PERSPECTIVES: IMMUNOLOGY

Translating Affinity into Response

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The T cell receptor (TCR) present on T cells scans the surface of other cells for peptides resulting from the degradation of pathogens. Another receptor, FcεRI, the high-affinity receptor for the Fc portion of immunoglobulin E (IgE) found on mast cells and basophils, initiates allergic responses. Both of these cell-surface protein-complexes, the TCR and FcεRI, are not simple binary switches, turned on when a ligand binds and off when it dissociates. Instead, depending on the nature of the ligand, they can trigger any one of a spectrum of cellular responses that range from maximal activation to a dominant negative signal. On pages 568 and 572 of this issue, Torigoe *et al.* (*1*) and Neumeister Kersh *et al.* (*2*) present striking results that offer clues to how the TCR and FcεRI can produce such a diverse range of responses.

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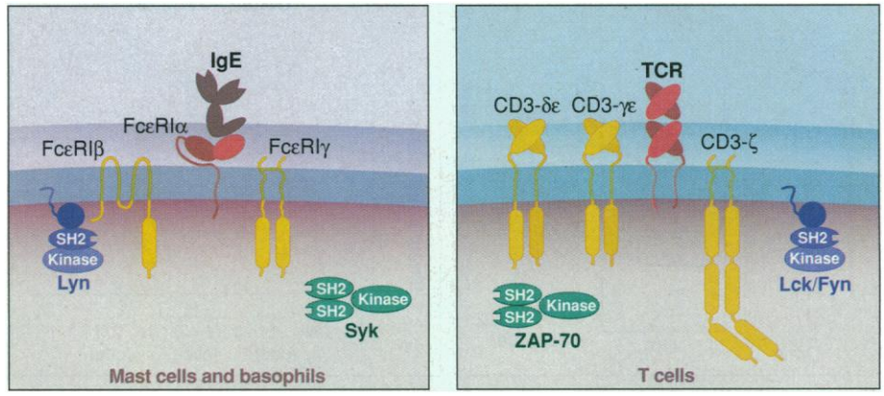
The TCR and FcεRI share structural and functional attributes (see the figure). For example, both of their antigen-binding modules are noncovalently associated with transducing subunits (CD3-γ, -δ, -ε, and -ζ in the case of the TCR; FcεRI-β and -γ in the case of FcεRI) that lack intrinsic enzymatic activity and contain one or multiple copies of a conserved sequence, the immunoreceptor tyrosine-based activation motif (ITAM), that is exposed to the cytoplasm. To exert their signaling functions, these subunits cooperate with nonreceptor tyrosine kinases belonging to the Src and Syk/ZAP-70 families. The TCR is triggered by complex recognition events that occur between an antigen-presenting cell and the T cell, which also requires additional cell-surface molecules such as CD4 and CD8. Triggering of FcεRI, a more tractable event, takes place when the FcεRI-bound IgE molecules are aggregated by soluble, multivalent antigens. A model of the early events after antigen-induced FcεRI aggregation has emerged within recent years (*3*, *4*). Before aggregation, the tyrosine kinase Lyn is already associated with the FcεRIβ subunit by a weak interaction that does not depend on phosphorylation. The increase in local molecular concentration that follows FcεRI aggregation allows the bound Lyn molecules to transphosphorylate the tyrosine residues on the approximated FcεRI-β and -γ ITAMs. Once phosphorylated, the ITAMs act as high-affinity docking sites for the SH2 domains of Lyn and Syk. Finally, the kinase activity of the recruited Syk increases as a result of both phosphorylation by Lyn and trans-autophosphorylation and contributes to the successful propagation of intracellular signals.



Signaling by two receptors. Composition of FcεRI **(left)** and the T cell receptor **(right)** complexes and of their proximal intracytoplasmic effectors. The immunoreceptor tyrosine-based activation motifs in the cytoplasmic tails of the transducing subunits are shown in red.

The biological potency of an individual FcεR receptor is determined by its lifetime within an aggregate, which is generally influenced by the dissociation rate constant of IgE. Thus, in the case of low-affinity IgE ligand, only a few early intracellular activation events are likely to take place before the receptor dissociates from its ligand and moves out of the aggregate. The new work by Torigoe *et al.* (*1*) demonstrates that, as compared with FcεRI aggregated by high-affinity ligands, FcεRI aggregated by low-affinity ligands is ineffective at stimulating distal steps of the signaling cascade. This finding is reminiscent of the partial T cell responses that follow TCR stimulation with less-than-optimal ligands (*5*) and suggests that both the TCR and FcεRI function according to a "kinetic proofreading" mode in which the cascade of intracellular events that follow interaction with a ligand unfolds as long as the initiating reaction is maintained (*6*).

Studies of rat basophilic leukemia (RBL) cells suggest that the amount of the kinase Lyn available to the FcεRI is limiting. The noncovalent, reversible interactions existing between FcεRI and such a limited pool of Lyn results in a flexibility in signaling properties that is not found in surface receptors in which a covalent linkage freezes the stochiometry of the ligand-binding and catalytic units. For example, upon aggregation of FcεRI, Lyn redistributes rapidly from the pool of free Lyn and from low-affinity, nonphosphorylated FcεRIβ binding sites to the high-affinity docking sites that constitute the newly phosphorylated FcεRIβ ITAMs (*4*). An important consequence of this redistribution is that an antigenic ligand capable of inducing an extensive aggregation of FcεRI can abrogate an ongoing response to a weak, noncross-reactive antigen by recruiting Lyn away from the previously formed FcεRI aggregates (*7*). The fact that discrete aggregates of FcεRI compete for limited amounts of Lyn provides a basis for the novel mechanism of ligand antagonism proposed by Torigoe *et al.* (*1*). Using RBL cells, they showed that despite their inability to use Lyn productively and activate the temporally most distal events of the signaling cascade, aggregates of FcεRI induced by an excess of low-affinity ligand could sequester Lyn, so that a smaller number of aggregates simultaneously induced by a higher affinity ligand become deprived of Lyn and thus unable to initiate the signaling cascade.

The molecular mechanisms proposed by Torigoe *et al.* may also function in other receptors that are subject to kinetic proofreading and that must interact with a limiting extrinsic initiating trigger. Indeed, the ability of some antigenic peptides to weaken or prevent a simultaneous T cell response to an optimal peptide has been indirectly correlated with their capacity to

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dissociate rapidly enough from the TCR so that a productive signal cannot occur but so that a limiting intracellular effector can nonetheless be depleted (*8*). TCR engagement by such fast-dissociating ligands induces a unique pattern of intracellular events that is not observed after stimulation with any concentration of an optimal ligand (*5*, *9*, *10*). With three concatenated ITAMs and six potential tyrosine phosphorylation sites, CD3-ζ can generate many discrete phospho-species and constitute a very appropriate gauge of the early tyrosine phosphorylation events that follow TCR engagement. Previous reports showed that resting T cells express a 21-kD phosphoform of CD3-ζ (p21)' (*5*, *9*). After full stimulation of their TCR, the phosphorylation of p21 increases and a 23-kD species (p23) appears. CD3-ζ phosphorylation also occurs after stimulation with suboptimal TCR ligands. In these cases, however, p21 is produced in large excess over p23.

Using antisera specific for each of the six phosphotyrosine motifs present in CD3-ζ, Neumeister Kersh *et al.* (*2*) have now deciphered the sites of phosphorylation in p21 and p23 and have made several important observations. First, p21 consists of two or three monophosphorylated ITAMs, an unexpected finding in view of the fact that the ZAP-70 tyrosine kinase is constitutively associated with p21 and preferentially associates with biphosphorylated ITAMs. Second, all six tyrosines in p23 are phosphorylated, allowing it to form a 1:3 complex with ZAP-70 molecules. Third, the reactions leading from basal to full phosphorylation of CD3-ζ proceed in a hierarchal mode and by way of discrete intermediates. These new results suggest that CD3-ζ is a molecular processor that can translate the variations in the half-lives of the TCR-ligand interactions into discrete phosphoforms and, by inference, distinct biological outcomes. Interestingly, as shown by Torigoe *et al.*, when stimulated with high- and low-affinity ligands, the FcεRI can also produce a diverse range of responses, but without incorporating a transducing subunit with concatenated ITAMs or relying on differential ITAM phosphorylation.

The data of Torigoe *et al.* and Neumeister Kersh *et al.* clearly reveal how antigen receptors translate quantitative differences in ligand binding into qualitatively different biological responses. We are still far from a quantitative understanding of the activation events triggered by those receptors. Nevertheless, the next steps—understanding how these various molecular components interact under the more compartmentalized circumstances of an intact cell—will be a story worth following.

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NOTA BENE: COMPUTERS

Softening Support for Macintoshes

The overall market share of Apple Computer's popular Macintosh system has been declining in recent years, falling from 6.7% of the total personal computer market in 1996 to 4.1% in 1997 (*1*). Although the popularity of the Macintosh computer remains high in scientific laboratories, even this previously secure niche of Apple's computer market may now be in trouble.

MacSciTech is an independent professional organization promoting use of the Macintosh computer in laboratories. With its broad range of services, the group has its finger on the pulse of scientific Macintosh computing. From MacSciTech's offices comes the popular *SciTech Journal*, which reaches 60,000 scientists and engineers each year, and Web sites geared to Macintosh users in laboratories (www.scitechcomp.org, www.macscitech.org). They also produce CD-ROMs to assist with data acquisition, imaging, math and simulation, earth-space sciences, and engineering, as well as the annual "Top 10 Wish List for Science and Engineering," which is a popular missive to Apple from scientists on their needs for Macintosh software.

MacSciTech has had a long history, which parallels Macintosh scientific usage. It sprang from the Consortium for Laboratory and Industrial Applications of the Macintosh (CLIAM), a trade organization formed independently of Apple to promote the use of the Macintosh in science and engineering. CLIAM's early success convinced Apple to provide financial and personnel resources to technical markets. In 1991, CLIAM spawned MacSciTech, which grew rapidly, ultimately reaching a core membership of 2000 highly influential scientists, engineers, and information systems (IS) professionals.

Partly as a result of MacSciTech's efforts, Macintosh sales have always been disproportionately higher to scientists than to the general public. Industry insiders point out that Macintosh scientific software makes up 40% of some markets, with particular strength in molecular biology, cognitive psychology, and chemistry. Between 1996 and 1997, however, academic market share for Macintoshes fell from 41.6% to 27.1% of total personal computer sales (*1*). MacSciTech speculates that this may be because IS departments of many research-based organizations do not buy Macintoshes because they require less support than Windows-based computers, threatening the departments' power base (*2*). Other factors may include Apple's opposition to Macintosh clones and a perceived price differential compared to Intel-based systems.

Another reason for the declining sales may be related to Apple's diminishing support for scientific developers and organizations. In the past, powerful Mac-only software products with unique features, such as Textco's Gene Construction Kit, have provided justification for the purchase of Macintosh equipment in otherwise hostile environments. Reduced support from Apple is driving Macintosh-only software developers to make dual-platform products, removing Apple supporters' historically powerful wedge to justify purchases of Macintoshes. Some developers, such as The MathWorks (*3*) are dropping Macintosh software support altogether. MacSciTech itself will soon reorganize as SciTech Computing Association, a new entity that will continue to support the Macintosh but will also support UNIX, Windows, and Linux systems. MacSciTech's decision comes on the heels of an announcement by Merck & Co. that it will eventually convert its 6000-plus base of lab Macintosh machines to Windows NT systems (*4*).

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