Towards the isolation of esterase and amidase catalytic antibodies: Examination of germinal center diversity by cloning and sequence analysis of genes encoding monoclonal antibodies elicited by a transition state analogue

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Abstract

In this study, a transition state analogue (TSA) approach was employed for the isolation of catalytic antibodies with esterase or amidase activity. The TSA used in this study, phosphonamidate, mimics the transition state of a corresponding amide or ester hydrolysis reaction. We applied hybridoma technology to immortalize the germinal center B cells that were induced by the TSA, and antibodies and their genes were isolated from more than a hundred hybridoma cell lines. ^ Degenerate primers were designed for “universal” amplification of mouse Ig genes. By using these degenerate primers, we have achieved a 100% amplification rate for more than a hundred Vκ and VH mouse Ig genes we have sampled. The germinal center (GC) diversity in response to the TSA was addressed by sequence analysis of 63 Vκ and 54 VH Ig genes isolate from GC derived and TSA positive hybridoma cell lines. Germline genes for all the Vκ and for some of the VH cDNAs were identified by aligning them to Vκ and VH germline databases. It was found that multiple germline families of Ig genes were involved in the immune response to the TSA. Somatic hypermutations were introduced into those Ig genes isolated from the hybridoma cell lines which had undergone isotype switching. Canonical conformations of the CDR for the sequenced antibodies were predicted by the available software. Preliminary data on the kinetic study on several TSA positive antibodies had showed that moderate esterase activities with kcat/Km up to 1,000M have been detected. ^ Our results showed that the immune response to a specific TSA immunization was highly diverse, demonstrated by the multiple Ig germline gene involvement, unrestricted Vκ and VH combination to form an antibody, and extensive somatic hypermutations on the class switched antibodies. Thus, GC B cells provide an diverse repertoire of antibody molecules and the limitation of isolating catalytic antibodies by the TSA approach is how to efficiently screen this huge immune repertoire. ^

Subject Area

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