

## How to Get Rid of Inhibitors

Jean-Marie Saint-Remy

Centre for Molecular and Vascular Biology, University of Leuven, Belgium

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“Getting rid of inhibitors is no longer a dream,” said Dr. Jean-Marie Saint-Remy. “For the past 18 years or so, we have deciphered all the mechanisms involved in the production of factor VIII (FVIII) antibodies – in this case, inhibitors.” The key to understanding approaches to eliminating inhibitors, he said, is to understand those mechanisms and to examine the possibilities of intervening at various stages of inhibitor development and activity.

When an antigen is introduced to an organism:

1. It must first be processed by an antigen-presenting cell (APC)
2. The APC presents it to a T-cell
3. When activated, the T-cell will interact with a B-cell to produce antibodies.

There are distinct portions of this process where intervention could eliminate or prevent the development of inhibitors: influencing FVIII itself, influencing the T-cell, or influencing B-cells.

Dr. Saint-Remy explored the possibility of rendering FVIII invisible to the immune system, perhaps through PEGylation (attaching a molecule called polyethylene glycol). This process works extremely successfully with small molecules but unfortunately, FVIII is quite large. Moreover, Dr. Saint-Remy expressed concern about the degree to which PEGylation might interfere with the functioning of FVIII and the effect of PEGylated FVIII on the cells that had taken it up.

Point mutation of T-cells could also reduce their interaction with FVIII, he said, effectively causing them to ignore it when it is introduced into the bloodstream. The expertise acquired in other fields, mostly with small molecules, suggests that a large number of mutations must be introduced to have an impact on T-cell recognition of a molecule. For a large molecule such as FVIII, hundreds of point mutations would probably be required, which would not likely be feasible.

Inhibitors are produced when B-cells differentiate into plasmocytes. At the B-cell level, it is also possible, in principle, to alter the binding site of an antibody through point mutation. This alteration, Dr. Saint-Remy said, would prevent the B-cells from recognizing FVIII. However, a very large number of B-cells are produced by the human body on an ongoing basis, and they are relatively short-lived. The development of inhibitors is a random event. This variability makes it impractical to target generic B-cells in the hope of producing tolerance to FVIII.

One promising approach is to use genetically modified antigen-presenting cells, Dr. Saint-Remy said. Using a retrovirus to transfect B-cells with a specific peptide that is present in non-activated B-cells, it is possible to keep the presentation of those peptides under control. In mouse models, this modification has proved to be highly stable, persisting for months. Indeed, the gene transfer leads the B-cell to produce interleukin-10 (IL-10), which is able to suppress the antigen-presenting capacity of APCs.

In addition, these genetically modified B-cells are ideally located in the same niches as T-cells, and are relatively easy to characterize and purify. However, it is still unclear how many of the modified B-cells would need to be introduced to induce tolerance or how often they would need to be injected, he said; this makes it a less than ideal potential intervention.

Intervening at the T-cell level also appears theoretically promising, Dr. Saint-Remy said. Using current technology, it is possible to produce anergy (absence of an immune reaction) in these cells, but this state is reversible. It is also possible to induce apoptosis (programmed cell death), he said. However, this approach is impractical because of the large number of T-cells and the difficulty of guaranteeing appropriate target specificity.

A much better approach, he said, is to focus on the extrinsic characteristics of regulatory T-cells (Tregs). If Tregs with antigen-specific suppression capacity could be introduced, it might be possible to eliminate inhibitors. However, despite significant progress, therapeutic applications of this approach will not be practical until more research adequately characterizes the appropriate Tregs and better methods of purification are developed.

A more practical and promising way to interfere with B-cell response than those already discussed, Dr. Saint-Remy said, is to target memory B-cells. Once B-cells acquire memorization, inhibitor production is much more likely to be sustained than transient. Memory B-cells are important targets for three reasons:

- When memory B-cells activate into plasmocytes, they locate in the bone marrow and in the spleen and are fairly inaccessible to therapy.
- Plasmocytes survive for much longer than normal B-cells (for weeks or even months), producing inhibitors the whole time.
- Because of the presence of Toll-like receptors (TLRs) on the surface of memory B-cells, they can continue to trigger inhibitor production long after the cognate antigen (in this case, FVIII) is no longer present. (This third characteristic may explain why some hemophilia patients continue to produce high inhibitor titers even when they have not been exposed to FVIII for years.)

If a patient needs FVIII, Dr. Saint-Remy said, they will most likely need it on a regular basis. Therefore, effective approaches for inducing tolerance must go beyond targeting “naive” B-cells and have an impact on memory cells. By using specific B-cell deletion – either by exposing a memory B-cell to FVIII or by introducing an antibody epitope – it is possible to induce Fas-dependant cell death.

The conventional therapeutic approach for inducing tolerance involves using large doses of FVIII for prolonged periods, Dr. Saint-Remy explained. This approach induces apoptosis of memory B-cells. However, it also has the potential to recruit newly formed B-cells as they emerge from the bone marrow, which is counterproductive.

However, use of an anti-idiotypic antibody to interfere with B-cell receptor signalling could possibly block the signalling pathway and effectively switch off targeted memory B-cells, Dr. Saint-Remy said. The B-cell receptors of the target cells possess specific idiotypes that are easily recognizable by anti-idiotypic antibodies. It has been possible to use human monoclonal

antibodies from inhibitor patients to isolate and produce several of the anti-idiotypic antibodies. Preliminary research suggests that the anti-FVIII immune response is less diverse than anticipated, therefore permitting fairly rapid progress *in vitro* toward developing a possible therapy for inhibitor elimination.

Dr. Saint-Remy also said that a significant proportion of the FVIII inhibitors derived from actual patients are able to bind and inhibit FVIII while in germline configuration. Research suggests this ability is a characteristic of the anti-C2 antibodies, which would explain why anti-C2 inhibitors are so readily eliminated by FVIII infusion, while other antibodies are more resistant.

Another promising development is the creation of a transgenic mouse model that expresses human B-cell receptors, he said. This model is allowing *in vivo* and *in vitro* observations of the fate of specific B-cells, the circumstances under which antibodies are produced, and the mechanisms by which tolerance is induced.

In summation, Dr. Saint-Remy noted that the use of gene therapy for identifying patients with inhibitor susceptibility and preventing it holds great promise. While he agreed that it will likely soon be possible to predict gene polymorphisms that increase the probability of inhibitor development, he discounted the likelihood of useful clinical therapies resulting from that direction. Instead, he stressed the use of complementary approaches focused on the elimination of memory B-cells, which he described as “a target we can hit,” and the induction of adaptive Tregs, such as IL-10-producing Tr1 cells.