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Response to “Mutation frequency vs. mutation patterns: a comparison of the results in spleen and Peyer’s patches”

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The role of DNA polymerase (pol) θ in somatic hypermutation of immunoglobulin genes has been fraught with controversy from the year that two reports were published--each claiming a different role for the polymerase. Casali and co-workers [1] reported that pol θ had a dominant role in hypermutation, since mice deficient for the polymerase had a 60–80% decrease in the frequency of mutations. Wang and colleagues [2] reported that pol θ contributed to mutations of C:G base pairs, and had a 20% drop in the total mutation frequency. Later publications from the Wang group [3,4] confirmed the 20% decrease in frequency, which now affected mutations of A:T pairs, as well as C:G. What was pol θ doing?

Pol θ may extend mismatches created by other polymerases during hypermutation, in accord with biochemical evidence showing its potential in primer extension from mismatches [5]. DNA pol η has been shown by many groups to generate mutations opposite A:T pairs during somatic hypermutation. Thus, one obvious scenario would be for pol θ to extend the mismatches generated by pol η . Mice deficient for both polymerases should have a much lower frequency of mutation. Experiments by Masuda et al. [4] and our group [6] found that pol η -deficient mice and pol η /pol θ -double deficient mice had the same frequency of mutation. Therefore, one could hypothesize that either pol η and pol θ work together, or pol η can function by itself and pol θ is unnecessary.

If a polymerase is involved in generating mutations in vivo, one would hope to see a more dramatic effect in the absence of the polymerase. This has not been the case with pol θ . B cells from Peyer’s patches, which are located on the small intestine, have similar frequencies of mutation in mice with and without pol θ [6,7]. These results indicate that pol θ does not play a crucial role in hypermutation. However, pol θ might still contribute to the process in a limited extent, as B cells from immunized spleens have a modest decrease in its absence [3].

Perhaps researchers in the field would be better served by coming to an agreement on the best way to measure mutation frequencies to assess what is relevant. A potential solution would be for everyone to analyze their data the same way. This would involve making the following choices: (1) cells would be from either spleen or Peyer’s patches; (2) if spleen is chosen, one common antigen should be used for immunization and the spleen should be harvested after the primary or secondary boost; (3) a defined number of sequences or mutations should be collected for the data to be significant; (4) mice on the same genetic background—C57BL/6--should be compared; (5) wild type mice should come from the same animal facility as the test mice and

preferably be littermates; (6) mice should be the same age and sex, etc. Because antibodies are under intense selection in vivo, it is hard to compare frequencies unless defined criteria are set.

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