HASTE MAKES WASTE

WHAT WENT WRONG WITH CENTOXIN

ANAHEIM, Calif.—Every young immunologist knows the thrill of a positive result when testing antisera raised against a "difficult" antigen. Too often that initial exuberance—making the months or years of work look like it paid off—turns to crushing disappointment when the experiment is repeated with the proper controls and a more highly purified cut of the antibody. "Sticky" immunoglobulin Ms (IgMs) are often the culprit. They bind the antigen. But they'll also bind the plastic ELISA plate—given the chance.

Centocor (Malvern, PA) may have fallen into just such a trap with HA-1A, the company's anti-lipopolysaccharide (anti-LPS) IgM monoclonal antibody—marketed in Europe as Centoxin. While the embarrassment of recanting the original result may seem mortifying to a young academic, the potential retraction of a product is disastrous for a biotech company.

What went wrong? "The company went too fast because of the competition," says Jean-Daniel Baumgartner of Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland), who extensively tested the French-licensed version of HA-1A from the original hybridoma. "It didn't take the time to study the antibody itself."

HA-1A alarm bells

Baumgartner began sounding the alarm about HA-1A over two years ago when his results indicated that the antibody had an undefinable epitope and no experimentally repeatable demonstration of protection in animals. Baumgartner says his presentations at conferences, published papers, and direct contacts with representatives of Centocor gained little attention.[1]

Baumgartner thinks much of the confusion over HA-1A started in Henry Kaplan's lab at Stanford University (Palo Alto, CA)—where the hybridoma was developed and licensed. Kaplan's group used crude preparations of hybridoma supernatant to demonstrate that IgMs bind to endotoxin *in vitro*. The group published striking data suggesting that the monoclonal protected both rabbits and mice against the effects of gram-negative bacteria, the source of endotoxin.[2]

Buoyed by these results, Centocor licensed the hybridoma and pushed immediately for a clinical trial. It purified the monoclonal from the supernatant.

The other licensee, Merieux Institute (Lyon, France), opted for a more conservative approach. It sought to first understand how the IgM binds. When Merieux couldn't reproduce any of Kaplan's data with its own purified version of the antibody, it turned to Baumgartner's group. Baumgartner's qualifications extended beyond his background in antibodies against endotoxins. He had spent a year in the lab of Elizabeth Ziegler of the University of California at San Diego. Ziegler directed Centocor's HA-1A clinical trials.

Misleading results

Baumgartner found surprising behavior *in vitro*. The antibody not only stuck to lipid A, but to anything hydrophobic—cardiolipins, lipoprotein, cell membranes, and gram-positive bacteria, which contain no endotoxin. He concluded that Kaplan's original paper may have been misleading because the proper controls hadn't been run. He also suggested that the crude supernatant extract may have had properties different from the purified antibody.

"Centocor went too fast because of the competition."

Baumgartner published his results—the first to contradict the anti-endotoxin-antibody concept—during Ziegler's first clinical trial of HA-1A. According to Baumgartner, Centocor's representatives were "very open at the beginning and said they would try to do suggested experiments." But Centocor refused to give him enough HA-1A to conduct his own animal tests—and to compare the antibody to the Merieux IgM antibody—for "legal reasons," he says. Finally, with Centoxin's release on the European market, Baumgartner bought a vial and confirmed that HA-1A had the same properties as the Merieux antibody.

Since the Xoma (Berkeley, CA) E5 IgM antibody—also an anti-endotoxin monoclonal—hasn't been made available to outsiders, and the experiments in animals have never been published in detail, it's difficult to tell whether Xoma has repeated Centocor's mistakes. But Baumgartner says Xoma's second clinical study, requested by the U.S. Food and Drug Administration (FDA, Bethesda, MD), asks a precise question, "Does E5 improve survival in gram-negative patients without shock?" The answer, says Baumgartner, is no.

There are other considerations to weigh in evaluating IgMs' sepsis-treating future. IgM hybridoma cell lines are notoriously more difficult to maintain than cell lines of comparative immunoglobulin Gs, and they produce smaller yields. These factors cause IgM costs to skyrocket.

IgM cost effectiveness is questionable, moreover. A recent cost-effectiveness and efficacy analysis of HA-1A and E5 by Richard Wenzel of the University of Iowa College of Medicine (Iowa City) states that a "second look at the data suggest that they would be of marginal benefit in practice."[3] The reply of the University of California's Ziegler, written before the most recent FDA ruling on HA-1A, defends the antibody on the basis of the earlier FDA decision.[4]

Second-generation products

If IgM products aren't the answer to sepsis, what is? Indeed, the biotech pipeline is filled with non-IgM therapies.

Start-up Incyte (Palo Alto, CA), for instance, recently presented data at the FASEB meeting in Anaheim, CA, comparing its bactericidal/permeability-increasing protein (BPI) to HA-1A. BPI is a naturally occurring protein found in neutrophils that apparently binds and neutralizes anti-LPS, facilitating its clearance. Incyte's animal data suggest that BPI is more effective than HA-1A in protecting CD-1 mice against endotoxin challenge at clinically relevant dosages. Genentech (S. San Francisco, CA) recently bought into the idea, signing a $14-million agreement with Incyte to commercialize BPI. Xoma also has a BPI program to back up E5. Curiously, Centocor rejected an option to purchase BPI rights in its recent buy out of Invitron. In a remarkable turn of events, the rejection opened the door for Invitron scientists, already convinced of BPI's potential, to acquire BPI rights and form Incyte.

As the second wave of anti-sepsis drugs prepares to enter clinical trials, the lessons of anti-sepsis monoclonals shouldn't be forgotten. Perhaps it's better to scrutinize early results and risk mortifying embarrassment than to have biotech companies risk stockholders losing faith in the promise of sepsis treatments. **—Stephen M. Edgington**

References

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