



The antibody web

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Within the past few years, the worldwide web (WWW) has emerged as a major source of information for the immunologist, creating a powerful influence on the researcher's strategies for acquiring scientific information. There are two reasons for this. First, the technology offers a solution to a problem that has hindered research and the use of monoclonal antibodies (mAbs) since interest grew in the early 1980s: many interesting monoclonals were reported, produced and offered by companies, but researchers often found it difficult to locate a particular source. Today, search engines permit the immediate location of a reference¹ or a supplier for a particular mAb clone², and hybridomas from the American type culture collection (ATCC) can be easily found at the ATCC site³. Search engines that specialize in commercially available antibodies are also useful, but are restricted by comparison with universal search engines because they require companies to actively submit their product data⁴. Nevertheless, the most comprehensive of these services⁵ offers access to more than 350 online catalogues. It is always worth trying a combination of several different search engines, including universal ones like Altavista. Altavista is not able to access a large fraction of catalogues posted on the web (cgi.bin type), but might help you to find the most recent entries. Companies present detailed data sheets for their products on the web, providing sufficient information for rapid selection of the mAb best suited to the researcher's needs. Link lists with websites of individual companies are available as well⁶. Most companies have their own domain; the standard internet address is 'www.companyname.com' – which is always worth trying if search engines don't give a result.

Second, a new information format has emerged: specialized websites devoted to particular scientific areas. These sites usually feature online articles in review style, optimized to give a quick overview, sometimes with multimedia elements that exceed the

The internet has evolved to play a central role in the lives of immunologists. Powerful search engines facilitate the location of references or suppliers at the click of a mouse. Specialized websites provide reviews for many immunological topics and links for fast navigation to related information. This article highlights websites related to monoclonal and recombinant antibodies.

bounds of printed scientific publications. Many of these sites feature a links section, connecting the user to related web resources in one mouse click. Good sites offer links to cited publications, allowing the reader access to the reference on screen – rendering the walk to the library obsolete. Significantly, the credit for most of these websites must go to individual researchers who have collected and examined internet links for their own use, rather than to research facilities or other institutions. A prominent example is 'Pedro's'⁷, a vast collection of molecular biology links, which also contains useful information for immunologists (in particular, databases and web-based software).

These developments illustrate the move towards online technology and the changing ways in which knowledge is acquired; above all, they highlight the undeniable driving force of the internet: convenience for the scientist. In this respect, users of web-based information should keep in mind that information on websites is not peer reviewed – and frequently reflects the preferences and opinions of the individual who presents it. However, when looking back on the development of websites with scientific contents, it appears that the immediate worldwide audience of the internet generates a quite reliable control force against low quality – at least for specialized topics with a limited audience, such as the scientific community of immunologists. It is not always easy to

find the good websites by using search engines. Therefore, a selection of websites that may serve as starting points for the scientist interested in monoclonal antibody technologies and their recent offspring, recombinant antibody engineering, are presented here. All websites mentioned in this article can be accessed directly via a section provided on my own website⁸.

Resources for teachers and students

The internet offers a vast body of information for teachers and students of immunology. Most of these sites offer basic introductions that rarely reach the extent of information provided by a textbook. Their attraction lies in their extensive use of graphical^{9,10} or multimedia elements, as in a virtual laboratory for the development of an ELISA assay¹¹ or in a beautiful introduction to antibody ultrastructure¹².

Resources for researchers

The number of informative websites for the experimental scientist or the clinician interested in antibodies is limited. In this area, it is most evident that the available material is the result of individual initiatives. The most comprehensive collection of links to antibody-related sites can be found on the *Antibody Resource Page*¹³. It offers a comprehensive list of contract and custom antibody suppliers, online databases, researchers' and teachers' resources, among many other related topics. General immunology links are available from Harvard¹⁴ or the *Immunology Link Pages*¹⁵; the latter offering, for example, a table of CD antigens, thus neatly illustrating how the availability of hybridoma technology gave rise to an entire new branch of immunology.

Quite a number of sites offer detailed laboratory protocols for the generation and use of antibodies; flow charts and protocols on hybridoma generation^{16,17}, for example. Perkin Elmer's *Antibody Manual*¹⁸ does not

cover the generation of monoclonal antibodies specifically, but provides helpful hints on antigen conjugation, immunization and antibody purification. An introductory review and comprehensive bibliography of adjuvants has been collected by the United States Department of Agriculture¹⁹. Protocols for developing an ELISA (Ref. 20) or for immunostaining tissue²¹ are available, as well as detailed protocols for FACS (Refs 22,23) and a large collection of FACS related links²⁴.

Antibody engineering

Recombinant antibody technology has revolutionized the generation of monoclonal antibodies by offering access to human monoclonal antibodies that recognize almost any antigen, and by allowing the construction of genetic fusion proteins that add new functions to the antigen binding site. A broad and comprehensive introduction to the technology, as well as particular aspects (bi-specific antibodies, diabodies, single-chain diabodies and intrabodies) and an excellent description of phage display can be found on Roland Kontermann's web pages²⁵. A list of sites related to recombinant antibody engineering is available from Jos Raats' pages²⁶, which also offers a brief graphical introduction into the phage display technology. My own website²⁷ offers an introduction to antibody engineering, frequently updated information on related conferences and, among other related information, a list of companies that sell, use or produce recombinant antibodies. Sites offering particular tools for the antibody engineer are given in the following section.

Antibody structure, sequence analysis and modelling

The classical *Kabat* database of antibody primary structure²⁸ allows the generation of alignments, variability plots, subgroup identification, among others. The *V-base*²⁹ database provides a complete set of germline genes of human variable regions, with alignments. A site that provides mouse germline V region genes is under construction³⁰. An excellent and huge collection of information related to antibody genes is provided by the *ImMunoGeneTics* database³¹, offering, among

many other topics, chromosomal locations, alignments of alleles, information on immunoglobulin gene structures and promoters, as well as search and alignment software. A detailed introduction to antibody V region sequence elements, amino acid nomenclature and identification of complementarity-determining region (CDR) regions is offered by Andrew Martin³². In addition, his website provides online access to unique software that lets the researcher check a new antibody sequence for conformity with known structures and to identify the CDR canonical structure. Software for the generation of molecular models of the variable regions by homology modelling is also available online^{33,34}.

Andreas Plückthun's lab provides nice slide shows that lead us deep into the antigen binding site – uncovering the molecular basis of antibody specificity³⁵. A detailed introduction to humanization strategies for mAbs is available^{36,37} – a technology considered to be beneficial for generating humanized antibodies of mouse origin for therapeutic or diagnostic *in vivo* application. Researchers working on human therapeutic antibodies should visit Mike Clark's *Therapeutic Antibody Human Homology Project*³⁸, with information on attempts to identify any correlation between the efficacy of therapeutic antibodies and the sequences of their variable regions (see also Mike Clark's article in this issue, p. 397). A comprehensive and searchable collection of atomic coordinate (PDB) files of the ~300 known structures of antibodies analysed by X-ray crystallography or nuclear magnetic resonance (NMR) is growing rapidly; according to the collection's authors, 78 new structures are expected by the end of this year³⁹.

Related resources

An increasing amount of knowledge on the molecular basis of antibody–antigen interactions is gained by epitope mapping techniques. Detailed protocols, as well as the necessary reagents for phage display, are available from G.P. Smith's website⁴⁰. Peptide spot assays are described elsewhere^{41,42}.

Antibody technologies, both hybridoma-based and recombinant, gave rise to hundreds of patent applications; thus, valuable

information may be obtained from patents⁴³. Clinical studies with antibody therapeutics can be identified on the *Centerwatch* server⁴⁴, which supplies a searchable database of industry-sponsored clinical trials worldwide, as well as those supported by the US National Institutes of Health (NIH). Information on regulatory issues – for example, the recommendations of the US National Research Council concerning the use of laboratory animals for monoclonal antibody production⁴⁵ – can be conveniently accessed online as well. *Yahoo News*⁴⁶ supplies recent articles from news agencies, with its main focus on biotech and pharmaceutical companies and their press releases. Finally, it should be mentioned that a variety of e-mail newsgroups may provide help in solving everyday experimental problems. The participant can post questions and present his own problems, or comment on submissions by others. The individual quality of a particular newsgroup, however, depends solely on the input, qualification and volunteer activity of the contributing members.

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Hybridoma technology: a view from the patent arena

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The arrival of hybridoma technology in 1975 threw down immediate challenges to patent laws, patent practitioners and biologists. From the outset, the role of patents in the technology was unclear. For the patent practitioner, the central issue concerned the flexibility of the existing patent system and its ability to protect biological products in a manner that was equitable to all concerned. For many biologists, the notion that a biological product, such as a hybridoma, could be turned into intellectual property through the patent system was new and challenged cherished beliefs and practices.

In principle, anyone is free to use anyone else's ideas, unless they are protected by some form of intellectual property right. In industry, patents are the main vehicle used to protect new ideas. Patents are granted by governments to stimulate innovation and encourage investment in research and development. They do so by giving the patentee the right to stop others from using an idea. The right is not automatic but is only granted for ideas that are new, non-obvious and industrially useful. Additionally, the

The patenting of biological molecules, such as hybridomas and monoclonal antibodies, has become an important issue in biology. While challenging cherished beliefs and practices of many biologists, it has been a central issue for hybridoma and monoclonal antibody research.

patentee must accept publication of the idea and describe it in sufficient detail so that it can be reproduced. The extent to which these requirements can be met will determine the breadth of the monopoly obtained.

Issues of novelty, obviousness and, in particular, how to describe hybridoma technology in a sufficiently reproducible manner, were still to the fore in 1981 when the Banbury Conference considered the patenting of life forms [Plant, D.W. *et al.* (1982) *Patenting of Life Forms* (Banbury Report; 10), Cold Spring Harbor Laboratory]. Unanswered questions included the issue of novelty – monoclonal antibodies pre-existed in the animal and on this basis were not new; obviousness – would every monoclonal antibody be patentable, would distinct cells

making the same antibody be considered unique?; and breadth of patent protection – could all monoclonal antibodies to an antigen be claimed when all that had been generated was a hybridoma that produced an antibody to a specific epitope? More fundamentally, as anyone could probably make a chemically different monoclonal that could carry out the same task, did it matter whether or not a monoclonal was patented? Finally, would it be the antigen rather than the antibody that would most often be patented?

Undeterred by such considerations, it did not take Academia and Industry long to flood the patent system with patent applications relating to hybridoma technology. As early as 1977, the Wistar Institute (Philadelphia, PA, USA) was filing broad patent applications claiming antibodies to viral and tumour antigens, with mixed success^{1,2}. By 1984, the patent literature was awash with claims of varying breadth to monoclonal antibodies recognizing a variety of antigens and to methods and cells for their production. Patent applications existed for every conceivable application where antibodies could be used to identify, analyse, quantify