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Tracking and Trapping Somatic Mutations in Ig Genes

Marko Radic[1]

By the early 1980s, the question of how the immune system generates diverse Ag receptors of (what we now call) the primary repertoire had been solved: Recombination between different gene segments, Vκ and Jκ or Vλ and Jλ for the Ig L chain and V_H, D_H, and J_H for the H chain, achieves an astounding diversity (1). In contrast, the mechanism of somatic, or intraclonal, diversification of Ag receptors remained mired in controversy. We know now that the diversification of receptors during the growth of a B cell clone is essential for affinity maturation, a defining feature of secondary immune responses. In secondary Ag responses, affinity increases over time as B cells with mutations that improve binding to Ag gain a selective advantage over cells with receptors that contain deleterious mutations or no mutations.

In 1970, Martin Weigert and colleagues came close to proving that somatic mutations diversify Ab receptors (2). At that time, Weigert was a postdoc with Mel Cohn at the Salk Institute. Weigert had learned bacterial genetics from Alan Garen and had picked up crucial knowledge about mutations and protein chemistry. Together, Weigert and Garen had unambiguously determined the base composition of the nonsense codons UAG and UAA (3). When Weigert joined Cohn, they agreed that Darwinian evolutionary principles must also apply to B cell Ag receptors and they passionately confronted the germline hypothesis of Ab diversity. Proponents of the germline hypothesis maintained that B cell immunity might be able to get by with 10,000 different Abs, a repertoire that could be achieved by combining 100 different L chains with 100 different H chains. To counter this deterministic view, Weigert et al. (2) decided to test the idea that Ab mutations are integral to B cell Ag responses.

It is hard to overemphasize the elegance and impact of the 1970 paper (2). In this highly cited paper, Weigert and colleagues determined the amino acid sequences of λ light chains from 19 murine myelomas and found that 12 sequences were identical, whereas the others differed from the consensus sequence at one, two, or three positions. Each of the differences was unique, thus leading the investigators to the bold conclusion that there is only one germline Vλ1 gene and that variants must arise by somatic mutations. Having communicated with Elvin Kabat and Tai Te Wu before the publication of their paper, Weigert et al. observed that the mutations affected residues located in CDRs of the L chain (4). This emphatically confirmed Weigert and Cohn's idea that somatic mutations diversify B cells such that selection may favor B cells with improved binding to Ag. This paper led Tonegawa to count the number of λ genes in the germline and, in accord with the conclusion of Weigert et al. (2), to find only a single copy of the Vλ1 gene (5).

The fact that κ L chains also mutate and are selected by Ag was shown by using Id-restricted Abs to identify structurally related L chains, determining the genomic complexity of the L chain group and comparing it to the number of expressed sequences as was done for Vκ21 L chains (6, 7). Subsequent efforts were directed at defining the precursor-product relationship between particular germline genes and their potential mutant versions expressed in B cells. Progress was made in characterizing V genes that participate in immune responses to haptens such as (4-hydroxy-3-nitro-phenyl)acetyl (NP; Ref. 8), phosphorylcholine (PC; Ref. 9), *p*-azophenylarsonate (Ars; Ref. 10), or 2-phenyl oxazolone (Ox; Ref. 11). It was observed that replacement mutations tended to be more abundant in isotype-switched than IgM Abs (12) and that the mutations had a profound effect on Ag binding (13). Nonetheless, important questions about somatic mechanisms of receptor diversification remained, such as: when and how do somatic mutations occur and what is the nature and the role of mutations in the Ag response?

With this as a backdrop, it is easy to appreciate the conceptual advance gained in the studies reprinted in this issue of *The Journal of Immunology* (14). This 1984 landmark paper stands out above its contemporaries in that it tracked and trapped somatic mutations. McKean and colleagues analyzed L chain sequences from clonally related B cells that were captured as hybridomas. They made the astounding observation that B cells accumulated mutations at a rate high enough to generate distinct sequences in different hybridomas. This feat was made possible by establishing criteria for the identification of clonal relatives among the immortalized B cells; that is, the relatives shared productive and nonproductive V gene alleles. Now, the authors could use the somatic mutations that arose in individual members of an expanding B cell clone to trace the evolution of the clone. This study demonstrated that mutations arose with an average rate of 10^{-3} per base pair per generation. This rate was many orders of magnitude greater than the normal spontaneous mutation rate and closely agreed with the intrinsic rate of 3×10^{-4} determined in cell culture (15). McKean et al. also showed that the mutations accumulated sequentially, both following and preceding isotype switch, and that replacements clustered preferentially in a CDR.

How was this breakthrough achieved? Keys to success lay in the choice of the experimental system and in the skill of the

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investigators. Although anti-hapten responses offered the advantage of an easy screen due to the limited numbers of V genes that form a suitable combining site, Lou Staudt and Walter Gerhard decided to focus on a naturally complex Ag, the influenza virus (16). They had selected a panel of 12 epidemic virus isolates and 39 mutants of the H1N1 strain, such that each virus differed from every other virus by a single amino acid substitution in the hemagglutinin (HA) coat Ag. This allowed the authors to screen 125 anti-HA mAbs from 14 individual BALB/c mice and identify groups of Abs in each mouse that shared epitope specificity yet exhibited subtle differences in binding. Staudt and Gerhard correctly predicted that the hybridomas represented variants arising from "within the clonal progeny of a precursor B cell" (16).

The results obtained by McKean et al. were confirmed by determining the mRNA sequences of H and L chains of the clonally related Abs to influenza HA (17). Importantly, Clarke et al. (17) found that ratios between replacement and silent mutations were different for CDR and framework domains, further arguing that competition and selection among clonal relatives shapes the evolution of a B cell clone. A subsequent study on Abs to oxazolone (18) extended these findings to another system. The experiments performed by immunologists during the 1970s and 80s, such as those highlighted here, transformed immunology from a clinical subspecialty to a vibrant branch of biology and contributed to the broad advance of our discipline.

Many current examples of research have their foundations in these elegant studies. Notably, Shlomchik et al. analyzed clonally related Abs to establish the paradigm that autoreactive B cells are selected for binding to auto-Ags and that mutations contribute to the pathogenesis of autoimmune disease (19, 20). Further examination of auto-Abs revealed a more extensive receptor diversification mechanism relying on additional rounds of V gene recombination to edit specificity and restore tolerance (21). Interestingly, the story that began with McKean et al. is not finished yet, as the molecular mechanism that introduces mutations into the H and L chain coding segments remains to be discovered (22).

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