

## Mechanisms of Corticosteroid Insensitivity in Asthma

Seminar 5001

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Current NIH/NHLBI guidelines for the management of persistent asthma recommend the use of corticosteroids (CS) for treatment of airway inflammation. However, multiple NIH clinical trials have reported that almost 50% of asthmatics do not respond to inhaled CS (ICS) therapy<sup>1</sup>, and up to 20% do not adequately respond to oral CS<sup>2</sup>. CS insensitivity is associated with persistent airway inflammation and increased airway remodeling<sup>3,4</sup>. CS insensitivity can therefore increase the severity of asthma and may account for much of the morbidity and cost of the disease due to use of costly medications and frequent hospitalizations. This seminar will review the current knowledge about the molecular mechanisms that result in CS insensitivity and the clinical implications of these findings. This knowledge is necessary to the development of biomarkers and new management strategies to improve CS responsiveness.

Corticosteroids include glucocorticoids and mineralocorticoids, which are produced in the adrenal cortex, and synthetically manufactured CS used as therapeutic medications<sup>5</sup>. They exert important biological functions, including control of various inflammatory processes. Glucocorticoids, often used as synonym for CS, act through binding to the glucocorticoid receptor (GCR), a specific 94-kDa cytoplasmic receptor protein, i.e. GCR-alpha, that functions as a ligand-dependent transcription factor. The GCR is expressed in almost all human cells. Clinically relevant insensitivity to CS can be caused by different pathogenic mechanisms that reduce their biological effects, including (1) genetically determined CS insensitivity through mutations in the glucocorticoid receptor (GCR) gene<sup>6</sup>, (2) altered expression of GCR splice forms associated with CS insensitivity<sup>7,8</sup>, (3) post-translational modifications of the GCR<sup>9,10</sup>, (4) conditions with decreased GCR binding affinity, (5) changes in regulation of CS-responsive genes, (6) altered CS metabolism, and (7) other factors interfering with CS function<sup>11-13</sup>.

In this seminar, we will review the molecular mechanisms of the GCR function. We will demonstrate the receptor function under CS-responsive conditions, and describe GCR regulation of the CS-responsive genes to enhance transcription (transactivation) or GCR-dependent direct sequestration of proinflammatory transcription factors to inhibit transcription of proinflammatory cytokine genes (transrepression)<sup>2,14,15</sup>.

Our lab has been NIH funded to examine cellular responses to CS and studies of molecular mechanisms of CS resistance. We will review the current understanding of the molecular pathways that lead to CS insensitivity, as we have found that a wide variety of factors can contribute to CS insensitivity including infection, obesity, allergen exposure, and ethnicity. These factors can select for a variety of airway cells which are either naturally CS insensitive, e.g. neutrophils<sup>16</sup> or CD8+ T cells<sup>17</sup>, or acquire a CS insensitive phenotype (including macrophages following microbial stimulation)<sup>18,19</sup>. We have elucidated various mechanisms by which CS insensitivity occurs, e.g. a relative decrease in GCR-alpha or overexpression of its dominant negative isoform, GCR-beta<sup>20</sup>. Other mechanisms include decreased nuclear translocation<sup>20</sup> and posttranslational modifications of GCR-alpha<sup>11</sup>. We recently reported that in asthmatics, reduced vitamin D levels are associated with impaired lung function, increased CS requirement and reduced CS response *in vitro*, signifying that supplementation with vitamin D in patients with asthma may improve multiple parameters of asthma severity and treatment response<sup>12,13,21</sup>.

Steroid insensitivity and persistent airway inflammation pose a significant management problem in asthma and other inflammatory disorders. We will review the causes and differential diagnosis of CS-resistance in asthma. In asthmatic patients, inadequate response to prolonged oral CS therapy and significant CS side effects raise concern about clinically relevant steroid insensitivity, prompting further evaluation and consideration of alternative therapies. Lack of CS side effects, e.g. weight gain and cushingoid features, in patients on oral CS, and poor response to inhaled CS (ICS) raise concerns about patient incompliance with CS therapy, or incorrect use of ICS. A morning plasma cortisol concentration will help determine the level of adrenal suppression and potential poor adherence to CS therapy.

The ability to identify patients that are likely to respond to CS is an important part of effectively treating asthma. We will review *in vitro* and *in vivo* methods to assess CS-responsiveness. The *in-vivo* response to oral CS can be determined by FEV1 improvement after a one-week course of prednisone, 20 mg p.o. twice daily. An FEV1 improvement of  $\geq 12\%$  is expected in CS-sensitive patients. Similarly, the response to ICS therapy over a 6- to 8-week period has been studied. Non-invasive biomarkers, e.g. assessment of sputum eosinophils and exhaled nitric oxide measurements may help predict the response to oral CS and ICS<sup>6,22</sup>. In specialized centers, steroid pharmacokinetic studies are used to assess the metabolism of oral CS used in asthma therapy. Decreased absorption of prednisone and abnormal interconversion to prednisolone may help explain a decreased response to therapy, and delayed clearance of prednisolone may place patients at an

increased risk for severe CS-side effects with long-term treatment. Inhibition of phytohaemagglutinin-induced proliferation of lymphocytes by different oral CS and ICS can be used to study the CS responsiveness of asthmatic patients. The IC<sub>50</sub> value, which reflects the amount of a given CS to inhibit the activation of lymphocytes by 50%, is used to quantitatively measure CS responsiveness of asthma. When managing asthmatic patients with suspected CS insensitivity, environmental and nutritional factors contributing to a decreased response to oral and ICS need to be taken into consideration and modified, if possible. Choice and dosing schedule of an oral CS may be adjusted based on results of steroid pharmacokinetic studies and assessment of in-vitro steroid responsiveness of patients' lymphocytes. Similarly, the ICS with the most favorable IC<sub>50</sub> should be chosen to optimize therapy. Alternative therapeutic strategies should be considered based on the phenotype of an asthmatic patient, and we will discuss emerging therapies that may be beneficial in CS-insensitive patients.

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