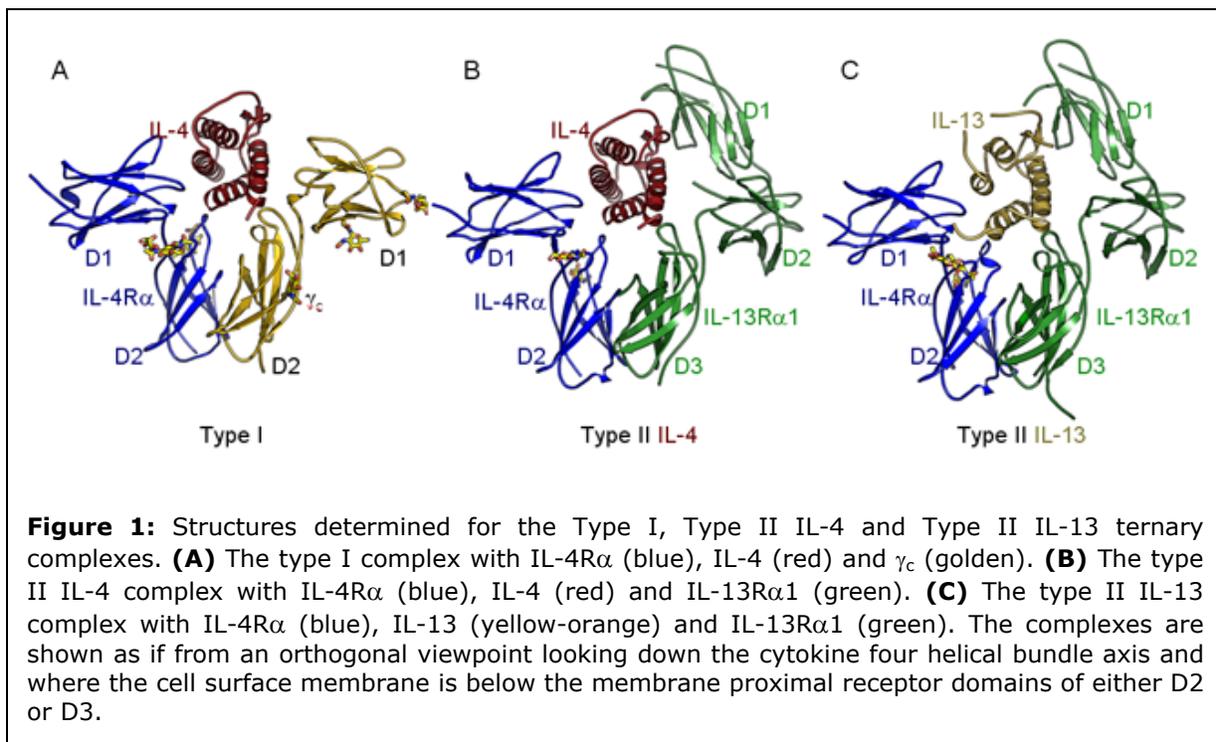


Snapshots of the IL-4 Receptor Ternary Complexes: An Opportunity to Visualize the Basis of Cytokine Receptor Pleiotropy in the Immune System

Cytokines are a group of proteins and peptides that are employed in complex multi-cellular organisms as signaling compounds produced by individual cells to transmit information from one cell to another. The distances across which these cytokine signals may travel varies from within the neighborhood of a tissue or organ to remote tissues far away from the cytokine source via circulating through the blood. They are variously named as interleukins, lymphokines, and chemokines as well as other “factors” and with the names based upon the presumed function at the time of discovery. These cytokines act by binding to a cell surface receptor in either of two ways. In the first case a cytokine binds the extracellular domain of a receptor and recruits additional receptors where a receptor is composed of three domains: extracellular ligand binding domain, a single trans-membrane helix domain and an intracellular domain; in this case, cytokine binding and subsequent extracellular domain rearrangements change the spacing and orientation of the intracellular domains resulting in signal transduction across the cell membrane in a deliberate manner. In the second case a cytokine binds to a multi-spanning transmembrane protein causing movements within the transmembrane region, which conveys the signal more directly across the membrane, where this latter mechanism is beyond the scope of our research results. Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory, and infectious diseases. When the body is fighting pathogens, cytokines activate and recruit immune cells to travel to the site of infection, for example. These cytokine-mediated processes are known to go awry in some diseases.

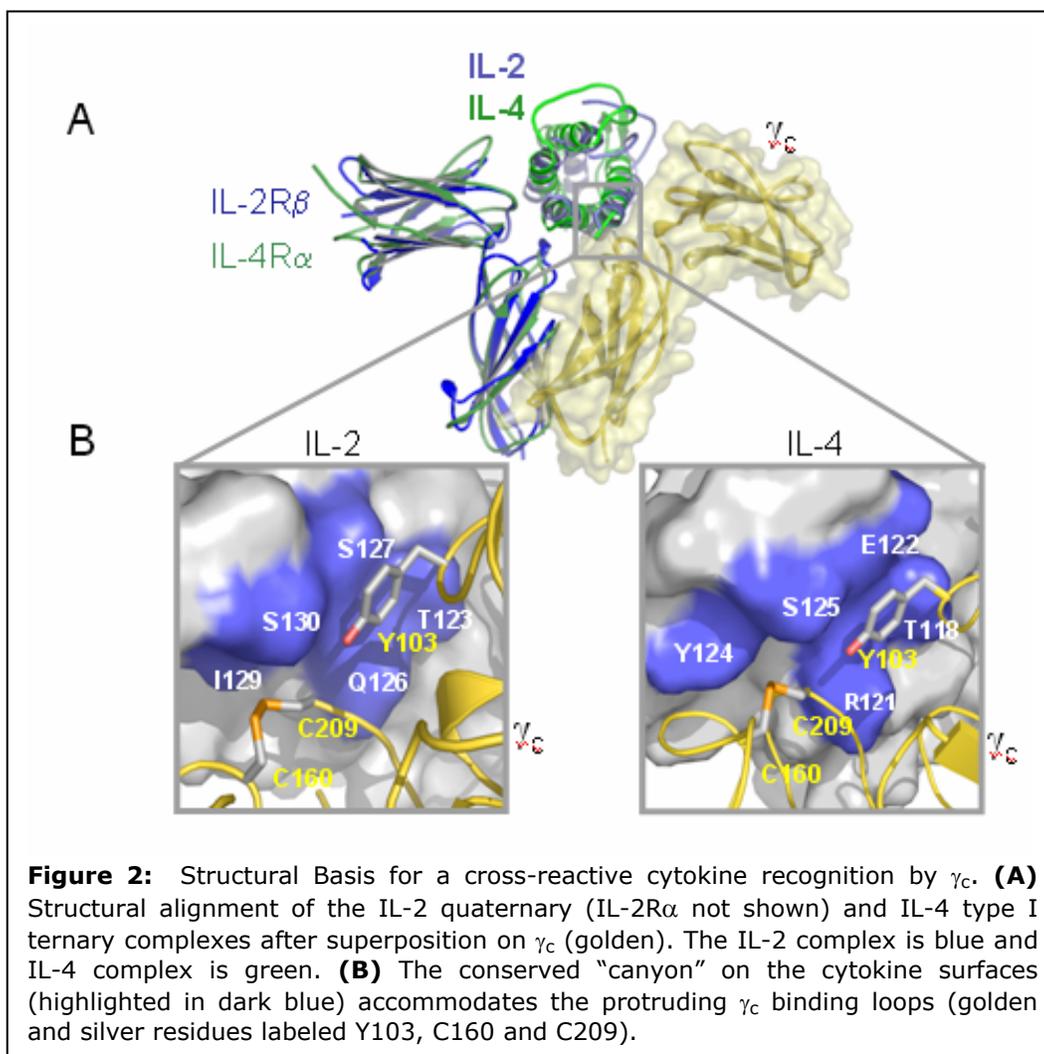


Interleukin-4 (IL-4) and Interleukin-13 (IL-13), cytokines critical to the development of T cell-mediated humoral immune responses to pathogens and are also associated with allergic asthma, exert their actions through different combinations of shared cell-surface receptors

the three-domain receptor signaling model. In this effort, the x-ray crystal structures were determined for three cytokine-receptor ternary complexes that comprise the IL-4/IL-13 system, providing molecular resolution insight into the manner in which signaling specificity is attained by these cytokines. These interleukin cytokines in different complexes with their receptor extracellular domains (ECDs) revealed features of a complete set of interactions for the signaling system including, IL-4R α (IL-4 receptor alpha), γ_c (IL-2 receptor gamma, also known as "common" gamma receptor) and IL-13R α 1 (IL-13 receptor alpha 1). Beyond the structural analysis using synchrotron radiation x-ray diffraction, further biochemical study identified different assembly properties and signaling potencies of the receptor complex in response to each cytokine, suggesting that the extracellular cytokine-receptor interactions are modulating intracellular membrane-proximal signaling events.

Each complex is composed of three proteins of modest size with one cytokine of either IL-4 (15.8 kDa) or IL-13 (14.5 kDa) and two different receptor ECDs of IL-4R α (23.9 kDa) and either γ_c (24.1 kDa) or IL-13R α 1 (37.1 kDa). Depending on the cell presenting the receptors on its cell surface membrane, the complex is either of Type I (IL-4R α / γ_c /IL-4) or Type II (IL-4R α /IL-13R α 1/IL-4, IL-4R α /IL-13R α 1/IL-13) signaling complex. In order to visualize the molecular interactions between the cytokines and receptors, each cytokine and receptor ECD was expressed and purified by recombinant methods utilizing insect cells. Stable complexes were further purified and relatively delicate, plate-like protein crystals on the order of 100 μ m in length by 100-300 μ m in width by 10-50 μ m in depth were grown for each complex and frozen to protect them from synchrotron x-ray radiation damage during data collection. X-ray diffraction experiments were completed using SSRL Beam Line 11-1 for the type I and type II IL-13 complexes. Additionally, for the type I complex experiments, we employed the Stanford Automated Mounting (SAM) robot along with remote-control data acquisition to increase the speed of crystal diffraction screening. The diffraction resolution for all data sets used to determine the structures was on average 3.0 Ångstroms.

The type I complex conforms to the canonical cytokine-receptor structural paradigm, whereas the type II complexes utilize an unusual top-mounted Ig-like sub-domain (D1) on IL-13R α 1 for a novel mode of cytokine engagement, rationalizing the evolutionary divergence of IL-13R α 1 and γ_c from a common ancestor (Figure 1). The recognition of several cytokines by a common receptor, as well as the engagement of multiple receptors by the same cytokine, is achieved through substantially different recognition chemistries, which appear similar on molecular models refined with data collected to 3.0 Å resolution. We also find that, in comparison to IL-2, the type I IL-4 complex appears to reveal the semblance of a γ_c -cytokine recognition 'motif' (Figure 2). Superposition of the IL-2 quaternary complex (from Wang, Rickert & Garcia, 2005) upon the type I IL-4 complex using the backbone carbon-alpha atoms of γ_c from both complexes may suggest how γ_c cross-reacts and is therefore able to be shared with six different cytokines throughout the immune system while these cytokines have less than 15% primary amino acid sequence identity. Both IL-2 and IL-4 bury similar surface areas ($\sim 1000\text{Å}^2$) with a similar ratio of polar to non-polar atoms at the same binding site superposed on γ_c . The γ_c binding sites on IL-2 and IL-4 exhibit apolar "canyons" that receive the protruding elbow of γ_c through near-ideal shape complementarity towards γ_c residues Y103 and a disulfide bond between C160-C209. The external interacting positions on these helices appear to be held in place by conserved hydrophobic residues in the core positions consistent across all 6 cytokines as a γ_c "recognition motif". Overall, we report on the IL-4/IL-13 signaling system that diverged from a common role in the innate immune response to an adaptive immune response. Understanding the signal transduction resulting from these complexes forming is important, in part, because it may serve a role when the immune system goes awry in diseases such as asthma.



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Additional Reference:

Xinquan Wang, Mathias Rickert, K. Christopher Garcia (2005). "Structure of the Quaternary Complex of Interleukin-2 with Its α , β , and γ_c Receptors" *Science* **310**(5751): 1159-63.

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