Factors influencing complement-dependent cytotoxicity by rituximab in 2D and 3D-cultured B-cell lymphoma

Sandra Kleinau, Sandra Lara, Juliane Heilig, Alexander Virtanen.
Dept. of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden.

The CD20-targeting monoclonal antibody rituximab (RTX) has a significant impact on the treatment of B-cell malignancies. However, a substantial proportion of $CD20^+$ B-cell lymphomas are unresponsive to RTX. This has highlighted the need to understand how RTX work and how different lymphoma subtypes hold resistance.
We systematically explored factors influencing complement-mediated tumor cell killing using different RTX isotypes; IgG1, IgG3, IgA1 and IgA2 on four human B-cell lymphoma cell lines, with differing levels of CD20 and complement regulatory protein CD59. Complement-dependent cytotoxicity (CDC) was assessed on 2 and 3-dimensional (D) cultured lymphoma cells by trypan blue, Annexin V and propidium iodide staining. Anti-CD59 antibody was used to investigate CD59 in RTX-mediated CDC.
Three out of 4 lymphomas were susceptible to RTX-mediated CDC when cultured in 2D, while only 2 out of 4 when grown in 3D. RTX-IgG3 induced the greatest CDC, followed by RTX-IgG1 and RTX-IgA2, while RTX-IgA1 displayed no complement activation. Superior CDC activity was seen in lymphoma cells with a high CD20/CD59 expression ratio. These lymphomas were also sensitive to RTX when grown in 3D, although the CDC was substantially reduced compared to 2D cultures. Complement-activated spheroids showed apoptosis and necrosis essentially in the outer cell-layers. Neutralization of CD59 overcame resistance to RTX-mediated CDC in 2D, but not in 3D-cultured lymphoma. Notably, CD59 expression increased in B cell lymphoma cells following 3D culturing.
In summary, CDC outcome in B-cell lymphoma is influenced by choice of RTX isotype, CD20 antigen density, tumor structure and CD59 expression.