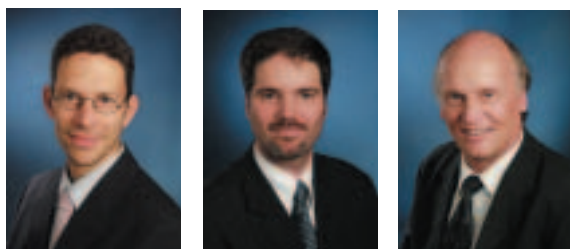


A shortcut from genomics to drug development and therapy

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The spotlight in biomedical research is shifting from genomics to proteomics. Now, the challenge is to translate the wealth of genomic information into useful basic and applied knowledge and, ultimately, into novel methods for the diagnosis, prevention, and therapy of diseases. For the prevailing task of identifying and characterizing interesting functional molecules, antibodies are ideal analytical tools.

Antibodies are an essential component of the immune defense of higher vertebrates. Due to their virtually infinite recognition potential, their binding specificity and affinity towards their antigens, they recognize and label foreign and aberrant molecules or pathogenic agents for subsequent degradation, neutralization, or killing. These characteristics make antibodies valuable tools for proteomic research and applications.

For example, antibodies can function as the key elements of protein chips, a modern proteomic screening platform. These special types of biochips called 'antibody microarrays' use a panel of antibodies with different specificities, which are arranged in a microarray format on a solid carrier, as capture probes for their matching protein counterparts.

Antibody-based methods are widely used in drug discovery and development: they are used in preclinical target identification and target validation studies in order to evaluate the clinical and commercial potential of target molecules. In addition to being used as analytical tools, antibodies are subject to development into diagnostic and therapeutic agents themselves. In medical therapy, antibodies have significant advantages compared to conventional chemical compound-based therapies.

They have fewer side effects due to their high specificity and affinity for the disease target and show favorable pharmacotoxicity and pharmacokinetics. In terms of persistence in vivo, the IgG class of antibodies is the most stable and has a serum half-life of 20 days. Furthermore, antibodies trigger effector functions such as complement-dependent cytotoxicity, and recruit immune effector cells to initiate destruction of the target by phagocytosis and cytolysis.

Genetic immunization

At the start of the 90s, it was shown that the application of plasmid DNA, carrying a reporter gene under the control of a eukaryotic promoter, into the muscle or the skin of an animal can lead to the expression of the introduced genetic information. In fact, the intracellularly synthesized foreign protein can act as an antigen and elicit a humoral and cellular immune response in the animal. GENOVAC, based in Germany, has standardized, optimized and further developed this technology of genetic immunization and combined it with the hybridoma technology for the generation of monoclonal antibodies. The experimental steps of this procedure involving proprietary immunization and screening methods are shown in the flow chart in Figure 1.

The GENOVAC technology

Native antigens formed

Our procedure takes advantage of an animal's cellular protein synthesis and processing machinery. Starting from cDNA, which has been cloned in specific expression vectors, the foreign antigen is administered and intracellularly synthesized in its natural conformation with correct post-translational modifications and 3D folding. The animal's immune system is naturally stimulated by the native protein expressed in low quantities to produce high-affinity antibodies recognizing the native form of their antigens.

Protein purification unnecessary

Conventional immunization technologies usually generate antibodies against purified proteins or against synthetic peptides based on amino acid sequences derived from DNA sequence data. Proteins are either isolated directly from cells or tissues or in a recombinant form after expression of the cDNA in bacteria, insect, yeast, or mammalian cells and purification is often hampered by protein degradation, low yields and inadequate purity. Relying on our novel approach, purification of the target protein is neither required for the immunization nor for the in vitro analysis and selection of the hybridoma clones.

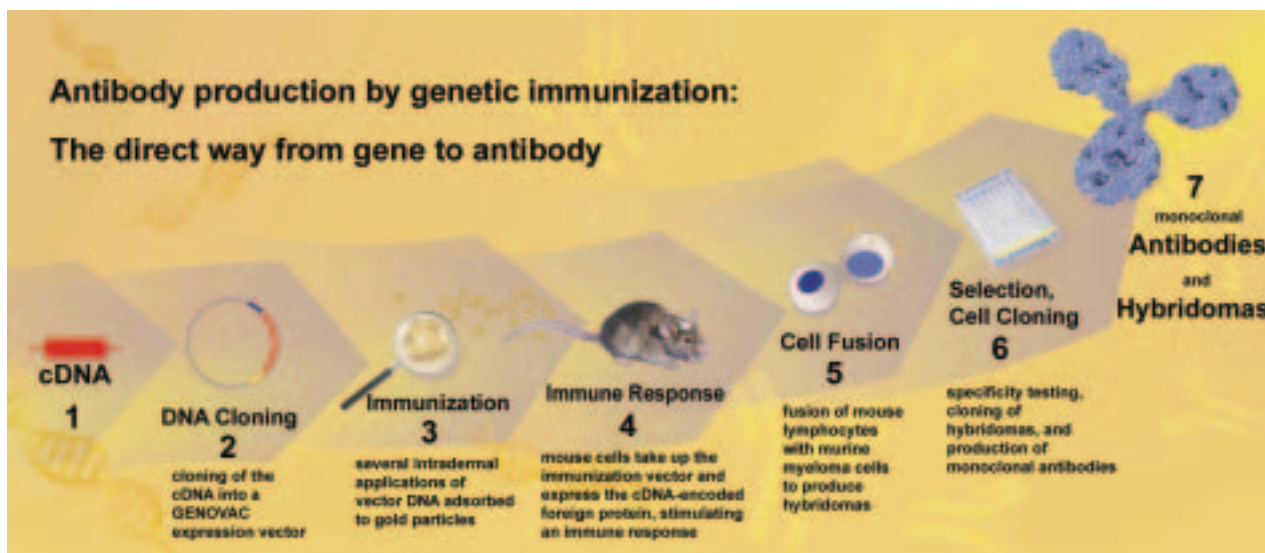


Figure 1. The GENOVAC technology for the production of polyclonal (steps 1-4) and monoclonal (steps 1-7) antibodies.

Our patented screening system for determining the specificity and the amount of polyclonal and monoclonal antibodies contained in immune sera and hybridoma supernatants relies on mammalian cell culture systems transfected with the original cDNA cloned into special screening vectors. This way, it is feasible to produce antibodies with proven specificity without needing to isolate the target protein.

Quick and inexpensive

By optimizing all experimental steps and applying high-throughput technologies, specificity-tested polyclonal antibodies can be generated in 2-3 months and mono-

clonal antibodies, starting from the same immunization constructs, in 3-6 months. A comparison of this method with standard technologies for the production of monoclonal antibodies is given in Figure 2. The largest time differences between the different immunization protocols originate from the preparation time for the immunizing agents, the antigens. When protein purification is required, it takes approximately 1-2 months to isolate recombinant proteins from bacteria, 2-3 months to purify them from insect cells, and 4-6 months to obtain them from mammalian cells. The synthesis of peptides for peptide immunization and the construction of the

cDNA vector for genetic immunization both take 10-20 days, but the quality of the antibodies generated by DNA immunization is considerably better. Peptide-specific antibodies rarely recognize native proteins (Figure 3) and generally have a lower affinity. In contrast to the wide time variation observed in antigen production between the different protocols, the time required for antibody development is almost constant. Here, all methods rely on time-consuming animal immunization and standard cell culture techniques. The relative costs for antigen generation correlate roughly with the required time. The costs for antibody development are slightly higher for the novel technology due to the special transfection-based screening method used in the absence of purified antigen.

Antibody quality

Distinct advantages of antisera generated by DNA immunization are their high binding affinity, which has been reported to be 100- to 1000-fold stronger than that of antisera raised against the corresponding recombinant proteins. Using this method monoclonal antibodies have been developed with affinities in the sub-nanomolar range.

Conventional immunization protocols using purified proteins can never rule out problems with contaminating proteins inducing cross-reacting antibody speci-

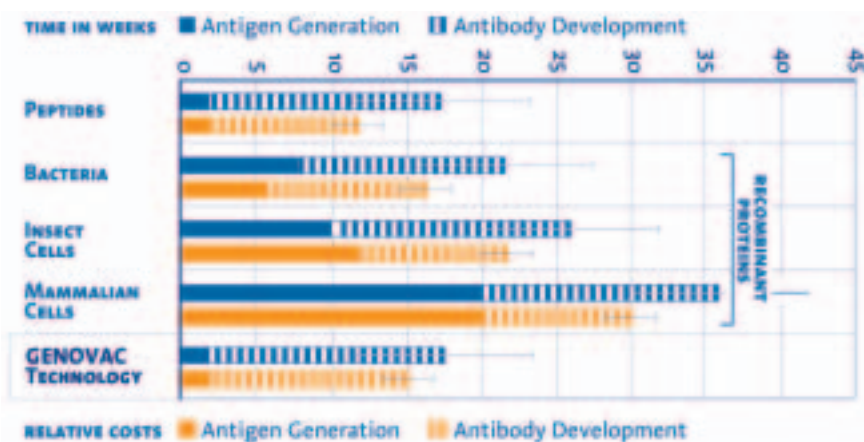


Figure 2. Comparison of time and relative costs between different monoclonal antibody production techniques. Estimated values for antigen generation and antibody development are indicated as solid and hatched parts of one bar.

cities. DNA, on the other hand, can easily be obtained in a pure form without protein or DNA contaminations so that highly specific antibodies against the desired protein are produced.

Tailor-made antibodies

Targeted manipulation of DNA is easy to perform, so that tailor-made antibodies can be designed that recognize, for instance, single protein domains, mutated regions, detoxified variants, iso- or pro-forms of enzymes, or specific viral and bacterial strains. The implications for drug development are obvious. It is possible, for example, to direct antibodies against functional binding domains of ligand or receptor molecules in order to activate or to inhibit receptor-mediated signaling. Genetic engineering of the underlying expression vectors holds all options for co-immunization with DNA sequences encoding immune-modulating or -enhancing factors. This way, the triggered immune response can be steered in desired directions. Currently, the problem that antibodies against single subunits of a molecular complex, for example a heterodimer or -trimer, do not necessarily recognize the assembled complex is addressed by simultaneous immunization of one animal with several subunit-specific cDNAs.

Diagnostics and therapeutics

Antibodies originating from genetic immunization usually recognize their native target proteins and can therefore be used in immunological methods investigating living cells (Figure 3). Together with the high specificity and affinity of the antibodies this is a prerequisite for the identification of the corresponding antigens in complex mixtures and environments such as blood or body tissues, where the targets are often present in low concentrations. The combination of all these properties renders it possible to use these antibodies in vivo as delivery vehicles of toxins, drugs, drug-activating enzymes, radioactive isotopes, and photo-labels to target specific disease sites in the body.

Membrane-bound and secreted proteins are the most interesting targets for diagnosis and therapy. Secreted proteins often serve as target structures for diagnostics to avoid invasive techniques in acquiring test samples from patients. Therapeutics, on the other hand, are often directed against membrane-bound proteins which are extracellularly accessible from blood or lymph. Both classes of proteins create problems in the conventional protein and

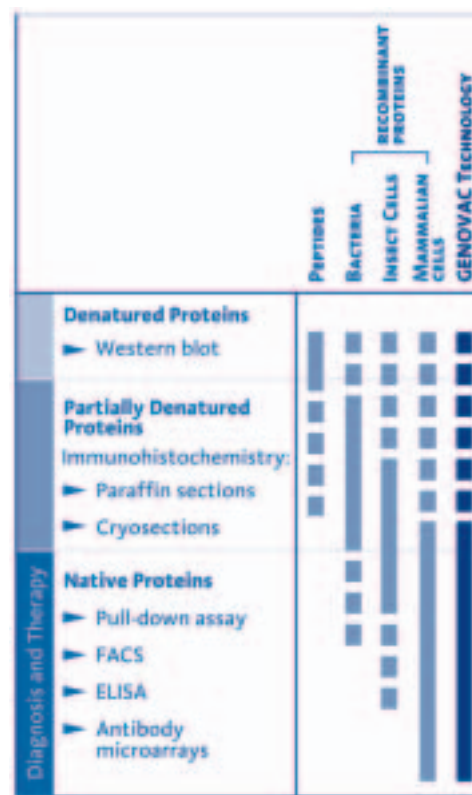


Figure 3. Performance of antibodies raised by different immunization techniques in information-sensitive analytical methods.

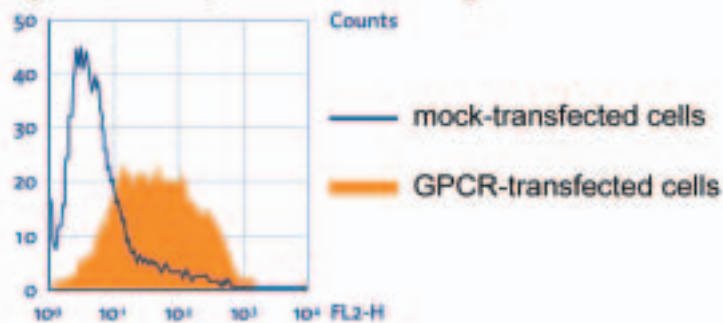
peptide immunization since they are subject to pronounced post-translational modifications and characterized by strong hydrophobic properties, respectively.

Protein localization/origin	Fraction of total projects (n=150)	Successful antibody production
Secreted	28%	88%
Membrane-bound: type I, type II	31%	92%
Membrane-bound: multiple spanning (GPCRs, ion channels)	11%	47%
Other localization (eg cytoplasm, nucleus, lysosomes, unknown)	22%	83%
Viral proteins	8%	67%
Overall success rates: antigen expression		95%
antibody production		84%

Table 1. Track record of antibody production by genetic immunization projects at GENOVAC.

FACScan Analysis

Hybridoma supernatant tested against



Immunofluorescence Analysis

HEK (human embryonic kidney) cells

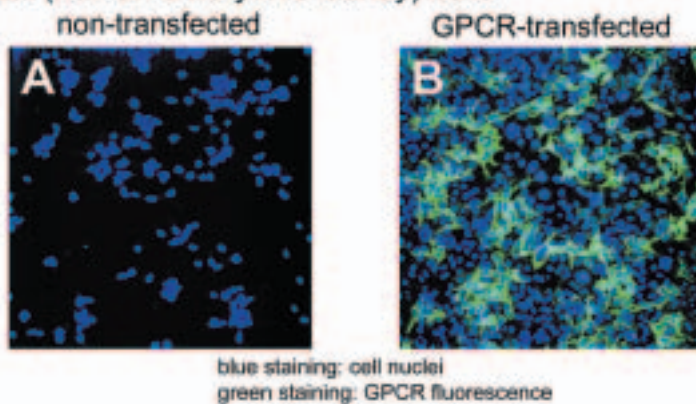


Figure 4. Specificity testing of an anti-GPCR monoclonal antibody. Transfected cells and adequate control cells were examined for receptor recognition by FACScan and immunofluorescence analysis.

Track record of projects

Projects involving antibody production against secreted and membrane-bound antigens constitute approximately 70% of all in-house and service projects completed at GENOVAC. The success rates for different antigen categories are indicated in Table 1.

The overall success rate for antibody production is constantly around 85%. Membrane-bound receptors and ion channels belong to the biologically most difficult, but clinically most attractive targets.

Rates for the successful production of antibodies against these membrane-buried proteins with extremely small protruding extramembraneous loops have been permanently improved and now reach 47%.

In a proof-of-principle study initiated by CuraGen, the objective was to generate polyclonal antibodies in rats against 10 chosen target proteins starting from cDNA clones or DNA sequence information. The targets were specifically chosen to represent various degrees of difficulty in antibody production. Our technology delivered tested high-quality antibodies against 9 of the 10 targets within 10 weeks. One of the known antigens was human Ras protein, yielding an immune serum which still gave a measurable signal at a 1:500,000 dilution in ELISA. This is remarkable, since there is a high sequence identity of 98% with an exchange of only 4 amino acids between the human and the rat Ras protein. In conclusion, the technology is well suited to produce antibodies even against highly conserved proteins.

The study has also proven that it is a fast and reliable method for target identification in the absence of purified protein. The positive outcome of the proof-of-principle test has resulted in a 5-year collaboration contract between both companies.

Antibodies blocking receptor function

G protein-coupled receptors are seven-transmembrane-domain receptors constituting the largest family of cell-surface molecules involved in signal transduction. They play key physiological roles, for instance in cell cycle control, metabolism, and hormone action, and their dysfunction results in a wide spectrum of diseases.

GPCRs are the target of 50-70% of the current therapeutic agents on the market with annual worldwide sales exceeding \$30 billion in 2001. The Human Genome Project has identified approximately 150 'orphan' GPCRs as potential new drug targets. Accordingly, members of the GPCR family represent up to 30% of the R&D portfolio of many pharmaceutical companies. They are the main targets to identify novel approaches for pharmacological intervention with inhibitory or stimulatory agents.

By applying our technology, a monoclonal antibody has been produced which recognizes native membrane-bound GPCR as documented by FACS and immunofluorescence analysis of transfected cells (Figure 4). Furthermore, antagonistic antibodies against transmembrane receptor proteins blocking the function of, for instance, cytokine (IL-13R, TNF α R-superfamily) and scavenger receptors were successfully raised. Currently, the R&D department is setting up a collaboration network in order to address the production of functional antibodies in more detail.

Product portfolio

Besides custom-tailored service and collaborative product development in the context of strategic alliances with partners from the biotechnology or pharmaceutical industries, GENOVAC also develops its own product portfolio of monoclonal antibodies. Based on its longstanding

experience with genetic immunization, the company has established a diverse spectrum of antibodies relevant for basic research as well as for the biopharmaceutical industry. This collection includes – among many others – different specific antibodies against members of the carcinoembryonic antigen (CEA) family, apoptosis-related antigens such as members of the granzyme family, FAS and ICOS ligand, as well as receptors such as the IL-13 receptor $\alpha 1$. The company continues to expand this portfolio on the basis of medical and commercial value.

Humanized and human antibodies

Mouse-generated antibodies are of limited therapeutic benefit due to their immunogenic potential in humans, resulting in reduced efficacy, rapid clearance from the circulation and, potentially, allergic reactions or other complications. AERES Biomedical specializes in the humanization of antibodies.

The company uses antibody engineering by complementarity-determining regions (CDR) grafting to remove the inherent immunogenicity of murine antibodies while retaining their affinity, specificity and their therapeutic activity, thereby enhancing their clinical utility.

Another approach to achieve immunocompatibility is to produce fully human antibodies in mice. Companies such as Medarex, Abgenix, and Kirin own transgenic strains of mice, whose murine antibody genes have been inactivated and replaced with the entire human immunoglobulin genetic repertoire. Theoretically it should be possible to offer a complete service starting from the cDNA and yielding specificity-tested, fully human antibodies for use in human therapy in 3 to 4 months.

Biovation based in Aberdeen, UK, follows an entirely different approach to reducing immunogenicity in therapeutic antibodies, thus improving their safe use in patients. Biovation's proprietary Delimmunisation technology identifies and removes helper T cell epitopes from antibody therapeutics. This way, the administered antibodies are not causing an immune response during the treatment, because the activation of helper T cells,

required to initiate and sustain antibody production by B cells, is prevented.

To date, most of the antibodies approved by the FDA for clinical use are chimeric or humanized. Humira, an anti-TNF α antibody from Cambridge Antibody Technology and Abbott, is the first fully human antibody originating from in vitro selection from a human antibody library in phage (phage-display technology), which has been approved for therapy. Together with fully human antibodies derived from transgenic mice these antibodies comprise most of the recently developed antibodies, which are now being tested in clinical trials.

Implications for drug development

Antibodies advance the fast transition from the information level of the genes to the function level of the proteins in the post-genomic era. By streamlining preclinical target identification and validation processes, they have the potential to accelerate the time-consuming and expensive drug discovery and development processes, to raise R&D productivity, minimize clinical failures, and increase the return on investment. In contrast to chemical drugs, diagnostic and therapeutic antibodies are naturally evolved molecules and therefore less likely to be toxic. Generally, they have shorter preclinical and clinical development times than new chemical entities.

Parallel to the transition from genomics to proteomics, antibodies are gaining more and more importance in clinical research and therapeutical practice. To date, the FDA has approved 13 monoclonal antibody therapies, mostly related to transplant rejection and the treatment of cancer and autoimmune diseases. More than 400 monoclonal antibodies are currently being tested in clinical trials worldwide. The antibody therapeutics market is expected to grow 30% annually, reaching over \$7

billion by 2004. A top-selling therapeutic antibody can generate over \$1 billion in annual sales.

How can drug development companies profit from the value added by antibodies and the enormous progress made in antibody production and engineering techniques? Contract antibody production gives the pharmaceutical industry access to the skills and technical expertise of specialized service companies, which are using state-of-the-art technology for the generation of high-quality antibodies. Outsourcing is a time- and cost-efficient alternative to establishing the experimental set-up and the necessary facilities in house.

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FURTHER INFORMATION

Clinical trials:

www.clinicaltrials.gov

Drugs approved by the FDA:

www.centerwatch.com/patient/drugs/druglist.html

FURTHER READING

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