

RITUXIMAB – HOW IT WORKS AND WHY RESISTANCE OCCURS

by Sue Herms

We have all heard of rituximab and many of us have been treated with it. But how does it work? Why does it work well for some of us but poorly or not at all for others? And what “new and improved” versions of rituximab are on the horizon?

Rituximab was developed by Idec Pharmaceuticals and initially approved by the Food and Drug Administration in 1997 for B-cell lymphomas that did not respond to other chemotherapy treatments. But now it has become standard therapy, alone or in combination with other drugs, for initial treatment of B-cell lymphomas as well as for certain auto-immune diseases such as rheumatoid arthritis, multiple sclerosis, and systemic lupus.

Rituximab is sold under the trade names of Rituxan in the U.S. and MabThera overseas and is currently marketed by Biogen Idec and Genentech in the U.S. and by Roche in the European Union. It is an engineered mouse/human monoclonal antibody directed against the CD20 antigen located on the surface of most B-cells. The CD20 antigen does not circulate freely in the blood nor is it normally shed from the surface of the B-cell. It should be noted that, because rituximab acts against all B-cells with the CD20 antigen, it targets normal B-cells as well as lymphoma B-cells.

The CD20 antigen appears early in maturing pre-B cells and remains as they mature but is lost or minimally present when B-cells develop into plasma cells. For this reason, it is a good “marker” for B-cell development and for B-cell lymphomas such as WM. Although the function of CD20 is still relatively unknown, it is currently thought to play a role in the movement of calcium across the B-cell membrane, maintaining the concentration of calcium in the cell and allowing the activation of B-cells during the body’s normal immune response.

How Does It Work?

How exactly does rituximab cause B-cell destruction? Those of us who have been treated with Rituxan surely wonder what is occurring as we watch the clear fluid dripping into our veins, hour after hour. This simple question is not easily answered. There are several mechanisms proposed to describe rituximab’s effect on B-cells, but they are still not completely understood. These processes have been studied in tumor cell lines and in animal models, but there are obvious difficulties with determining how they work in the human body. Probably all of them play some role in the destruction of B-cells, but it has not yet been determined which is the dominant mechanism, if indeed there is one.

Some studies suggest that rituximab interferes with the internal function of B-cells. Such studies indicate that rituximab can induce apoptosis (cell death) directly by interfering with CD20 calcium regulation and elevating the amount of calcium in the B-cells to abnormal levels. It may also interfere with various cell factors, causing outright cell death or inhibiting cell growth. Among these cell factors

is Bcl-2, a member of a small family of closely related genes that can be divided into death-inhibiting genes, such as Bcl-2 and Bcl-xL, and death-promoting genes, such as Bax and Bad. The balance between death-promoting and death-inhibiting gene expression is critically important in both B- and T-cells, because these populations are regulated so that a person will, in the absence of infection, maintain a constant level of B- and T-cells despite the production and death of many of these cells each day. Typically, Bcl-2 and Bcl-xL are over-expressed in lymphomas, and rituximab appears to be able to interfere with their signaling pathways and reduce their expression. The over-expression of Bcl-xL may also play a role in making B-cell lymphomas more resistant to other types of chemotherapy; therefore adding rituximab to the therapeutic regimen appears to increase the effectiveness of the therapy.

There are also three main ways in which rituximab acts in concert with the body's own immune system cells once the rituximab antibody has entered the blood stream and attached to a B-cell. The mouse portion on one end of the rituximab antibody is the part that targets the CD20 antigen on the B-cell, while the other end of the antibody is human IgG, kappa type. When the mouse portion "locks" onto the CD20 "docking site" on the B-cell, the human IgG portion on the other end attracts or "recruits" the body's own immune system cells, also called effector cells, to respond. These effector cells include macrophages, neutrophils, and natural killer cells, and they attach to the rituximab antibody at a specific location called the Fc receptor site. This Fc receptor site is important and will be referred to later in the discussion on rituximab resistance. In this context, one can think of rituximab as a "bridge" that brings together the B-cell and the effector cell so that the B-cell can be destroyed.

One way or mechanism by which the B-cell is destroyed is through direct ingestion of the B-cell by the effector cell in a process called antibody-dependent phagocytosis. The effector cells in this scenario are usually macrophages, which are activated monocytes. Several studies have shown that this scenario occurs in laboratory cell lines, but other studies have suggested that this plays a relatively minor role in B-cell destruction in the body.

A second and more important mechanism appears to be antibody-dependent cellular cytotoxicity (ADCC). In this case, the intact B-cell is not ingested or phagocytosed. Instead, certain effector cells (usually natural killer cells and possibly also macrophages) are triggered to release pore-forming proteins that penetrate the B-cell membrane and proteolytic enzymes that break up its structure and degrade its chromosomes. This process ultimately causes cell death through lysis (destruction of a cell by damage to its outer membrane).

A third important mechanism for B-cell destruction is referred to as complement-dependent cytotoxicity (CDC). Complement is a system of small proteins circulating in the blood that, when stimulated, form a series of enzymes which can directly attack the cell membrane or target the cell for destruction by phagocytosis. Its activity is said to "complement" the activity of antibodies, hence its name. Rituximab is capable of binding to complement proteins to initiate this response, in this case against the B-cells that it attaches to. It has been demonstrated that, when rituximab is infused into a patient, complement in the bloodstream is temporarily consumed or used up because of this process.

Another theory suggests a fourth way that Rituxan cooperates with the immune system. The suggestion is that B-cells coated with rituximab are capable of stimulating dendritic cells, which are special cells

that are able to recognize antigens, present them to T-cells, and activate T-cells to attack the antigens, in this case the coated B-cells. This may be one reason why rituximab appears to work for several months after actual treatment, even though blood and tissue levels of the drug may no longer be in the therapeutic range.

It bears repeating these words from the first paragraph in this section: the proposed mechanisms are still not completely understood and probably all of them play some role in the destruction of B-cells, although it is not yet determined which is the dominant mechanism.

Why Is There Resistance?

Unfortunately, depending on the type of lymphoma, 30%-50% of patients show no response to rituximab, either initially or upon re-treatment. The problem of resistance to rituximab has been a continuing source of interest and speculation and is the target of much research. Mechanisms for tumor resistance are complex and can be host-related or tumor-related.

Host-related factors can include such things as the efficiency of the complement system and the various effector cells in eradicating B-cells. One fairly recent development in this regard is the discovery that some individuals respond better to rituximab because of the genetic makeup of some of their effector cells (macrophages and natural killer cells). In particular, this involves the Fc receptor site of the effector cell that attaches to the IgG portion of the rituximab molecule mentioned above. Think of this as a locking mechanism—the better the effector cells fits and binds to rituximab, the better it will work. Sequences of amino acids make up the genes that code for this Fc site, and the sequences can vary somewhat from one individual to another. It has been demonstrated that one part of the Fc site, called FcR3a, can have either the amino acid valine or the amino acid phenylalanine in position 158 of its gene sequence. Since a person receives one gene from his father and one from his mother, a person who has two valine amino acids at this position has a better response to rituximab (40% of WM patients in one study) than a person who has two phenylalanines (9% of WM patients), and a person with one valine and one phenylalanine has an intermediate response between the two. The valine seems to confer a better binding site or “locking” mechanism for the effector cell. In this connection, a test has been developed that can determine the makeup of a patient’s FcR3a and thus possibly predict his response to rituximab therapy.

Tumor-related factors that can influence response to rituximab include the location or the environment in which the tumor cells are located. Typically, bulky lymph nodes are difficult to treat with rituximab because it cannot penetrate easily. Generally speaking, a higher tumor burden (and/or a higher IgM level in WM patients) may be more difficult to treat with rituximab than a lower tumor burden. It has been suggested that B-cell lymphomas with greater CD20 expression, such as diffuse large cell lymphoma, respond somewhat better than lymphomas with lower CD20 expression, such as small lymphocytic lymphoma. Follicular lymphoma and WM appear to fall somewhere in the middle. Other cells and cell factors can influence the growth of lymphomas and thus may complicate the treatment issue. In WM, for example, mast cells have been implicated as important for stimulating the growth of WM cells. Some WM patients have demonstrated tumor cells that exhibit resistance to the effects of complement, a phenomenon probably true for other lymphomas as well.

Just as bacteria can mutate and become resistant to antibiotics with prolonged treatment, tumor cells may become resistant to rituximab. Some B-cell lymphomas may diminish in their CD20 expression upon repeated exposure to rituximab, or the actual structure of the CD20 molecule may be altered. If tumor cells do not already show resistance to complement, they may develop it. They may also compensate by developing increased expression of Bcl-2 or Bcl-xL or may develop other protective factors that diminish the ability of the body's immune system to kill them.

Fortunately, researchers are exploring ways to improve the action of rituximab and other similar anti-CD20 antibodies to get around these various resistance mechanisms. These improvements will be the subject of an article to appear in the next issue of the *Torch*.

This discussion is by no means an exhaustive or all-inclusive one. Anyone interested in additional or more technical information regarding these topics is encouraged to contact the author at suenchas@bellsouth.net. The author wishes to acknowledge with thanks an earlier article by Guy Sherwood in the Summer 2006 *Torch* entitled "Monoclonal Antibody Therapy for Waldenstrom's Macroglobulinemia - A Brief Literature Review."

IWMF Board Member Sue Herms is chair of the Publications Committee, member of the Research Committee, and Associate Editor of the Torch. Her column Medical News Roundup appears in every issue of the IWMF newsletter. Sue Herms holds degrees in Zoology and in Medical Technology from Ohio University and from the Medical University of South Carolina, respectively, and is affiliated with Roper Hospital in Charleston, South Carolina.

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