Monoclonal or Polyclonal… or Neither?

Antibodies are essential weapons in the immune system because they seek out and stick to specific targets, cell membrane proteins of invading microbes. Though this property also makes them very useful for finding target molecules in the lab, there is also a pressing need for refinement in antibody-based techniques. Dr Greer Arthur explains why.

A vast array of research methods harness the power of the immune system to identify molecules of interest (called antigens). That is, in a lab, if you want to track a particular molecule in a sample, one of the best things you can do is apply a special protein that is known to recognise your target antigen (called an antibody) and see whether it sticks. Successful "sticking" can be visualised and quantified by labelling the antibody with a fluorescent dye, either by direct fusion or by application of a fluorescently-labelled secondary antibody. While this seems simple enough, we rely heavily on assumptions about the specificity of the antibody for the target molecule, and it's here that our decision to use either monoclonal or polyclonal antibodies can have a huge impact. But which is better? Or is there a third option?

Strong and specific defences

Antibodies are neat little proteins with a unique ability to seek out, recognise and bind to particular antigens. More specifically, antibodies bind to explicit regions of the antigen, known as epitopes. Antigens and their epitopes are not exclusively proteins; polysaccharides and lipids can also behave like antigens, for example. Antigens are simply a group of molecules that would, *in vivo* (in the body), be recognised as foreign by the immune system, and would therefore trigger an immune response.

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In humans, we manufacture our own antibodies based on which antigens we're exposed to, and thereby what our immune system realises it needs to protect us against. When we encounter an antigen for the first time, our immune system designs and assembles the antibodies from fresh, so that we become primed and prepared for the next assault.

Importantly, we only produce antibodies as and when we need them, a quirk that remains useful for a number of clinical applications, such as vaccinating against specific infectious agents. By injecting an individual with an antigenic substance, the immune system is encouraged to produce a targeted response, including antibodies with a specific affinity for that exact antigen.

Antibodies for the lab

For the purposes of research, this phenomenon has been harnessed by challenging animals with target molecules, which act as antigens, and then harvesting the ensuing reservoir of target-specific antibodies. For decades, this is how polyclonal antibodies have been produced. An animal, such as a rabbit or sheep, for instance, is injected with an antigen, and after allowing an immune response to build, the serum containing the antibodies is collected. Next, the antibodies are (often) purified according to what they're attracted to, and then packaged up and used as a reagent for immunostaining in an experimental or diagnostic setup.

Room for improvement?

As efficient and valid as this process seems, it bears several inherent problems. As described by Andrew Bradbury and Andreas Plückthun in the scientific journal *Nature* last year, with the support of over one hundred co-signatories, only a small proportion of antibodies in the polyclonal "soup" are specific for the original injected target, since the collected serum will inevitably contain other predestined antibodies. Furthermore, little normalisation of the proportion and specificity of antibodies can be achieved between each animal and each immunisation, making batches highly variable and challenging to validate. Selectivity of the polyclonal serum can be gauged, to some extent, by comparing their binding ability with that of control antibodies to the same sample. Control antibodies are a mixture of antibody proteins generated in the same species of animal, but unlike the primary antibody, they were not raised against the same, specific antigen, therefore should not bind to the target. However, since corresponding controls are not necessarily produced from the same animal prior to immunisation, the validity of this comparison is restricted.

Understandably, attempts have been made to improve the consistency and discrimination of antibody production. In 1975, the step up from polyclonal to monoclonal antibodies was made by creating a hybridoma: a B cell capable of antibody synthesis was taken from a mouse immunised with a particular antigen and then fused with a mouse myeloma cancer cell. This created a continual cell line that was restricted to resolutely manufacturing a fixed type of antibody, which could be harvested easily. With monoclonal reagents containing a stricter population of antibodies with improved specificity, the resultant reagents allow greater confidence when successful antibody binding is achieved. But even here, there are problems.

Sources of monoclonal antibodies are relatively fragile; hybridoma cell lines are as susceptible to dying or defrosting complications as any other type of cell, and re-establishing the same cell line capable of manufacturing exactly the same antibody is challenging. As noted by Bradbury and Plückthun in *Nature*, antibody genes can also be lost by the cells, and without vigilant characterisation, the spectrum of specificity of each batch of antibodies can risk being translated into unreliable and unreproducible research findings. In fact, the precise specificity of most monoclonal antibodies remains unknown. While authorities such as the US Food and Drug Agency ensure thorough validation before antibodies reach the human participants of clinical trials, the gargantuan remainder of the research world is still vulnerable to poor and variable antibody reagents.

Taking antibodies to the next level

According to Bradbury, Plückthun and their 110 co-signatories, another step up from monoclonal antibodies is long overdue. Delays and the persistence of poor reagents are becoming ever more costly to the advancement of research and the pockets of funding agencies. It is in all of our interests to adapt and move forward – the direction in which science was always meant to flow.

The answer? As well as filtering out all the poorly characterised, unreliable antibodies, leads in the field are stretching towards the design and commercial manufacture of recombinant antibodies. Unlike monoclonal antibodies, which still rely on a mouse's immune system to do all the designing, recombinant antibodies are engineered from precise DNA sequences encoding the exact antibody structure needed. If antibody sequences were made publicly accessible and validated to a high immunological standard, researchers would have absolute confidence in knowing what their antibody reagents were capable of binding to.

Rather than opting to simply trust the manufacturer's validation procedures, or even turning a blind eye to the possibility that a better antibody is needed for the sake of convenient data, researchers can enforce this transition by choosing higher quality reagents: recombinant over monoclonal, and monoclonal over polyclonal. Likewise, publishers and funding administrations could impose a minimum requirement on the standard of reagents. Just as databases such as the UCSC Genome Browser have indisputably enriched genetics-based research, scientists and biotech companies could join forces to produce databases with characterised, published recombinant sequences. This would in turn enhance all of our data and get us the real results we're all looking for.

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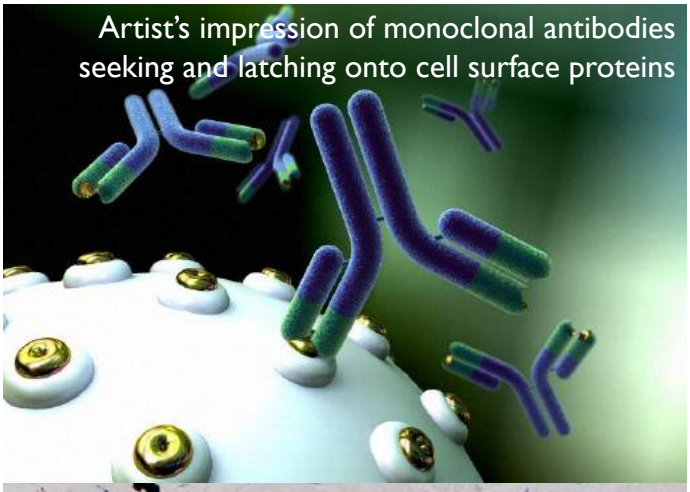
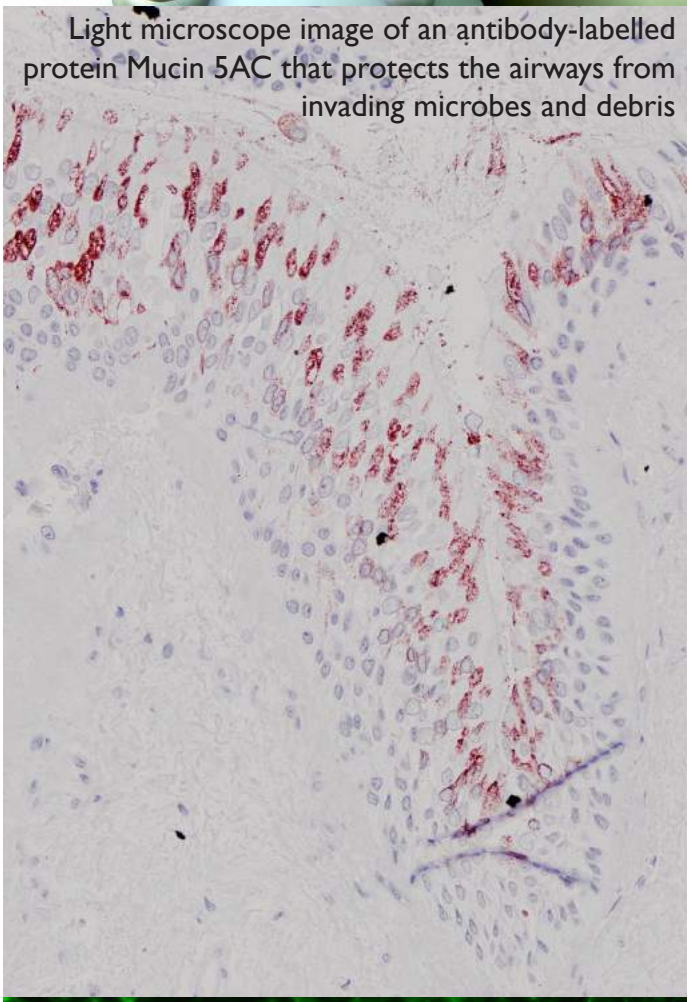
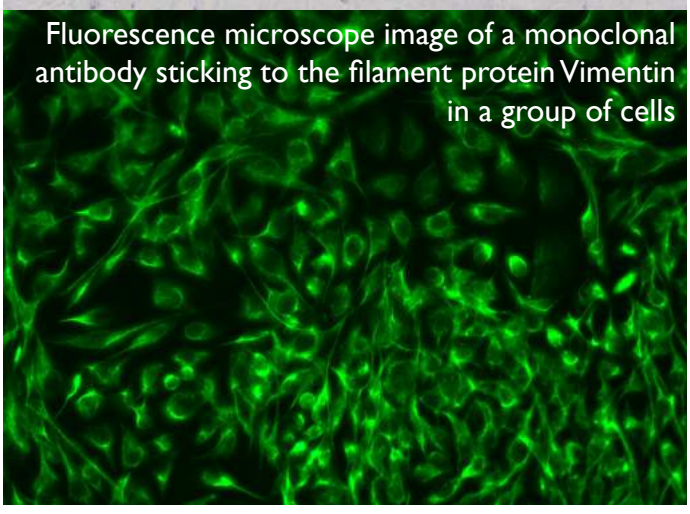
Reference: http://www.nature.com/news/reproducibility-standardize-antibodies-used-in-research-1.16827



Artist's impression of monoclonal antibodies seeking and latching onto cell surface proteins



Light microscope image of an antibody-labelled protein Mucin 5AC that protects the airways from invading microbes and debris



Fluorescence microscope image of a monoclonal antibody sticking to the filament protein Vimentin in a group of cells

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