University of Utah
UNDERGRADUATE RESEARCH JOURNAL

Multivalent Human Serum Albumin – Anti-CD20 Fab' Conjugates Induce Apoptosis in Lymphoma Cell Analogs

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Non-Hodgkin lymphoma (NHL) is an immune disease mostly of B-cell origin (eighty-five percent of the time) as well as the ninth leading cause of cancer death in the United States. Although treatments for NHLs greatly improved following the FDA approval of Rituximab (RTX), refractive malignancies still occur that are nonresponsive and/or resistance to current therapies in at least a third of all patients. This has been attributed both to the inability of immune effector cells (eg., macrophages, natural killer cells) to hypercrosslink ligated monoclonal antibodies (mAbs), and to Fc receptor (FcR)-mediated endocytosis or "trogocytosis" of CD20 antigens. In order to address these clinical obstacles, we designed a novel paradigm in macromolecular therapeutics that can specifically kill cancer cells without a drug. This paradigm is based on the use of anti-CD20 Fab' fragments in a multivalent system. Crosslinking of CD20 receptors leads to receptor clustering, transfer to lipid rafts, opening of a calcium channel, and ultimately apoptosis. Additionally, the removal of the Fc fragment resulted enticingly in both the rendering of the system to be immune dependent and in decreasing the numerous adverse effects.

In this study, we have used human serum albumin (HSA) as the multivalent carrier of RTX based Fab' fragments. We have covalently attached multiple Fab' fragments to HSA, characterized the nanoconjugate's physiochemical properties, and evaluated its efficacy to induce apoptosis of Raji B cells in vitro. The efficacy of the nanoconjugate to induce apoptosis was determined with Annexin V assay and flow cytometry. The interaction of the nanoconstruct with Raji cells was characterized using confocal microscopy of Cy5 labeled conjugates.

As predicted, the HSA-$(Fab')_x$ conjugate was able to induce cell death in vitro. The results of the Annexin V apoptosis assay showed that 38.9 percent of the cell population treated with the conjugate became apoptotic, while 13.6 and 15.7 percent of the cell populations untreated and treated with whole RTX mAb became apoptotic respectively **(see Figure 1)**. Furthermore, images recorded by use of confocal microscopy suggest that the attachment of HSA-$(Fab')_x$ conjugate to the cell membrane is CD20 specific **(see Figure 2)**. While not conclusive, the combination of these results suggest that the mechanism of action involves cross-linking of the CD20 receptor, which subsequently induces apoptosis. We believe these results warrant further investigation of the mechanism of action of HSA-(Fab')x, as well as the treatment potential of this nanoconjugate.

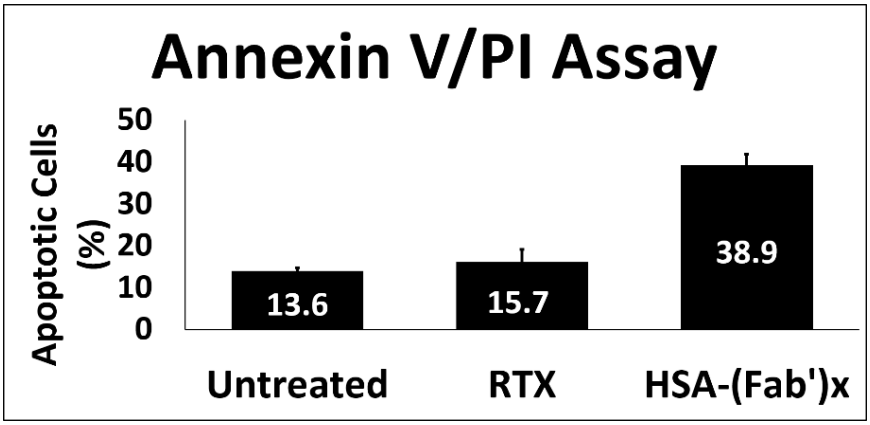

Figure 1: Cell death was measured by flow cytometry evaluation of Annexin V binding (n=3). Data shown as mean ± SD. Statistical comparison was performed by comparing experimental group (1 μM Fab' equivalent of nanoconjugate HSA-(Fab')x) with control groups (cells treated with 1 μM Fab' equivalent of RTX and cells untreated) (*: $p < 0.05$).

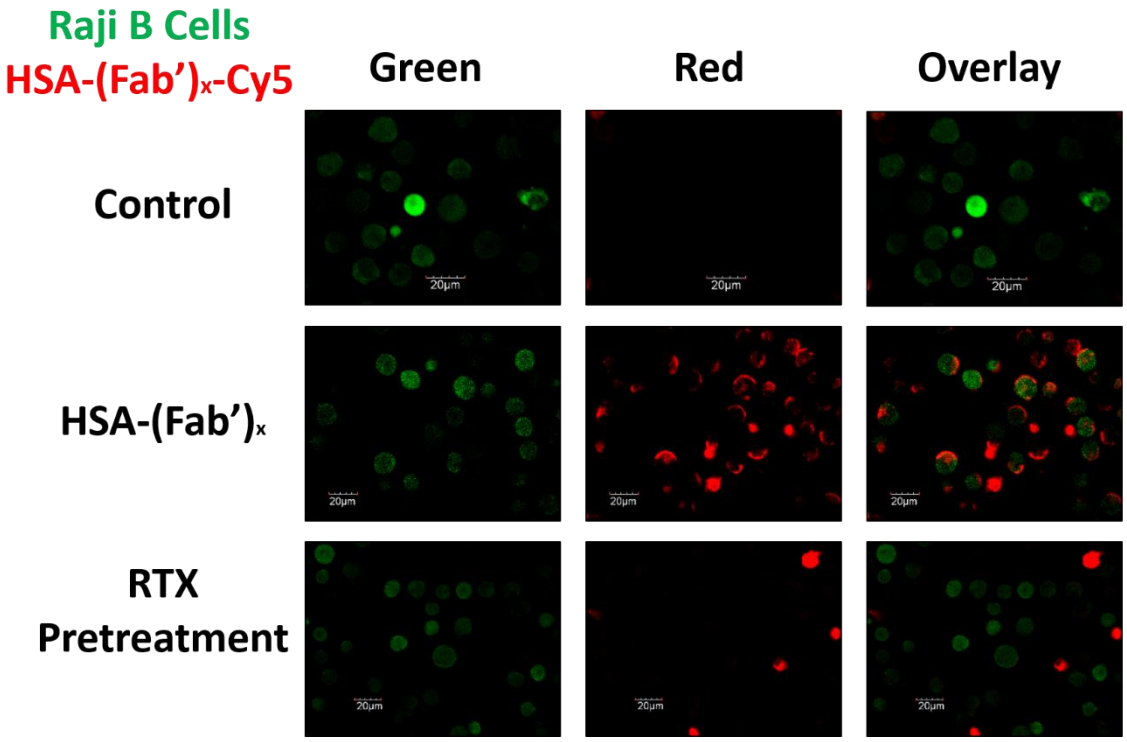

Figure 2: Confocal fluorescence microscopic images of green-fluorescent CD20 positive Raji B cells exposed to HSA-(Fab')x labeled with Cy5 red fluorescent label. Two separate experimental conditions consisted of cells that were pretreated with RTX and those that weren't. Cells treated with these two conditions, as well as cells that were untreated, were then imaged and compared as shown above.