03

Cytokine receptors

03-01/O

X-LINKED SUSCEPTIBILITY TO MYCOBACTERIA IS CAUSED BY MUTATIONS IN THE NEMO LEUCINE ZIPPER DOMAIN THAT IMPAIR CD40-DEPENDENT IL-12 PRODUCTION

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Mendelian susceptibility to mycobacterial disease (MSMD) is a clinical syndrome caused by germline mutations in at least five autosomal genes, resulting in impaired IL-12-dependent, IFN-γ-mediated immunity. An X-linked recessive form of MSMD (XR-MSMD) was clinically described in 1994, but its molecular basis remained unknown. We report neighboring mutations in NF-κB essential modulator (NEMO) in three unrelated kindreds with XR-MSMD. Three-dimensional modeling of the NEMO oligomerization domain showed that these mutations (E315A and R319Q) affect residues that are likely to form a salt bridge in the leucine zipper (LZ) domain. The mutant proteins were normally expressed in blood and fibroblastic cells. The patients' monocytes however presented an intrinsic defect in T cell-dependent IL-12 production, resulting in an extrinsic defect of IFN-γ secretion by T cells. Moreover, IL-12 production was impaired due to a specific defect in NEMO-mediated CD40 signaling upon monocyte and dendritic cell stimulation by CD40L-expressing T cells and fibroblasts, respectively. However, the CD40-dependent up-regulation of co-stimulatory molecules of monocytes and the proliferation and immunoglobulin class switch of B cells were normal. Moreover, the patients' blood and fibroblastic cells responded normally to other NF-κB activators, such as TNF-α, IL-1β, and LPS. These two mutations in the NEMO LZ domain thus provide the first genetic etiology of XR-MSMD. They also demonstrate the importance of the T cell- and CD40L-triggered, CD40- and NEMO-mediated induction of IL-12 by monocyte-derived cells for protective immunity to mycobacteria in humans.

03-02/P

DIFFERENTIAL RESPONSIVENESS TO IFN-ALPHA AND IFN-BETA OF HUMAN MATURE DC THROUGH MODULATION OF IFNAR EXPRESSION.

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In human dendritic cells (DC), infection with *Mycobacterium tuberculosis* and viruses, or stimulation with TLR-3 and -4 agonists cause the release of type I IFN. This autocrine IFN may have critical effects on DC biology. IFN has been shown to promote the differentiation of human blood monocytes into DC with potent T cell stimulatory activities and to contribute to DC maturation. Although DC represent one of the major source of type I IFN and are also key responders, only few studies have addressed the question of their responsiveness at different stages of maturation. The signaling activities of two IFN subtypes, IFN-gamma2 and IFN-beta, in immature and maturing human DC have been studied. IFN-beta released upon stimulation with LPS or poly I:C is responsible for a rapid and sustained STAT-1 and -2 activation and expression of IFN-stimulated genes, such as the transcription factor IRF-7 and the chemokine CXCL-10. The autocrine production of IFN-beta from LPS and poly I:C matured DC induced a state of temporary desensitization to a further challenge with IFN-gamma or IFN-beta and this correlated with a marked decline in the level of the two IFN receptor (IFNAR) subunits. Interestingly, we found that upon clearing of the released cytokines, LPS-stimulated DC reacquired full responsiveness to IFN-beta but only partial responsiveness to IFNs-gammaβ while their maturation process was unaffected. Monitoring of surface and total levels of the receptor subunits showed that maximal expression of IFNAR2 resumed within 24 h of clearing, while IFNAR1 expression remained low. Thus, mature DC can modulate their sensitivity to two IFN subtypes through a differential regulation of the IFNAR subunits. These results shed light on a new physiological mechanism that differentially tunes the response to type I IFN subtypes of a specialized leukocytic population, such as DC, that regulates multiple aspects of the immune response.

03-03/P

IL-17-MEDIATED GENE EXPRESSION: IMPLICATIONS FOR UNDERSTANDING SIGNALING BY A UNIQUE CYTOKINE RECEPTOR

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IL-17 is the founding member of an emerging family of inflammatory cytokines whose activities remain poorly defined. IL-17 has been implicated in autoimmune diseases including rheumatoid arthritis and psoriasis, but IL-17 is also highly important for host defense to infectious agents, including yeast and gram negative bacteria. The IL-17 receptor (IL-17RA) is unique in sequence from other cytokine receptor families, and consequently very little is known about its signal transduction mechanisms. We performed microarray experiments in order to identify endpoints of IL-17-induced signal transduction, which can ultimately be used to dissect receptor-mediated signaling. We subsequently performed bioinformatics analysis and empirical promoter studies of the target gene promoters in order to identify consensus modes of IL-17-mediated gene regulation. The hallmark gene target of IL-17 is the cytokine IL-6, which we previously demonstrated to be regulated by the transcription factors NF-kappaB and C/EBP. However, IL-6 is also regulated at the level of mRNA stability, and the contributions of IL-17 signals to IL-6 transcriptional induction are relatively weak. More recently, we identified the acute phase response gene 24p3/lipocalin 2 as another strongly-induced IL-17 target gene, which is controlled almost entirely at the level of transcription. Promoter analyses and DNA binding assays revealed that 24p3 expression is also regulated by NF-kappaB and C/EBP. Comparative promoter studies of the proximal regulatory regions in a collection of IL-17-induced genes revealed a statistical enrichment of NF-kappaB, C/EBP and STAT binding sites, suggesting a conserved "IL-17-response element." The mechanism by which IL-17RA induces these transcription factors is unknown. Interestingly, the C/EBP-beta and C/EBP-delta genes are also transcriptional targets of IL-17, suggesting a positive feedback loop by which IL-17 amplifies target gene expression. Moreover, because the 24p3 promoter is so strongly activated by IL-17, we can use this promoter as a tool to monitor the function of IL-17RA mutants in an IL-17RA-null background.

03-04/P

LIGAND-DEPENDENT AND INDEPENDENT PATHWAYS MEDIATING UBIQUITINATION AND DEGRADATION OF TYPE I INTERFERON RECEPTOR

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Down regulation of cytokine receptors in response to their ligands plays a key role in restricting the extent of cytokine signaling. Ubiquitination of the IFNAR1 subunit of the Type I interferon (IFN) receptor mediates the IFNAR1 degradation (*1*). This ubiquitination requires the recruitment of β-Trcp ubiquitin ligase in a manner that relies on IFNAR1 phosphorylation on Ser535 within the destruction motif (*2*). Here we report that IFNAR1 ubiquitination and degradation as well as phosphorylation of IFNAR1 on Ser535 can occur via either ligand-dependent or independent pathway. Ser535 phosphorylation, ubiquitination and degradation of endogenous IFNAR1 expressed at low levels are robustly stimulated by the ligand treatment. Tyk2 catalytic activity is essential for this stimulation (*3*). However, the major cellular Ser535 IFNAR1 kinase activity detected in vitro does not require either IFNα treatment or Tyk2 catalytic activity suggesting the existence of an alternative pathway that mediates IFNAR1 phosphorylation on Ser535. Indeed, high levels of accumulation of endogenous IFNAR1 in the cells treated with lysosomal inhibitors exhibit Ser535 phosphorylation in the absence of the ligand treatment. Furthermore, phosphorylation, ubiquitination and degradation of exogenous IFNAR1 expressed at high levels depend neither on ligand treatment nor on Tyk2 catalytic activities. Given that high levels of IFNAR1 are known to suppress cell proliferation even in the absence of IFNα (*4*), we propose that the ligand/Tyk2-independent pathway plays an important role in limiting the cellular levels of IFNAR1.

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03-06/P

ADMINISTRATION OF INTERLEUKIN-11 INCREASES SERUM STNFRI AND CRP LEVELS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCY

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Interelukin-11 is a cytokine with known anti-inflammatory activities. We have previously shown that in patients with haematological malignancy, chemotherapy-induced mucositis is reduced by the administration of rhIL-11. Preservation of the mucosal barrier in IL-11-treated patients was associated with a significant reduction in bacteraemia. In the present study, we measured the daily serum concentrations of sTNFRI, IL-6, IL-8, TNFα and CRP following the administration of either rhIL-11 or placebo throughout chemotherapy. Significantly higher sTNFRI levels [mean pg/ml (95% CI)] were detected in patients receiving rhIL-11 compared to placebo, [1749.7 (1626-1882.9) vs. 1038.5 (953.3-1131.3)], respectively ($p=0.01$) throughout the observation period. Higher sTNFR levels were also observed during days without infection [1406.6 (1266.1-1563) vs. 871.3 (774.9-979.6), $p<0.001$] in rhIL-11 vs. placebo arm, respectively. A similar pattern in CRP concentrations was observed. Multivariate analysis indicated rhIL-11 was associated with elevated sTNFRI or CRP independent of infectious episodes and other factors. 7 patients (all receiving placebo) of 40 had elevated TNFα levels. IL-6 and IL-8 levels were raised during infectious episodes in both sets of patients and were not substantially affected by rhIL-11 treatment. Bacteremia, fungal infections and fever of unknown origin (FUO) were reduced in rhIL-11 treated patients. Given the role of sTNFRI in dampening the deleterious effects of a hyperactive TNF-α environment, rhIL-11-induced upregulation of sTNFRI shedding is a potentially important mechanism for modulating immune and inflammatory responses in humans.

03-07/P

INSTRUCTIVE ROLE OF IL-2 RECEPTOR SIGNALING IN MYELOID LINEAGE DIFFERENTIATION

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Cytokines play crucial roles in the growth, survival and differentiation of developing hematopoietic cells. During hematopoiesis, multiple steps are required for lineage commitment of hematopoietic stem cells (HSCs). However, it is still under debate whether cytokines simply support proliferation/survival or they have instructive actions on lineage decision of developing cells from HSCs. Common lymphoid progenitors (CLPs) are lymphoid lineage-committed progenitor that can give rise to all classes of lymphocytes but not myeloid cells. However, IL-2 stimulation through ectopic IL-2 receptor (IL-2R) can initiate a latent myeloid differentiation program in CLPs, suggesting that IL-2R can exert influential effect on lineage commitment and differentiation. Although known signals via IL-2R and IL-7 receptor (IL-7R) are mostly the same, IL-7R fails to initiate lineage conversion in CLPs. This discrepancy suggests that IL-2R can activate distinctive signaling pathways that are absent in IL-7R. In this study, we investigated how IL-2R delivers such unique signals to affect lineage decision in CLPs. Our results show that the lineage conversion ability lies in IL-2Rβ cytoplasmic domain and can be transferred to IL-7R by transplanting A or H region of IL-2Rβ to IL-7Rα C-terminus. In addition, we identify that Shc is one of the major mediators of IL-2Rβ to initiate lineage conversion. We would also like to discuss the presence of unique signaling pathway(s), which is necessary for initiation of the latent myeloid differentiation in CLPs.

03-08/P

STRUCTURAL AND FUNCTIONAL DIFFERENCES BETWEEN HUMAN AND NON-HUMAN CELL EXPRESSED HUMAN TNF RII-FC CHIMERA.

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Glycosylation is the major post translational modification of human proteins and can have a significant impact on the function and antigenicity of the protein. Glycosylation is extremely complex, with the potential for a protein to have N-, O- and/or C-linked glycans and for each site of glycosylation to have multiple glycan structures attached to it. Glycosylation of recombinant proteins is dependent on the machinery of the cell line in which they are made. Thus a protein made in human, NS0, or CHO cells may have significantly different glycosylation structures, and hence have different functions and immunogenicity effects. TNF RII is a receptor with high affinity for TNF-alpha. This receptor is a type 1 transmembrane glycoprotein that is cleaved by proteolytic processing to produce a soluble form. TNF RII-Fc chimera is produced by fusing the DNA sequence encoding the signal peptide and extracellular domain of human TNF RII to the Fc region of human IgG1. A commercially available form of TNF RII-Fc produced in CHO cells has been shown to be effective in treating a variety of immune diseases, such as rheumatoid arthritis and psoriasis, diseases characterized by the over-expression of TNF-alpha. In this study we compare the glycosylation structures on human cell expressed (hcx™) human TNF RII-Fc to those on NS0 and CHO expressed human TNF RII-Fc. We also present preliminary data showing immunogenicity differences *in vivo*.

03-09/P

CROSS-REACTIVITY OF IL-10 HOMOLOG CYTOKINES, IL-19 AND IL-22 WITH HUMAN IL-10 RECEPTOR 1

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Background. A family of IL-10-related cytokines has been recently identified; these include IL-19, IL-20, IL-22, IL-24, and IL-26 as well as several cytokines encoded by viral genomes (e.g. ebvIL-10 or cmvIL-10). The predicted helical structure of these homodimeric molecules is conserved. Some of the receptor subunits are shared among the cytokine homologs, but the extent of cross-reactivity has not been entirely defined. We tested the interaction of human IL-10 homolog cytokines with the IL-10R1 as well as with the IL-10R1-S138G variant that is present in about 15% of Caucasian alleles (*J Immunol* 2003; 170: 5578-82).
Methods. Recombinant cytokines were expressed in HeLa and supernatants were collected. Single cell clones carrying either IL-10R1 wild-type or IL-10R1-S138G were treated with various concentrations of IL-10, IL-19, IL-20, IL-22, IL-24, IL-26 and mock supernatant for 30 min at 37°C, cells were harvested and STAT3 phosphorylation at Y705 was analyzed by Western blotting. To ensure receptor specificity, clones were pre-incubated with a neutralizing anti-IL-10R1 antibody (100ug/mL, R&D) prior to exposure to cytokines.
Results. STAT3 phosphorylation was observed with IL-10, IL-19, IL-22 and somewhat weaker also IL-20, but not IL-24, IL-26 or mock supernatant. The receptor specificity was confirmed by neutralizing STAT3 phosphorylation with anti-IL-10R1 antibody. In clones carrying IL-10R1-S138G, only moderate activation of STAT3 was detected when compared to the IL-10R1-WT clones.
Conclusion. Both IL-10R1 wildtype and S138G variant receptor can be activated by certain IL-10 homolog cytokines such as IL-19, IL-20, and IL-22. Our finding suggests that these cytokines may either compete with IL-10 for its receptors or activate cells that carry IL-10R1 in the absence of IL-10 or in the absence of their specific receptors (i.e. IL-20R1, IL-22R1). Our observations add to the complexity of interactions of IL-10 homologs with the cytokine receptor family type 2.

03-10/P

THE IL-22 RECEPTOR MEDIATES IL-10-LIKE INHIBITORY ACTIVITY WHEN EXPRESSED IN A MACROPHAGE CELL LINE

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IL-22 is a T cell-derived class-2 cytokine that shares a low but significant degree of sequence homology with IL-10. Macrophages express IL-10 receptors (IL-10R1), but they do not normally express IL-22 receptors (IL-22R1). Therefore, IL-10 but not IL-22 can signal transduce in macrophages. Although macrophages do not express IL-22R1, they do express the accessory chain, IL-10R2, which is an essential component of both the IL-10 and IL-22 receptor complexes. IL-10 inhibits expression of pro-inflammatory cytokines in macrophages. In contrast, IL-22 up-regulated expression of many of the same genes in IL-22 receptor-positive cell types such as keratinocyes and hepatocytes. To determine if the IL-22 receptor can mediate anti-inflammatory activity when expressed in macrophages, we generated RAW264 cell transfectants that stably express the IL-22R1 chain. IL-10 but not IL-22 inhibited expression of LPS-inducible genes such as IP-10, KC and TNF-α in wild-type RAW264 cells. However, both IL-10 and IL-22 inhibited cytokine gene expression in IL-22R1-transfected RAW264 cells. In contrast, although keratinocytes constitutively express IL-22 receptors and IL-22 induces activation of STAT3 in these cells, IL-22 did not inhibit cytokine gene expression in keratinocytes. These findings demonstrate that the IL-22R1 chain can mediate IL-10-like anti-inflammatory activity when expressed in macrophages but not in keratinocytes. These findings also suggest that the gene(s) that mediates IL-10-inducible inhibition may be preferentially expressed in macrophages.

03-11/P

FROM INSIGHTS IN LEPTIN RECEPTOR ACTIVATION TO THE DESIGN OF NOVEL LEPTIN ANTAGONISTS

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Leptin was originally identified as an adipostat that signals the body fat stores to the brain. Since then, several peripheral activities were documented, i.e. on the immune system. Leptin contributes to the pathogenesis of certain autoimmune diseases, providing a rationale for the development of leptin antagonists.
Leptin shows strong similarities to the long-chain α-helical cytokines interleukin 6 (IL-6) and granulocyte colony-stimulating factor (G-CSF). Its receptor (LR) belongs to the cytokine receptor family with an extracellular portion composed of two Cytokine Receptor Homology domains (CRH1 and CRH2), separated by an Immunoglo bulin-like domain (Ig-like) and followed by two membrane-proximal Fibronectin Type III domains (FN-III). Based on detailed mutagenesis data, we propose a hexameric leptin/receptor complex consisting of two leptin and four LR molecules. Each leptin ligand interacts with three LRs, whereby Site II is the major leptin binding site (interacting with CRH2) and Site III is strictly required for LR activation (interacting with Ig-like). Leptin induces a conformational change in the LR complex leading to the direct interaction of the FNIII domains, and subsequent iuxtaposition and activation of the cytosolic JAK kinases. We previously reported the design of a leptin mutein with antagonistic properties. Through elimination of binding site III, this mutant is a competitive inhibitor of leptin binding, but is by itself incapable of activating the LR complex. Consistent with our model, we have now obtained three different types of "nanobodies" directed against the LR that are capable of neutralising leptin activity. The first two nanobody panels interfere with Site II and III interactions, respectively. Nano bodies belonging to the third panel target the FN-III domains and block the ligand-induced conformational switch without interfering with leptin binding. Since nanobodies do not cross the blood-brain barrier, such antagonists may selectively inhibit leptins' peripheral activities, i.e. suppression of immune functions without inducing weight gain.

03-12/O

CHARACTERIZATION OF THE IL-6 INHIBITOR IL-6-RFP: FUSED RECEPTOR DOMAINS ACT AS HIGH-AFFINITY CYTOKINE-BINDING PROTEINS

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Most cytokines signal through heteromeric receptor complexes consisting of two or more different receptor subunits. Fusion proteins of the extracellular parts of receptor subunits termed cytokine traps turned out to be promising cytokine inhibitors useful in anti-cytokine therapy. We developed a fusion protein consisting of the ligand binding domains of the IL-6 receptor subunits IL-6Rα and gp130 that acts as a highly potent IL-6 inhibitor. Gp130 is a shared cytokine receptor that is also used by the IL-6-related cytokines oncostatin M (OSM) and leukemia inhibitory factor (LIF). In this study we show that the IL-6 receptor fusion protein (IL-6-RFP) is a specific IL-6 inhibitor. IL-6-RFP does not block the OSM or LIF mediated induction of a reporter gene under the control of the α2-macroglobulin promoter. Furthermore, we characterized the complex of IL-6-RFP and fluorescently labelled IL-6 (YFP-IL-6) by blue-native PAGE and gel filtration. A two-fold molar excess of IL-6-RFP over IL-6 is sufficient to entirely bind IL-6 in a complex with IL-6-RFP. As shown by treatment with urea, the complex of IL-6 and IL-6-RFP is more stable than a complex of IL-6, soluble IL-6Rα and soluble gp130. The apparent molecular mass of the IL-6/IL-6-RFP complex determined by blue-native PAGE and gel filtration suggests that IL-6 is trapped in a structure analogous to the native hexameric IL-6 receptor complex. Thus, fusion of the ligand binding domains of heteromeric receptors leads to highly specific cytokine inhibitors with superior activity. Futhermore, novel receptor fusion proteins for the inhibition of murine and human LIF will be presented.

03-13/P

SOLUBLE IFNAR2 TRANS-SIGNALING POTENTIATES IFNβ MEDIATED EFFECTS IN SEPTIC SHOCK

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The type I interferons (IFNs) bind to a common receptor complex consisting of IFNAR1 and IFNAR2. Alternative splicing of the *Ifnar2* gene results in a transmembrane (tmIFNAR2) and a soluble (sIFNAR2) isoform. Increase in serum sIFNAR2 concentration is seen in certain chronic viral infections and cancers. Recombinant sIFNAR2 acts as an antagonist by inhibiting type I IFN activity *in vitro* and could also modulate IFN effects via the pharmacokinetic properties of IFN. High sIFNAR2 levels are seen in mouse body fluids and organs. Western analysis of organs from perfused mice showed surprisingly low levels of sIFNAR2, suggesting that blood cells are a major source of sIFNAR2. Indeed, Peripheral Blood Leukocytes (PBL) from blood was shown to be a major producer of sIFNAR2. In order to determine the effects of sIFNAR2 we have initiated a program to generate transgenic mice. A minigene construct composed of 8kb of the 5' flanking region, exon 1, intron 1 and the remainder of sIFNAR2 cDNA to generate transgenic mice (the SolOX mouse) that overexpress the soluble receptor. Expression levels in the SolOX mice were demonstrated by Northern and Western analysis of organs and serum and transgene copy number was determined by quantitative southern blot. The effect of over expression on immune cell populations and susceptibility to viral and bacterial infections, ability to alter pharmacodynamic properties of IFN are being analyzed in these SolOX mice. These transgenic mice are phenotypically normal except for decreased thymus cellularity. IFNβ is produced in the thymus and potentiation of IFN effects by high sIFNAR2 concentrations may influence proliferation or apoptosis of immature thymocytes. IFN β which is produced during sepsis is a key factor involved in lethality due to septic shock. SolOX mice show an increased susceptibility to bacterial lipopolysaccharide (LPS) mediated septic shock. When *in vivo* IFN signaling was investigated faster and enhanced IFN mediated STAT phosphorylation was observed in SolOX mice suggesting transsignaling by sIFNAR2. These studies will have important implications for the role of sIFNAR2 in disease and resistance to IFN therapy.

03-14/P

TWO ISOFORMS OF THE IL-3Rα RECEPTOR REGULATE DIFFERENTIATION AND SELF-RENEWAL OF MULTI-POTENTIAL HAEMATOPOIETIC CELLS

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The balance between self-renewal and differentiation of haematopoietic stem cells is critical for normal blood cell formation and in leukaemia. Interleukin-3, the earliest acting of the colony stimulating factors regulating haematopoiesis, promotes both self-renewal of early multi-potential progenitors and also their differentiation but little is known of the mechanisms involved. Recently, in our laboratory, we have identified an alternatively spliced form (SP2) of IL-3Rα, present in mouse and human haematopoietic cells. RT-PCR and Western blot analysis of 10 mouse and 6 human hematopoietic cells or cell lines indicated that IL-3Rα SP2 is widely expressed. Expression of hIL-3Rα SP2/hβc and mIL-3Rα SP2/mβIL-3 supports the growth of CTLL-2 cells, though higher concentrations of IL-3 are required compared with IL-3Rα SP1 (the originally published isoform). Expression of hIL-3Rα SP1/hβc promotes multi-potential FDCP-mix cell self-renewal, however SP2 promotes differentiation of FDCP-mix cells. Studies of the differentiated cells using light and electron microscopy and characterization of their surface markers by FACS suggests they are basophil-like consistent with the *in vivo* function of IL-3. The study gives new insights into the regulation of self-renewal and differentiation by IL-3 and may help improve our understanding of the role of IL-3 signalling in immune responses, myeloid leukaemia and several other diseases.

03-15/P

RECRUITMENT AND ACTIVATION OF STAT3 BY THE IL-22R THROUGH A NOVEL TYROSINE-INDEPENDENT MECHANISM

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IL-22 is an IL-10-related cytokine that plays a role during liver injury, acute phase response as well as in proliferation and differentiation of keratinocytes. IL-22 signals through a receptor that is composed of two chains from the class II cytokine receptor family: IL-22R and IL-10Rβ (CRF2-4), which is also involved in IL-10 signalling. The IL-22R chain is also involved in the activities of IL-20 and IL-24 and could play a role in psoriasis. In this study, we analyzed the signal transduction pathways activated in response to IL-22 in various cell types. Among the multiple signalling pathways activated by IL-22, and including phosphorylation of STAT1, STAT3 and STAT5, as well as activation of MEK1/2, ERK1/2, p90RSK, JNK and p38 kinases, STAT3 phosphorylation seems to play a major role in IL-22 activities. Although IL-22R contains several tyrosines with the consensus surrounding sequence to recruit and activate STAT3, we found that mutation or deletions of all tyrosines of IL-22R only marginally affects STAT3 activation. Moreover, we could show that a specific region of IL-22R can recruit STAT3 in the absence of IL-22, and allows for IL-22-induced phosphorylation of STAT3 independently of IL-22R tyrosines. This constitutive association of STAT3 with IL-22R results from a direct and specific interaction between the cytoplasmic domain of IL-22R and the coiled coil domain of STAT3, and does not

require the SH2 domain. Deletion of the region of IL-22R involved in this interaction significantly affects the ability of the receptor to mediate IL-22 activities, indicating that this new mechanism of STAT3 recruitment might play a key role in signalling by this receptor.

03-16/P

LIGAND-INDUCED CONFORMATIONAL CHANGE OF IFNAR1 SUGGESTS VERTICAL SIGNAL PROPAGATION BY THE TYPE I INTERFERON RECEPTOR

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The type I interferon (IFN) receptor plays a key role in innate immunity against viral and bacterial infections. All type I IFNs recruit the same receptor subunits, ifnar2 and ifnar1, yet activate differential response patterns. It appears that the interaction with the low affinity subunit ifnar1, which is probably only recruited on the cell surface, is responsible for differential signal activation. We have studied the recognition of IFNs by ifnar1 in vitro using label-free and fluorescence detection techniques. All three N-terminal Ig-like domains of the ectodomain of ifnar1 (ifnar1-EC) are responsible for ligand recognition, yet the fourth, membrane-proximal domain is absolutely essential for signal activation. Using a novel fluorescence quenching assays in solution and on surfaces, we identified substantial conformational changes of ifnar1-EC upon ligand binding. Similar conformational and changes were observed for both IFNα2 and IFNβ, corroborating a similar architecture of the signalling complexes for both interferons. Temperature-dependent kinetic analysis of ligand interaction yielded a negative activation energy of the ligand dissociation kinetics, confirming the role of conformational isomerization during ligand binding. Strikingly, this conformational change is propagated to the membrane-proximal domain of ifnar1-EC, which is not involved in ligand recognition, yet essential for signal activation. This effect was further confirmed by electron transfer-based fluorescence quenching and by single molecule fluorescence life-time and autocorrelation measurements. Taken together, our results suggest that ifnar1 may propagate a ligand-induced conformational change vertically through the membrane. This feature has been already proposed for several other cytokine receptors, but never been experimentally shown so far.

03-17/O

DYNAMICS OF TYPE I INTERFERON RECEPTOR ASSEMBLING IN LIVE CELLS

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Cytokine receptors are promising drug targets for fighting complex diseases such as cancer, autoimmune diseases or allergy. However, the mechanism of signal activation by cytokines is currently not well understood, making it difficult to systematically design pharmaceutically active compounds. Here, we report first steps towards studying ligand-induced assembling of the type I interferon receptor in life cells using fluorescence recovery after photobleaching (FRAP), fluorescence quenching and single particle tracking. Ifnar1 and ifnar2 were fluorescence labeled using autofluorescent proteins, as well as different techniques for posttranslational fluorescence labeling. Thus, selective labeling of the receptor in the plasma membrane with different fluorescence probes was achieved. The mobility of the receptor subunits was studied by FRAP. Free diffusion of the receptor subunits with a diffusion coefficient of ~0.02 $\mu m^2/s$ was observed, which was also confirmed by single molecule fluorescence tracking. Strikingly, similar diffusion properties were observed for the full-length proteins compared to variants with truncated cytoplasmic domains. Ligand binding studies and changes in the mobilities of the receptor subunits upon ternary complex formation confirmed a ligand-induced cross-linking of the receptor subunits. Using novel multivalent chelator heads, the interaction between the receptor subunits were studied by fluorescence quenching assays, and correlated with ligand binding events. Using ligand chasing experiments, the mechanism of ligand-induced receptor assembling and dynamics of the interactions was quantitatively assessed. These studies were complemented with monitoring STAT recruitment and translocation in real time by fluorescence resonance energy transfer.

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FUNCTIONAL REDUNDANCY BETWEEN ELF-1 AND GABP COMPLEX ENSURES CD25 EXPRESSION DURING ACTIVATION OF HUMAN PERIPHERAL T-CELLS

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The interleukin 2 (IL-2) receptor a-chain/CD25 (IL-2Ra) is essential for assembly of low-, pseudo-high and high-affinity IL-2 receptors. Initially activation of resting peripheral T-cells results in a transient low-level transcriptional up-regulation of IL-2Ra expression mediated through the proximal promoter/enhancer element. This is followed by IL-2 dependent autocrine up-regulation of IL-2Ra transcription mediated through the conserved distal IL-2 responsive enhancer (IL-2RE), whose activity depends on a synergistic action of IL-2 induced STAT5a/b, GATA and T-cell specific Ets family protein Elf-1. However, normal hematopoietic development and T-cell functions in Elf-1 deficient mice suggest transcriptional compensation by other Ets-family protein(s) with similar DNA-binding requirements. Here we show that lymphoid-specific Ets-family factor Elf-1 and ubiquitously expressed GABPa/b complex compete for a common critical *ets* DNA binding elements within the proximal promoter and IL-2RE of human IL-2Ra gene. Expression levels of Elf-1, GABPa and in particular GABPb are significantly increased during activation of resting human peripheral $CD4^+$ and $CD8^+$ T-cells, and overexpression of either Elf-1 or GABPa/b resulted in an increased expression of IL-2Ra protein. RNA-interference mediated downregulation of both Elf-1 and GABPa in resting human T-cells strongly reduced activation-dependent increase of IL-2Ra expression. These data suggest that Elf-1 and GABPa/b complex are essential redundant transcriptional regulators of IL-2Ra expression in lymphoid cells.