The Antibody Society's 2022 Science Writing Competition

Post-doctoral fellow winner

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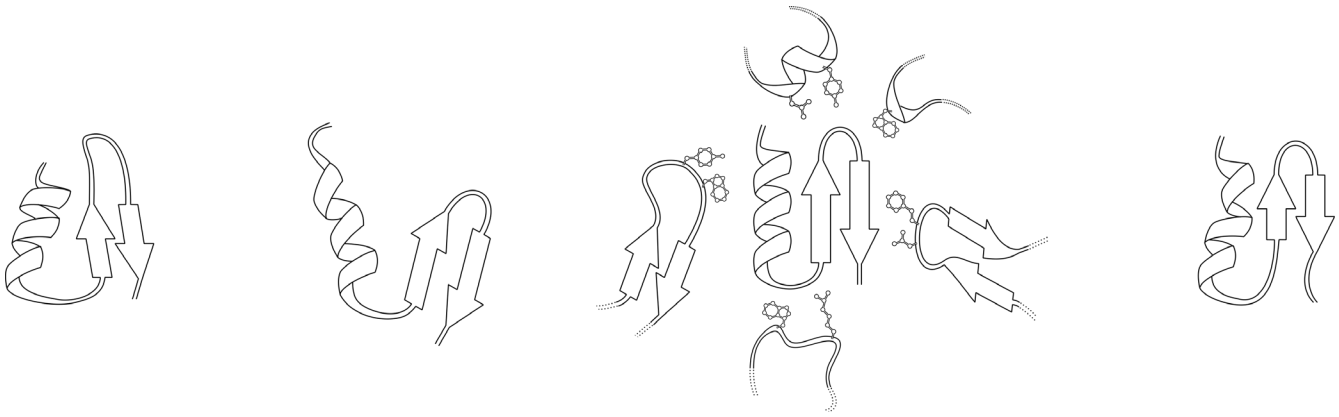
Antibody Discovery's Diversity Problem



There is perhaps no greater test of a biological theory, than to reconstruct from its principles, the complexity they simplify. The aspirations of antibody discovery, which are to obtain "antibodies" that specifically recognize an "antigen", are ostensibly practical. They are well aligned to the ambitions of adaptive immunity, which seeks to design molecules that can identify pathogens. So, to discover antibodies, it is often the case that we must realize in tubes and glassware, what evolution has wrought in adaptive immunity. Which is antibody discovery's unseen beauty. That its technical mystique may be understood as a few simple problems, whose answers elaborate immunology's doctrine.

Immunize with an antigen, and chances are, given a little time, you will find antibodies in the blood. Indeed, such a procedure established antibody discovery before antibodies were discovered.[1] But immunize one hundred animals with one antigen and you will likely find, in the form of antibodies, a thousand different answers. Though they might serve the immune system equally well, they betray the investigator, who must be concerned with reproducibility. It is the founding problem of modern antibody discovery: that although we can immunize and sometimes obtain antibodies to our liking, they are proteins, and for reasons of evolution, difficult to copy.

Strange, for a product of evolution, whose habit is messy efficiency, that the immune system rather neatly organizes its antibody repertoire. That antibodies of the blood are made by plasma cells. Which belong to the B cell lineage of the bone marrow. That each B cell is the custodian of a single antibody conceived in DNA, and built into protein. And together they curate a library numbering billions. Whose pages are torn out and written anew in perpetuity, by naive B cells guessing at unknown foes.

But most antibodies never meet their complement. So, most B cells die. Which is the constraint that designs antibody discovery, whose task will be to fish a few specific antibodies from billions. And trace the DNA that bore them, to a mortal B cell.

Hybridoma

The problem is that of genotype and phenotype. Assigning to a phenotype, the antibody, which can be assayed but not easily copied; a genotype, the antibody's DNA, which can be copied but not easily assayed. The B cell, by safeguarding an antibody's DNA, from which it also constructs and secretes the antibody, would solve the problem eloquently were its life not so short. But it is for reasons of safety that it must be so. Since in generating their antibody, B cells are permitted the rare privilege of editing their own DNA. In the absence of signals otherwise, a B cell's impulse must be toward self-destruction. Lest by some unfortunate happening, its editing extend to more selfish ends.

It is with some irony, then, that in antibody discovery, the B cell's savior is a myeloma cell, which shares the same lineage, but whose aberrant genome has granted it eternal life. With the right solution, the right polymers, a little electricity, mortal B cell and neighboring immortal myeloma cell will fuse.[2] The resulting hybridoma will take from the B cell a unique antibody, and from the myeloma cell time. Time enough that the pool of hybridomas, each with their own antibody, can be diluted, one cell per culture well. Then divide and multiply, secreting an antibody now into culture medium instead of blood. Which can be assayed and traced back to a well, and a hybridoma lineage, and antibody-encoding DNA. Copied and edited, preserved so that it can then be produced, in one form or another, by some unfamiliar cell.

Of course, the fusion of myeloid cell and B cell is itself an occult practice, whose efficiency, in real terms, will struggle to reach a few percent. So, most B cells, their constitutions weak by design, perish in the electrofusion chamber. That hybridoma works well at all, reflects an immune repertoire skewed momentarily by the insult of immunization.

Phage Display

If attachment of genotype and phenotype is all you need, then you needn't bother with life. A virus will do.[3] Specifically, a phage, whose brief genome orders a protein coat. That upon replicating and being assembled in a bacterial host, will encapsulate its genome again. And set off to propagate. Ad infinitum. It is hard to die, when you are already dead. So, the phage proves a hardy steward for a genome.

Insert instructions for an antibody into the coat protein's DNA, and the phage, not knowing better, will replicate as a particle decorated by an antibody, whose contents contain DNA instructions for the same. For the purposes of assay, antibody fused to phage will, in all

likelihood, attach to its antigen just as well as the original antibody. So take a pool of phage, each displaying its own antibody, and show it your antigen. Wash away those that cannot recognize it, and no matter how few remain, if they can infect bacteria, then they can be amplified. And if they can be amplified, you will have discovered an antibody.

To construct such a pool of antibody-displaying phage, however, a library of antibody DNA must be cloned. It then must be introduced into bacteria. Analogous to fusion in hybridoma, transformation of bacteria is inherently inefficient. It is compounded with the need to clone antibody DNA *en masse*, which typically necessitates mixing up the separate heavy and light chain pairs that describe a single antibody. It all serves to limit library size, typically a few orders of magnitude short of an entire immune snapshot. So, phage display too, benefits from the propensity of certain antibodies to dominate responses to immunization. But it also reflects on the antibody's phenotype, that recognition is not an equal contribution of chains, so that unfamiliar heavy and light chains may function just as well as their cognate pairings.

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From these (approximate) solutions, we derive the context of modern antibody discovery. Which narrates a similar tale to hybridoma in single cell approaches. That attempt to preserve B cells just long enough to generate antibodies to assay. And then freezes instead of fusing them. So that upon reanimation, the DNA of some interesting antibody can be amplified, and preserved from a single B cell.[4] Modern antibody discovery also tells the epilogue to phage display in mRNA display, which sheds the constraint of a phage particle, and tethers antibody directly to its genetic material. In doing so, capturing orders of magnitude more antibodies.[5]

True obsolescence is rare in science. With time, it is more common that old techniques find new niches. Look today and you will find phage rather successfully displaying camelid antibodies, which lack light chains. And hybridoma, assisted by robotics, playing a numbers game that squeezes ever more details from the immune response. When time is short, you will even find, in serum therapy, antibodies harvested from immunization with little concern for their genotype. But in the era of therapeutic antibodies, the task of antibody discovery has evolved from simply finding antibodies that recognize, to antibodies that modulate. And they are considerably rarer.

For much of antibody discovery's history we have been content to preserve a small fraction of the 10^{12} or so antibodies in a typical repertoire. But it is a snapshot. Record a life time and it will grow by an order or magnitude. And with a population of individuals perhaps several orders more. Our trick used to be that immunization would sort out the important ones for us to preserve. But now it is possible to sequence single cells, and dream, *in silico*, entirely new repertoires, which can be synthesized and captured *in vitro*.[6] To a good approximation, antibody diversity is modern antibody discovery's maxim. In the absence of better theoretical prediction,

it is believed that if you increase the unique antibody sequences in your library, you will increase your chances of finding something rare. But add one more template, and by pairing with every other one, your library will grow exponentially. Soon you will have more antibodies than the universe can make.

So, antibody discovery now considers the problem of genotype and phenotype again. And attempts to connect the two computationally. Though to only modest success. So, it also looks to immunology, which having sketched the B cell's life, now struggles with the fine details of their selection in the germinal center. And faces the same problem. Because there are only so many mice to immunize, and so many antigens to immunize with. We can manipulate the components of adaptive immunity with more freedom than ever, but to judge their effect on antibody generation, our readout is almost always genetic sequences. And it is phenotype which immunity optimizes. So, now it is antibody discovery, recapitulating principles in tubes and glassware, which reunites with immunology, to write a new doctrine.

References

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