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Anti-CD20 mediated apoptosis in B-cell lymphoma impairs “don’t-eat-me” CD47 signaling and boosts Fc receptor-mediated phagocytosis

Oanh Nguyen[1], Sandra Lara[1], Giovanni Ferro[1], Matthias Peipp[2], Sandra Kleinau[1]
[1]Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden, [2]Division of Antibody-Based Immunotherapy, University Hospital Schleswig-Holstein, Kiel, Germany

The IgG1 anti-CD20 monoclonal antibody rituximab (RTX) is a well-established treatment for B-cell non-Hodgkin lymphomas (NHLs). However, not all B-cell NHLs respond to RTX treatment. Thus, there is an urgent need for new strategies to enhance RTX-mediated anti-tumor immune responses.

The efficacy of RTX depends crucially on the phagocytosis of antibody-opsonized tumor cells by monocytes/macrophages. The phagocytic process is regulated by the “don’t-eat-me” CD47 molecule, which dampens the phagocytic activity. In this study, we demonstrate that by impairing CD47 signaling, we can significantly enhance RTX-induced phagocytosis of human $CD20^+$ B-cell lymphoma cells by human monocytes.

Our results reveal that an IgG2 isotype of RTX (RTX-IgG2) in combination with RTX-IgG1 or RTX-IgG3, can significantly induce enhanced phagocytosis of $CD20^+$ B-cell lymphoma cells in comparison with single use of RTX-IgG1 or RTX-IgG3. This effect was associated with CD20-dependent apoptosis by RTX-IgG2. Likewise, the apoptosis inducer staurosporine (STR) could enhance phagocytosis of lymphoma cells when combined with RTX-IgG1 or RTX-IgG3. Notably, we observed that apoptosis mediated by RTX-IgG2, as well as by STR, downregulated CD47 expression on the lymphoma cells. Indeed, microscopic analysis revealed that the expression of CD47 was decreased and scattered in patches on the cell membrane of RTX-IgG2 or STR-treated lymphoma cells. In contrast, RTX-IgG1 and RTX-IgG3 did not induce apoptosis and alteration of CD47 expression on the lymphoma cells. Aligned with this finding, we show that CD47-blocking antibodies, either with a functional or silenced Fc domain, on lymphoma B-cells enhance the phagocytic activity induced by RTX-IgG1 or RTX-IgG3 in monocytes.

Based on this principle, we further demonstrate that RTX-IgG2 can enhance the efficacy of other tumor-targeting antibodies, such as IgG1 anti-PD-L1, in stimulating phagocytosis of $CD20^+$ B-cell lymphoma cells.

In summary, our study demonstrates that RTX-IgG2 enhances Fc receptor-mediated phagocytosis of $CD20^+$ B-cell lymphoma cells through CD20-dependent apoptosis and downregulation of CD47. This finding emphasizes the potential of RTX-IgG2 as a valuable agonist in B-cell NHL treatment.

Combining RTX-IgG2 with RTX-IgG1, or potentially any other tumor targeting antibody, offers an exclusive target-specific approach to boost the efficacy of $CD20^+$ B-cell lymphoma therapies.