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LYMPHOID NEOPLASIA

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Unique approach for B lymphoma therapy

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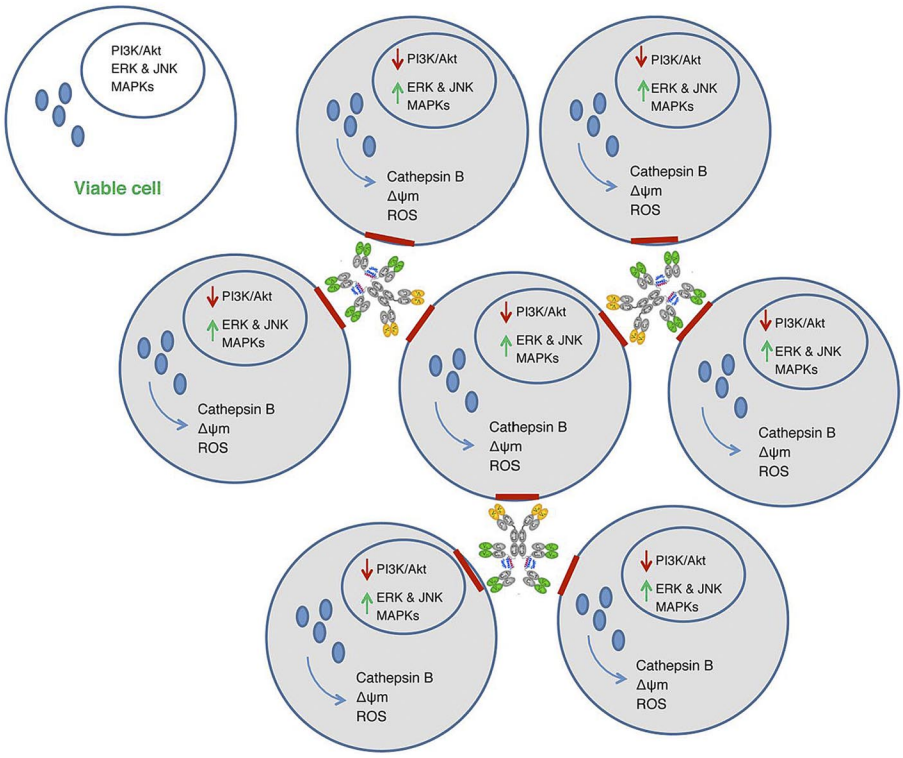
Genetic engineering has substantially improved antibodies used in the treatment of cancer and related diseases, initially by providing more compatible reagents (chimeric, humanized, human) for patients but more recently by improving their clinical efficacy.[1] In this issue of *Blood*, Gupta et al have used a novel genetic engineering method, termed "dock and lock,"[2-4] to construct a HexAb bispecific antibody directed against 2 B-cell epitopes (CD20, CD74). By so doing, they have tapped into a distinctive B-cell homeostatic mechanism that controls B-cell growth and proliferation.[5]



Biologic effect of cross-linking multivalent anti-CD20/CD74 bispecific antibodies in B-cell lymphomas. Aggregation of lymphoma cells by bispecific antibodies causes reorganization of actin and migration of antigen (red) into lipid rafts that in turn induces the release of lysosomal cathepsin B and reactive oxygen species (ROS) and influx of calcium ions into mitochondria. Swollen lysosomes and mitochrondria are accompanied by the deactivation of the PI3K/Akt signaling pathway and the rapid and sustained activation of ERK, JNK, and MAPKs associated with cell death. Nonaggregated cells remain viable in the absence of antibody binding.

Earlier it was observed that the agglutination of malignant B cells by certain antibodies induced the movement of bound antigens into cell surface lipid rafts located sporadically on B cells to give a speckled membrane pattern of antibody binding by immunofluorescence microscopy.[6] For example, it is now known that anti-CD20 antibodies fall into 2 groups: type I and type II.[7,8] Type I antibodies represented by Rituxan and Veltuzumab used in this study produce a ring pattern on lymphoma cells after binding and by themselves do not produce significant apoptosis. By comparison, type II anti-CD20 antibodies represented by tositumomab produce a speckled membrane pattern and are very effective in inducing cell death by a caspase-independent mechanism. Type I antibodies can be induced to produce this type of apoptosis after cross-linking by antibodies that bind Rituxan or using multivalent antibody polymers to simulate this cross-linking phenomenon.[9]

In the paper by Gupta et al, the authors have found another method of inducing B lymphoma cell death using a bispecific antibody that cross-links 2 different B-cell antigens, CD20 and CD74.[2] By testing different compositions of HexAbs, they determined that 2 combinations are clinically active, necessitating additional studies to determine which is most efficacious in patients. As shown in the figure, the cross- linking of CD74, which is directed against the invariant chain of the HLA-Dr molecule,[10] and CD20, which is highly expressed on B-cell neoplasms but whose function is still not fully understood, produced cell clustering that is associated with actin reorganization, antigen migration into lipid rafts, swelling of mitochondria and lysosomes to cause influx of calcium and release of capthepsin B, respectively, down-regulation of Bcl-xL and pAkt, and the rapid and sustained phosphorylation of ERK and JNK second signals culminating in cell death.

Because Veltuzumab is a type I anti-CD20 and by itself is not apoptotic, it would be of interest to determine whether substituting a

type II anti-CD20 in the HexAb might be more effective. CD74 is a good choice for the HexAb because it binds a critical molecule for B-cell survival and proliferation.[10] Nonetheless, the emergence of this novel genetically engineered bispecific antibody should be a welcome new product for the treatment of mantle zone lymphoma, chronic lymphocytic leukemia, and other difficult-to-treat B-cell tumors expressing both CD20 and CD74 antigens. The ingenuity and creativity displayed in this study demonstrates further the ever-increasing role of genetic engineering in the development of promising new antibody reagents for cancer immunotherapy.

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Avoiding intuxication

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Why primary autoimmune hemolytic anemia (AHA) is more refractory to standard "frontline" treatment than immune thrombocytopenia (ITP) remains unclear. The response of AHA to steroids, splenectomy, and especially intravenous immunoglobulin is inferior to that seen in patients with ITP, making reliance on immunosuppression more important.

This observation is especially intriguing in patients with "warm" immunoglobulin G (IgG)–mediated AHA that is not primarily complement-mediated. Almost all patients with "warm" IgG-mediated AHA also show a brisk reticulocytosis, unlike ITP in which ineffective platelet production contributes to thrombocytopenia. Perhaps the size and rigidity of the antibody, complement in the case of "cold," IgM-coated red blood cells, and the location and developmental expression of antigens on red cell precursors and on mature red cell surface permits more efficient clearance by tissue macrophages than comparably opsonized platelets. Clinical disease might develop at lower antibody density more rapidly after autoantibodies form, when they might be more susceptible to immunosuppression.[1] Lower rates of spontaneous or treatment-derived responses may also relate to the site of the underlying immune defect as AHA or Evans syndrome is more common in patients with constitutive defects, such as the autoimmune lymphoproliferative syndrome.[2]

This conundrum brings us to the observations of Barcellini et al in their article in this issue of *Blood*.[3] They report that each of 14 patients with primary warm AHA and 5 of 9 patients with primary cold AHA responded to 4 weekly intravenous injections of low-dose rituximab (100 mg/wk × 4), which they substituted for conventional dosing (375 mg/m^2/wk × 4). The choice of a lower dose was based on the reasoning that less drug would be needed to ablate a smaller number of autoreactive B cells in AHA than is required to induce remission in B-cell lymphoma. Similar findings of efficacy in ITP have been reported in single-arm studies, but only 20% of patients appear to maintain a complete response more than 3 years after low or conventional doses of rituximab.[4] The results of Barcellini et al compare very favorably to other series of adults with warm AHA treated with rituximab using conventional dosing.[4] However, more time will need to elapse to determine whether patients with AHA treated with "low-dose" rituximab relapse at the same rate as those with ITP. Likewise, it will be important to see whether the reduced dosage leads to better preservation of pretreatment B- and T-cell repertoires and preserves response to vaccination[5] and lowers risk of infection. In addition, paraproteins (if present) may serve as useful surrogate markers of efficacy and mechanism of effect.

Why does ITP or AHA recur despite prolonged and virtually complete B-cell depletion in the peripheral blood? Potential reasons include down-regulation, internalization or proteolytic shedding of CD20, failure to ablate "CD20 low"–expressing early B-cell progenitors, plasma cells and long-lived memory cells, and cell sanctuaries in the bone marrow, spleen, and lymphoid organs where the effect on B cell–T cell cooperation and induction of T-regulatory suppressor cells may be incomplete.[6] The mechanism of action of rituximab is complex, incompletely understood, and may even involve induction of an idiotype-specific T-cell response that sustains remission.[7] It is possible that low-dose rituximab invokes an immunomodulatory response that does not require complete B-cell depletion.

The article by Barcellini et al also reminds us that we know frightfully little as to how red cell or platelet autoantibodies arise, how rituximab works, or how it should be administered and monitored. The premise underlying immunosuppression for AHA and ITP is that the responsible autoreactive B and T cells are more susceptible to eradication or are simply less abundant in the immune repertoire than those responsible