Biotechnology in Blood Transfusion

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FOREWORD

This symposium is devoted to Biotechnology in Blood Transfusion; there are 22 experts discussing the state of the art in the application of monoclonal antibodies, recombinant DNA technologies and heterologous expression systems to the improvement and sometimes replacement of blood products, characterization of blood constituents, and the effect of these developments on blood transfusion procedures.

Ten and maybe five years ago the title of a symposium such as this would have been *Biosciences in blood transfusion*, informing what basic developments in molecular biology, biochemistry and human physiology might pertain to blood transfusion in the distant future. That future is getting closer, and not only one is interested in basic developments in immunology, recognition and identification of viral and bacterial components and products, tissue and bloodgroup typing, but also in the potential application of these developments and their economic perspectives.

That is what biotechnology is all about: basic science tells us where and how we might look for new technologies, and the development of such technologies is only possible if there is a perspective for improvement in quality, safety, acceptance or performance to cost ratio. This means that working under the flag of biotechnology is both exciting and frustrating: exciting because there is much to be done over a very wide range, from very fundamental to applied studies, and even venturing into commercialization and new business development; frustrating because despite the seemingly endless possibilities, considerable energy and steadfastness are needed to realize the apparent economic potential. In the end economic feasibility is a harsher judge of reality than a granting committee.

Some of these prospects which are already being judged or will soon be judged, will be discussed during this symposium. They include such topics as:

1. large-scale production of monoclonal antibodies;
2. applications of monoclonal antibodies for quality control and the detailed characterization of blood and other tissues;
3. applications of monoclonal antibodies in the ultrapurification of blood proteins and other biologicals;
4. application of ultrapure blood proteins for therapeutic purposes, and
5. production of human blood proteins in large-scale mammalian cell bioreactors using heterologous recombinant DNA expression systems.

If we look a little further into the future, several other developments may well become important. For instance: the production of human blood proteins or appropriate substitutes not only in animals and in animal or microbial cell

bioreactors, but also in suitable plants; the increased use of synthetic peptides and perhaps even proteins, as organic chemists improve their technologies inspired by the life sceinces and natural models; the application of protein engineering to alter blood proteins which are to be used therapeutically – I am thinking here of properties such as altered temperature or pH stability, smaller or larger size, antigenic epitopes tailored to specific patient groups and last but not the least, the development of ultrasensitive biosensing systems for the rapid and increasingly complete characterization of blood.

However, without the perspective of economic development biotechnology is nothing, and although this development can certainly occur spontaneously, it may be useful to create an atmosphere and an infrastructure which stimulate biotechnology based economic developments. Every industrial nation does this to some extent, using tax incentives, subsidies, specific programs or a combination thereof.

The Netherlands recognized this need in the late 70's and approached it in various formal and informal ways. University researchers came together and developed research and teaching programs in close co-ordination. This saved the government the unpleasant and difficult task of dividing limiting amounts of funds over large numbers of competing laboratories. Meanwhile, the Dutch Biotechnological Society was founded; its membership grew rapidly and is now well beyond 1000, which makes it one of the largest societies of the European Federation of Biotechnology. Contacts between universities, industries and government are intense, frequent and informal and the first phase of the government stimulated development can clearly be said to have been successfull.

The second phase is now upon us. Having established an excellent R&D infrastructure, the government has recently launched a program of support for industries which are interested in developing or expanding their R&D base in collaboration with university and institute laboratories. A similar approach is now being followed by the government in several other areas with economic potential, such as material sciences, information sciences, and of relevance to this symposium, biomedical technology.

In the university city of Groningen, a number of institutes and research centres has been developed and some of these are relevant to the topics discussed at this symposium. These include the Groningen Biotechnology Centre, the Biomolecular Science Centre, the BIOSON institute, the research laboratories of the Medical School, and of course, our host: the Red Cross Blood Bank Groningen-Drenthe.

Prof.Dr. Bernard Witholt
Dean Faculty of Biochemistry
President Science Park
University of Groningen

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OPPORTUNITIES FOR BIOTECHNOLOGY IN TRANSFUSION MEDICINE

L.R. Overby

Biotechnology is approaching its 15th anniversary. The importance of recombinant DNA technologies was recognized in 1974 when scientists recommended that ethical, environmental, technical and commercial issues surrounding genetic engineering should be assessed broadly before rapid exploitation of the impending breakthroughs. Most of these issues have been clarified and we have seen amazing progress in applying biotechnologies to problems in human health, to animal and plant life, and to areas of energy and the environment.

In this conference, we are highlighting existing and potential applications of biotechnology to blood transfusion and hemotherapy. If we look at progress in molecular biology over the past 15 years and its relation to blood transfusion issues, we can project that the future holds a bright promise for increased safety and efficacy for blood and blood products and for new discoveries that will increase the role of hemotherapy in human healt care.

In assessing the potential influences of biotechnology on blood transfusions, this review is organized into the following sections: (1) *Direct Products* that contribute to safety and efficacy of blood and blood products; (2) recombinant derived *Replacement Products* for materials purified from plasma; and (3) *Indirect Products and Knowledge* that may affect the supply and use of blood and blood products in hemotherapy. The review is not all-inclusive, but representative examples are discussed in each case that illustrate the positive role of biotechnology in transfusion medicine.

Genetically engineered products that directly affect safety and efficacy

Transfusion-associated infections are major concerns of transfusion medicine. Most infections have been minimized over the last decade but have not been eliminated. Two major approaches have contributed to reduction in diseases transmitted by transfusion. In one case, blood is screened prior to use and interdicted from use if known infectious agents are probably present. Equally important is donor selection that minimizes donations from high-risk groups with the possibility of blood-borne infectious agents. In the case of infectious agent screening recombinant-derived antigen, antibodies and nucleic acids have led to improved diagnostic products that identify and diagnose blood donors with carrier states of hepatitis and AIDS viruses. In the case of high-risk donor groups recombinant-derived vaccines can serve to provide protective immunity and thereby ensure safety of blood from an immunized donor.

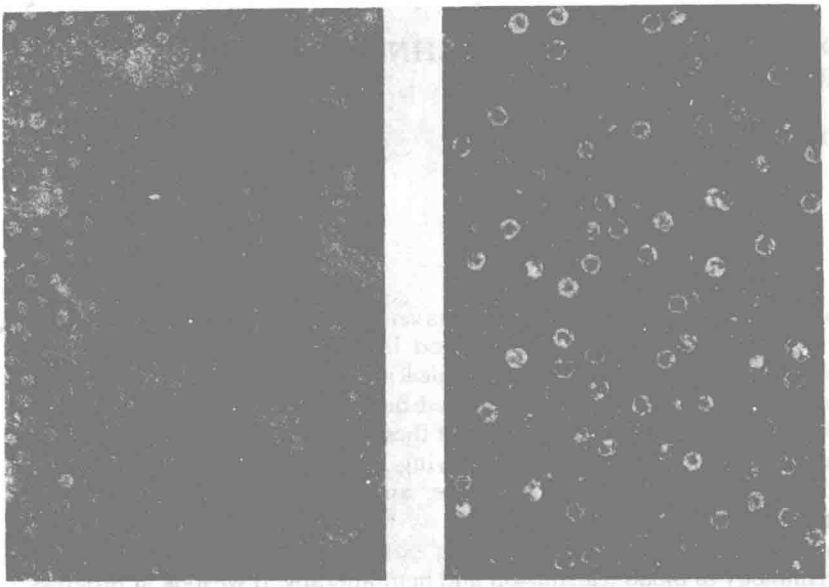

Figure 1. Electron microscope visualizations of HBsAg particles produced in yeast (left) and HBcAg particles produced in E. coli (right). Final magnification: 130,000×.

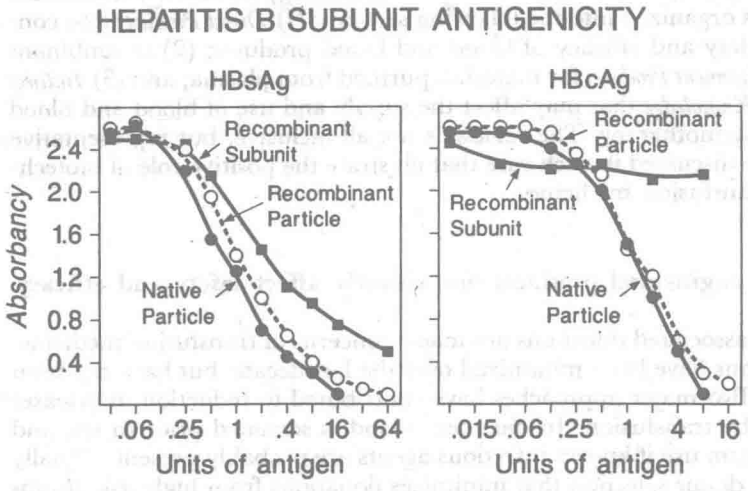

Figure 2. Comparison of native and recombinant HBsAg (left) and HBcAg (right) particles for reactivity with anti-HBs and anti-HBc, respectively, in human sera. Sera were tested for antibodies in an enzyme immuno assay using serum derived antigen. The sera were then retested after absorption with increasing quantities of native or recombinant antigens. A unit of antigen was arbitrarily defined as the quantity giving a 50% reduction in absorbancy.

Hepatitis B and AIDS diagnostics and vaccines

Hepatitis B. The hepatitis B virus (HBV) was one of the first human pathogens to be cloned and sequenced and studied at the molecular level for producing viral antigens in heterologous organisms. Valenzuela et al., in 1982, cloned the subunit polypeptide gene for the surface antigen (HBsAg), and produced HBsAg particles in yeast, identical in all respects to native particles in plasma of infected individuals [1]. Earlier, Stahl et al. expressed the HBV core antigen (HBcAg) gene in E. coli and produced core antigen particles identical to the HBV nucleocapsid particles [2]. Electron microscopic visualizations of these two genetically engineered HBV antigens are shown in Figure 1. Biophysically the particles are identical to those found in human plasma during HBV infection.

The convenient sourcing of HBsAg and HBcAg by production in microorganisms has led to the use of the recombinant materials as diagnostic reagents. Immunologic identity of the native virus antigens and the corresponding recombinant antigens is illustrated in Figure 2. As shown in this comparison, native and recombinant HBsAg and HBcAg particles were equally competitive for anti-HBs and anti-HBc, respectively, in human antiserum. The dissociated HBsAg subunit protein was also reactive with anti-HBs. However, HBsAg subunit protein was not reactive with anti-HBc. Diagnostic accuracy and the quality of commercial products should be substantially increased through the use of genetically engineered antigen particles to test for anti-HBs and anti-HBc. Currently, all commercial tests for anti-HBc use E. coli produced HBcAg. This test is now being used in the United States as a surrogate screening marker for non-A non-B (NANB) hepatitis based on two prospective studies showing a correlation of HBcAg in donor blood and NANB hepatitis in recipients [3,4].

Delta hepatitis. The delta-hepatitis agent (HDV) does not appear to be a problem of safety in blood transfusions since the agent is found only during co-infection with HBV [5]. It is presumably a defective RNA virus that requires ongoing HBV replication for its own life cycle [6,7]. Superinfection of HBV with HDV almost always results in a more severe pathologic consequence. The agent appears to be a new class of human pathogen. The techniques of molecular biology have been key to understanding the nature of HDV. Wang et al. have cloned and sequenced the single stranded, circular RNA genome of HDV [8]. They also identified that the immunogenic 'delta antigen' was produced from the anti-genomic (positive) strand, and could be readily produced in bacteria. Figure 3 illustrates the possible open reading frames and candidate virus-coded proteins for HDV. Thus far, only the proteins derived from reading frame 5 in the anti-genomic strand have been found to react with antibodies present during HDV infection. Further work at the molecular level should lead to a more complete understanding of this new hepatotropic agent. Thus far, it appears that the agent represents a new class of mammalian viruses, with some properties suggestive of plant viroids and virusoids.

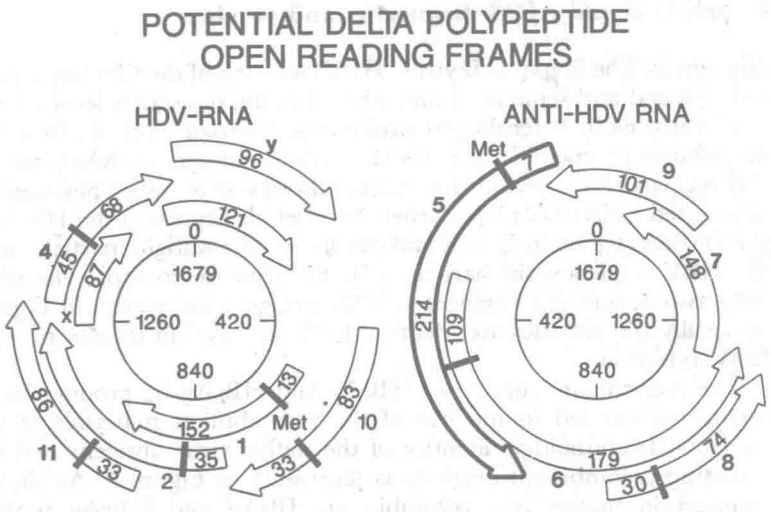

Figure 3. Map of the open frames of hepatitis delta virus genomic RNA (left) and the complementary RNA (right). Only the protein derived from reading frame in the complementary strand was found to react with human anti-delta sera.

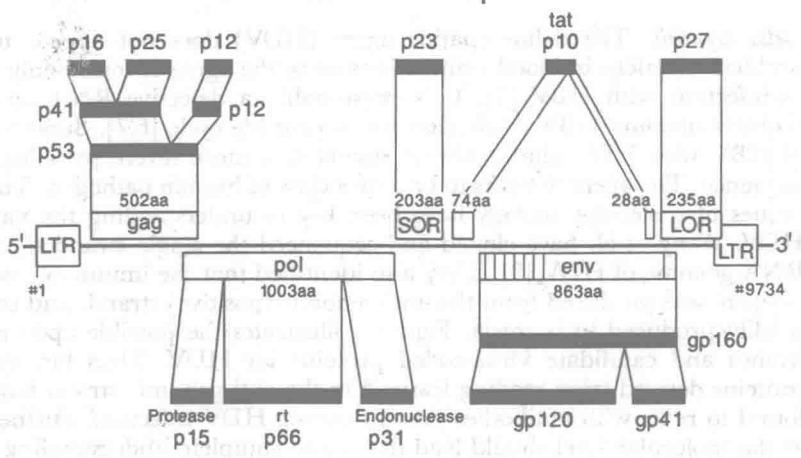

Figure 4. Genomic map of the typical human immunodeficiency virus showing the major virus proteins and their approximate molecular weight in kilodaltons.

AIDS. The Human Immunodeficiency Viruses (HIV) are currently one of the highest priority problems in public health. Transfusion associated AIDS is of special importance for blood transfusion services, and all blood collections in North America and Western Europe are routinely screened for antibodies to HIV. The initial commercial products for testing for anti-HIV have used partially purified viral lysates as antigen sources. Standardization of virus purity and control of false positive reactions have been difficult problems for these virus based tests. It is likely that recombinant derived proteins will be the method of choice for future generations of diagnostic tests for HIV antibodies and antigens.

Several HIV isolates were cloned and sequenced in 1985 [9]. The genomic map for HIV is illustrated in Figure 4, showing the major gene products of the virus. All of the virus protein studied thus far have been shown to induce antibodies in a large percentage of infected persons. These studies have been possible only as a result of producing each of the individual proteins through biotechnology processes and constructing immunoassays with the purified materials. Our laboratory estimated the percentage of sera from infected persons that reacted with each of nine HIV proteins [10]. As shown in Table 1, the reactive rates of sera varied from 37% to 99% for the individual antigens. We selected four recombinant proteins representing p-24 *gag*, p-31 *pol*, gp-41 *env* and gp-120 *env* as candidates for diagnostically relevant antigens. The four proteins were configured into a banded immunoblot format for detection of individual antibodies. Figure 5 shows the comparison of five sera tested with the recombinant immunoblot and a typical Western blot using purified HIV. The recombinant proteins showed equal immunoreactivity along with a simple and easily interpretable pattern. The procedure will be useful for rapid and sensitive confirmation of test results and for research on prognostic and diagnostic significance of antibody profiles. The availability of recombinant antigens will permit the procedures to be performed in many laboratories unable to routinely perform Western blots.

Table 1. Reactive rates of anti-HIV positive sera with individual virus proteins.

Polypeptide designation	Gene source	Percentage of sera reactive
gp120	env	89
gp41	env	98
p53	gag	87
p25	gag	76
p16	gag	41
p12	gag	not tested
p31*	pol	92
p66**	pol	80
p15***	pol	not tested
p27	LOR	37
p20	3'ORF	43
p10	tat	not tested

* endonuclease; ** reverse transcriptase; *** protease

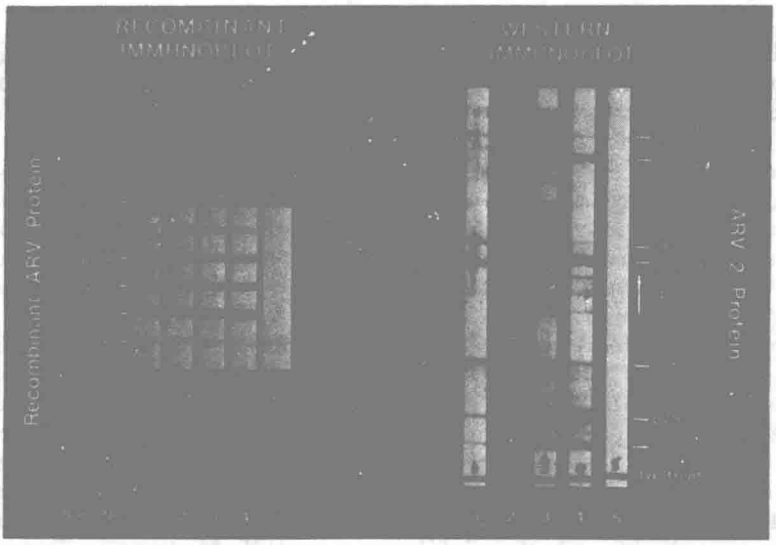

Figure 5. Comparisons of typical Western blot analyses using purified virus (right) and a recombinant immunoblot using four recombinant antigens (left) for antibodies in human immunodeficiency virus (HIV) sera. Sera 1-4 were from known infections. Serum 5 was a negative control.

Table 2. Comparison of anti-HIV reactive rates of 2013 normal blood donors in ELISA tests using recombinant antigens and purified virus.

	Recombinant antigen	Purified virus
No. of sera reactive	5	14
No. of sera confirmed positive	1	1
No. of sera non-reactive	2007	1998

Table 3. Comparison of dilution sensitivity of ELISA tests for anti-HIV with recombinant antigens and purified virus.

Serum dilution	Recombinant antigen		Purified virus	
	Absorbancy	+/−	Absorbancy	+/−
0	>3.000	+	2.320	+
1:2	>3.000	+	2.163	+
1:4	>3.000	+	1.542	+
1:8	>3.000	+	0.990	+
1:16	>3.000	+	0.520	−
1:32	2.025	+	0.361	−
1:64	1.323	+	0.230	−
1:128	0.570	−	0.157	−