STRONG CHILDREN'S RESEARCH CENTER

Summer 2014 Research Scholar

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ABSTRACT

Title: Isolating hybridoma isotype variants using fluorescence-activated cell sorting

Background: *Pneumocystis jirovecii* is an opportunistic fungal pathogen that affects immunocompromised patients. The Gigliotti lab has generated B cell hybridoma monoclonal antibodies that are cross-reactive in both mouse and human species of *Pneumocystis*, enhancing the usefulness of mouse models and offering potential avenues of vaccine development. The current set of available monoclonal antibodies is limited to relatively few isotypes. Isotype switching is a process by which antibodies change their heavy-chain constant domain; it occurs spontaneously at a frequency of 10^{-5} to 10^{-6}, making isolation of switch variants labor-intensive using well-established ELISA spot assays.

Objective: Determine if flow cytometry can be used to identify rare switch variants, and isolate switch variants using fluorescence-activated cell sorting.

Results: By mixing 4F11 (producing IgM antibody) and 4F11G_1 (producing IgG_1 antibody) at varying ratios and analyzing with a BD LSRII Flow Cytometer, we demonstrate that an APC-conjugated anti-Mouse IgG_1 antibody differentiates between the IgM and IgG1 antibody isotypes of B cell hybridomas. To test the ability of our fluorochrome to detect rare positive events,4F11G1 was added to a culture of 4F11 at ratios ranging from $1:10^2$ to $1:10^6$. Detection of IgG1 cells was attainable in a range similar to that expected during spontaneous isotype switch events. Finally, a BD FACSAriaII was used to screen $3x10^7$ IgM hybridomas for spontaneous IgG_1 switch variants, resulting in 720 cells being sorted out of the total culture for further analysis with ELISA.

Conclusion: Flow cytometry offers a rapid approach to isolating isotype switch variants and is significantly less resource and time-intensive than the standard methods of using cycles of ELISAs and limiting dilutions. In the literature, the estimated spontaneous isotype switch rate is approximately 10^{-5} to 10^{-6}, while we sorted suspected switch variants from the culture at approximately 1 per $3.6x10^5$ cells using flow cytometry. Further work will be performed to determine the discriminatory power of this method of using fluorescent-activated cell sorting versus ELISA, as well as including a broader panel of fluorescent dyes to isolate other isotype variants. This method of isolating isotype switch variants can be improved by using mutagenic agents that are known to increase the switch frequency.