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Pillars of Immunology

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After the discovery of the Ag/MHC-binding TCR for Ag and associated CD3 chains, the race was on to understand how these proteins function to control T cell activation. By 1994, when the featured paper by Iwashima et al. (1) was published, many of the now-familiar players in this process had been identified. Thus, it was known that the ζ-chain (previously known as CD3ζ) of the TCR complex, as well as the CD3 γ-, δ-, and ε-chains, could be phosphorylated on tyrosine residues (2). It was also clear that these chains contained most, if not all, of the information needed to link the TCR to (at the time poorly defined) signaling pathways that control T cell activation. A key piece of information in this hunt was the realization (first by Michael Reth; subject of a recent *Pillars of Immunology* commentary) (3, 4) that the cytoplasmic regions of the CD3 and ζ proteins (and related proteins associated with surface BCR and IgE-binding FcεRI, among others) contain a conserved motif, sometimes referred to as the ARAM (Ag receptor activation motif), but now known as the ITAM. Meanwhile, a large number of studies had shown that the tyrosine kinase Lck was intimately involved in early TCR-dependent signaling, in addition to being critical for T cell activation and development (see Ref. 5 for an early review). Art Weiss and others had also shown that the cytoplasmic tails of ζ or the CD3 ε protein (or Igα or Igβ in B cells) could at least partially substitute, in the context of a chimeric molecule, for Ag receptor signals (6–9). The other critical piece of this puzzle was the identification of Zap70, again by Weiss and colleagues (10), as a cytoplasmic tyrosine kinase that could be recruited directly to the cytoplasmic tail of ζ (hence its full name, ζ-associated protein of 70 kDa).

Thus, at this point many of the now-familiar players were assembled, but they were still in need of stage directions. For instance, although Zap70 was known to be recruited to the TCR ζ-chain, it was not clear whether TCR crosslinking was sufficient for this, or whether some additional signaling process had to occur to facilitate Zap70 recruitment. Additionally, although Lck had been shown to be required for T cell activation, its relationship to Zap70 was not clear. Thus, it was not known whether Lck and Zap70 resided at the apex of parallel signaling pathways to control T cell activation or whether they might function in a linear pathway. There was also an incomplete understanding of the relative roles of the kinase and Src homology 2 (SH2) domains of the two proteins. Of particular interest was the observation that Zap70 (similar to its cousin Syk) contains two SH2 domains, as opposed to Lck and other Src family kinases, which possess only a single SH2. Thus, were both of these SH2 domains required for signaling and TCR recruitment?

On the basis of five compact figures, without the benefit of online supplementary material, Iwashima et al. drew the following conclusions. First, association of Zap70 after TCR triggering requires expression of Lck. Second, the kinase activity of Lck, but not its SH2 domain, is required to promote the Zap70–ζ interaction. Third, both SH2 domains of Zap70 are required for its interaction with ζ. Fourth, both tyrosines within an ITAM must be phosphorylated to recruit Zap70. Finally, kinase-deficient Zap70 can still associate with Lck-phosphorylated ζ, but this is not sufficient for tyrosine phosphorylation of downstream substrates.

There are several reasons why I think this paper is worthy of inclusion in *Pillars of Immunology*. First, it describes a fundamental principle of Ag receptors, that is, that they use Src and Syk family tyrosine kinases in a stepwise fashion to control early events in cellular activation. This is now a textbook model, appearing in any introductory course that covers signaling by cells of the immune system. Second, the paper is elegant and economical in its approach and presentation. Experiments appear straightforward (at least in retrospect), and the totality of the data presented consists of five figures, with a total of 10 panels. Third, the conclusions reached, based on the data presented, have held up remarkably well over time (now >20 y). Finally, the paper contains several prescient predictions, which have also proven to be true. For example, the kinase activity of Zap70 was postulated to be critical for phosphorylation of multiple "downstream" substrates involved in T cell activation. We now know the identity of a number of these direct Zap70 substrates, including the adaptor proteins LAT and SLP-76 and the enzyme PLC-γ1.

Although it is fashionable to be somewhat dismissive of signaling studies performed in cell lines, papers such as this one point out the power of cell line–based approaches for discovery of basic signaling mechanisms. Thus, in this case the authors used Jurkat T cells and COS-18 cells in their experiments. Jurkat T cells in particular have been profoundly useful for the discovery of the basic rules of TCR signaling (11). Certainly there are caveats to be considered, such as the lack of PTEN expression by these cells, but it cannot be overemphasized that our current understanding of TCR signaling

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Abbreviations used in this article: SH2, Src homology 2; Zap70, ζ-associated protein of 70 kDa.

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was largely worked out in Jurkat T cells. In that light, this particular *Pillars of Immunology*–worthy study contributed key findings on which much subsequent research has been based.

Disclosures

The author has no financial conflicts of interest.

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