

Manufacturing antibodies

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New antibodies and recombinant proteins with a key signaling role in immune response to disease have been produced through collaboration between molecular immunology institutes in the Czech Republic and Germany and a private company. The proteins have their own direct uses in immunization and are also the starting point for production of novel, highly specific antibodies with a wide range of biomedical applications. All of the new products are already being marketed commercially.

Belgian EUREKA project has developed a range of unique and highly specific monoclonal and polyclonal antibodies - the proteins produced in the blood which counteract bacteria, viruses or [cancerous cells](#). This was achieved by first producing a number of [recombinant proteins](#) which are important components of cellular signalling pathways. These proteins themselves have direct uses in immunisation and experimental studies. A further key advance is the incorporation of novel fluorochrome dyes with specific monoclonal antibodies, which can then be used in diagnosis of leukaemia and rheumatic diseases; also in oncology and haematology research.

The human immune system protects the body from disease by identifying and destroying the agents of disease - bacteria, viruses and also its own cells if they become transformed into a potentially cancerous tumour. The immune system depends on the activity of antibodies, which are naturally produced within its white blood cells. The structure of antibodies has many millions of variations; each capable of recognising and marking a specific antigen, for example from a specific [bacterium](#), so that the bacterium of that strain can be identified

and destroyed by other types of [white blood cells](#). If a molecule from a specific bacterium binds to a receptor protein on the surface of the white blood cell, the protein, which is an important component of the signalling pathway, triggers a response within the cell. In addition, antibodies can serve as an extremely useful research and diagnostic tool, as they can bind with great specificity and sensitivity to their target structures and then can be visualised by staining with specific dyes.

The Antibodies fabric

Modern molecular techniques now enable in vitro production of some of the receptor proteins. The RECAN project used recombinant techniques to produce them - combining defined DNA sequences with the DNA of bacteria to alter the coding for specific traits, and then harvesting the altered protein derived from that recombinant DNA.

The recombinant protein was then used to immunise test subjects using standard hybridoma technology. This involves fusion of specific antibody-producing cells with cancer cells to form hybrid cell lines, growing them in tissue culture, and retaining and purifying the antibodies produced. In this project, a first test group was used to produce antibodies derived from monoclonal antibodies, and another to produce antibodies derived from polyclonal antibodies.

"We used a standard technology" says Professor Vaclav Horejší of the Institute of Molecular Genetics in Prague (IMG). "But what's unique about our project is the products - especially the monoclonal antibodies, which are of unique specificity, with great commercial potential and in some cases also useful for diagnostics."

Fluorescent labelling

The RECAN project has also made important advances in cytofluorometry, which is the use of specific fluorescent markers to distinguish between types of cells. The project partners developed methods to prepare monoclonal antibodies bound to several types of a new range of fluorochrome dyes. Antibodies bound to these dyes can readily be distinguished from those labelled with more conventional dyes.

One particular focus of this part of the project was to develop methodology for the immunophenotyping of leukaemia, and Exbio aims to be one of the first European companies to offer use of these novel fluorochrome dyes to screen leukaemia patients and those with rheumatoid arthritis. One of the monoclonal antibodies recognizes an important signalling protein called ZAP70, which is a characteristic marker of certain types of leukaemia and therefore can be used for diagnostic purposes. Although this method is already established, new monoclonal antibodies are still necessary to develop a standard protocol for routine diagnosis of this type of leukaemia, using the best reagents.

A ready market; a fruitful collaboration

The products generated as a result of the RECAN project are already commercially available worldwide through Exbio. The recombinant protein products can be used for immunisation in the production of antibodies, and also as specific internal standards for their production and determination. Production of the specific monoclonal and polyclonal antibodies makes it possible to target new antigens, which will contribute to the development of new immunochemical assays. Finally the fluorescently-labelled antibodies will find numerous applications in both diagnosis of conditions, and also in research studies in aspects of haematology, oncology, immunology and other areas of biomedical research.

The RECAN project made optimum use of existing techniques by applying them to a new area. In doing so, it produced a new range of products which make significant improvements over existing possibilities. Professor Horejší comments that collaboration was important - the project partners were in touch before RECAN began, but they were each able to contribute different skills. The IMG laboratory developed most of the recombinant proteins and used them for immunisation and the production of the hybridoma cell lines delivering the monoclonal antibodies. The Magdeburg immunology laboratory was responsible for independent testing of several of the monoclonal antibodies and evaluating their qualities in specialised immunochemical techniques. Exbio applied its unique expertise in fluorescent labelling of [monoclonal antibodies](#). The company also prepared several batches of polyclonal antibodies and prepared them for commercialisation, including the optimisation of large scale production, purification and stabilisation.

Provided by EUREKA

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