Respiratory Innate Immune Factors Regulate Steroid-resistant Airway Hyperreactivity and Asthma

By

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THESIS STATEMENTS

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Jingjing Li
January 2014
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<table>
<thead>
<tr>
<th>ABBREVIATION</th>
<th>DEFINITION</th>
</tr>
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<tbody>
<tr>
<td>AAL(S)</td>
<td>2-amino-4-(4-heptylophenol)-2-methylbutanol</td>
</tr>
<tr>
<td>AAVs</td>
<td>adeno-associated viruses</td>
</tr>
<tr>
<td>Ant-9</td>
<td>antagonomir-9</td>
</tr>
<tr>
<td>AHR</td>
<td>airway hyperresponsiveness</td>
</tr>
<tr>
<td>AP-1</td>
<td>activator protein 1</td>
</tr>
<tr>
<td>APCs</td>
<td>antigen presenting cells</td>
</tr>
<tr>
<td>ARG1</td>
<td>arginase-1</td>
</tr>
<tr>
<td>BALF</td>
<td>broncho alveolar lavage fluid</td>
</tr>
<tr>
<td>pCAF</td>
<td>p300/CBP-associated factor</td>
</tr>
<tr>
<td>CBP</td>
<td>cyclic AMP response element-binding protein</td>
</tr>
<tr>
<td>CDK</td>
<td>cyclin-dependent kinase</td>
</tr>
<tr>
<td>CSF-1</td>
<td>colony-stimulating factor</td>
</tr>
<tr>
<td>DAMPs</td>
<td>damage associated molecular patterns</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>DEX</td>
<td>dexamethasone</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco's Modified Eagle Medium</td>
</tr>
<tr>
<td>EBI3</td>
<td>EBV-induced gene 3</td>
</tr>
<tr>
<td>FCS</td>
<td>fetal calf serum</td>
</tr>
<tr>
<td>FEV1</td>
<td>forced expiratory volume in one second</td>
</tr>
<tr>
<td>FGR</td>
<td>familial GC resistance</td>
</tr>
<tr>
<td>FIZZ1</td>
<td>resistin-like molecule-α</td>
</tr>
<tr>
<td>GCs</td>
<td>glucocorticoids</td>
</tr>
<tr>
<td>GM-CS</td>
<td>granulocyte macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GLIZ</td>
<td>GC-induced leucine zipper protein</td>
</tr>
<tr>
<td>GP</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>GREs</td>
<td>GC response elements</td>
</tr>
<tr>
<td>GSK3</td>
<td>glycogen synthase kinase 3</td>
</tr>
<tr>
<td>HAT</td>
<td>histone acetyltransferase</td>
</tr>
<tr>
<td>HAD</td>
<td>CHAT activity and recruit histone deacetylase</td>
</tr>
<tr>
<td>HDM</td>
<td>house dust mite</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank's buffered salt solution</td>
</tr>
<tr>
<td>HPRT</td>
<td>hypoxanthine-guanine phosphoribosyl transferase</td>
</tr>
<tr>
<td>Hsps</td>
<td>heat shock proteins</td>
</tr>
<tr>
<td>IκB</td>
<td>inhibitor of nuclear factor-κB</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>interferon-gamma</td>
</tr>
<tr>
<td>IFNγR</td>
<td>IFN-γ receptor</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IRFs</td>
<td>interferon regulatory factors</td>
</tr>
<tr>
<td>i.t.</td>
<td>intratracheally</td>
</tr>
<tr>
<td>KC</td>
<td>keratinocyte-derived chemokine</td>
</tr>
<tr>
<td>LBD</td>
<td>ligand-binding domain</td>
</tr>
<tr>
<td>lipo-C12MDP</td>
<td>liposome-encapsulated clodronate</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>MCP-1</td>
<td>monocyte chemotactic protein-1</td>
</tr>
<tr>
<td>miR</td>
<td>microRNA</td>
</tr>
<tr>
<td>miRISC</td>
<td>miRNA-induced silencing complex</td>
</tr>
<tr>
<td>MIP</td>
<td>macrophage inflammatory protein</td>
</tr>
<tr>
<td>MKP</td>
<td>mitogen-activated protein kinase phosphatase</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor-kappaB</td>
</tr>
<tr>
<td>iNOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NLRs</td>
<td>NOD-like receptors</td>
</tr>
<tr>
<td>NTHi</td>
<td>non-typeable <em>Haemophilus influenzae</em></td>
</tr>
</tbody>
</table>
OR  olfactory receptor
OVA  ovalbumin
PAMPs  pathogen associated molecular patterns
PBMC  peripheral blood mononuclear cell
PMN  polymorphonuclear neutrophil
PP2A  protein phosphatase 2A
PPP2R5D  protein phosphatase 2, regulatory subunit B
PRRs  pattern recognition receptors
Raw  airway resistance
RLRs  RIG-I-like receptors
pri-miRNA  primary RNA
RSV  respiratory syncytial virus
RT  room temperature
SABAs  beta2-adrenoceptor agonists
SCFAs  short chain fatty acids
SEB  staphylococcus aureus enterotoxin B
SLPI  secretory leukoprotease inhibitor
SRC  steroid receptor co-activator
STATs  signal transducer and activator of transcriptions
Th  T helper cell
Ticam-1  Toll-interleukin 1 receptor domain (TIR)-containing adaptor molecule-1
TIR  Toll / interleukin-1 receptor-like domain
TLRs  Toll-like receptors
(TNF)-α  tumor necrosis factor-α
Tirap  TIR domain-containing adaptor protein
Trif  TIR-domain-containing adapter-inducing interferon
UTR  untranslated regions
ABSTRACT

Asthma is a chronic inflammatory disease of the airways and a combination of genetic and environmental factors underpin the pathogenesis. The clinical symptoms of asthmatics most mild to moderate, allergic asthma patients can be effectively managed by combination therapy with broad-spectrum anti-inflammatory agents and bronchodilators (typically inhaled glucocorticoids and long acting β-agonists). Indeed, glucocorticoids remain the forefront therapeutical approaches for the treatment of asthma. However, 5-10% of asthmatics who have severe asthma do not respond to treatment, and these patients account for almost 50% of asthma-related healthcare costs. Thus it is essential to understand the pathogenesis of steroid resistance in severe asthma for the development of more efficient therapies for those patients. With well-established animal models of steroid resistant airway hyper-responsiveness (AHR, a hallmark feature of asthma) and in vitro culture systems of pulmonary macrophages, the underlying mechanisms regulating steroid resistance and exacerbation of asthma have been thoroughly investigated, particularly on the causative roles of innate immune factors. This thesis consists of three publications. The first publication identifies changes in the expression of key innate immune molecules and their signalling pathways in a mouse model of steroid-resistant AHR and demonstrates the central role of pulmonary macrophages in the induction of steroid-resistant AHR. The second publication investigates the expression of olfactory receptors in the respiratory system and on immune cells in response to innate immune activation, and identifies a potential
role of olfactory receptors in regulating the function of pulmonary macrophages. The final publication discusses the modulation of small non-coding RNAs, microRNA, expression by innate immune activation in a steroid-resistant mouse model of asthma and evaluates the role of key microRNAs involved in the induction of steroid-resistant AHR by regulating the activity of a critical phosphatase, protein phosphatase-2A, which further affected the function of glucocorticoid.