Bioinformatic analysis of the antibody repertoire induced in young and old donors in response to yellow fever vaccination

A.S. Obraztsova, M. Shugay, D.M. Chudakov

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences
117997, Russian Federation, Moscow, GSP-7, Ulitsa Miklukho-Maklaya, 16/10, alcedooo@gmail.com

Humoral immunity in humans deteriorates with age leading to an increased susceptibility to infections and an impaired response to vaccination [1]. Recent advances in high-throughput sequencing techniques has allowed us to comprehensively study this phenomenon. Using the immune repertoire sequencing technology and our optimized protocol [2] we were able to obtain more than a million of full-length immunoglobulin (Ig) sequences from unvaccinated donors of various ages and compare them to more than 10,000 Ig sequences of memory B-cells induced in young and old donors in response to yellow fever (YF) vaccination.

One of the mechanisms that is critical for forming an efficient immune response is the affinity maturation of B-cells, a process during which somatic hypermutations (SHMs) are introduced into the Ig sequence followed by subsequent rounds of antigen-driven selection in germinal centers of lymph nodes. The present work solves two main technical hurdles that complicate the analysis of SHMs. First, the background noise of PCR and sequencing errors that can interfere with SHM analysis is eliminated using the molecular tagging technique and an extension of our previously published algorithm [3] to full-length Ig sequences. The second difficulty lies in the identification of SHMs that fall into the complementarity-determining region 3 (CDR3) of the Ig sequence that doesn't have any germline reference sequence. To solve the latter problem, we have developed a novel algorithm that can accurately identify and cluster CDR3 sequences that are far more similar than can be expected from random sampling of CDR3 repertoire. This approach allows us to accurately reconstruct the clonal trees of full-length Ig sequences present in the sample using the parsimony principle.

Our results reveal a pattern of V-D-J rearrangements in YF-specific Ig sequences which is substantially different from the one observed in the peripheral blood of unvaccinated donors and donors who have undergone a flu vaccination, which is in line with the fact that the YF virus is generally novel for european population. We also were able to identify significant differences in the substitution patterns of SHMs and isotype usage between old and young donors. Moreover, using the number of SHMs as a molecular clock, we demonstrate that

there are age-related differences in class-switch recombination dynamics. Interestingly, the presence of a relatively high number of hypermutations in the root sequences of clonal trees suggests that at least some of the YF-specific Ig sequences observed in old donors were already subject to some affinity maturation process earlier in the donor's live. Taken together our results suggest that aging has a profound effect

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