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The Glucocorticoid Receptor in Asthma and why it Can not Cure the Disease

Michael Roth

The Woolcock Institute for Medical Research, Royal Prince Alfred Hospital, University of Sydney, Australia and Pulmonary Cell Research, University Hospital Basel, Switzerland

Glucocorticoids are one of the key drugs for therapy of inflammatory diseases including asthma. Glucocorticoids are assumed to enter cells via diffusion, and not by active transport. Once in the cytosol glucocorticoids bind to the glucocorticoid receptor (GR). The GR is kept inactive by a complex formed with heat shock proteins, FKBP5, and several other co-factors. For activation one glucocorticoid molecule binds to one GR molecule, the heat shock proteins are released, and two GR form a dimer. FKBP51 is than replaced by FKBP52, which couples the complex to dynein which in turn hooks the complex onto tubuli and moves them into the nucleus. In the nucleus the GR-dimer recognises a specific DNA consensus sequence, the glucocorticoid response-element (GRE: AGA ACA NNN TGT TCT), and functions as transcription factor.

However, in recent years four additional mechanisms of glucocorticoid and GR function have been described: (a) an active GR-molecule binds to other transcription factors including AP-1 and NF_KB and blocks their function, (b) they downregulate NF_KB activity by upregulating its inhibitor I_KB, (c) they form complexes with the CCAAT/enhancer binding proteins (C/EBP)-α and -β, and as a complex bind to the CCAAT DNA consensus sequence, and finally (d) glucocorticoids inhibit Raf in the MAPK-kinase signalling cascade. It is unclear whether the various actions/interactions of the active GR occur as independent parallel functions or if it is the cell type, or the stage of cell development that dictates which pathway is affected. However, none of the glucocorticoid actions has a lasting effect. The short lived action of glucocorticoids is based on the short lifespan of the active GR. While animal models suggested recycling of the GR-dimer after being released from the consensus DNA sequence, this observation was never been confirmed in human cells. Recent data indicate a rapid degradation of the GR dimer within six hours after activation.

These molecular biological properties of glucocorticoids explain why asthma patients have to regularly inhale glucocorticoids and a long lasting therapeutic effect can not be observed. Recently developed glucocorticoids promise longer activity, therefore, reduced dose regimens, and may cause less side effects. One type of new glucocorticoids is inactive and is slowly activated locally by enzymes released by epithelial cells. The onset of drug action is therefore slightly slower than that of the common glucocorticoids, but longer lasting. However, the effect of the active glucocorticoid metabolite on the GR is as short as the classical drug. In contrast, a lipophylic form of glucocorticoids has been proven to reduce the frequency of inhalations, results in better quality of life, and reduces asthma symptoms. Molecular biological analysis revealed that lipophylic glucocorticoids activate the GR as fast as the classical drugs, but keep the GR-dimer at least three times longer active. It is yet unclear whether this effects depends on a different binding affinity of the drug with the GR, or on a more stable binding of the GR-glucocorticoid complex to the DNA consensus sequence, or on delayed degradation of the GR-dimer.

However, glucocorticoids can not cure asthma as such. One pathological feature of the asthmatic airway is a significant increase in the mass of bronchial smooth muscle cells (BSMC), manifesting in hypertrophy and hyperplasia. This pathology of asthma occurs very early in life and has been described in airways of young children.

We have earlier reported that BSMC obtained from the bronchus of asthmatic patients proliferate 2.5 faster than those obtained from non-asthmatic controls. This difference can also be observed after several passages in cell culture and must therefore be either genetically determined or imprinted by a yet unknown mechanism, such as antigen exposure.

Glucocorticoids downregulate the growth of BSMC in culture, but there was no evidence that they reduce the mass of bronchial smooth muscle bundles in the asthmatic bronchus. Investigating the signalling pathway in asthmatic and non-asthmatic BSMC we could prove that two distinct signalling pathways control the anti-inflammatory and the anti-proliferative action of glucocorticoids. While glucocorticoids downregulated the synthesis of pro-inflammatory mediators such as IL-6 and IL-8 in asthmatic and non-asthmatic cells, they did not block the proliferation of BSMC obtained from asthmatic patients.

To exert their anti-proliferative function the activated GR has to form a complex with C/EBP-α. The complex migrates into the nucleus and binds to the CCAAT DNA consensus sequence located in the promoter of the cell cycle inhibitor $p21^{(Waf1/Cip1)}$, which is consequently upregulated, and activated. Active $p21^{(Waf1/Cip1)}$ blocks the progression of the cell cycle at the restriction point between the G1 and the S-phase.

We could show that in asthmatic BSMC lines (n=20) C/EBP-α is not expressed and therefore the GR can not form the complex to activate the $p21^{(Waf1/Cip1)}$ gen. When these cells were transfected with a human C/EBP-α expression vector they became sensitive to the anti-proliferative action of the glucocorticoids and their spontaneous proliferation rate was significantly reduced. On the other hand transient transfection of non-asthmatic cells with C/EBP-α antisense oligonucleotides reduced their sensitivity to the anti-proliferative action of glucocorticoids significantly. To prove a disease specific pattern we included BSMC lines from: lung tumour (n=15), emphysema (n=10), lung transplantation donors (n=10), and form bronchial biopsies of healthy volunteers (n=12) as controls. All these cell lines expressed C/EBP-α upon stimulation with 5% fetal calf serum.

Preliminary data indicate that the lack of C/EBP-α in asthmatic BSMC is cell type specific since circulating peripheral blood lymphocytes from asthmatic probands expressed C/EBP-α. Furthermore, our studies revealed that the lack of C/EBP-α in asthmatic bronchial smooth muscle cells is caused by a defect in the translation of C/EBP-α messenger RNA into the respective protein and not in a gene mutation or gene defect.

In summary, glucocorticoids can not cure asthma, but they downregulate the inflammatory response caused by antigens. Long acting glucocorticoids can reduce the dose of inhaled steroids significantly. Asthma may be cured if the translation of C/EBP-α mRNA could be reactivated.