MOLECULAR PATHOGENESIS OF NON-EOSINOPHILIC ASTHMA

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Publications


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<tbody>
<tr>
<td>AHR</td>
<td>Airway Hyperresponsiveness</td>
</tr>
<tr>
<td>APCs</td>
<td>Antigen Presenting Cells</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar Lavage</td>
</tr>
<tr>
<td>CAM</td>
<td>Cellular Adhesion Molecule</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complimentary Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>CF</td>
<td>Cystic Fibrosis</td>
</tr>
<tr>
<td>cRNA</td>
<td>Complimentary Ribonucleic Acid</td>
</tr>
<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EA</td>
<td>Eosinophilic Asthma</td>
</tr>
<tr>
<td>ECM</td>
<td>Extracellular Matrix</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno Sorbent Assay</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced Expiratory Volume in 1 Second</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced Vital Capacity</td>
</tr>
<tr>
<td>GINA</td>
<td>Global Initiative for Asthma</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte – Colony Stimulating Factor</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Granulocyte Macrophage - Colony Stimulating Factor</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hanks Balanced Salt Solution</td>
</tr>
<tr>
<td>ICAM</td>
<td>Intercellular Adhesion Molecule</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled Corticosteroids</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
</tr>
<tr>
<td>IKK</td>
<td>IkB kinase</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IRAK</td>
<td>IL-1R associated kinase</td>
</tr>
<tr>
<td>IVT</td>
<td>In Vitro Transcription</td>
</tr>
<tr>
<td>JAM</td>
<td>Junction Adhesion Molecule</td>
</tr>
<tr>
<td>LBP</td>
<td>LPS Binding Protein</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LTA</td>
<td>Lipoteichoic Acids</td>
</tr>
<tr>
<td>LTB₄</td>
<td>Leukotriene B₄</td>
</tr>
<tr>
<td>LTRAs</td>
<td>Leukotriene Receptor Antagonists</td>
</tr>
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</table>
MACS Magnetic Cell Separation
MD-2 Myeloid Differentiation-2
MIP Macrophage Inflammatory Protein
mg Milligram
MGG May Grunwald Giemsa
mL Millilitre
mRNA messenger RNA
MMP Matrix Metalloproteinase
MPO Myeloperoxidase
NA Neutrophilic Asthma
NE Neutrophil Elastase
NEA Non-eosinophilic Asthma
NF-κB Nuclear Factor κB
NOD Nucleotide Binding Oligomerisation Domain
OCS Oral Corticosteroids
OD Optical Density
PAF Platelet Activating Factor
PBS Phosphate Buffered Saline
PAMPs Pathogen Associated Molecular Patterns
PBMCs Peripheral Blood Mononuclear Cells
PEF Peak Expiratory Flow
PCR Polymerase Chain Reaction
PGA Paucigranulocytic Asthma
PMA Phorbol Myristate Acetate
PRR Pattern Recognition Receptor
RANTES Regulated on Activation Normal T cell Expressed and Secreted
RNA Ribonucleic Acid
ROS Reactive Oxygen Species
RSV Respiratory Syncytial Virus
SP-A Surfactant Protein-A
TH T Helper
TIMP-1 Tissue Inhibitor of Metalloproteinases-1
TLR Toll-like Receptor
TNF-α Tumor Necrosis Factor-alpha
ABSTRACT

Asthma involves chronic inflammation of the airways that is heterogeneous in nature. Eosinophilic airway responses are well described in asthma, however non-eosinophilic subtypes of asthma have been recently reported, and can involve the influx of neutrophils into the airways (neutrophilic asthma). Neutrophils are important effector cells of the innate immune system. These cells are the first to migrate to inflammatory sites, where they contain and eliminate pathogenic microorganisms. Neutrophils also release cytokines and chemokines that initiate and amplify inflammatory responses.

The mechanisms of neutrophilic asthma remain largely unknown; however activation of the innate immune response is implicated, particularly increased levels of proinflammatory cytokines Interleukin (IL)-8 and IL-1β and gene expression of Toll Like Receptor (TLR)-4 and TLR2 have been demonstrated in induced sputum samples. This thesis examines innate immune responses of airway and circulating neutrophils, with a focus on neutrophilic asthma. Innate immune neutrophil activation occurs in response to exposure to Lipopolysaccharide (LPS), which activates TLR4. The activation response consists of the release of preformed granule associated mediators such as Matrix Metalloproteinase (MMP)-9 and Oncostatin M (OSM), new gene transcription and release of inflammatory cytokines such as IL-8, IL-1β and Tumor Necrosis Factor (TNF)-α, and new gene transcription of TLR2 & TLR4 which serve to amplify neutrophil responses. In addition, this thesis examines whole genome gene expression profiles of circulating neutrophils in neutrophilic and eosinophilic asthma. The aims of this thesis are based on the hypothesis that dysregulation of innate immune neutrophil responses occurs with ageing and airway disease, particularly neutrophilic asthma and chronic obstructive pulmonary disease (COPD).

With advancing age, there were alterations in the innate immune responses of neutrophils, which were characterised by enhanced spontaneous activation of both airway and circulating neutrophils, and a decreased response of circulating neutrophils to LPS. There was a decreased activation of airway neutrophils in airway disease that was most pronounced in neutrophilic asthma and COPD, with decreased production and release of proinflammatory cytokines most likely due to a downregulation of TLR4. TLR2 was downregulated in resting and LPS stimulated circulating neutrophils in
asthma, particularly neutrophilic asthma. Circulating neutrophils had a decreased spontaneous release of total MMP-9, and downregulation of OSM, TLR2 and TLR4 at rest in COPD. However when stimulated with LPS, subjects with COPD had an enhanced proinflammatory cytokine release, with increases in IL-8 and TNF-α compared to subjects with asthma or healthy controls. Analysis of whole genome gene expression of circulating neutrophils in asthma revealed distinct gene profiles relating to asthma subtype. There was upregulation of genes relating to cell motility, inhibition of apoptosis and the NF-κB in neutrophilic asthma, which would contribute to their accumulation in the airways.

The innate immune response is critical in controlling infections by bacteria and viruses. The reduced innate immune response of airway neutrophils in airway disease could contribute to impaired local defense, which may lead to an increased susceptibility to infection by invading pathogens. Systemically, the molecular mechanisms of neutrophilic asthma are distinct from eosinophilic asthma and may involve the enhancement of neutrophil chemotaxis and survival, contributing to their accumulation in the airways.