A Clue to Antigen Receptor Tails

Bryan Irving and Arthur Weiss

J Immunol 2014; 192:4013-4014; doi: 10.4049/jimmunol.1400660
http://www.jimmunol.org/content/192/9/4013

References
This article cites 22 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/192/9/4013.full#ref-list-1

Why The JI? Submit online.

- Rapid Reviews! 30 days* from submission to initial decision
- No Triage! Every submission reviewed by practicing scientists
- Fast Publication! 4 weeks from acceptance to publication

*average

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts

The Journal of Immunology is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2014 by The American Association of Immunologists, Inc. All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.
A Clue to Antigen Receptor Tails

Bryan Irving* and Arthur Weiss†,‡

In science, context is essential for interpreting and integrating new observations into current paradigms. Without sufficient context, the significance of an important or insightful discovery may go unrealized until additional information is gleaned that brings its value to light. One such example was an observation made by Michael Reth during the early days of exploration into mechanisms of Ag receptor signaling in a correspondence to Nature in 1989 (1). In his very short note titled “Antigen receptor tail clue,” Reth drew attention to a consensus motif present in the cytoplasmic tails of several components of Ag receptors including invariant subunits of the TCR and BCR complexes, in addition to subunits of the FcεRI receptor and the envelope protein of the bovine leukemia virus. The tyrosine and leucine (isoleucine)-based motif consists of only six consensus residues that occur in remarkably conserved spacing; it includes two acidic residues (Asp, Glu) with defined spacing upstream of two YxxL(I) groups separated by 7 aa (see figure 1 of Ref. 1). Because this motif occurs in components of multisubunit receptors with similar capacities to initiate signaling when cross-linked, Reth speculated presciently that “on cross-linking of receptor, the conserved amino acids from several chains may come together to form a binding site for the putative proteins that generate the signals resulting in proliferation and differentiation of the B and T cells . . . .”

At the time of publication, this letter, despite its title, inspired curiosity but provided little in the way of clues or mechanistic insight into Ag receptor signaling. Today the letter is viewed as prophetic and the “Reth motif” is widely considered the key to understanding the function of the immune tyrosine-based activation motif (ITAM) that serves as the basis for initiating signal transduction upon ligand engagement of several hematopoietic Ag receptors and a means by which viruses exploit Ag receptor signaling pathways to their benefit. The conserved YxxL pairs represent sites for phosphorylation by Src family kinases that, once phosphorylated, become stable docking sites for the tandem Src homology 2 (SH2) and ZAP70 cytoplasmic tyrosine kinases. These kinases cooperate to facilitate further signaling cascades that culminate in cellular activation.

So why did this letter not receive more attention and immediately expedite the field’s discovery of the ITAM? Reth pointed out that the motif is present in the CD3γ and CD3ζ subunits, but absent from CD3ε, the subunit that was then most implicated in signaling, as it was the target of most known mitogenic Abs directed against the TCR, for example, 2C11, OKT3, UCHT1, and Leu4. This unfortunate conclusion likely resulted from the publication of an erroneous CD3ε sequence in 1984 that was later corrected via a published erratum (2). At the time, the prevailing data in TCR signaling supported a role for the tyrosine phosphatase CD45 that had recently been demonstrated (4), it was the following year (1990) that tyrosine kinase inhibitors were shown to block initiation of TCR signaling (5, 6). Moreover, the requirement for CD45 confused the issue, because absence of a tyrosine phosphatase would be predicted to promote tyrosine phosphorylation. Finally, the discovery of the SH2 domain reactivity with PO4-Tyr, the key feature of the ITAM YxxL(I) motif, was still a year away (7).

Work from several laboratories led to an appreciation of the signaling function of the ITAM motif. Unlike the CD3 chains, ζ had virtually no extracellular domain but became phosphorylated on multiple tyrosine residues upon receptor ligation (8, 9). This was reminiscent of the tyrosine kinase growth factor receptors whose cytoplasmic tails similarly contained multiple tyrosine residues that became auto-phosphorylated upon ligand binding. Ligand-induced phosphorylation of these receptors led to their association with signaling molecules to initiate the signaling cascade. Despite the structural complexity of the TCR and its absence of a catalytic domain, we hypothesized it might ultimately initiate signaling in a similar manner through its phosphorylated ζ dimer, with the other chains perhaps serving some sort of regulatory function.

We tested this hypothesis by replacing the transmembrane and short extracellular domain of ζ with that of another dimeric protein, CD8, thereby enabling its separation from the TCR and independent expression of ζ in a chimeric form. We then assessed the signaling capacity of the ζ-chain independent of the TCR and even in a T cell line devoid of endogenous TCR expression. Surprisingly, oligomerization of the CD8/ζ chimera was sufficient to induce both early and late events associated with stimulation of the multichain TCR (10). At the same time, an independent study from the Seed laboratory utilizing a similar chimeric strategy demonstrated the capacity of ζ to induce mobilization of intracellular calcium and mediate cytolytic activity (11). These data demonstrated...
for the first time a signaling capacity of one of the TCR invariant chains, and they pointed to the ζ dimer as the relevant signaling apparatus of the TCR. However, that hypothesis was short-lived and in need of modification, for shortly thereafter experiments from the Malissen laboratory suggested a comparable signaling function for the CD3 module in the context of a TCR essentially devoid of ζ cytoplasmic sequences and the Klausner group showed TCR-independent signaling by CD3ε-containing chimeras (12, 13). Mutation of ζ and CD3 modules led to “re-identification” of the conserved motif originally described in the Reth letter and revealed the functional importance of the properly spaced YxxL pairs (13, 14). The motif is derived from an evolutionarily conserved two-exon structure that is used for signaling in several invariant chains associated with Ag receptors (12).

Although these studies demonstrated sufficiency of the motif for T cell activation, they revealed little of the mechanistic basis for the consensus motif and spacing or the functional relevance of its multiple representations within the receptor. Were the motifs functionally redundant or did some possess unique functions? Triplication of a single motif in a chimeric context did confer enhanced function, supporting a role for amplification in the multiplicity (15). However, this result did not preclude the potential for unique properties within individual motifs that might synergize for optimal signaling in the native TCR context. Also, whereas the functional importance of the conserved tyrosine residues in the motif had been demonstrated, the role of their phosphorylation was still unclear. Both positive and negative roles had been proposed for ζ phosphorylation in regulating TCR function (12).

Studies of the Src family kinase, Lck, and a 70-kDa ζ-associated protein helped reveal the true mechanistic function of the motif. A signaling defective mutant derived from Jurkat T cells (JCAM.1) was revealed to be deficient in Lck, and its signaling could be restored upon reconstitution with Lck (16). These data were consistent with the profound defect reported in T cell development in Lck-deficient mice (17), and they helped clarify the earlier results demonstrating potent inhibition of T cell activation by tyrosine kinase inhibitors (5). They also helped to resolve the apparent paradox of the positive regulatory requirement for the tyrosine phosphatase CD45 in initiation of TCR signaling. CD45 is critical for activating Lck through dephosphorylation of its C-terminal negative regulatory tyrosine (18). Despite the requisite presence of Lck for TCR function, a stable association between it and the TCR could not be shown. Instead, a 70-kDa tyrosine phosphorylated protein was reproducibly induced to associate with ζ shortly after TCR stimulation (19). Purification and cloning of this protein identified ZAP70 as a 70-kDa tyrosine kinase that contains two SH2 domains upstream of its kinase domain (20). Studies identifying a requisite sequential function for Lck and ZAP70 revealed the role of the conserved residues in the ITAM motif and refined our mechanistic understanding of early TCR signaling (21, 22). First, Lck phosphorylates tyrosine residues within the cytoplasmic ITAMs. These Lck-mediated phosphorylations provide the basis for recruitment of ZAP70, whose interaction is stabilized via its two tandem SH2 domains interacting with the doubly phosphorylated YxxL pair that comprises the ITAM motif. The conserved spacing of 7–8 aa between the YxxL pairs is important to provide stable binding by ZAP70. Once recruited to an ITAM, ZAP70 becomes phosphorylated by Lck to become fully activated to initiate the downstream signaling cascade that culminates in T cell activation.

In retrospect, and armed today with a mechanistic understanding of early TCR signaling, one might question why Reth’s observation was not more obvious. The conserved spacing of the consensus residues was striking and their presence in multiple Ag receptor subunits certainly compelling. However, the letter’s significance could not be realized until new experimental work was conducted that provided the necessary context for our current, albeit still incomplete, understanding of ITAM function.

**Disclosures**

The authors have no financial conflicts of interest.

**References**