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Resolving The Mechanism(s) of Immunological Recognition and its Coupling to Cellular Response(s)

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Our main goal is resolving the mechanism(s) of immunological recognition and the biochemical cascade coupling it to the respective cellular response. To this end we are pursuing the process of T-cell receptors recognition of its targets, the complexes of MHC encoded molecules with respective antigen derivative, e.g. peptides.

As a model system for an immunological stimulus-response coupling cascade, we are investigating that which is initiated by the type 1 Fcε receptors (FcεRI) on mast cells and are focusing currently, mainly on mechanisms of its control and regulation.

A distinct additional research topic pursued in our lab is that of investigating how internal electron transfer is taking place in proteins.

T-cell receptor recognition of its ligand

The key parameters controlling T-cell activation are proposed to be those of the kinetics of interactions between the T cell receptor (TCR) for antigen and its ligand. Analysis of kinetic data has so far produced conflicting insights and here we offer a novel consideration of this problem. As a model system, association and dissociation of a soluble T-cell receptor (sT1) and its specific ligand, an azido-benzoic acid (ABA) derivative of the peptide SYIPSAEK(ABA)I, (residues 252-260 from *Plasmodium berghei* circumsporozoite protein) bound to the class I Major Histocompatibility Complex H-2K^{d} encoded molecule (MHCp) were studied by the Surface Plasmon Resonance (SPR). The TCR-ligand association rate constant was found to be relatively slow and markedly temperature dependent, decreasing from 7.0×10^{3} at 25°C to 1.8×10^{2} $M^{-1}s^{-1}$ at 4°C. Hence it is suggested that the observed slow rate constants are the result of unresolved elementary steps of the association process. Indeed, a rigorous analysis of the kinetic data shows that the time-courses of TCR – MHCp interaction can be fitted well to either one of two different, yet closely related mechanisms, where an induced-fit of or a pre-equilibrium between two ligand free TCR conformers are operational. Both these mechanisms may provide a rationale for the reported conformational flexibility of the TCR binding site and its unusual ligand recognition properties, which combine high specificity with considerable cross-reactivity.

Regulation and control of an immunoreceptor stimulus-response coupling cascade; The FcεRI case

Aggregation of the type 1 Fcε receptors (FcεRI) on mast cells initiates a cascade of biochemical processes culminating in secretion of both stored and *de novo* synthesized inflammatory mediators. Understanding the regulation of this cascade is still relatively limited. We have therefore concentrated on identifying the key players in the negative control of the response to the FcεRI in the mucosal-type mast cells of the RBL-2H3 line.

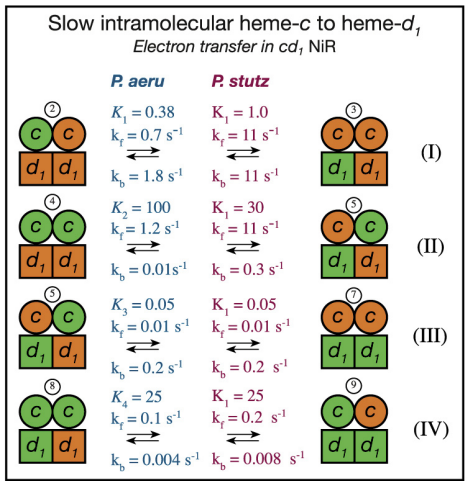
We have previously shown that the FcεRI mediated degranulation can be efficiently suppressed upon clustering an inhibitory receptor discovered in our lab. This receptor named Mast cell function-associated antigen (MAFA) is a C-type lectin, with a mannose binding capacity. Its intracellular tail contains a sequence related to the Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM), which plays an essential role in MAFA's action. We have later shown that MAFA clustering leads to a rapid phosphorylation of its ITIM's tyrosyls, creating a docking site for several SH2 domain containing molecules, including two phosphatases; Namely, the inositol-phosphatase (SHIP) and the protein tyrosine phosphatase (SHP-2). Both, once recruited to the plasma membrane, were found to counteract the activating signaling induced by the FcεRI.

More recently, we have examined the molecular mechanism of MAFA's inhibition of the late phase of secretory response to the FcεRI clustering; i.e. *de novo* synthesis and secretion of leukotrienes or cytokines. We found that MAFA clustering selectively suppresses gene transcription of cytokines including IL-1, IL-4, IL-6, IL-10, IFNγ and TNF-α, while that of IL-3 and IL-5 is unaffected. By studying the upstream molecular mechanisms of this inhibition we found that MAFA interferes with FcεRI induced p38 and Erk-1/2 activation. Moreover, the two adaptor proteins – Dok1 and Dok-2 were shown to be involved in MAFA's inhibitory action

Significatly,these two proteins were also found to control directly the response to FcεRI clustering which caused their elevated, relatively long lasting, tyrosine phoshorylation with the concomitant increase in their binding to RasGAP (and to other adaptors). This suggested Dok's role in modulating the Ras activity initiated by FcεRI aggregation. Indeed, we have further shown that the FcεRI mediated Ras/Raf1/Erk signaling cascade, and concomitantly the *de-novo* TNF-α synthesis are markedly reduced in RBL-2H3 cells over-expressing Dok-1. We therefore propose that Dok-1 is an important element of MAFA's inhibitory action as well as a part of a built-in an auto-regulatory apparatus of the FcεRI stimulus-response coupling cascade. Taken together, Dok-1 may have a considerable significance in modulating the FcεRI induced late phase allergic response.

Allosteric control of intra-protein electron transfer

Investigation of thermodynamics and kinetics of the intramolecular electron transfer processes among the active centers of both the copper or heme containing enzymes nitrite reductase have been carried out. While in the former enzyme electron distribution among the sites and their rate constants were found to be controlled by the free energy differences between them; In the heme containing cd_1 nitrite reductases, allosteric interactions among the sites provide an additional control mechanism



Selected Publications

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Acknowledgements:

Israel Pecht is the Dr. Morton and Anne Kleiman Professor of Chemical Immunology.

a. Israel National Science Foundation; b. Minerva Foundation;Munich, Germany c. EC 5th framework