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Antibody production branches out

Alan Dove

Antibodies, the molecular workhorses of protein research, have traditionally been one of the most difficult reagents to procure. Using innovative new technologies, though, a burgeoning antibody production industry is turning these molecules into commodities.

Kids today do not know how good they have it. Not long ago, generating a research-grade antibody to a protein was a major ordeal. From expressing and purifying the antigen to immunizing a suitable animal, generating hybridoma cells and purifying the final product, the process could occupy months of a graduate student's or postdoc's time.

Not anymore. "There are so many shops [that] will synthesize the peptides for you, immunize a mouse or a rabbit or a rat, whatever you want, and they'll send you the serum," says Tillman Gerngross, cofounder and CEO of Adimab. He adds that "getting polyclonal antibodies [to] something, or even monoclonals—that's a commoditized business at this point."

Commodity antibodies cover most research needs quite well, but for some targets and applications, they simply will not do. Because they come from standard laboratory mammals, for example, commercial antibodies can only target a limited set of epitopes; the injected animal recognizes conserved mammalian antigens as 'self', blunting or blocking its immune response to them. Scientists who have an eye toward clinical applications may also need better control over the antibody's structure and production than a commercial source can provide.

Fortunately, several groups have been developing novel antibody production platforms for these special cases, ranging from simple do-it-yourself expression systems to sophisticated bioreactors capable of making clinical-grade material.

An artist's rendering shows a yeast cell presenting antibodies on its surface, which bind antigen for detection and cell sorting by flow cytometry. Image courtesy of Adimab.

Brewing B cells

At Gerngross's company, the focus is on a very specific—and very valuable—question in clinical research. "Here's an antigen, how quickly can I get to a panel of fully humanized genes [to] that antigen? It is that metric that we sort of tried to make progress against," says Gerngross.

To do that, Adimab took their work completely outside the animal kingdom, to the yeast *Saccharomyces cerevisiae*. Gerngross and his colleagues generated a complete synthetic library of the human preimmune repertoire and engineered the yeast to make human immunoglobulin gamma (IgG) molecules representing that repertoire. Each yeast cell produces a specific IgG and presents it on its surface, like a B cell. The result is a fungal version of the human spleen, containing antibodies to every possible antigen.

With the system built, the team can now present antigens to it. "Once you have [IgG] on the surface, then you can use [flow cytometry] or magnetic bead sepa-

ration to find those yeasts that make an antibody that is presented on the surface that is [to] your antigen," says Gerngross. Unlike a spleen, however, the yeast cells have not been preselected to eliminate self-reactive antibodies. As a result, the system contains a wide range of antibodies to the conserved mammalian epitopes that traditional animal-based schemes miss.

The strategy also gets around a limitation of yeast as an antibody production platform. "Different sequences will in some

cases express very well in yeast and in other cases not express at all," Gerngross explains. Previous efforts that selected antibodies in mammals, then cloned the locus encoding IgG into yeast for expression, yielded mixed results. Because Adimab has built the entire preimmune repertoire into yeast, the company automatically preselects antibodies that will express well in that system.

With Gerngross's approach, generating a complete panel of antibodies to a given antigen takes about 3–4 weeks, after which the yeast can be transferred to a different medium that causes the cells to secrete their IgG molecules instead of presenting them on the surface. "You can purify [the antibody] out of a 24-well plate and that will typically yield somewhere between 50 and a few hundred micrograms," says Gerngross. Those quantities are sufficient for small-scale experiments, and though the yeast culture could be scaled up for larger batches, users can also move the selected antibody genes into other

A Coomassie-stained gel shows the purity of antibodies produced by a yeast expression system. Image courtesy of Adimab.

systems. The Adimab yeast strain itself is proprietary, but Gerngross says the antibody genes the company provides could be moved into another strain of yeast for production, or into mammalian cells.

Hunt and peck

Whereas Adimab has focused on duplicating the functions of B cells in simple fungi, other companies prefer to rely on a ready-made immune system for antibody selection. At Crystal Bioscience, for example, researchers are using chickens to generate antibodies.

According to Robert Etches, the company's president and CEO, the birds provide many of the same advantages as a yeast system: "because of the solid genetic distance between chickens and mice and humans and so on, the chicken can see antigens that other animals don't, because there is too great a similarity between a mouse protein and a human protein the mouse doesn't recognize it as nonself, whereas a chicken will."

Etches admits that it is not an entirely new idea. Indeed, chickens were one of the first model organisms used for immunological research, and investigators have long used them to generate antibodies to targets that are highly conserved in mammals. "The problem has been that there hasn't been a good way of making a chicken monoclonal antibody. That is the part of the technology that we've developed here at Crystal Bioscience. Our in-house technology is aimed at the production of monoclonal antibodies from immunized chickens," says Etches.

To do that, the team collects the spleen cells from immunized birds, then puts the cells inside small agarose capsules, isolating each B cell in its own sphere. Each sphere also contains antigen-coated beads. The beads trap antigen-reactive antibodies inside the spheres containing the B cells that produced them. Fluorescently tagged IgY, the chicken equivalent of

mammalian IgG, then highlights all of the spheres whose B cells produce antibodies to that antigen.

Once they have sorted out the right cells, Etches and his colleagues isolate the genes encoding those cells' antibody variable regions. "You can then reconstruct that about any way you'd like: you can put it onto a human constant region; you can put it onto a mouse constant region; you can put it back onto a chicken constant region; you can basically make any kind of antibody that you want using those chicken [variable] sequences," he says.

Although working with whole animals is somewhat slower than using libraries of yeast cells, Etches argues that his system has some compensatory advantages: "if you use an animal such as a chicken, you gain access to all of the affinity maturation processes that are in the humeral immune system to give you a really good antibody,... whereas in nonvertebrate systems, you don't have that."

Both the Crystal Bioscience and the Adimab platforms can be used to produce research-scale quantities of antibodies, but neither company is particularly interested in larger-scale production. Instead, both companies provide the antibody genes, which customers can then express in their system of choice. "We're not in the production business, we're in the information business. What we ultimately give you is sequence [encoding] an antibody that when you express it in [Chinese hamster ovary (CHO) cells] or wherever you wish to manufacture the antibody, [the sequence] encodes that binding event that elicits a desired therapeutic effect. How you make it is totally your problem," says Gerngross.

Turning over a new leaf

Researchers confronting the antibody production problem often turn to cultured mammalian cells, but they may soon find themselves shopping for expression sys-

tems in the greenhouse instead. "Plants have all the machinery necessary for not only the production of the proteins but also for the assembly of them correctly into the classic IgG [and] also the more complex [antibody classes] like secretory IgA [molecules], which also have the joining chain and the secretory component," says George Lomonosoff, professor of biological chemistry at the John Innes Centre in Norwich, UK.

Because plants express the proteins so well, researchers have long sought to use plants as an antibody expression platform. Trials in the late 1980s established that transgenic plants can do the job at least as well as mammalian cells in culture. "The problem with the transgenic approach is that it is quite slow, and you have to decide what you want to make, and then it's a whole process of transformation, regeneration, crossing and eventually getting true breeding lines [that]

Infiltrating a leaf with a plasmid containing a gene encoding a red fluorescent protein causes the leaf to express the protein at levels high enough to be seen visually in extract from the leaf and on a Coomassie-stained gel. Image courtesy of G. Lomonosoff.

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have high-enough expression levels,” he says.

Instead of making an entire transgenic plant, Lomonosoff and his colleagues introduce the antibody genes into the leaves of an unmodified plant. “You can actually insert the genes encoding the heavy and the light chain...on the same plasmid, and simply put the plasmids into *Agrobacterium* sp. and flood the cells, the leaf tissue, with those constructs,” Lomonosoff explains, adding that “you can actually get very high levels of expression within a few days in leaf tissue.”

Because the process is so quick, scientists can make multiple versions of an antibody and express them in different leaves, then select the ones with the high-

est affinity. For small-scale laboratory use, a few inoculated leaves can produce milligram quantities of protein, so even researchers without large plant facilities should find the technique straightforward.

The system can also be used to produce other types of complex proteins, including viral subunits that assemble into complete virion cores. One company, Medicago, is using that approach to produce an experimental H5N1 influenza vaccine, and Lomonosoff says he has received many reagent requests from academic researchers as well: “I think I sent four lots of plasmids off just today. We send a little sort of expression kit;... the idea is to get it as widely used as possible.”

Relatively small bioreactors, such as these in the protein production core facility at EPFL, can produce large quantities of antibodies from traditional mammalian cell cultures. Image courtesy of F. Wurm.

Investigators who hope to commercialize an antibody may also find plants appealing, as plant expression is relatively easy to scale up, but Lomonosoff cautions against visions of field-grown pharmaceutical antibodies: “You can imagine if you grew stuff in a field, there’d be all sorts of things like bird droppings and earthworms and insects coming into your product potentially, and that concerns regulatory agencies.”

Tried and true

Even with careful containment, new systems such as plants may never be practical choices for researchers who hope to see their antibodies used clinically. “The regulatory agencies are not very innovative and provocative,” says Florian Wurm, professor of biotechnology and head of the laboratory of cellular biotechnology at the Ecole Polytechnique Federale de Lausanne (EPFL) in Lausanne, Switzerland. Wurm adds that “if you want today to get your product into the clinic in the fastest way, you don’t experiment around with a system [that] has not been used before because you lose two years of time, minimum.”

Pharmaceutical production problems may not interest academic scientists in the early phases of a project, but Wurm, who runs the core protein production facility at EPFL, advocates getting useful antibodies into industrial-grade systems as early as possible. “More frequently than I sometimes expect, I find people doing weird things, coming with very strange cell lines to us and saying ‘can I make out of this a manufacturing process?’... Well, yes you can, in principle,... but I would advise you rapidly to switch to CHO” cells.

CHO cells have long been unfashionable in basic research laboratories, where

the decades-old system is regarded as a creaky industrial workhorse that desperately needs to be replaced. Wurm begs to differ: “there is truly no communication between the two worlds. There’s so much innovation...in this technology, the old one, but it’s not sexy enough to publish in a leading journal.”

Through incremental advances, industrial researchers have progressively increased the density and longevity of a typical CHO cell culture. In the 1980s, getting a suspension culture with 2 million cells per milliliter to survive for a week was a triumph. Today, antibody production facilities routinely culture 15 million cells per milliliter for three weeks in chemically defined medium.

Wurm explains that a modern bioreactor filled with CHO cells and serum-free medium can yield 2 grams per liter of antibody, with very few contaminants. He adds that “in spite of the fact that [*Escherichia coli*] grows faster, in spite of yeast being a wonderful organism, you cannot get this purity and you cannot get the yield out of these microbial systems as we have achieved now out of mammalian cells.”

Although each system has its adherents and detractors, most agree that it is a good time to be working on antibodies. “Antibody products and similar products for therapy are growing every year between 10 and 15 percent in spite of the economic downturn, which no other industry in the world does, so I think it’s a very exciting period to be in and trying to contribute to this field,” says Wurm.

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SUPPLIERS GUIDE: COMPANIES OFFERING ANTIBODIES AND ANTIBODY SERVICES

Company	Web address
AbD Serotec	http://www.ab-direct.com/
Abgent	http://www.abgent.com/
Active Motif	http://www.activemotif.com/
Adimab	http://www.adimab.com/
AnaSpec	http://www.anaspec.com/
Assay Designs	http://www.assaydesigns.com/
BioGenes	http://www.biogenes.de/
Charles River	http://www.criver.com/
Chemicon (Millipore)	http://www.chemicon.com/
DIAsource Immuno Assays S.A.	http://www.diasource-diagnostics.com/
Epitomics	http://www.epitomics.com/
Fitzgerald	http://www.fitzgerald-fii.com/
GeneTel	http://www.genetel-lab.com/
GENOVAC	http://www.genovac.com/
GenWay Biotech	http://www.genwaybio.com/
GTC Biotherapeutics	http://www.gtc-bio.com/
Harlan Sera-Lab	http://www.harlanseralab.co.uk/
Innovative Research	http://www.innov-research.com/
Lonza Biologics	http://www.lonzabiologics.com/
Mabtech	http://www.mabtech.com/
Maine Biotechnology Services	http://www.mainebiotechnology.com/
MorphoSys	http://www.morphosys.com/
New England Peptide	http://www.newenglandpeptide.com/
Novus Biologicals	http://www.novusbio.com/
OriGene	http://www.origene.com/
ProSci Inc	http://www.prosci-inc.com/
Research & Diagnostic Antibodies	http://www.rdabs.com/
SDI	http://antibodies.sdix.com/
SouthernBiotech	http://www.southernbiotech.com/
Zymed (Invitrogen)	http://www.zymed.com/

Corrigendum: Antibody production branches out

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In the version of this article initially published, the images used in the first two illustrations were incorrectly attributed to T. Gerngross instead of Adimab. The error has been corrected in the HTML and PDF versions of the article.