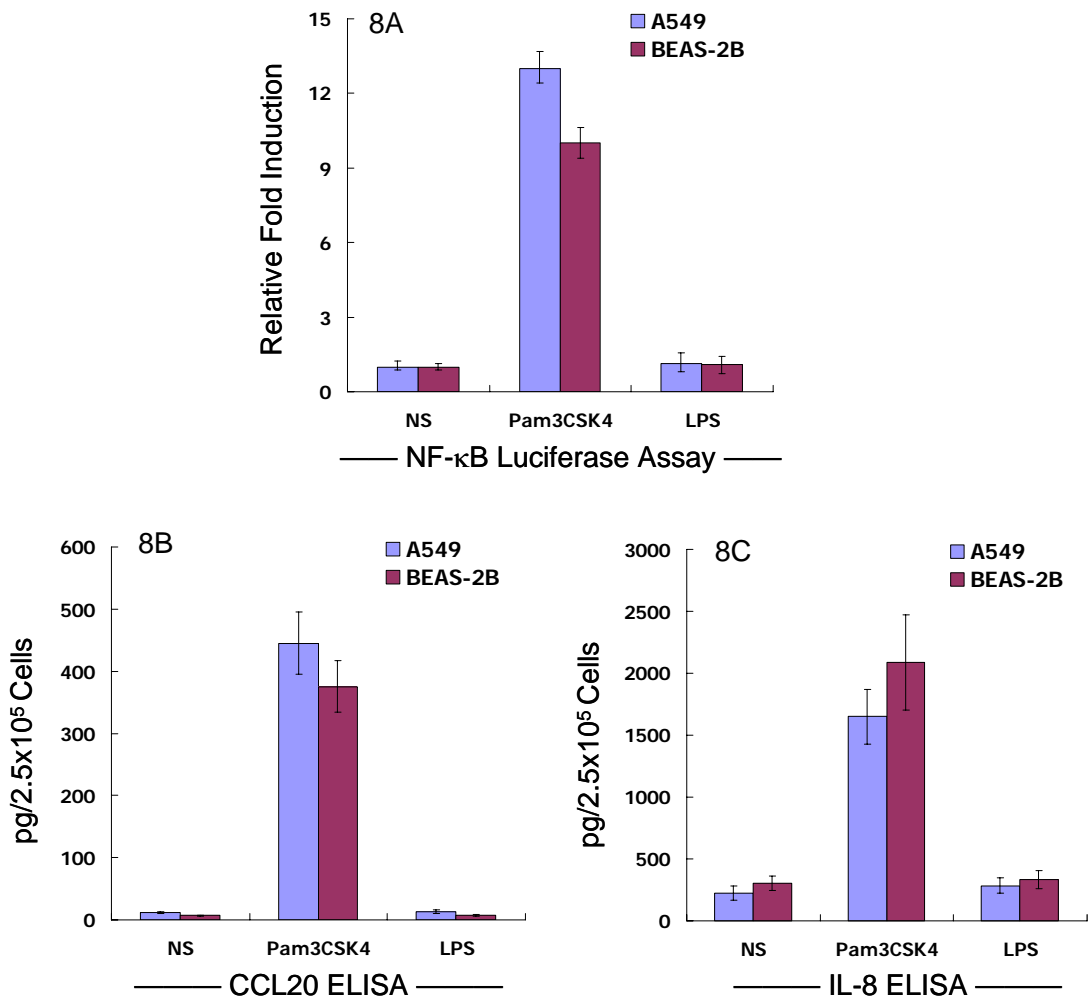


Figure 8. Specific ligand for TLR2, Pam3CSK4 (1 $\mu\text{g}/\text{mL}$), and TLR4, UP-LPS (1 $\mu\text{g}/\text{mL}$), were used to stimulate A549 and BEAS-2B cells. In both cells, NF- κ B activation (8A) were measured by dual luciferase assay, while CCL20/MIP-3 α (8B) and CXCL8/IL-8 (8C) production were analyzed by ELISA. Data presented are the mean and the standard error of the mean of four independent experiments.

CD14 and MD2 are required to activate TLR4-mediated signaling and enhance TLR2-mediated responses

To examine the critical role of CD14 and MD2 in TLRs signaling, we used the HEK293 cell line, which is a human embryo kidney epithelial cell that does not express endogenous TLRs, CD14 or MD2. Stably transfecting HEK293 cells with expression plasmids, we selected and cultured a series of cell lines expressing TLR2, TLR4 or TLR4/CD14/MD2. Empty vector pcDNA3.1 was transfected into the cells to generate a control cell line. FCM was used to examine the cell surface expression of each receptor. There was no expression of TLR2, TLR4 or CD14 in the HEK293-vector cell line (Fig. 9A and 9D), or in untransfected HEK293 cells (data not shown). Each stable transfected cell line expresses the appropriate TLR on their cell surfaces (Fig. 9B, 9C and 9E). Compared to HEK293-TLR4/CD14/MD2 cells, HEK293-TLR4 cells did not respond to Pam₃CSK₄ or LPS with NF- κ B activation and CCL20/MIP-3 α mRNA expression, only showing a low-level response to *M. catarrhalis* (Fig. 10A and 10B). In contrast, triply transfected cells responded very well to both LPS and *M. catarrhalis* (Fig. 10A and 10B), indicating that CD14 and MD2 are necessary for activation of the TLR4 signaling pathway. To further investigate the important roles of CD14 and MD2 in TLR signaling, we compared the effect of conditioned culture media of HEK293-TLR4 or HEK293-TLR4/CD14/MD2, which contains a large amount of soluble CD14 and MD2, on HEK293-TLR2 and HEK293-TLR4 cells. Without CD14 and MD2, only HEK293-TLR2, but not HEK293-TLR4 cells, responded significantly to Pam₃CSK₄ and *M. catarrhalis*

Figure 9. Expression of TLR2, TLR4, CD14 and MD2 on stable transfected HEK293 cells.

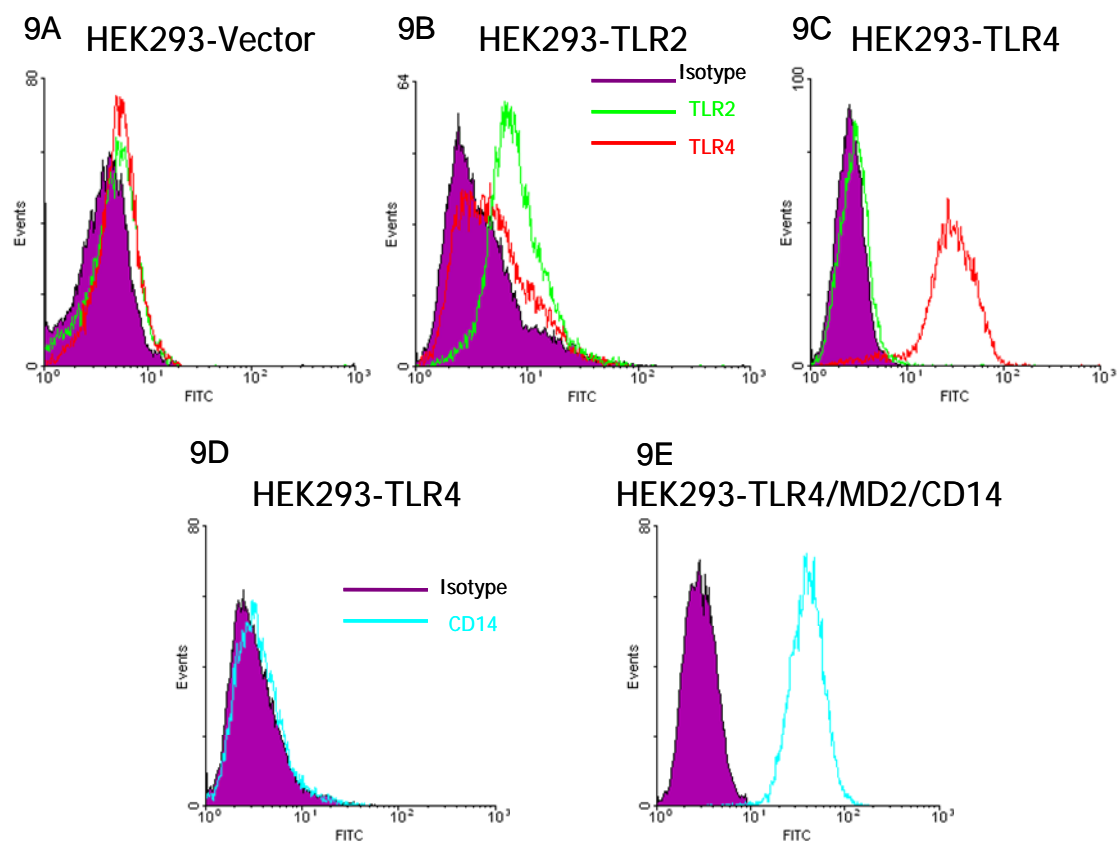


Figure 9. Expression of TLR2 and TLR4 on HEK293-vector, HEK293-TLR2 and HEK293-TLR4 cells were measured by FCM (9A-C). Expression of CD14 on HEK293-TLR4 and HEK293-TLR4/MD2/CD14 cells were measured by FCM (9D and 9E).

stimulation (Fig. 10D and 10G), as indicated by NF- κ B activation and CCL20/MIP-3 α mRNA expression. HEK293-TLR4 cells can not be activated by various TLRs agonists or *M. catarrhalis* (Fig. 9E and 9H), and exhibited a similar pattern to the control cell line (Fig. 10C and 10F). However, in presence of soluble CD14 and MD2, LPS or *M. catarrhalis* induced NF- κ B activation and CCL20/MIP-3 α mRNA expression in HEK293-TLR4 cells (Fig. 10E and 10H), while no significant change was observed in HEK293-cDNA3.1 cells, presumably due to

Figure 10. CD14 and MD2 in TLR2- and TLR4-mediated signaling.

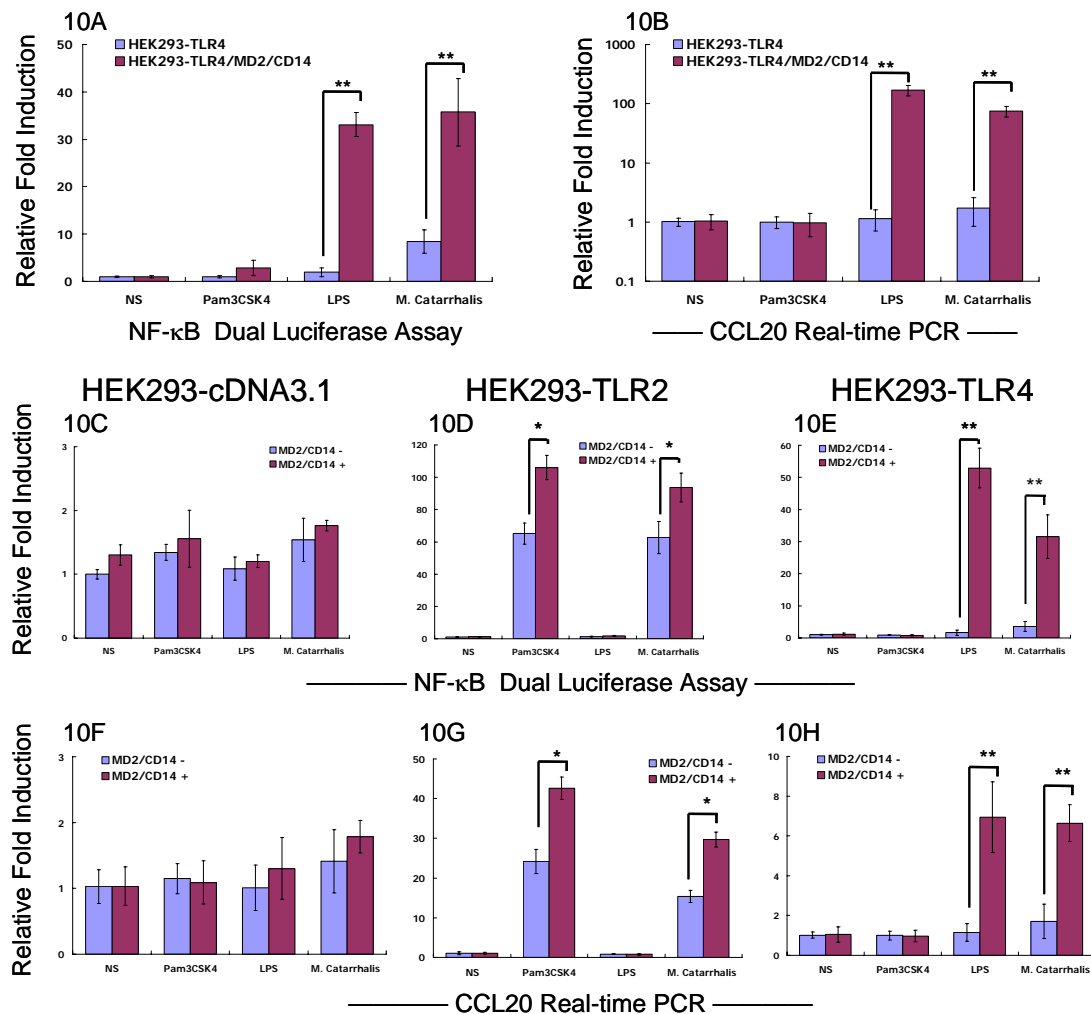


Figure 10. Pam3CSK4 (100ng/mL), UP-LPS (1 μ g/mL), and *M. catarrhalis* (MOI 10) were used to stimulate HEK293 cells stably transfected with TLR4 or TLR4/MD2/CD14. NF- κ B activation (10A) and CCL20/MIP-3 α mRNA expression (10B) in each cell line were measured by dual luciferase assay and real-time PCR, respectively. HEK293 cells stably transfected with cDNA3.1, TLR2 or TLR4 were incubated with culture medium of HEK293-TLR4 or HEK293-TLR4/MD2/CD14. Pam3CSK4 (100 ng/mL), UP-LPS (1 μ g/mL), and *M. catarrhalis* (MOI 10) were then used to stimulate the cells. NF- κ B activation (10C-E) and CCL20/MIP-3 α mRNA expression (10F-H) in each cell line were measured by dual luciferase assay and real-time PCR, respectively. Data presented are the mean and the standard error of the mean of three independent experiments. * $P < 0.05$. ** $P < 0.01$ by Student's *t*-test.

the lack of TLR2 and TLR4 (Fig. 9C and 9F). Interestingly, conditioned media from HEK293-TLR4/CD14/MD2 could also generate enhanced responses in HEK293-TLR2 cells to Pam₃CSK₄ and *M. catarrhalis* (Fig. 9D and 9G), consistent with the interpretation that CD14 forms a co-receptor complex with TLR2, facilitating the recognition of TLR2 ligand and thus increasing the TLR2-mediated responses.

VDR and TLRs induces both membrane-bound and soluble CD14 expression on THP1 cells, but does not change MD2 expression

In the human body, CD14 and MD2 are mainly expressed by cells of the myeloid lineage, especially monocytes and macrophages. To determine whether increased CD14 and MD2 enhance TLR-mediated inflammatory responses, we first examined the expression of these two co-receptors in THP1 cells. The naïve monocytic cells, THP1, express low levels of CD14 on their surface, and (3.9±1.2)% of total cells were positive as shown by FCM (Fig. 11A, top left and 11B), and no soluble CD14 was detected in their culture medium (Fig. 11D). Recent study suggests that 1 α , 25-dihydroxy VD₃, the active form of vitamin D₃, specifically induces CD14 expression on monocytes through the vitamin D₃ receptor (VDR) (Liu et al., 2006; Schaubert et al., 2007). Upon stimulating THP1 cells with 1 α , 25-dihydroxy VD₃, membrane-bound CD14 was upregulated (Fig. 11A-C) and soluble CD14 was significantly induced (Fig. 11D): (80.0±7.5) % of cells stained positive, and soluble CD14 reached more than 5 ng/mL. Similarly, Pam₃CSK₄ and *M. catarrhalis* could

Figure 11. 1α , 25-dihydroxy VD3 induces both membrane-bound and soluble CD14 expression, but does not change MD2 expression on THP1 cells.

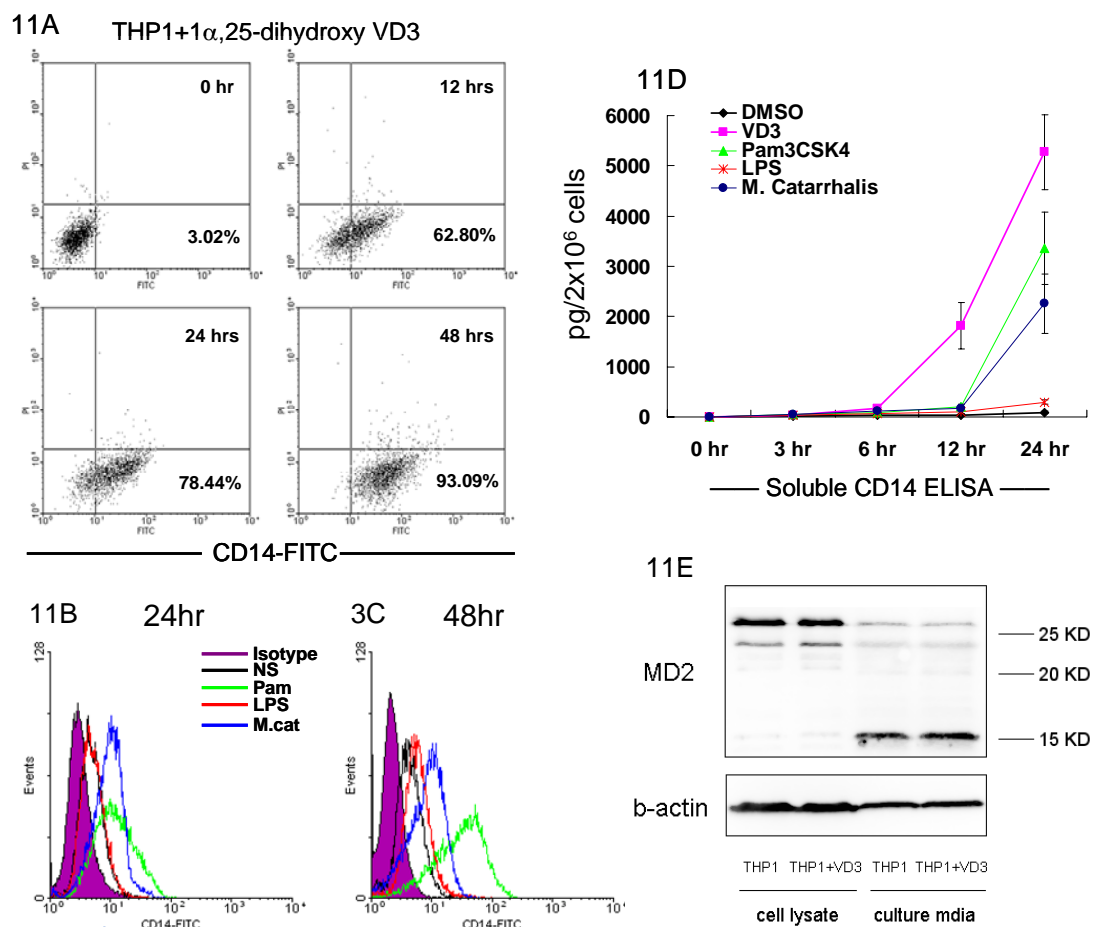


Figure 11. Time course of membrane-bound CD14 expression on 1α , 25-dihydroxy VD3 (100 nM) treated THP1 cells (11A), staining the cells with FITC conjugated antibody for CD14 at indicated time points and analyzed by FCM. Similarly, membrane-bound CD14 on THP1 cells treated with Pam3CSK4 (1 μ g/mL), UP-LPS (1 μ g/mL) and *M. catarrhalis* (MOI 10) for 24hrs and 48hrs were studied by FCM (11B). Time course of soluble CD14 secretion from THP1 cells treated with Pam3CSK4, UP-LPS, *M. catarrhalis* and 1α , 25-dihydroxy VD3 were measured by ELISA (11C). MD2 in the cell lysate and culture media of 1α , 25-dihydroxy VD3 (100 nM) treated THP1 was detected by western blotting (11D), compared to DMSO treated THP1 cells. Data presented are the mean and the standard error of the mean of three independent experiments.

also induce the maturation of THP1 cells through TLR2; while LPS showed only weak effects (Fig. 11B-D). Western blotting showed that MD2 exists as different forms in cell lysates and culture media. In cell lysates, MD2 mainly consists of two forms about 25 kDa, due to the differentially glycosylated forms. In culture media, monomeric MD2 around 15 kDa is the major form. In contrast to CD14, MD2 expression was not changed after 1α , 25-dihydroxy VD_3 stimulation (Fig. 11E), suggesting that VDR is not involved in regulating MD2 expression, at least not in THP1 cells.

CD14 increases TLR2- and TLR4-mediated inflammatory responses in THP1 cells

We next examined whether increased CD14 expression would enhance TLR2 and TLR4-mediated inflammatory responses in monocytes, THP1 cells were pretreated with 1α , 25-dihydroxy VD_3 for 24 h, and then stimulated with Pam₃CSK₄ or LPS. CCL20/MIP-3 α and CXCL8/IL-8 secretion were measured in a time course of 24 h. In DMSO treated THP1 cells, LPS only induced a very low level of CCL20/MIP-3 α and CXCL8/IL-8 production, both less than 100 pg/mL within 24 h post stimulation (Fig. 12B and 12D). Pam₃CSK₄ could effectively activate the cells through TLR2 pathway (Fig. 12A and 12C). On the other hand, 1α , 25-dihydroxy VD_3 primed group showed amplified responses after 6 h post stimulation by Pam₃CSK₄ or LPS (Fig. 12A-D). LPS, even in the presence of CD14, did not induce to the same level as Pam₃CSK₄; it did increase the CCL20/MIP-3 α and CXCL8/IL-8

Figure 12. CD14 increases TLR2- and TLR4-mediated inflammatory responses in THP1 cells.

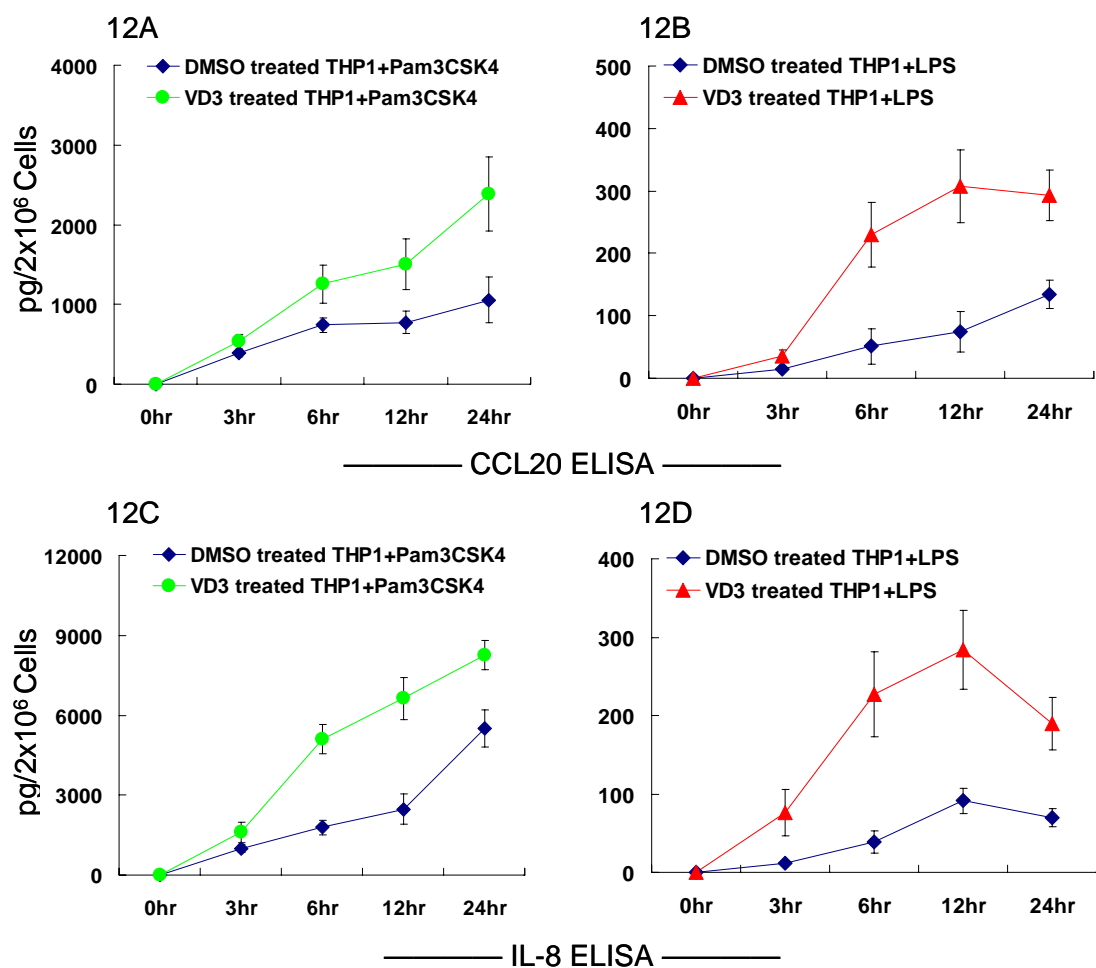


Figure 12. Naïve THP1 cells were pretreated with 1 α , 25-dihydroxy VD₃ (100 nM) for 24 h, with DMSO treated cells as control. Each group of cells was stimulate with Pam3CSK4 (1 μ g/mL) or UP-LPS (1 μ g/mL) for another 24hrs, and culture medium was collected at the indicated times. Time course of CCL20/MIP-3 α (12A and 12B) and CXCL8/IL-8 expression (12C and 12D) between naïve and mature THP1 cells, was analyzed by ELISA. Data presented are the mean and the standard error of the mean of four independent experiments.

expression considerably, level compared to DMSO primed cells. To exclude the possibility that TLR expression might be upregulated and contribute to the increased inflammatory responses, we examined TLR2 and TLR4 expression by FCM. No significant difference was seen between DMSO and 1 α , 25-dihydroxy VD₃ treated

Figure 13. $1\alpha, 25$ -dihydroxy VD_3 does not change TLR2 and TLR4 expression on THP1 cells.

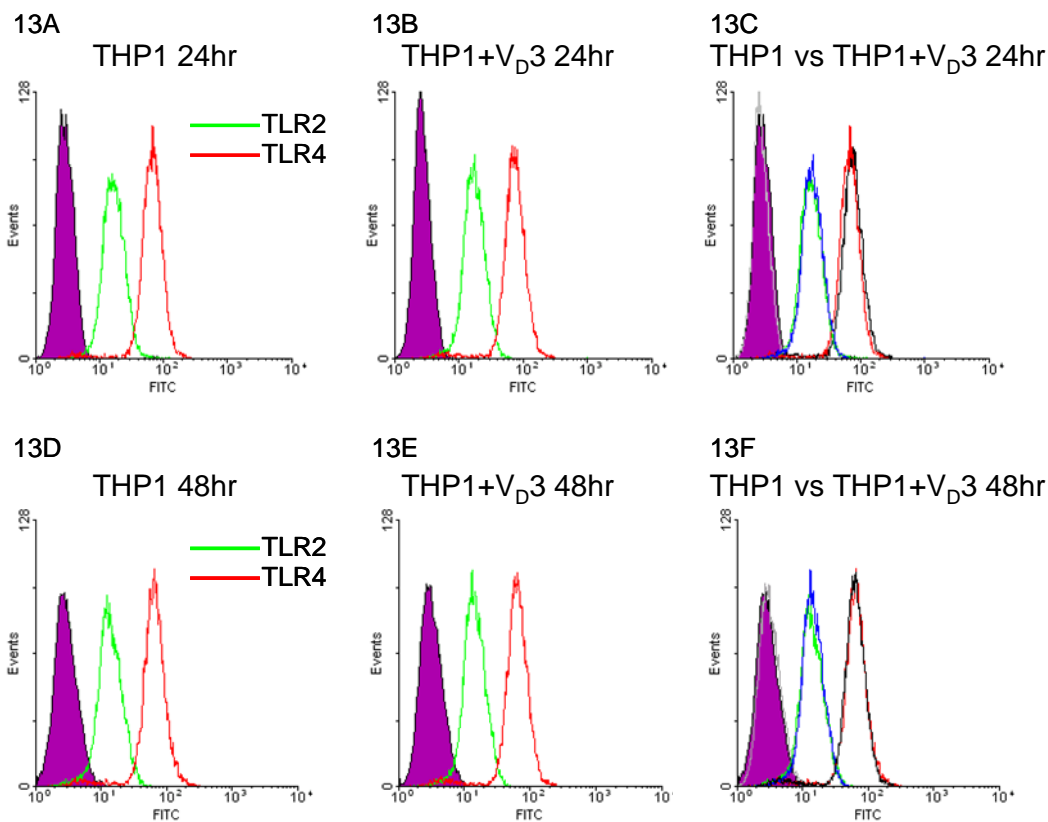


Figure 13. Naïve THP1 cells were stimulated with $1\alpha, 25$ -dihydroxy VD_3 (100 nM) for 24h (13B) or 48 h (13E), with DMSO treated cells as control (13A and 13D). At end of each time point, THP1 cells were collected and stained with TLR2-FITC or TLR4-FITC antibody, isotype-FITC antibody was use as control. FCM were conducted to analysis the TLR2 and TLR4 expression on THP1 cells. Merged figures were used to compare the expression of TLR2 and TLR4 between DMSO and $1\alpha, 25$ -dihydroxy VD_3 treated THP1 cells at each time point (13C and 13F).

THP1 cells (Figure 13). These data suggest that the highly elevated CD14 is responsible for increased TLR2- or TLR4-induced inflammatory responses in THP1 cells. The CD14 plays an important role as an autocrine messenger in monocytes: it could be upregulated by VDR or TLRs and then facilitates the ligands association with TLR2 or TLR4, enhancing each TLR-mediated inflammatory response.

Soluble CD14 increases TLR2- and TLR4-mediated inflammatory responses in lung pulmonary epithelial cells

Since A549 and BEAS-2B do not express CD14 or MD2, we are interested in whether soluble forms of CD14 and MD2, released by myeloid cells, could restore the TLR4 signaling and augment the TLR2 responses in pulmonary epithelial cells. Conditioned culture media were collected from THP1 cells pretreated with DMSO or $1\alpha, 25$ -dihydroxy VD_3 for 24 h. We then applied either Pam₃CSK₄ or LPS, in addition to conditioned media, to stimulate A549 or BEAS-2B cells for 12 h. According to CCL20/MIP-3 α and CXCL8/IL-8 production, Pam₃CSK₄-induced TLR2 signaling pathway was significantly upregulated by CD14 in both cell types (Fig. 14A-D). LPS-induced TLR4 responses were also activated, compared to cells incubated with control conditioned medium (Fig. 14A-D). These data suggests that CD14 can participate in paracrine signaling: after released by monocytes, it circulates and binds to neighboring cells that do not express CD14, such as pulmonary epithelial cells, thus modulating the TLRs signaling on those cells.

Anti-CD14 antibody inhibits *M. catarrhalis*-induced inflammatory responses between mature monocytes and pulmonary epithelial cells

M. catarrhalis is one of the major pathogens that cause exacerbations in patients with COPD. Our previous data have shown that *Moraxella catarrhalis* induces CCL20/MIP-3 α in airway epithelial cells mainly through TLR2-MyD88-TRAF6-NF- κ B/MAPK signaling pathway. However, during COPD, infiltrated monocytes would presumably create an environment highly abundant in

Figure 14. Conditioned medium from 1α , 25-dihydroxy VD₃ treated THP1 cells increases TLR2- and TLR4-mediated inflammatory responses in human pulmonary epithelial cells.

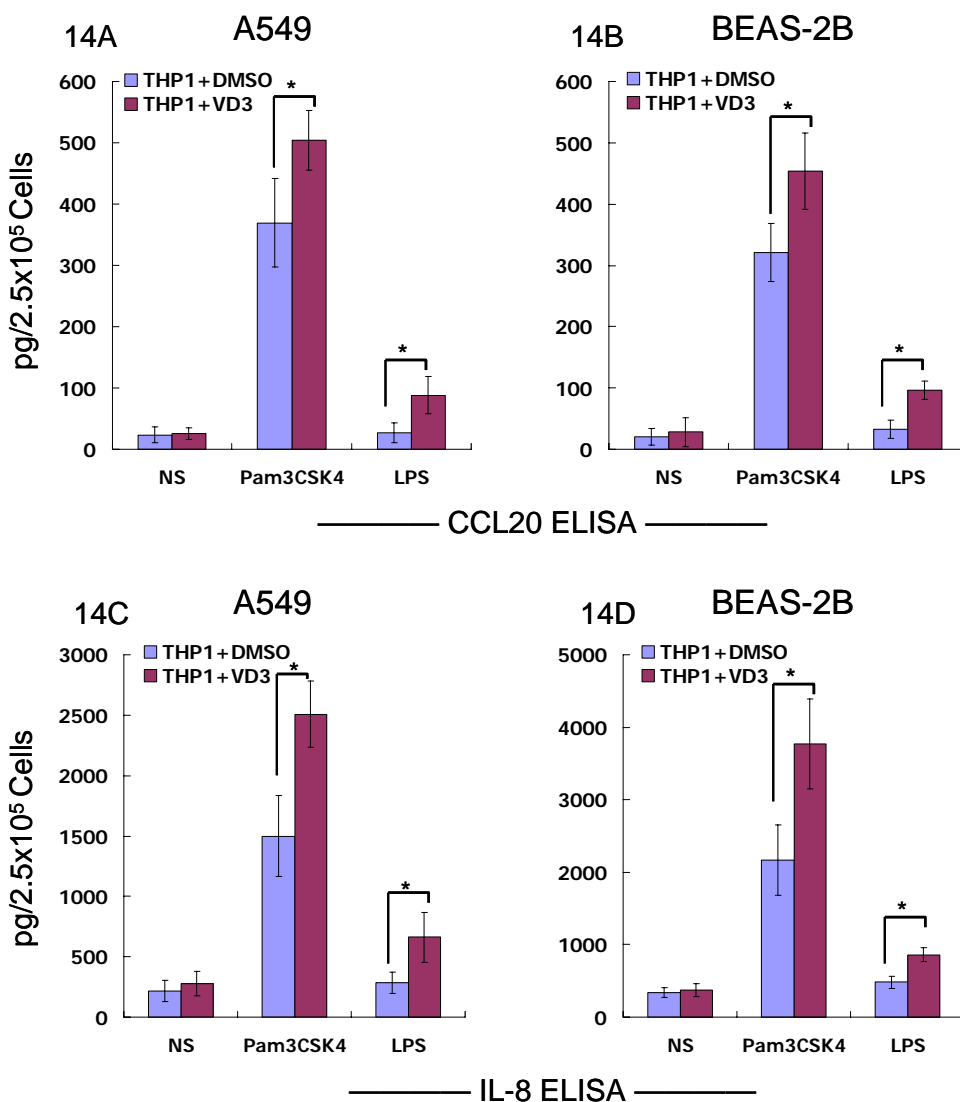


Figure 14. Culture medium of THP1 cells pretreated with DMSO or 1α , 25-dihydroxy VD₃ cells for 24 h were collected by centrifuge at 500xg for 5 min. In the presence of conditioned culture media, A549 and BEAS-2B cells were stimulated with Pam3CSK4 (1 μ g/mL) or UP-LPS (1 μ g/mL) for 12hrs. CCL20/MIP-3 α (14A and 14B) and CXCL8/IL-8 secretion (14C and 14D) were measured by ELISA. Data presented are the mean and the standard error of the mean of four independent experiments. * $P < 0.05$ by Student's t -test.

Figure 15. CD14 antibody inhibits *M. catarrhalis*-induced inflammatory responses in mature THP1 cells and conditioned media primed pulmonary epithelial cells.

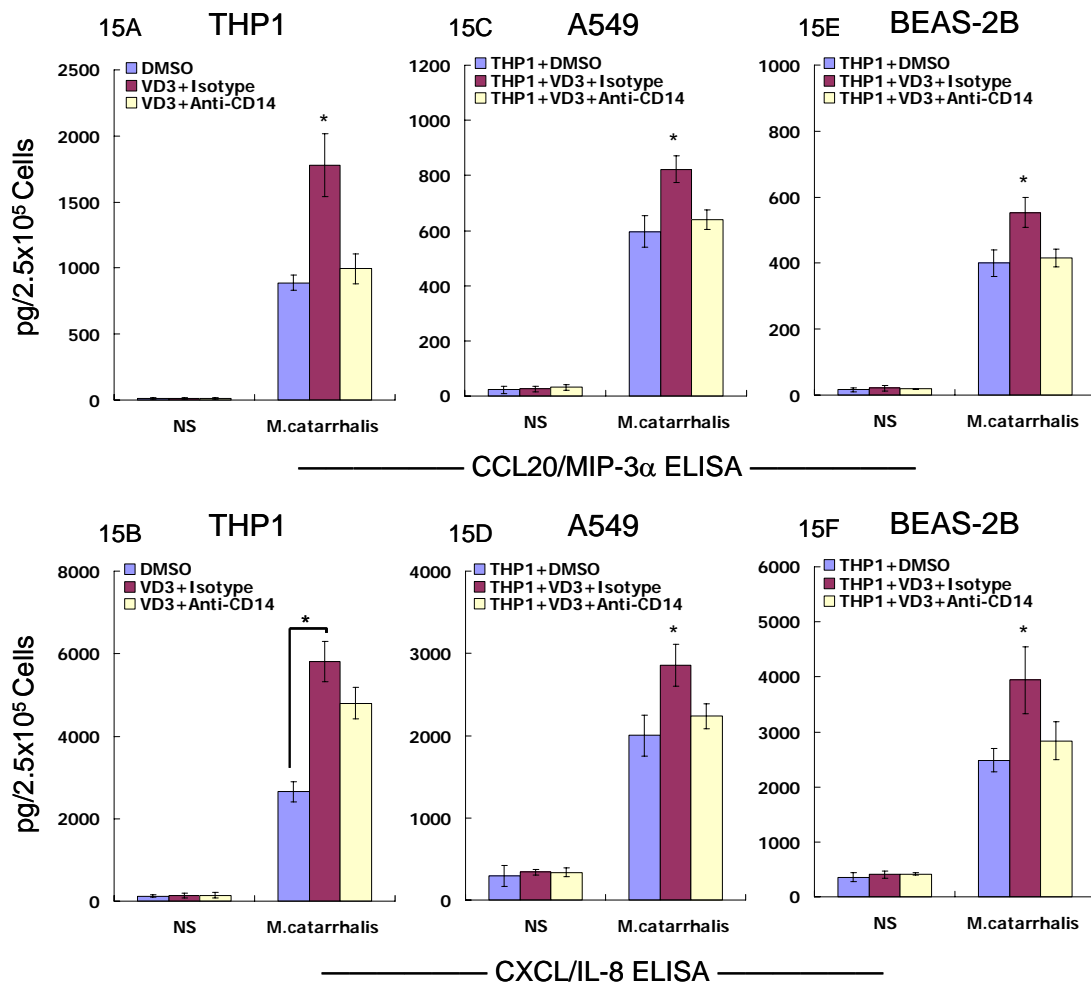


Figure 15. Naïve and mature THP1 cells were pretreated with CD14 antibody (20 µg/mL) for 1 h, or isotype antibody as control, then incubated with MOI 10 of *M. catarrhalis* for 12 h. Then supernatant was collected and ELISA was used to detect the level of CCL20/MIP-3α (15A) and CXCL8/IL-8 (15B). A549 and BEAS-2B cells were incubated with conditioned media of THP1 cells for 1 h, with CD14 antibody (20 µg/mL) or isotype antibody, and then incubated with MOI 10 of *M. catarrhalis*. 12 h post infection, the culture media was collected and analyzed CCL20/MIP-3α and CXCL/IL-8 production in A549 (15C and 15D) and BEAS-2B cells (15E and 15F) by ELISA. * $P < 0.05$. ** $P < 0.01$ by Student's *t*-test.

CD14 at the site of the infection. The soluble CD14 may create a crosstalk between monocytes and epithelial cells, amplifying the inflammatory responses in both cells and thus developing into exacerbation. To confirm the important role of CD14, we

pretreated naïve or mature THP1 cells with anti-CD14 antibody for 1 h and then incubated with *M. catarrhalis* for another 12 h. *M. catarrhalis* could induce higher CCL20/MIP-3 α and CXCL8/IL-8 release in 1 α , 25-dihydroxy VD₃ treated THP1 cells than in naïve THP1 cells, and this effect could be partially inhibited by CD14 antibody (Fig. 15A and 15B). Similarly in A549 and BEAS-2B cells, conditioned medium from 1 α , 25-dihydroxy VD₃ pretreated THP1 cells, compared to that from naïve THP1 cells, enhanced the *M. catarrhalis*-induced CCL20/MIP-3 α (Fig. 15C and 15E) and CXCL8/IL-8 (Fig. 15D and 15F) production in airway epithelial cells. Such amplified inflammatory responses can be attenuated by CD14 antibody (Fig. 15C-F). All these data indicate that CD14 plays a crucial role in both monocytes and pulmonary epithelial cells in fulfilling the complete function of TLR2 and TLR4 signaling. Soluble CD14 could thus mediate crosstalk between monocytes and pulmonary epithelial cells to create an amplification loop in inflammatory responses, which is an ideal therapeutic target for bacterial infection induced exacerbations in patients with COPD.

DISCUSSION

COPD is now the fourth leading cause of death in United States, accounting for 122,000 deaths, 661,000 hospitalizations, 2 million emergency department visits and more than 17 million physician or hospital outpatient visits annually (CDC, 2006). However, there is still no effective treatment to control the disease progression and reduce its mortality rate. Most common treatments, such as bronchodilators, only improve the symptoms but do not suppress the underlying inflammations. So our study focused on understanding the molecular basis of bacteria-induced inflammatory responses on pulmonary epithelial cells and monocytes would help to identify new therapeutic target for COPD.

Pulmonary epithelial cells have been found to respond poorly to LPS stimulation (Cowland et al., 2003; Tsutsumi-Ishii and Nagaoka, 2003), controversy is still remaining about whether TLR4 could mediate sufficient response on these cells. Our data have confirmed that only Pam₃CSK₄, but not LPS, induces proinflammatory responses, such as NF- κ B activation and secretion of CCL20/MIP-3 α and CXCL8/IL-8 in A549 and BEAS-2B cells. Also, only antibody for TLR2, but not for TLR4, could inhibit *M. catarrhalis*-induced responses in two airway epithelial cell lines. Such poor response to LPS is not due to the lack of TLR4 expression because both flow cytometry and western blotting data have confirmed TLR4 expression on both A549 and BEAS-2B cells. Our hypothesis is that missing CD14 and MD2 on pulmonary epithelial cells might contribute to the “silence” of TLR4 signaling. By

using stably transfected HEK293 cells, we confirmed that only with CD14 and MD2, can the TLR4 signal pathway be activated by LPS or *M. catarrhalis*. In addition, CD14 could enhance the TLR2-mediating responses, suggesting CD14 could serve as an effective mediator for both TLR2 and TLR4 signaling.

CXCL8/IL-8 is a useful inflammation marker as its level is highly induced in the sputum of patients with COPD, especially in exacerbation caused by bacteria infection (Yamamoto et al., 1997; Hill et al., 1999). In our study, we found that CCL20/MIP-3 α , could be highly induced in both pulmonary epithelial cells and monocytes through TLR2 or TLR4 signaling pathway. By using loss of function assays, we identified that production of CCL20/MIP-3 α in pulmonary epithelial cells is mainly through TLR2-MyD88-TRAF6-NF- κ B signaling pathway. CCL20/MIP-3 α has also been found only highly induced in the patients with COPD, compared with never-smokers or smokers without COPD, and contributed to the infiltration of dendritic cells (DCs) (Demedts et al., 2007; Tsoumakidou et al., 2008). Thus CCL20/MIP-3 α might be taken as a new marker to monitor the development of inflammation in patients with COPD.

CD14 is mainly expressed on cells of myeloid lineage including monocytes and macrophages, and also at lower level on neutrophils. In the patients of COPD, with the infiltration of these inflammatory cells into the lung, CD14 level has been found significantly increased in bronchoalveolar lavage (BAL) (Hollander et al., 2007; Regueiro et al., 2009). In normal conditions, the human lung maintains a homeostasis state between commensal microbes and epithelium. Pulmonary epithelial cells

constitutively release some antimicrobial peptides, such as defensins, at basal level into the lumen of airways (Bals et al. 2004), inhibiting the colonization of bacteria at lower respiratory tract while not inducing inflammation. However, when host defense fail to maintain the balance, increased exposure of pathogen will first activate pulmonary epithelial cells. Large amount of chemokines and cytokines are secreted into the submucosa to recruit phagocytes to the site of infection. Based on our results, naïve monocytic THP1 cells only express very low level of mCD14 and sCD14 that both can be induced by 1α , 25-dihydroxy VD_3 , Pam₃CSK₄, LPS and *M. catarrhalis*. Increased mCD14 amplified both TLR2 and TLR4-mediated inflammatory response on THP1 cells. These data suggests that without CD14, TLR2 and TLR4 signaling can not be fully activated. It has been shown that association of lipopeptides or LPS with CD14 is the first step before the complex is transported to TLR2 or TLR4-MD2, respectively (Manukyan et al., 2005; da Silva et al., 2001). However, the mechanism of how CD14 amplify TLR-mediated signaling is still not clear. One possible explanation is that ligand-activated CD14 binds to TLRs and translocates to membrane lipid rafts, where is rich in src kinases and G protein binding molecules (Pfeiffer et al., 2001; Schmitz and Orsó, 2002; Finberg and Kurt-Jones, 2006). These kinases will facilitate the downstream signaling adaptors such as MyD88, TRAM and Trif binding to the TIR domain of TLRs and subsequently initiate the production of chemokines and cytokines. Interestingly, sCD14 seems to play an important role in mediating crosstalk between airway epithelial cells and monocytes. Introducing the sCD14 could restore the TLR4 signaling on both A549 and BEAS-2B cells and also

enhanced the TLR2-mediated responses. Antibody for CD14 could partially block *M. catarrhalis*-induced inflammatory responses in pulmonary epithelial cells primed by conditioned media from CD14 bearing monocytes. Such effect might suggest the existing of a vicious circle during the exacerbation of COPD. Activated pulmonary epithelial cells initiate the chemoattractant of monocytes and macrophages, which would help to clear the invading pathogens by phagocytosis and producing proinflammatory mediators. Once the infection is controlled, these newly recruited monocytes and macrophages will differentiate back to a quiescent form (Lambrecht, 2006); otherwise largely released sCD14 could amplify the activation form of pulmonary epithelial cells and themselves, inducing an uncontrollable acute inflammation and eventually exacerbation (Fig. 16). Altogether, as its essential role in modulating inflammation in different cellular component in the lung, CD14 could be a new therapeutic target in pathogenesis of COPD or exacerbation.

Current development of therapeutic strategy for COPD is mainly focus on anti-inflammation approaches (Barnes and Stockley, 2005). Specific antibody for cytokines such as TNF- α or IL-6 has been proven to be effective in some chronic inflammatory diseases (Reimold, 2002; Nishimoto and Kishimoto, 2004). Thus applying of such antibody might be extended in treatment of COPD. However, a clinical study by using CXCL8/IL-8 neutralize antibody failed to achieve significant improvement in dyspnoea (Mahler et al., 2004). These results remind us that inflammation is a complicated process, which is involved with lots of different inflammatory mediators that all have their contributions. It may not surprise us that

single antibody to block one of these mediators can not achieve therapeutic goals. Meanwhile, some specific inhibitors for transcriptional factors such as NF- κ B, MAPK and PI3K were tested in inhibiting these signaling pathways induced inflammation (Barnes and Stockley, 2005). It seems that such approach, by inhibiting the release of inflammation mediators in a broad spectrum, could overcome the shortcoming of the antibody therapy. However, inhibition of these transcriptional factors could also impair the host defense system, increasing the chance of infectious disease. Also there might be some unpredicted side effects because these transcriptional factors are also important in regulating a large number of genes that are critical for apoptosis, tumorigenesis, and autoimmune diseases. Taken all together, CD14 might be a potential target to control the inflammation in COPD. Antibody of CD14 could only partially decrease the TLR2-mediated inflammatory responses, because TLR2 itself can still bind with its ligands and mount sufficient responses. While for TLR4, it seems that CD14 is more essential because the response scale to LPS is relatively low compared to Pam₃CSK₄. Thus fully inhibition of CD14 could block the crosstalk between pulmonary epithelial cells and monocytes and suppress the enhanced responses in both cells, while TLR2 could still mount certain level of responses to maintain the innate immunity. Interestingly, recent study has shown that CD14 also helps the cellular uptake of Poly I:C and subsequently enhances Poly I:C-mediated TLR3 activation (Lee et al., 2006). It suggests that CD14 might also get involved in virus-induced responses through TLR3. Respiratory virus such as rhinovirus,

Figure 16. sCD14 mediates crosstalk between pulmonary epithelial cells and monocytes.

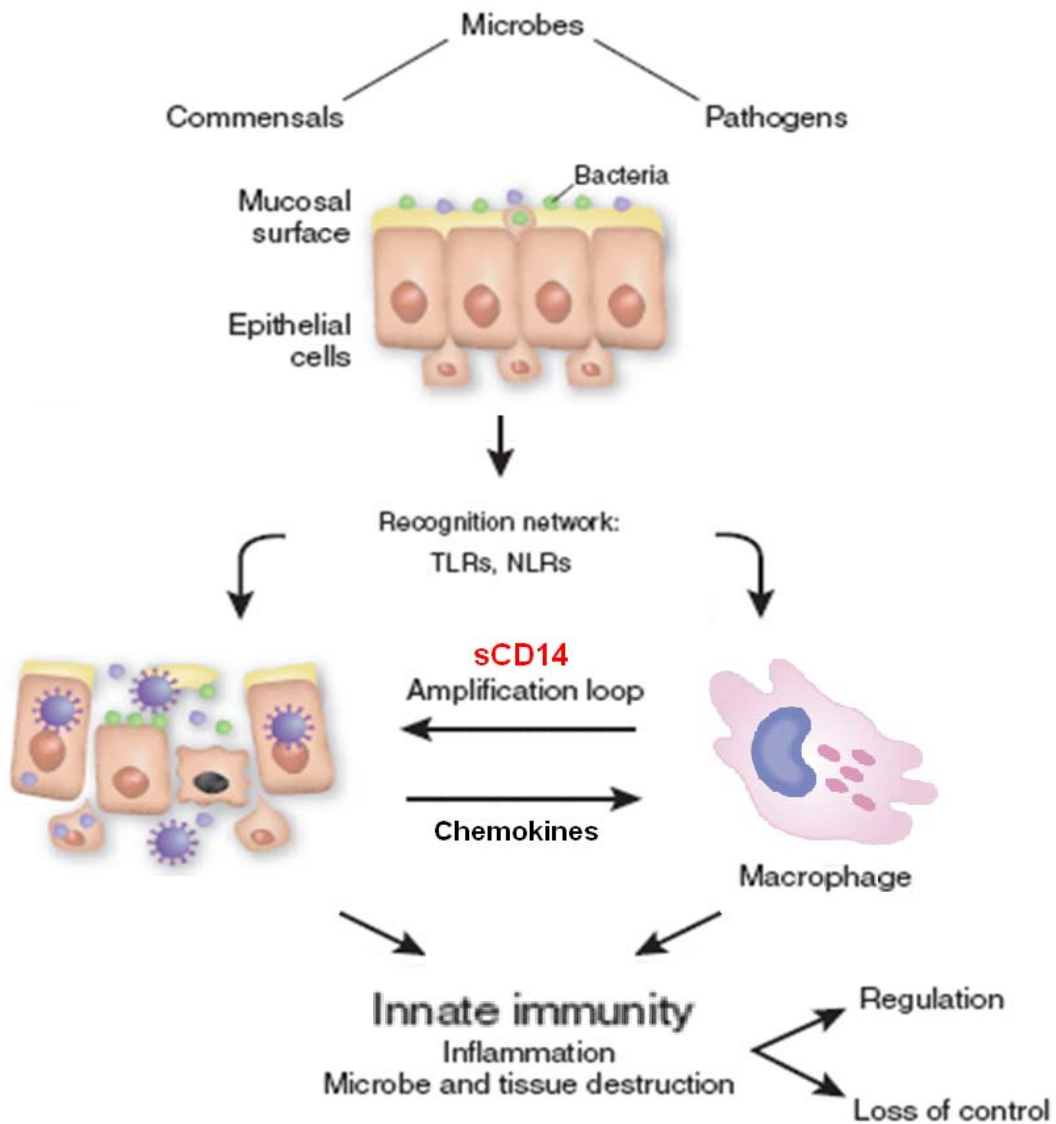


Figure 16. In the patients with COPD, activated pulmonary epithelial cells initiate the chemoattractant of monocytes. Activated monocytes not only help to clear the invading pathogens by phagocytosis and producing proinflammatory mediators, but also largely release sCD14 that could amplify the inflammatory responses of pulmonary epithelial cells, inducing an uncontrollable acute inflammation and eventually exacerbation. (Adapted and modified from Sansonetti, 2006 and modified).

influenza and respiratory syncytial virus (RSV) have all been found increased during the exacerbation (Sapey and Stockley, 2006). Inhibition of CD14 could also have potential effect on suppress virus-induced inflammation. Future study could also focus on understanding the mechanism of how sCD14 is released from monocytes or macrophages. Until now, it has been shown that sCD14 could be cleaved off from membrane by protease and phospholipase or directly secreted without GPI anchor (Bazil and Strominger, 1991; Bufler et al., 1995; Kirkland and Viriyakosol, 1998). It would be interesting to indentify the specific inhibitor that can block the release of sCD14 and test its potential application for the treatment of COPD.

CONCLUSIONS

1. *M. catarrhalis* can induce inflammatory responses on human pulmonary epithelial cells, such as CCL20/MIP-3 α expression and NF- κ B activation.
2. *M. catarrhalis*-induced CCL20/MIP-3 α expression is mainly through TLR2-MyD88-TRAF6-NF- κ B signaling pathway.
3. TLR4 signaling pathway is not activated because two important mediators, CD14 and MD2, are missing on A549 and BEAS-2B cells.
4. 1 α , 25-dihydroxy VD₃ induces both membrane and soluble CD14 expression on naïve monocytic THP1 cells, but has no effect on MD2 expression.
5. Increased CD14 expression enhances the TLR2-mediated responses and activates TLR4 signaling pathway on THP1 cells.
6. Conditioned media from 1 α , 25-dihydroxy VD₃ primed THP1 cells induces elevated inflammatory responses on pulmonary epithelial cells, which can be inhibited by CD14 antibody.

REFERENCES

- Aaron SD, Angel JB, Lunau M, Wright K, Fex C, Le Saux N, Dales RE. (2001) Granulocyte inflammatory markers and airway infection during acute exacerbation of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 163: 349–355.
- Akira S, Takeda K. (2004) Toll-like receptor signalling. *Nat Rev Immunol.* 4, 499-511.
- Akira S, Uematsu S, Takeuchi O. (2006) Pathogen recognition and innate immunity. *Cell.* 124, 783-801.
- Akira S. (2003) Toll-like receptor signaling. *J Biol Chem.* 278(40):38105-8.
- Aldonyte R, Jansson L, Piitulainen E, Janciauskiene S. (2003) Circulating monocytes from healthy individuals and COPD patients. *Respir Res.* 4:11.
- Alexopoulou L, Thomas V, Schnare M, Lobet Y, Anguita J, Schoen RT, Medzhitov R, Fikrig E, Flavell RA. (2002) Hyporesponsiveness to vaccination with *Borrelia burgdorferi* OspA in humans and in TLR1- and TLR2-deficient mice. *Nat Med.* 8(8):878-84.
- Anderson KV, Jürgens G, Nüsslein-Volhard C. (1985) Establishment of dorsal-ventral polarity in the *Drosophila* embryo: genetic studies on the role of the Toll gene product. *Cell.* 42(3):779-89.
- Au BT, Teixeira MM, Collins PD, Williams TJ. (1998) Effect of PDE4 inhibitors on zymosan-induced IL-8 release from human neutrophils: synergism with

- prostanoids and salbutamol. *Br J Pharmacol* 123:1260–1266.
- Babusyte A, Stravinskaite K, Jeroch J, Lötvall J, Sakalauskas R, Sitkauskiene B. (2007) Patterns of airway inflammation and MMP-12 expression in smokers and ex-smokers with COPD. *Respir Res.* 8:81.
- Bakri F, Brauer AL, Sethi S, Murphy TF. (2002) Systemic and mucosal antibody response to *Moraxella catarrhalis* following exacerbations of chronic obstructive pulmonary disease. *J Infect Dis.* 185:632–640.
- Ball P. Epidemiology and treatment of chronic bronchitis and its exacerbations. *Chest.* 1995 Aug;108(2 Suppl):43S-52S.
- Bals R, Hiemstra PS. (2004) Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J.* 23(2):327-33.
- Bals R. (2000) Epithelial antimicrobial peptides in host defense against infection. *Respir Res.* 1(3):141-50.
- Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. (2000) Immunobiology of dendritic cells. *Annu Rev Immunol.* 18:767–811.
- Baraldo S, Lokar Oliani K, Turato G, Zuin R, Saetta M. (2007) The Role of Lymphocytes in the Pathogenesis of Asthma and COPD. *Curr Med Chem.* 14(21):2250-6.
- Barczyk A, Pierzchała W, Kon OM, Cosio B, Adcock IM, Barnes PJ. (2006) Cytokine production by bronchoalveolar lavage T lymphocytes in chronic obstructive pulmonary disease. *J Allergy Clin Immunol.* 117(6):1484-92.

- Barnes PJ, Stockley RA. (2005) COPD: current therapeutic interventions and future approaches. *Eur Respir J.* 25(6):1084-106.
- Barnes PJ. (2000) Chronic obstructive pulmonary disease. *N Engl J Med.* 343(4):269-80.
- Barnes PJ. (2004b) Mediators of chronic obstructive pulmonary disease. *Pharmacol Rev.* 56(4):515-48.
- Barnes PJ. (2008a) Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol.* 8(3):183-92.
- Barnes PJ. (2008b) The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest.* 118(11):3546-56.
- Barnes, PJ. (2004a) Macrophages as orchestrators of COPD. *COPD.* 1, 59–70.
- Barton GM, Medzhitov R. (2003) Toll-like receptor signaling pathways. *Science.* 300(5625):1524-5.
- Bazil V, Baudys M, Hilgert I, Stefanová I, Low MG, Zbrozek J, Horejsí V.(1989) Structural relationship between the soluble and membrane-bound forms of human monocyte surface glycoprotein CD14. *Mol Immunol.* 26(7):657-62.
- Bazil V, Strominger JL. Shedding as a mechanism of down-modulation of CD14 on stimulated human monocytes. *J Immunol.* 1991 Sep 1;147(5):1567-74.
- Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. (2000) Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax* 55:114–120.
- Biernacki WA, Kharitonov SA, Barnes PJ. (2003) Increased leukotriene B4 and

- 8-isoprostane in exhaled breath condensate of patients with exacerbations of COPD. *Thorax* 58:294–298.
- Bowler RP, Barnes PJ, Crapo JD. (2004) The role of oxidative stress in chronic obstructive pulmonary disease. *COPD*. 1(2):255-77.
- Bracke KR, D'hulst AI, Maes T, Moerloose KB, Demedts IK, Lebecque S, Joos GF, Brusselle GG.(2006) Cigarette smoke-induced pulmonary inflammation and emphysema are attenuated in CCR6-deficient mice. *J Immunol*. 177(7):4350-9.
- Bradley JR, Pober JS. (2001) Tumor necrosis factor receptor-associated factors (TRAFs). *Oncogene*. 20(44):6482-91.
- Brogden KA. (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol*. 3(3):238-50.
- Bufler P, Stiegler G, Schuchmann M, Hess S, Krüger C, Stelter F, Eckerskorn C, Schütt C, Engelmann H. (1995) Soluble lipopolysaccharide receptor (CD14) is released via two different mechanisms from human monocytes and CD14 transfectants. *Eur J Immunol*. 25(2):604-10.
- Burge S, Wedzicha JA. (2003) COPD exacerbations: definitions and classifications. *Eur Respir J Suppl*. 41:46s-53s.
- Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV. (1996) TRAF6 is a signal transducer for interleukin-1. *Nature*. 383(6599):443-6.
- Capelli A, Di Stefano A, Gnemmi I, Balbo P, Cerutti CG, Balbi B, Lusuardi M, and Donner CF. (1999) Increased MCP-1 and MIP-1_α in bronchoalveolar lavage fluid of chronic bronchitis. *Eur Respir J*. 14:160–165.

- Casadevall C, Coronell C, Ramírez-Sarmiento AL, Martínez-Llorens J, Barreiro E, Orozco-Levi M, Gea J.(2007) Upregulation of pro-inflammatory cytokines in the intercostal muscles of COPD patients. *Eur Respir J.* 30(4):701-7.
- Celli BR, Barnes PJ. (2007) Exacerbations of chronic obstructive pulmonary disease. *Eur Respir J.* 29(6):1224-38.
- Chen D, McMichael JC, VanDerMeid KR, Hahn D, Mininni T, Cowell J, Eldridge J. (1996) Evaluation of purified UspA from *Moraxella catarrhalis* as a vaccine in a murine model after active immunization. *Infect Immun.* 64(6):1900-5.
- Churg A, Wright JL. (2005) Proteases and emphysema. *Curr Opin Pulm Med.* 11(2):153-9.
- Connors AF Jr, Dawson NV, Thomas C, Harrell FE Jr, Desbiens N, Fulkerson WJ, Kussin P, Bellamy P, Goldman L, Knaus WA. (1996) Outcomes following acute exacerbation of severe chronic obstructive lung disease. *Am J Respir Crit Care Med.* Oct;154(4 Pt 1):959-67.
- Cope LD, Lafontaine ER, Slaughter CA, Hasemann CA Jr, Aebi C, Henderson FW, McCracken GH Jr, Hansen EJ. (1999) Characterization of the *Moraxella catarrhalis* *uspA1* and *uspA2* genes and their encoded products. *J Bacteriol.* 181, 4026-4034.
- Cosio MG, Saetta M, Agusti A. (2009) Immunologic aspects of chronic obstructive pulmonary disease. *N Engl J Med.* 360(23):2445-54.
- Cowburn AS, Condliffe AM, Farahi N, Summers C, Chilvers ER. (2008) Advances in neutrophil biology: clinical implications. *Chest.* 134(3):606-12.

- Cowland JB, Sørensen OE, Sehested M, Borregaard N. (2003) Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha. *J Immunol.* 171(12):6630-9.
- Culpitt SV, Rogers DF, Shah P, De Matos C, Russell RE, Donnelly LE, Barnes PJ. (2003) Impaired inhibition by dexamethasone of cytokine release by alveolar macrophages from patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 167(1):24-31.
- Currie GP, Wedzicha JA. (2006) ABC of chronic obstructive pulmonary disease. Acute exacerbations. *BMJ.* 333(7558):87-9.
- da Silva Correia J, Ulevitch RJ. (2002) MD-2 and TLR4 N-linked glycosylations are important for a functional lipopolysaccharide receptor. *J Biol Chem.* 277(3):1845-54.
- de Boer WI, Sont JK, van Schadewijk A, Stolk J, van Krieken JH, and Hiemstra PS. (2000) Monocyte chemoattractant protein 1, interleukin 8 and chronic airways inflammation in COPD. *J Pathol.* 190:619–626.
- de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. (1998) Transforming growth factor beta1 and recruitment of macrophages and mast cells in airways in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 158(6):1951-7.
- Demedts IK, Bracke KR, Van Pottelberge G, Testelmans D, Verleden GM, Vermassen FE, Joos GF, Brusselle GG. (2007) Accumulation of dendritic cells and increased CCL20 levels in the airways of patients with chronic obstructive pulmonary

disease. *Am J Respir Crit Care Med.* 175(10):998-1005.

Deng L, Wang C, Spencer E, Yang L, Braun A, You J, Slaughter C, Pickart C, Chen ZJ. (2000) Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell.* 103(2):351-61.

Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), National Center for Health Statistics. (2006) Available from:<http://www.cdc.gov/nchs/about/otheract/hpdata2010/focusareas/fa24-rd2.htm/>

Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, Saetta M. (1998) Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med.* 158:1277–1285.

Diamond G, Legarda D, Ryan LK. (2000) The innate immune response of the respiratory epithelium. *Immunol Rev.* 173, 27-38.

Dockrell DH, Whyte MK. (2006) Regulation of phagocyte lifespan in the lung during bacterial infection. *J Leukoc Biol.* 79(5):904-8.

Donaldson GC, Wedzicha JA. (2006) COPD exacerbations .1: Epidemiology. *Thorax.* 61(2):164-8.

Doz E, Noulin N, Boichot E, Guénon I, Fick L, Le Bert M, Lagente V, Ryffel B, Schnyder B, Quesniaux VF, Couillin I. (2008) Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling

- dependent. *J Immunol.* 180(2):1169-78.
- Dunne A, Ejdeback M, Ludidi PL, O'Neill LA, Gay NJ. (2003) Structural complementarity of Toll/interleukin-1 receptor domains in Toll-like receptors and the adaptors Mal and MyD88. *J Biol Chem.* 278(42):41443-51.
- Ferrero E, Hsieh CL, Francke U, Goyert SM. (1990) CD14 is a member of the family of leucine-rich proteins and is encoded by a gene syntenic with multiple receptor genes. *J Immunol.* 145(1):331-6.
- Ferrero E, Jiao D, Tsuberi BZ, Tesio L, Rong GW, Haziot A, Goyert SM. (1993) Transgenic mice expressing human CD14 are hypersensitive to lipopolysaccharide. *Proc Natl Acad Sci U S A.* 90(6):2380-4.
- Finberg RW, Kurt-Jones EA. CD14: chaperone or matchmaker? (2006) *Immunity.* 24(2):127-9.
- Fitzgerald KA, McWhirter SM, Faia KL, Rowe DC, Latz E, Golenbock DT, Coyle AJ, Liao SM, Maniatis T. (2003a) IKKepsilon and TBK1 are essential components of the IRF3 signaling pathway. *Nat Immunol.* 4(5):491-6.
- Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, Brint E, Dunne A, Gray P, Harte MT, McMurray D, Smith DE, Sims JE, Bird TA, O'Neill LA. (2001) Mal (MyD88-adaptor-like) is required for Toll-like receptor-4 signal transduction. *Nature.* 413(6851):78-83.
- Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM, Golenbock DT. (2003b) LPS-TLR4 signaling to IRF-3/7 and NF-kappaB involves the toll adaptors TRAM and TRIF. *J Exp Med.* 198(7):1043-55.

- Frey EA, Miller DS, Jahr TG, Sundan A, Bazil V, Espevik T, Finlay BB, Wright SD. (1992) Soluble CD14 participates in the response of cells to lipopolysaccharide. *J Exp Med.* 176(6):1665-71.
- Gompertz S, O'Brien C, Bayley DL, Hill SL, Stockley RA. (2001) Changes in bronchial inflammation during acute exacerbations of chronic bronchitis. *Eur Respir J.* 17:1112–1119.
- Gordon S. (2002) Pattern recognition receptors: doubling up for the innate immune response. *Cell.* 111, 927-930.
- Goyert SM, Ferrero E, Rettig WJ, Yenamandra AK, Obata F, Le Beau MM. (1988) The CD14 monocyte differentiation antigen maps to a region encoding growth factors and receptors. *Science.* 239(4839):497-500.
- Greenberg SB, Allen M, Wilson J, Atmar RL. (2000) Respiratory viral infections in adults with and without chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* Jul;162(1):167-73.
- Groenewegen KH, Schols AMWJ, Wouters EFM. (2003) Mortality and mortality-related factors after hospitalisation for acute exacerbation of COPD. *Chest.* 124:459–67.
- Gu XX, Chen J, Barenkamp SJ, Robbins JB, Tsai CM, Lim DJ, Battey J. (1998) Synthesis and characterization of lipooligosaccharide-based conjugates as vaccine candidates for *Moraxella (Branhamella) catarrhalis*. *Infect Immun.* 66(5):1891-7.
- Gupta D, Kirkland TN, Viriyakosol S, Dziarski R. (1996) CD14 is a cell-activating

- receptor for bacterial peptidoglycan. *J Biol Chem.* 271(38):23310-6.
- Gutierrez-Ramos JC, Lloyd C, Kapsenberg ML, Gonzalo JA, Coyle AJ. (2000) Non-redundant functional groups of chemokines operate in a coordinate manner during the inflammatory response in the lung. *Immunol Rev.* 177:31–42.
- Haddad J (2004) Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. *Int Immunopharmacol* 4:475–493.
- Hajjar AM, O'Mahony DS, Ozinsky A, Underhill DM, Aderem A, Klebanoff SJ, Wilson CB. (2001) Cutting edge: functional interactions between toll-like receptor (TLR) 2 and TLR1 or TLR6 in response to phenol-soluble modulin. *J Immunol.* 166(1):15-9.
- Hammad H, Lambrecht BN. (2008) Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. *Nat Rev Immunol.* 8(3):193-204.
- Hashimoto C, Hudson KL, Anderson KV. (1988) The Toll gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell.* 52(2):269-79.
- Haziot A, Chen S, Ferrero E, Low MG, Silber R, Goyert SM.(1988) The monocyte differentiation antigen, CD14, is anchored to the cell membrane by a phosphatidylinositol linkage. *J Immunol.* 141(2):547-52.
- Haziot A, Ferrero E, Köntgen F, Hijiya N, Yamamoto S, Silver J, Stewart CL, Goyert SM. (1996) Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice. *Immunity.* 4(4):407-14.

- Haziot A, Rong GW, Silver J, Goyert SM. (1993) Recombinant soluble CD14 mediates the activation of endothelial cells by lipopolysaccharide. *J Immunol.* 151(3):1500-7.
- Heldin CH, Miyazono K, ten Dijke P. (1997) TGF-beta signalling from cell membrane to nucleus through SMAD proteins. *Nature.* 390(6659):465-71.
- Hieshima K, Imai T, Opdenakker G, Van Damme J, Kusuda J, Tei H, Sakaki Y, Takatsuki K, Miura R, Yoshie O, Nomiya H. (1997) Molecular cloning of a novel human CC chemokine liver and activation-regulated chemokine (LARC) expressed in liver. Chemotactic activity for lymphocytes and gene localization on chromosome 2. *J Biol Chem.* 272(9):5846-53.
- Hill AT, Bayley D, Stockley RA. (1999) The interrelationship of sputum inflammatory markers in patients with chronic bronchitis. *Am J Respir Crit Care Med* 160: 893–898.
- Hill AT, Campbell EJ, Hill SL, Bayley DL, and Stockley RA. (2000) Association between airway bacterial load and markers of airway inflammation in patients with stable chronic bronchitis. *Am J Med.* 109:288–295.
- Hill DJ, Virji M. (2003) A novel cell-binding mechanism of *Moraxella catarrhalis* ubiquitous surface protein UspA: specific targeting of the N-domain of carcinoembryonic antigen-related cell adhesion molecules by UspA1. *Mol Microbiol.* 48, 117-129.
- Hippenstiel S, Opitz B, Schmeck B, Suttorp N. (2006) Lung epithelium as a sentinel and effector system in pneumonia--molecular mechanisms of pathogen

- recognition and signal transduction. *Respir Res.* 7, 97.
- Hodge SJ, Hodge GL, Reynolds PN, Scicchitano R, Holmes M. (2003) Increased production of TGF- β and apoptosis of T lymphocytes isolated from peripheral blood in COPD. *Am J Physiol Lung Cell Mol Physiol.*
- Hoebe K, Du X, Georgel P, Janssen E, Tabeta K, Kim SO, Goode J, Lin P, Mann N, Mudd S, Crozat K, Sovath S, Han J, Beutler B. (2003) Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. *Nature.* 424(6950):743-8.
- Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, Cherniack RM, Rogers RM, Sciurba FC, Coxson HO, Paré PD. (2004) The nature of small-airway obstruction in chronic obstructive pulmonary disease. *N Engl J Med.* 350(26):2645-53.
- Hogg JC, Senior RM. (2002) Chronic obstructive pulmonary disease - part 2: pathology and biochemistry of emphysema. *Thorax.* 57(9):830-4.
- Hollander C, Sitkauskiene B, Sakalauskas R, Westin U, Janciauskiene SM. (2007) Serum and bronchial lavage fluid concentrations of IL-8, SLPI, sCD14 and sICAM-1 in patients with COPD and asthma. *Respir Med.* 101(9):1947-53.
- Holm MM, Vanlerberg SL, Sledjeski DD, Lafontaine ER. (2003) The Hag protein of *Moraxella catarrhalis* strain O35E is associated with adherence to human lung and middle ear cells. *Infect Immun.* 71, 4977-4984.
- Horng T, Barton GM, Flavell RA, Medzhitov R. (2002) The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors. *Nature.* 420(6913):329-33.
- Horng T, Barton GM, Medzhitov R. (2001) TIRAP: an adapter molecule in the Toll

- signaling pathway. *Nat Immunol.* 2(9):835-41.
- Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S. (1999) Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol.* 162(7):3749-52.
- Hu WG, Chen J, Battey JF, Gu XX. (2000) Enhancement of clearance of bacteria from murine lungs by immunization with detoxified lipooligosaccharide from *Moraxella catarrhalis* conjugated to proteins. *Infect Immun.* 68(9):4980-5.
- Hu WG, Chen J, Collins FM, Gu XX. (1999) An aerosol challenge mouse model for *Moraxella catarrhalis*. *Vaccine.* 18(9-10):799-804.
- Imai K, Dalal SS, Chen ES, Downey R, Schulman LL, Ginsburg M, D'Armiento J. (2001) Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. *Am J Respir Crit Care Med.* 163(3 Pt 1):786-91.
- Ioachimescu OC, Stoller JK. (2005) A review of alpha-1 antitrypsin deficiency. *COPD.* 2(2):263-75.
- Janeway CA Jr, Medzhitov R. (2002) Innate immune recognition. *Annu Rev Immunol.* 20, 197-216.
- Janeway CA Jr, Medzhitov R. (2002) Innate immune recognition. *Annu Rev Immunol.* 20, 197-216.
- Janssens S, Beyaert R. (2003) Functional diversity and regulation of different interleukin-1 receptor-associated kinase (IRAK) family members. *Mol Cell.* 11(2):293-302.

- Jiang Q, Akashi S, Miyake K, Petty HR. (2000) Lipopolysaccharide induces physical proximity between CD14 and toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-kappa B. *J Immunol.* 165(7):3541-4.
- Jiao X, Hirano T, Hou Y, Gu XX. (2002) Specific immune responses and enhancement of murine pulmonary clearance of *Moraxella catarrhalis* by intranasal immunization with a detoxified lipooligosaccharide conjugate vaccine. *Infect Immun.* 70(11):5982-9.
- Karalus R, Campagnari A. (2000) *Moraxella catarrhalis*: a review of an important human mucosal pathogen. *Microbes Infect.* 2(5):547-59.
- Karin M, Ben-Neriah Y. (2000) Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol.* 18:621-63.
- Kawai T, Adachi O, Ogawa T, Takeda K, Akira S. (1999) Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity.* 11(1):115-22.
- Kawai T, Takeuchi O, Fujita T, Inoue J, Mühlradt PF, Sato S, Hoshino K, Akira S. (2001) Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. *J Immunol.* 167(10):5887-94.
- Keatings VM, Collins PD, Scott DM, Barnes PJ. (1996) Differences in interleukin-8 and tumor necrosis factor- α in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med.* 153:530-534.
- Kim JI, Lee CJ, Jin MS, Lee CH, Paik SG, Lee H, Lee JO. (2005) Crystal structure of

- CD14 and its implications for lipopolysaccharide signaling. *J Biol Chem.* 280(12):11347-51.
- Kirkland TN, Viriyakosol S. (1998) Structure-function analysis of soluble and membrane-bound CD14. *Prog Clin Biol Res.*397:79-87.
- Kirschning CJ, Wesche H, Merrill Ayres T, Rothe M. (1998) Human toll-like receptor 2 confers responsiveness to bacterial lipopolysaccharide. *J Exp Med.* 188(11):2091-7.
- Kurashima K, Fujimura M, Myou S, Ishiura Y, Onai N, Matsushima K. (2006) Asthma severity is associated with an increase in both blood CXCR3+ and CCR4+ T cells. *Respirology.* 11(2):152-7.
- Kusunoki T, Hailman E, Juan TS, Lichenstein HS, Wright SD. (1995) Molecules from *Staphylococcus aureus* that bind CD14 and stimulate innate immune responses. *J Exp Med.* 182(6):1673-82.
- Lafontaine ER, Cope LD, Aebi C, Latimer JL, McCracken GH Jr, Hansen EJ. (2000) The UspA1 protein and a second type of UspA2 protein mediate adherence of *Moraxella catarrhalis* to human epithelial cells in vitro. *J Bacteriol.* 182, 1364-1373.
- Lambrecht BN. (2006) Alveolar macrophage in the driver's seat. *Immunity.* 24(4):366-8.
- Landmann R, Müller B, Zimmerli W. (2000) CD14, new aspects of ligand and signal diversity. *Microbes Infect.* 2(3):295-304.
- Lanone S, Zheng T, Zhu Z, Liu W, Lee CG, Ma B, Chen Q, Homer RJ, Wang J,

- Rabach LA, Rabach ME, Shipley JM, Shapiro SD, Senior RM, Elias JA. (2002) Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *J Clin Invest.* 110(4):463-74.
- Lee E, Lindo T, Jackson N, Meng-Choong L, Reynolds P, Hill A, Haswell M, Jackson S, Kilfeather S. (1999) Reversal of human neutrophil survival by leukotriene B(4) receptor blockade and 5-lipoxygenase and 5-lipoxygenase activating protein inhibitors. *Am J Respir Crit Care Med.* 160(6):2079-85.
- Lee HK, Dunzendorfer S, Soldau K, Tobias PS. (2006) Double-stranded RNA-mediated TLR3 activation is enhanced by CD14. *Immunity.* 24(2):153-63.
- Li S, Strelow A, Fontana EJ, Wesche H. (2002) IRAK-4: a novel member of the IRAK family with the properties of an IRAK-kinase. *Proc Natl Acad Sci U S A.* 99(8):5567-72.
- Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schaubert J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science.* 311(5768):1770-3.
- Lopez AD, Murray CC. (1998) The global burden of disease, 1990-2020. *Nat Med.* 4:1241-3.
- Lord KA, Hoffman-Liebermann B, Liebermann DA. (1990) Nucleotide sequence and expression of a cDNA encoding MyD88, a novel myeloid differentiation primary

- response gene induced by IL6. *Oncogene*. (7):1095-7.
- Lukacs NW, Hogaboam CM, Kunkel SL. (2005) Chemokines and their receptors in chronic pulmonary disease. *Curr. Drug Targets Inflamm. Allergy*. 4:313–317.
- MacNee W (2000) Oxidants/antioxidants and COPD. *Chest* 117 (Suppl 1):303S–317S.
- Mahajan B, Vijayan VK, Agarwal MK, Bansal SK. (2008) Serum interleukin-1beta as a marker for differentiation of asthma and chronic obstructive pulmonary disease. *Biomarkers*. 13(7):713-27.
- Mahler DA, Huang S, Tabrizi M, Bell GM. (2004) Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. *Chest*. 126: 926–934.
- Manukyan M, Triantafilou K, Triantafilou M, Mackie A, Nilsen N, Espevik T, Wiesmüller KH, Ulmer AJ, Heine H. (2005) Binding of lipopeptide to CD14 induces physical proximity of CD14, TLR2 and TLR1. *Eur J Immunol*. 35(3):911-21.
- Martin TR, Frevert CW. (2005) Innate immunity in the lungs. *Proc Am Thorac Soc*.2(5):403-11.
- McGettrick AF, O'Neill LA. (2004) The expanding family of MyD88-like adaptors in Toll-like receptor signal transduction. *Mol Immunol*. 41(6-7):577-82.
- Medzhitov R. (2001) Toll-like receptors and innate immunity. *Nat Rev Immunol*. 1(2):135-45.
- Meier PS, Troller R, Grivea IN, Syrogiannopoulos GA, Aebi C. (2002) The outer

membrane proteins UspA1 and UspA2 of *Moraxella catarrhalis* are highly conserved in nasopharyngeal isolates from young children. *Vaccine*. 20, 1754-1760.

Meja KK, Barnes PJ, and Giembycz MA (1997) Characterization of the prostanoid receptor(s) on human blood monocytes at which prostaglandin E2 inhibits lipopolysaccharide-induced tumour necrosis factor- α . *Br J Pharmacol* 122:149–157.

Miravittles M, Murio C, Guerrero T, Gisbert R; DAFNE Study Group. Decisiones sobre Antibioticoterapia y Farmacoeconomía en la EPOC. (2002) Pharmacoeconomic evaluation of acute exacerbations of chronic bronchitis and COPD. *Chest*. 121(5):1449-55.

Miura Y, Shimazu R, Miyake K, Akashi S, Ogata H, Yamashita Y, Narisawa Y, Kimoto M. (1998) RP105 is associated with MD-1 and transmits an activation signal in human B cells. *Blood*. 92(8):2815-22.

Miyake K, Shimazu R, Kondo J, Niki T, Akashi S, Ogata H, Yamashita Y, Miura Y, Kimoto M. (1998) Mouse MD-1, a molecule that is physically associated with RP105 and positively regulates its expression. *J Immunol*. 161(3):1348-53.

Monso E, Ruiz J, Rosell A, Manterola J, Fiz J, Morera J, Ausina V. (1995) Bacterial infection in chronic obstructive pulmonary disease: a study of stable and exacerbated outpatients using the protected specimen brush. *Am J Respir Crit Care Med* 152:1316–1320.

Montuschi P, Kharitonov SA, Ciabattini G, Barnes PJ. (2003) Exhaled leukotrienes

- and prostaglandins in COPD. *Thorax* 58:585–588.
- Moore B, Murphy RF, Agrawal DK. (2008) Interaction of tgf-beta with immune cells in airway disease. *Curr Mol Med*. 8(5):427-36.
- Mullen GE, Kennedy MN, Visintin A, Mazzoni A, Leifer CA, Davies DR, Segal DM. (2003) The role of disulfide bonds in the assembly and function of MD-2. *Proc Natl Acad Sci U S A*. 100(7):3919-24.
- Murphy TF, Brauer AL, Grant BJ, Sethi S. (2005) *Moraxella catarrhalis* in chronic obstructive pulmonary disease: burden of disease and immune response. *Am J Respir Crit Care Med*. 172(2):195-9.
- Murphy TF, Kyd JM, John A, Kirkham C, Cripps AW. (1998) Enhancement of pulmonary clearance of *Moraxella (Branhamella) catarrhalis* following immunization with outer membrane protein CD in a mouse model. *J Infect Dis*. 178(6):1667-75.
- Murphy TF. (1996) *Branhamella catarrhalis*: epidemiology, surface antigenic structure, and immune response. *Microbiol Rev*. 60(2):267-79.
- Murphy TF. (1998) Lung infections. 2. *Branhamella catarrhalis*: epidemiological and clinical aspects of a human respiratory tract pathogen, *Thorax*. 53:124–128.
- Muzio M, Ni J, Feng P, Dixit VM. (1997) IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science*. 278(5343):1612-5.
- Nagai Y, Akashi S, Nagafuku M, Ogata M, Iwakura Y, Akira S, Kitamura T, Kosugi A, Kimoto M, Miyake K. (2002) Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol*. 3(7):667-72.

- Nelson RT, Boyd J, Gladue RP, Paradis T, Thomas R, Cunningham AC, Lira P, Brissette WH, Hayes L, Hames LM, Neote KS, McColl SR. (2001) Genomic organization of the CC chemokine mip-3alpha/CCL20/larc/exodus/SCYA20, showing gene structure, splice variants, and chromosome localization. *Genomics*. 73(1):28-37.
- Nishimoto N, Kishimoto T. (2004) Inhibition of IL-6 for the treatment of inflammatory diseases. *Curr Opin Pharmacol*. 4: 386–391.
- Ohnishi T, Muroi M, Tanamoto K. (2001) N-linked glycosylations at Asn(26) and Asn(114) of human MD-2 are required for toll-like receptor 4-mediated activation of NF-kappaB by lipopolysaccharide. *J Immunol*. 167(6):3354-9.
- Oshiumi H, Matsumoto M, Funami K, Akazawa T, Seya T. (2003) TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction. *Nat Immunol*. 4(2):161-7.
- Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A. (2000). The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci U S A*. 97(25):13766-71.
- Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, Fabbri LM, Johnston SL. (2006) Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am J Respir Crit Care Med*. 173(10):1114-21.
- Patel IS, Seemungal TA, Wilks M, Lloyd-Owen SJ, Donaldson GC, Wedzicha JA.

- (2002) Relationship between bacterial colonisation and the frequency, character and severity of COPD exacerbations. *Thorax*. 57:759–764.
- Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS; (2001) GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am J Respir Crit Care Med*. 163(5):1256-76.
- Pavord ID, Tattersfield AE. (1995) Bronchoprotective role for endogenous prostaglandin E2. *Lancet* 344:436–438.
- Pearson MM, Lafontaine ER, Wagner NJ, St Geme JW 3rd, Hansen EJ. (2002) A hag mutant of *Moraxella catarrhalis* strain O35E is deficient in hemagglutination, autoagglutination, and immunoglobulin D-binding activities. *Infect Immun*. 70, 4523-4533.
- Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. (2005) Interpretative strategies for lung function tests. *Eur Respir J*. 26(5):948-68.
- Peng D, Hong W, Choudhury BP, Carlson RW, Gu XX. (2005) *Moraxella catarrhalis* bacterium without endotoxin, a potential vaccine candidate. *Infect Immun*. 73(11):7569-77.
- Perkins ND. (2007) Integrating cell-signalling pathways with NF-kappaB and IKK function. *Nat Rev Mol Cell Biol*. 8, 49-62.

- Pfeiffer A, Böttcher A, Orsó E, Kapinsky M, Nagy P, Bodnár A, Spreitzer I, Liebisch G, Drobnik W, Gempel K, Horn M, Holmer S, Hartung T, Multhoff G, Schütz G, Schindler H, Ulmer AJ, Heine H, Stelter F, Schütt C, Rothe G, Szöllösi J, Damjanovich S, Schmitz G. Lipopolysaccharide and ceramide docking to CD14 provokes ligand-specific receptor clustering in rafts. *Eur J Immunol.* 2001 Nov;31(11):3153-64.
- Pollard JA, Wallace RJ Jr, Nash DR, Luman JI, Wilson RW. (1986) Incidence of *Branhamella catarrhalis* in the sputa of patients with chronic lung disease. *Drugs.* 31:103–108.
- Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science.* 282(5396):2085-8.
- Pugin J, Heumann ID, Tomasz A, Kravchenko VV, Akamatsu Y, Nishijima M, Glauser MP, Tobias PS, Ulevitch RJ. (1994) CD14 is a pattern recognition receptor. *Immunity.* 1(6):509-16.
- Pugin J, Schürer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. (1993a) Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. *Proc Natl Acad Sci U S A.* 90(7):2744-8.
- Pugin J, Ulevitch RJ, Tobias PS. (1993b) A critical role for monocytes and CD14 in endotoxin-induced endothelial cell activation. *J Exp Med.* 178(6):2193-200.

- Quint JK, Wedzicha JA. (2007) The neutrophil in chronic obstructive pulmonary disease. *J Allergy Clin Immunol.* 119(5):1065-71.
- Qureshi ST, Larivière L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D. (1999) Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J Exp Med.* 189(4):615-25.
- Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, Zielinski J; (2007) Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med.* 176(6):532-55.
- Rahman I (2003) Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol.* 36:95–109.
- Rahman I, Adcock IM.(2006) Oxidative stress and redox regulation of lung inflammation in COPD. *Eur Respir J.* 28(1):219-42.
- Regueiro V, Campos MA, Morey P, Sauleda J, Agustí AG, Garmendia J, Bengoechea JA. (2009) Lipopolysaccharide-binding protein and CD14 are increased in the bronchoalveolar lavage fluid of smokers. *Eur Respir J.* 33(2):273-81.
- Reimold AM. (2002) TNF α as therapeutic target: new drugs, more applications. *Curr Drug Targets Inflamm Allergy.* 1: 377–392.
- Rodríguez-Roisin R.(2006) COPD exacerbations.5: management. *Thorax.* 61(6):535-44.
- Rossi DL, Vicari AP, Franz-Bacon K, McClanahan TK, Zlotnik A. (1997)

- Identification through bioinformatics of two new macrophage proinflammatory human chemokines: MIP-3alpha and MIP-3beta. *J Immunol.* 158(3):1033-6.
- Russell RE, Thorley A, Culpitt SV, Dodd S, Donnelly LE, Demattos C, Fitzgerald M, Barnes PJ (2002) Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine and serine proteases. *Am J Physiol Lung Cell Mol Physiol.* 283:L867–L873.
- Salvi SS, Barnes PJ. (2009) Chronic obstructive pulmonary disease in non-smokers. *Lancet.* 374(9691):733-43.
- Sansonetti PJ. (2006) The innate signaling of dangers and the dangers of innate signaling. *Nat Immunol.* 7(12):1237-42.
- Sapey E, Stockley RA. (2006) COPD exacerbations . 2: aetiology. *Thorax.* 61(3):250-8.
- Schauber J, Dorschner RA, Coda AB, Büchau AS, Liu PT, Kiken D, Helfrich YR, Kang S, Elalieh HZ, Steinmeyer A, Zügel U, Bikle DD, Modlin RL, Gallo RL. (2007) Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest.* 117(3):803-11.
- Schmitz G, Orsó E. (2002) CD14 signalling in lipid rafts: new ligands and co-receptors. *Curr Opin Lipidol.* 13(5):513-21.
- Schromm AB, Lien E, Henneke P, Chow JC, Yoshimura A, Heine H, Latz E, Monks BG, Schwartz DA, Miyake K, Golenbock DT. (2001) Molecular genetic analysis of an endotoxin nonresponder mutant cell line: a point mutation in a conserved

- region of MD-2 abolishes endotoxin-induced signaling. *J Exp Med.* 194(1):79-88.
- Schumann RR, Leong SR, Flagg GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ.(1990) Structure and function of lipopolysaccharide binding protein. *Science.* 249(4975):1429-31.
- Schutysse E, Struyf S, Van Damme J. (2003) The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev.* 14(5):409-26.
- Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. (1998) Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 157(5 Pt 1):1418-22.
- Sethi S, Evans N, Grant BJB, Murphy TF. (2002) New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. *N Engl J Med.* 347:465–471.
- Sethi S, Murphy TF. (2001) Bacterial infection in chronic obstructive pulmonary disease in 2000: a state of the art review. *Clin Microbiol Rev.* 14: 336–363.
- Sethi S, Murphy TF. (2001) Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. *Clin Microbiol Rev.* 14(2):336-63.
- Sethi S, Wrona C, Grant BJ, Murphy TF. (2004) Strain-specific immune response to *Haemophilus influenzae* in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 169:448–453.
- Shapiro SD, Goldstein NM, Houghton AM, Kobayashi DK, Kelley D, Belaouaj A. (2003) Neutrophil elastase contributes to cigarette smoke-induced emphysema in

- mice. *Am J Pathol.* 163:2329–2335.
- Shapiro SD, Senior RM. (1999) Matrix metalloproteinases. Matrix degradation and more. *Am J Respir Cell Mol Biol.* 20:1100–1102.
- Shapiro SD. (1999) The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 160:S29–S32.
- Sharma S, tenOever BR, Grandvaux N, Zhou GP, Lin R, Hiscott J. (2003) Triggering the interferon antiviral response through an IKK-related pathway. *Science.* 300(5622):1148-51.
- Shibuya H, Yamaguchi K, Shirakabe K, Tonegawa A, Gotoh Y, Ueno N, Irie K, Nishida E, Matsumoto K. (1996) TAB1: an activator of the TAK1 MAPKKK in TGF-beta signal transduction. *Science.* 272(5265):1179-82.
- Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, Kimoto M. (1999) MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med.* 189(11):1777-82.
- Simmons DL, Tan S, Tenen DG, Nicholson-Weller A, Seed B. (1989) Monocyte antigen CD14 is a phospholipid anchored membrane protein. *Blood.* 73(1):284-9.
- Slack JL, Schooley K, Bonnert TP, Mitcham JL, Qwarnstrom EE, Sims JE, Dower SK. (2000) Identification of two major sites in the type I interleukin-1 receptor cytoplasmic region responsible for coupling to pro-inflammatory signaling pathways. *J Biol Chem.* 275(7):4670-8.
- Soler N, Ewig S, Torres A, Filella X, Gonzalez J, Zaubet A. (1999) Airway inflammation and bronchial microbial patterns with stable chronic obstructive

- pulmonary disease. *Eur Respir J.* 14:1015–1022.
- Sugita S, Kohno T, Yamamoto K, Imaizumi Y, Nakajima H, Ishimaru T, Matsuyama T. (2002) Induction of macrophage-inflammatory protein-3 α gene expression by TNF-dependent NF-kappaB activation. *J Immunol.* 168(11):5621-8.
- Suzuki N, Suzuki S, Duncan GS, Millar DG, Wada T, Mirtsos C, Takada H, Wakeham A, Itie A, Li S, Penninger JM, Wesche H, Ohashi PS, Mak TW, Yeh WC. (2002) Severe impairment of interleukin-1 and Toll-like receptor signalling in mice lacking IRAK-4. *Nature.* 416(6882):750-6.
- Swantek JL, Tsen MF, Cobb MH, Thomas JA. (2000) IL-1 receptor-associated kinase modulates host responsiveness to endotoxin. *J Immunol.* 164(8):4301-6.
- Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. (2002) Distinct effects of T-bet in TH1 lineage commitment and IFN-gamma production in CD4 and CD8 T cells. *Science.* 295(5553):338-42.
- Takaesu G, Kishida S, Hiyama A, Yamaguchi K, Shibuya H, Irie K, Ninomiya-Tsuji J, Matsumoto K. (2000) TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Mol Cell.* 5(4):649-58.
- Takaesu G, Surabhi RM, Park KJ, Ninomiya-Tsuji J, Matsumoto K, Gaynor RB. (2003) TAK1 is critical for IkappaB kinase-mediated activation of the NF-kappaB pathway. *J Mol Biol.* 326(1):105-15.
- Takahashi S, Takeuchi K, Okabe S.(1999) EP4 receptor mediation of prostaglandin E2-stimulated mucus secretion by rabbit gastric epithelial cells. *Biochem*

Pharmacol. 58(12):1997-2002.

Takeda K, Kaisho T, Akira S. (2003) Toll-like receptors. *Annu Rev Immunol.* 21:335-76.

Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S. (1999a) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity.* 11(4):443-51.

Takeuchi O, Kaufmann A, Grote K, Kawai T, Hoshino K, Morr M, Mühlradt PF, Akira S. (2000) Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a toll-like receptor 2- and MyD88-dependent signaling pathway. *J Immunol.* 164(2):554-7.

Takeuchi O, Kawai T, Mühlradt PF, Morr M, Radolf JD, Zychlinsky A, Takeda K, Akira S. (2001) Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol.* 13(7):933-40.

Takeuchi O, Kawai T, Sanjo H, Copeland NG, Gilbert DJ, Jenkins NA, Takeda K, Akira S. (1999b) TLR6: A novel member of an expanding toll-like receptor family. *Gene.* 231(1-2):59-65.

Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, Modlin RL, Akira S. (2002) Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol.* 169(1):10-4.

Takizawa H, Tanaka M, Takami K, Ohtoshi T, Ito K, Satoh M, Okada Y, Yamasawa F, Nakahara K, Umeda A. (2001) Increased expression of transforming growth

- factor- β 1 in small airway epithelium from tobacco smokers and patients with chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med.* 163: 1476–1483.
- Tetley TD. (2002) Macrophages and the pathogenesis of COPD. *Chest.* 121:156S–159S.
- Timpe JM, Holm MM, Vanlerberg SL, Basrur V, Lafontaine ER. (2003) Identification of a *Moraxella catarrhalis* outer membrane protein exhibiting both adhesin and lipolytic activities. *Infect Immun.* 71, 4341-4350.
- Tobias PS, Soldau K, Ulevitch RJ. (1986) Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. *J Exp Med.* 164(3):777-93.
- Toshchakov V, Jones BW, Perera PY, Thomas K, Cody MJ, Zhang S, Williams BR, Major J, Hamilton TA, Fenton MJ, Vogel SN. (2002) TLR4, but not TLR2, mediates IFN-beta-induced STAT1alpha/beta-dependent gene expression in macrophages. *Nat Immunol.* 3(4):392-8.
- Traves SL, Culpitt S, Russell REK, Barnes PJ, and Donnelly LE (2002) Elevated levels of the chemokines GRO- α and MCP-1 in sputum samples from COPD patients. *Thorax.* 57:590–595.
- Tsoumakidou M, Demedts IK, Brusselle GG, Jeffery PK. (2008) Dendritic cells in chronic obstructive pulmonary disease: new players in an old game. *Am J Respir Crit Care Med.* 177(11):1180-6.
- Tsutsumi-Ishii Y, Nagaoka I. (2003) Modulation of human beta-defensin-2 transcription in pulmonary epithelial cells by lipopolysaccharide-stimulated

- mononuclear phagocytes via proinflammatory cytokine production. *J Immunol.* Apr 15;170(8):4226-36.
- Unhanand M, Maciver I, Ramilo O, Arencibia-Mireles O, Argyle JC, McCracken Jr. GH, Hansen EJ. (1992) Pulmonary clearance of *Moraxella catarrhalis* in an animal model. *J Infect Dis.* 165:644–50.
- Vanechoutte M, Verschraegen G, Claeys G, Van Den Abeele AM. (1990b) Serological typing of *Branhamella catarrhalis* strains on the basis of lipopolysaccharide antigens. *J Clin Microbiol.* 28(2):182-7.
- Vanechoutte M, Verschraegen G, Claeys G, Weise B, Van Den Abeele AM. (1990a) Respiratory tract carrier rates of *Moraxella* (*Branhamella*) *catarrhalis* in adults and children and interpretation of the isolation of *M. catarrhalis* from sputum. *J Clin Microbiol.* 28:2674–2680.
- Verduin CM, Hol C, Fleer A, van Dijk H, van Belkum A. (2002) *Moraxella catarrhalis*: from emerging to established pathogen. *Clin Microbiol Rev.* 15(1):125-44.
- Vernooy JH, Lindeman JH, Jacobs JA, Hanemaaijer R, Wouters EF. (2004) Increased activity of matrix metalloproteinase-8 and matrix metalloproteinase-9 in induced sputum from patients with COPD. *Chest.* 126(6):1802-10.
- Visintin A, Mazzoni A, Spitzer JA, Segal DM. (2001) Secreted MD-2 is a large polymeric protein that efficiently confers lipopolysaccharide sensitivity to Toll-like receptor 4. *Proc Natl Acad Sci U S A.* 98(21):12156-61.
- Wan YY, Flavell RA. (2007) Regulatory T cells, transforming growth factor-beta, and immune suppression. *Proc Am Thorac Soc.* 4(3):271-6.

- Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. (2001) TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature*. 412(6844):346-51.
- Wedzicha JA. (2004) Role of viruses in exacerbations of chronic obstructive pulmonary disease. *Proc Am Thorac Soc*. 1: 115–120.
- Wilson R. (1998) The role of infection in COPD. *Chest*. 113:242S–248S.
- Wright SD, Detmers PA, Aida Y, Adamowski R, Anderson DC, Chad Z, Kabbash LG, Pabst MJ. (1990a) CD18-deficient cells respond to lipopolysaccharide in vitro. *J Immunol*. 144(7):2566-71.
- Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. (1990b) CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*. 249(4975):1431-3.
- Wright SD, Tobias PS, Ulevitch RJ, Ramos RA. (1989) Lipopolysaccharide (LPS) binding protein opsonizes LPS-bearing particles for recognition by a novel receptor on macrophages. *J Exp Med*. 170(4):1231-41.
- Yamamoto C, Yoneda T, Yoshikawa M, Fu A, Tokuyama T, Tsukaguchi K, Narita N. (1997) Airway inflammation in COPD assessed by sputum levels of interleukin-8. *Chest*. 112:505–510.
- Yamamoto M, Sato S, Hemmi H, Hoshino K, Kaisho T, Sanjo H, Takeuchi O, Sugiyama M, Okabe M, Takeda K, Akira S. (2003a) Role of adaptor TRIF in the MyD88-independent toll-like receptor signaling pathway. *Science*. 301(5633):640-3.
- Yamamoto M, Sato S, Hemmi H, Uematsu S, Hoshino K, Kaisho T, Takeuchi O,

- Takeda K, Akira S. (2003b) TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol.* 4(11):1144-50.
- Yamamoto M, Sato S, Mori K, Hoshino K, Takeuchi O, Takeda K, Akira S. (2002) Cutting edge: a novel Toll/IL-1 receptor domain-containing adapter that preferentially activates the IFN-beta promoter in the Toll-like receptor signaling. *J Immunol.* 169(12):6668-72.
- Yang RB, Mark MR, Gray A, Huang A, Xie MH, Zhang M, Goddard A, Wood WI, Gurney AL, Godowski PJ. (1998) Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature.* 395(6699):284-8.
- Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaja M, Yim M, Du K, Singh S, Pike JW, Darnay BG, Choi Y, Wu H. (2002) Distinct molecular mechanism for initiating TRAF6 signalling. *Nature.* 418(6896):443-7.
- Yokomizo T, Izumi T, Chang K, Takuwa Y, Shimizu T. (1997) A G-protein-coupled receptor for leukotriene B4 that mediates chemotaxis. *Nature.* 387(6633):620-4.
- Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. (2000) A second leukotriene B4 receptor BLT2. A new therapeutic target in inflammation and immunological disorders. *J Exp Med* 192:421–432.
- Yoshioka A, Betsuyaku T, Nishimura M, Miyamoto K, Kondo T, Kawakami Y. (1995) Excessive neutrophil elastase in bronchoalveolar lavage fluid in subclinical emphysema. *Am J Respir Crit Care Med.* 152:2127–2132.

Yu S, Gu XX. (2005) Synthesis and characterization of lipooligosaccharide-based conjugate vaccines for serotype B *Moraxella catarrhalis*. *Infect Immun.* 73(5):2790-6.

Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese Jr RJ, Chapman Jr HA, Shapiro SD, Elias JA. (2000) Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest.* 106:1081–1093.

ABSTRACT

COPD is characterized by chronic inflammation in the lung, and its severe stage exacerbation is one of the major burdens of healthcare, causing diminished quality of life and highly increased mortality rate. Most common treatments for COPD could only improve the symptoms, but do not suppress the undergoing inflammations. Our study based on understanding the molecular basis of *M. catarrhalis*-induced innate immune responses on pulmonary epithelial cells and monocytes would help to identify new therapeutic target for COPD. *M. catarrhalis* has been proved to be the third leading cause of COPD. We found that *M. catarrhalis* could induce CCL20/MIP-3 α expression mainly through TLR2-MyD88-TRAF-6-NF- κ B/MAPK signaling pathway on pulmonary epithelial cells. Only TLR2, but not TLR4 signaling pathway can be activated on those cells during *M. catarrhalis* infection. Study on stable transfected HEK293 cells suggested that CD14 and MD2 are critical to activate TLR4 signaling; and sCD14 could enhance TLR2-mediated inflammatory responses. Thus our hypothesis is that lack of CD14 and MD2 might contribute to the silence of TLR4 signaling; and sCD14 released from monocytes could modulate TLR2- and TLR4-mediated inflammatory responses on airway epithelial cells. Both mCD14 and sCD14 expression levels are very low on naïve monocytic THP1 cells. 1 α , 25-dihydroxy VD₃ and specific ligands of TLR2 and TLR4 could increase mCD14 and sCD14 expression, which up-regulates both TLR2- and TLR4-mediated inflammatory responses on THP1 cells. Meanwhile, conditioned media from 1 α ,

25-dihydroxy VD₃ primed THP1 cells could activate TLR4 signaling pathway and enhance TLR2-mediated inflammatory responses on pulmonary epithelial cells under *M. catarrhalis* infection. Such amplified inflammatory responses could be abolished by CD14 antibody. All these data suggest that sCD14 could mediate a crosstalk between monocytes and pulmonary epithelial cells to mount an amplification loop through TLR2 and TLR4 during bacteria-induced inflammatory responses. Thus CD14 could be a potential therapeutic target to inhibit the abnormal inflammation during the pathogenesis of COPD or development of exacerbation.