Antibodies: friend or foe?

Romke Bron and Nigel W. Bunnett

Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia

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IT IS HARD TO OVERESTIMATE the importance of antibodies in biomedical research. They are the cornerstones of techniques that are commonly used to localize, identify, and quantitate messengers and mediators of physiological and pathophysiological processes, often with exquisite sensitivity and specificity. Antibodies are the workhorses of medical diagnostics, and humanized monoclonal antibodies are breakthrough treatments for important diseases. They have even been used to stabilize and facilitate structural studies of G protein-coupled receptors (GPCRs).

Many contributors to the American Journal of Physiology Gastrointestinal and Liver Physiology use antibodies for their research, often with great success, considering them a staple in their experimental arsenal. Yet caution is required in the use of antibodies in these heady days of scientific innovation, where it is possible to colocalize proteins beyond the theoretical resolution of light by superresolution microscopy, to define the structure of signaling complexes within a few Angstrom resolution, and to acquire the entire expression profile of a single cell with minimal time and cost. It is important to remember that many of the “bread and butter” technologies that many of us use have an Achilles’ heel: they all stand or fall with the quality of the antibodies.

Unfortunately, many of the everyday antibody tools are not very good. Approaches to generate specific antibodies are as much of a lottery today as they have been for decades and frequently fail to deliver the desired result. Antibodies are sometimes used without rigorous controls for specificity. For proprietary reasons, some companies fail to provide information about epitopes. For some classes of transmembrane proteins, including GPCRs, ion channels and transporters, the lack of reliable and fully characterized antibodies is particularly striking. Specific and properly characterized GPCR antibodies are a rarity, with many reports of GPCR antibodies failing to meet basic criteria for specificity (Ref. 8 and references therein). Some reviewers and editors are unaware of the pitfalls of using antibodies, which has compounded these problems. Together, they have lead to an accumulation of unreliable data in the published literature over the years, which has contributed to what has been decried as “a crisis of reproducibility” (1).

There is a growing literature, including several editorials, outlining the criteria and tests that are required to establish antibody specificity (2, 5, 8-10). These include disappearance of immunoreactivity after gene deletion (using knockout mice) or knockdown (using siRNA) and detection of a single band of the appropriate molecular weight in Western blots. An overlap between immunoreactivity and mRNA expression (assessed by in situ hybridization) is supportive. Identical results with antibodies from two different species is a good, but not foolproof, indication of specificity. Preabsorption with the antigenic peptide is not sufficient because it can prevent binding of the antibody to both specific and nonspecific targets. Omission of the primary antibody or its replacement with an isotype control is a wholly inadequate control for antibody specificity.

A related issue involves the reporting of antibody use. Important information includes the source of the antibody (catalog and lot number), the species in which it was raised, a detailed description of the antigen (antibodies raised against peptides of undisclosed sequence are not accepted by some journals because this information is essential for reproducibility), and antibody dilutions and incubation conditions. With these criteria in mind, we urge contributors, reviewers, and editors of the American Journal of Physiology Gastrointestinal and Liver Physiology to pay particular attention to the proper controls for the use of antibodies.

There are several resources that can help investigators select the best antibodies for their research. Before commencing experiments involving antibodies, researchers can now consult a number of rapidly expanding databases that list antibodies and their validated applications. For instance, the Journal of Comparative Neurology features a spreadsheet on its web site providing details for over 7,000 validated antibodies, with links to the studies documenting specificity testing. CiteAb is a web based database that ranks antibodies according to the number of times they have been cited (6). Of course, popularity is no guarantee for reliability, but peer-reviewed data on an antibody of interest is infinitely better than the minimal information that is often provided by antibody companies, and may often include experiments that properly validate the antibody. The online Journal F1000Research has also started a laudable initiative to collect and publish antibody validation studies (7), allowing researchers to claim credit for their efforts, even when the results are negative. Given the challenges in developing specific antibodies to certain classes of proteins, such as GPCRs, we encourage investigators to seek alternative approaches. For example, nonradioactive in situ hybridization is sensitive and straightforward, and can rapidly deliver results (3, 4). Because this technique allows localization of gene expression (and thus the likely presence of a protein) at cellular resolution, it may provide at least a provisional answer to many of the expression queries that immunohistochemistry has so far failed to address in a satisfactory manner.

We provide these comments to help our contributors select the optimal antibody tools for their research. Please send us your best work.

DISCLOSURES

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AUTHOR CONTRIBUTIONS

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