

## **Impact of B cell depletion with rituximab on antibody responses**

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Rituximab, chimeric anti-human CD20, is approved for treatment of B-cell lymphoma and most recently, rheumatoid arthritis, in adults. It is being used experimentally in other various immune related diseases such as immune thrombocytopenic purpura, systemic lupus erythematosus, myasthenia gravis, and rheumatoid arthritis. In transplant recipients, it is used for treatment of post transplant lymphoproliferative disease, to anecdotally to reduce pre-formed anti-HLA and anti-ABO antibodies and for the prevention and treatment of acute rejection.

Rituximab is directed at CD20+ B cells such that there is rapid clearance of circulating B cells (CD19<sup>+</sup>). Dosing with rituximab does not impact circulating leukocytes or CD3<sup>+</sup> T cells. Naive (CD19<sup>+</sup>CD5<sup>+</sup>) B cells recover more rapidly than memory B cells (CD19<sup>+</sup>CD27<sup>+</sup>), which remained below baseline for up to two years after dosing. Since there is little to no CD20 expressed on plasma cells, it is not expected that there would be a decrease in plasma cells, the source of circulating antibodies.

While it has been shown that rituximab almost completely eliminates circulating B cells, there is surprising little information on its effect on in vivo human antibody responses. For example rituximab has been suggested to decrease antibody responses of lymphoma patients to recall antigens and to decrease primary and secondary antibody responses to keyhole limpet hemocyanin in baboons. One established method used to assess in vivo antibody responses in humans is immunization with the T-cell dependent, neoantigen bacteriophage phiX174. In normal humans, following primary immunization, the resulting immune response consists of predominately IgM neutralizing antibody with the titer peaking at 2 weeks. After a secondary immunization, given six weeks later, the resulting antibody titer peaks at 1 week, is substantially higher, and consists of approximately equal amounts of IgM and IgG.

We studied the in vivo antibody response of chronic renal failure (CRF) patients to the neoantigen bacteriophage phiX174 given with or after ablation with rituximab. Rituximab significantly decreased peak  $K_v$  responses when compared to both historic non-CRF controls and to CRF subjects. CRF itself decreased peak  $K_v$  responses compared to non CRF controls. Percent-ratio of anti-phage IgM to IgG was significantly decreased in RIT treated subjects. One of 3 subjects treated with rituximab was found to have developed a partial B cell tolerance to phiX174 administration 2 years later. We have now returned to the bench by using transgenic mice that express human CD20 on their B cells. With selected immunization of these mice with T dependent and T independent antigens, with and without rituximab depletion, we have been able to identify that the effect of CD20 depletion depends on the type of immunogen. Finally, clinical trials are underway, designed to specifically test the impact of rituximab on in vivo antibody immune responses