fore the identification, biological characterization of endogenous Cynomolgus IFN-α subtypes and their accurate detection are of utmost importance for predicting the clinical outcome of effective human therapeutics. We have isolated full length IFN-α coding sequences from Cynomolgus genomic DNA using PCR methods. Sequence analysis revealed 11 unique IFN-α subtypes which were transiently expressed in Vero cells. The conditioned supernatants were tested in parallel with purified, *E. coli* produced Cynomolgus IFN-α2 for accurate quantitation. All samples tested on Cynomolgus cells exhibited strong antiviral activity. Additionally, the biological activities of the IFN proteins secreted in the tissue culture supernatants were characterized in several human cell-based assays including A549/EMCV cytopathic protective effect, OVCAR-3 antiproliferative and caspase-3/7 activation assays. All subtypes displayed activity on these separate human cell lines suggesting reasonable species cross-reactivity. Analysis in a human IFN-α ELISA further supported these observations. Separately, we analyzed the activity in a human cell-based reporter assay (iLite™ Human IFN-α Kit) including the use of an anti-IFN-α receptor neutralizing antibody. This assay correlated with the other human cell-based assays, providing an additional, more rapid measure of activity. Moreover, the activity could be potently neutralized by the anti-human IFNAR monoclonal antibody further suggesting similarities between Cynomolgus and human IFN-α receptor proteins. Therefore these reagents should prove useful in the detection and biological evaluation of Cynomolgus IFN-α proteins used for the better understanding of human viral diseases and their treatments.

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Multiple roles for the SOCS box

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SOCS proteins play a critical role in the negative feedback inhibition of cytokine signal transduction pathways. SOCS protein contain a C-terminal SOCS box domain. A similar SOCS box domain is found at the C-terminus of over 70 human proteins. The SOCS box domain is usually coupled to protein interaction module(s) that interact with a protein target. The SOCS box can interact with elongin and cullin molecules, leading to formation of an E3 ligase complex and proteasomal degradation of the target. In this way, SOCS proteins can direct activated cytokine receptor complexes to proteasomal degradation. We recently demonstrated that the SOCS box of SOCS proteins is implicated in the interaction of all SOCS proteins with SOCS2, 6 and 7. This interaction can lead to degradation of the targeted SOCS protein. SOCS2, 6 and 7 are thus able to control degradation of other SOCS proteins. We further demonstrated that the interaction of Cis with its substrate is critically dependent upon the integrity of its SOCS box and its interaction with elongins. The SOCS box thus emerges as a versatile module controlling cytokine signaling via multiple mechanisms.

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Design of high affinity interleukin-6 and gp130 variants: Implications for cytokine-receptor assembly

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Binding of human Interleukin-6 (IL-6) to its specific receptor IL-6R is a prerequisite for the activation of the signal transducing receptor glycoprotein 130 (gp130).IL-6 binds to the IL-6R via site I and makes contact to gp130 via site II and site III. The mode of assembly and the stochiometry of the signalling complex is not completely understood. We have used two different models to shed more light on the molecular make-up of the signalling IL-6R complex. In the first model we have analyzed the viral IL-6 (vIL-6) protein from Human herpes virus 8which unlike human IL-6 can bind to gp130 directly. We have identified the amino acids within vIL-6, which are responsible for direct binding to gp130 and we show that these amino acids when transferred into the human IL-6 protein also render human IL-6 independent of the IL-6R. In the second model we have identified amino acid residues within the gp130 molecule, which are of particular importance for binding to the IL-6/IL-6R complex. We have identified three amino acids, which, when mutated, give rise to gp130 molecules with higher binding affinities. Surprisingly, amino acids within vIL-6, important for direct gp130 binding and amino acids governing gp130 binding to the IL-6/IL-6R lie within site III, which has previously been thought to be responsible for the dimerization of two preassembled IL-6/IL-6R/gp130 trimers. We believe that the importance of site III for the assembly of the IL-6 receptor complex is underestimated and we will present an alternative model of receptor complex assembly which proposes a tetrameric instead of a hexameric IL6/IL6R/gp130 signaling complex.

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Erythropoietin and glucocorticoids exert antagonistic effects on STAT-5 phosphorylation in primary human erythroblasts

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Extensive clinical studies have established a direct correlation between numbers of red cells present in the blood and the concentration of erythropoietin. Erythropoietin interacts with a specific receptor, EPO-R, on the surface of erythroid cells. In the mouse, in addition to EPO and EPO-R, the glucocorticoid receptor (GR) controls erythropoiesis following stress. It is generally accepted that glucocorticoids enter cells and interact with GR within the cytoplasm. This binding induce receptor dimerization, STAT-5-phosphorylation and formation of GR/STAT-5 complexes, that migrate to the nucleus, bind to specific consensus sequences and activate the expression of target genes. In erythroid cells, GR stimulation regulates the expression of a subset of genes associated with induction of proliferation. Recent evidence indicates the existence of rapid glucocorticoid signalling mediated by membrane associated receptors. The possibility that GR might be associated with the plasma membrane of erythroid cells has not been investigated to date. In this study we describe a new biochemical pathway that might contribute to the reversible inhibition exerted by glucocorticoids on erythroid maturation and that may contribute to the genesis of polycythemia vera (PV). We show that dexamethasone antagonizes the effects of erythropoietin on maturation, but not on survival, of human proerythroblasts. While erythropoietin and dexamethasone alone induce STAT-5-phosphorylation, the combination was ineffective in promoting STAT-5-phosphorylation in normal erythroblasts. By contrast, STAT-5-phosphorylation was observed in erythroblasts from PV patients stimulated with erythropoietin and dexamethasone in combination. Growth factor deprivation induced significant levels of STAT-5-phosphorylation in erythroblasts from normal subjects and from PV patients. A significant correlation exists between levels of STAT-5-phosphorylation induced by growth factor deprivation and numbers of proerythroblasts generated in vitro from different donors. These data suggest that glucocorticoids interfere with EPO-induced maturation by antagonizing the STAT-5-phosphorylation activity of EPO-R and that this antagonism is altered in proerythroblasts from PV patients.

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Does S100A12 activate mast cells and monocytes/macrophages via rage?

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S100A12 is considered a DAMP thought to exert proinflammatory functions through ligation of RAGE1. Earlier experiments were based on effects of bovine S100A12 on BV-2 cells (murine microglial cells)1. Monocytes and THP-1 cells express RAGE. Using a homologous system, we assessed 9 batches of LPS-free recombinant S100A12, but found no induction of IL-1β, IL-6, IL-8 or TNF in PBMCs, or of IL-8 by undifferentiated/differentiated THP-1 cells. Although we showed that S100A12 undergoes structural changes with zinc, to form quatramers and hexamers, zinc did not alter the inability of S100A12 to activate monocytes. S100A12 is chemotactic for monocytes and mast cells (MC), induces MC degranulation and MC-dependent inflammation in vivo. Responses were partially blocked by sRAGE, a RAGE antagonist, although MC did not express RAGE mRNA or protein, suggesting an alternate receptor. S100A12 provoked chemotaxis of THP-1 cells in a biphasic manner, with optimal concentrations at 10–9 and 10–12 M; both were pertussis toxin sensitive. sRAGE only reduced migration of THP-1 cells in response to 10-9 M S100A12. PD98059, a selective ERK1/2 inhibitor which reduces RAGE-mediated responses, did not affect S100A12-mediated THP-1 cell migration at either concentration. Thus monocytes and MC may share a common G-protein coupled receptor that mediates S100A12-induced chemotaxis. These results suggest that the 'RAGE/S100 proinflammatory axis' theory should be re-evaluated. Because S100A12 binds zinc we investigated its influence