Repression of inflammatory genes by glucocorticoids and non-steroidal compounds

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Inflammatory gene promoters are complex promoter sequences, comprising many different responsive sequence elements; however, we found that only the inducible transcription factor NF-kB is responsible for gene induction in response to TNF. Other promoter-bound factors (like AP1, CREB, C/EBP, etc.) synergize with NF-kB and form all together a necessary platform for recruitment of additional nuclear, non-DNA-bound cofactors (co-activators), which determine the general levels of inflammatory gene expression, yet not the induction of it. Therefore, the inducible factor NF-kB can be regarded as the ultimate switch for inflammatory gene induction. Expression of inflammatory genes can be counteracted by inhibitors of the ERK and/or the p38 MAP kinase pathways; these inhibitors have, however, no impact on cytoplasmic NF-kB activation, or on NF-kB/DNA binding. Therefore, we hypothesized that the nuclear transactivation capacity of NF-kB might be disturbed. As neither ERK kinase nor p38 MAP kinase directly phosphorylate the NF-kB p65 subunit, we identifieded the Mitogen- and Stress-activated protein Kinase 1 (MSK1) to be the executing enzyme for p65 phosphorylation. Actually, this kinase, downstream of ERK and p38, is a pure nuclear kinase, which integrates upstream incoming signals for cell growth or stress. Blocking this particular kinase equals inhibition of inflammatory gene expression. MSK1 phosphorylates a specific residue (i.e. Ser 276) within the NF-kB p65 subunit, which is absolutely required for the formation of a transcription-competent enhanceosome, and consequently for driving inflammatory gene expresssion. MSK1 is not a novel kinase, but was described to specifically phosphorylate the Histon-3 tails within the nucleosomal structure. Modification of these tails is presently being considered as a local coding process within the chromatin, leading to relaxation. Therefore, MSK1 seems to serve a double function, i.e. as a specific NF-kB p65 kinase, as well as an important signaling intermediate for chromatin relaxation.

Glucocorticoids are known as effective drugs to combat inflammatory diseases, and are very widely used for this purpose. These lipophilic compounds bind to the glucocorticoid receptor (GR), which now acts as an activated transcription factor. The anti-inflammatory potential of glucocorticoids is, however, not being associated with the gene-inductive activities of the activated GR, but rather assumed to result from negative interference with inflammatory, i.e. NF-kB-driven gene expression. A few authors have pointed to the potential of glucocorticoids, i.e. of the activated GR, to upregulate expression of the gene coding for the NF-kB inhibitor molecule I-kB-a, but we have not found this effect in mouse fibroblast or endothelial cells. Other investigators considered nuclear GR as a competitor for activated NF-kB in assembling the necessary enhanceosome at gene promoters, resulting in decreased NF-kB gene transcription. We found that this is not the case either. Moreover, this hypothesis does not explain the specificity of glucocorticoids towards inflammation. In addition, we neither have

found any effect of glucocorticoids on the above mentioned phosphorylation cascades, nor on the activity of MSK1 itself, but clearly detected inhibition of the MSK1-mediated Histon-3 phosphorylation. Therefore, we hypothesize that the presence of GR inhibits the inflammation-specific kinase MSK1, although itself activated and fully enzymatically active, to be recruited to the inflammatory gene promoters, which thus forms the molecular basis for blocking inflammatory gene expression.

Non-steroidal, anti-inflammatory drugs (NSAIDs) or natural compounds also interfere with the activating kinase pathways, with enhanceosoom building and chromatin modification, rather than with the cytoplasmic activation mechanism of NF-kB, as is generally accepted. Although more detailed molecular studies are needed to pinpoint the various inhibitory steps of different compounds, they will certainly lead to more appropriate and combinatorial anti-inflammatory therapies.