



Recombinant glucocerebrosidase and Lyme disease vaccine Made by genetic engineering (No. 11 in a series of articles to promote a better understanding of the use of genetic engineering)

1. Cerezyme — recombinant protein treatment for Gaucher's disease

Gaucher's disease, the most common of the lysosomal storage diseases, is an autosomal recessive disorder resulting from a deficiency in glucocerebrosidase and the consequent abnormal accumulation of lipids within macrophage lysosomes. The lipid glucocerebroside accumulates in macrophages causing cellular enlargement. These cells, called Gaucher cells, are found in the spleen, liver and bone marrow, where they cause functional abnormalities of these organ systems.

Gaucher's disease affects approximately 5000 people worldwide, with a particularly high incidence in the Ashkenazi Jewish population. Although this disorder is potentially lethal, it can manifest itself through a wide range of clinical symptoms.

There are three principle types of Gaucher's disease. Type I disease refers to the non-neuronopathic adult form, which also has the highest prevalence and is the least understood of the three forms. Symptoms do not tend to surface until adulthood and, since the severity of disease varies so drastically from case to case, it is estimated that an unknown percentage of affected individuals are never properly diagnosed.

Type I disease, including bone problems and organ enlargement, can be successfully relieved through administration of mannose-terminated placental or recombinant glucocerebrosidase.

Types II and III, the acute neuronopathic or infantile and subacute neuronopathic or juvenile varieties, respectively, are more severe forms of Gaucher's disease.

These forms of Gaucher's disease typically involve neurologic signs, which currently available enzyme replacement therapies are unable to reverse.

The first data demonstrating the clinical effectiveness of weekly i.v. infusions of placental derived glucocerebrosidase for the treatment of Gaucher's disease were reported in 1990 by Barton et al. (Proc. Natl. Acad. Sci. USA 87, 1913–1916). In April of 1991, placental derived glucocerebrosidase (Ceredase) was approved by the FDA. By this time research into a recombinant means to express the protein was in progress. The driving force behind this effort was the anticipated lack of supply of placental derived material when full marketing would be achieved.

A recombinant production system utilizing a dihydrofolate reductase (DHFR) deficient Chinese hamster ovary (CHO) cell line was developed. The cDNA for human β -glucocerebrosidase

was obtained from Dr Ernest Beutler. This cDNA was isolated from a cDNA library derived from the W138 fetal human lung fibroblast cell line. A number of different plasmids suitable for transfection into mammalian cells were made, all derived in part from pSV2 DHFR described by Subramani et al. in 1981 (Mol. Cell. Biol. 1, 854–864).

The various vectors and transfection techniques generated more than 10 000 recombinant colonies. The colonies were systematically screened to detect production of glucocerebrosidase. Only the top 100 colonies were chosen for further development. These were expanded into cell lines and underwent gene amplification by having methotrexate added to the growth media. Following 2 months of culture, low producers were eliminated from further analysis.

At 6 months the gene amplification was complete and only six cell lines were considered to be candidates for recombinant glucocerebrosidase (rGCR) production.

The manufacturing process developed for production of rGCR (Table 1) is an anchorage dependent production system utilizing extended protein-free cultivation of the cells. The cells are initially propagated in a serum-based media formulation on microcarriers in spinner culture vessels. This dramatically increases the available surface area on which the cells can grow. When confluence is reached, the cultures are switched to a protein-free media formulation designed to maintain the cells in a viable, non-growing state. The protein-free nature of the media greatly facilitates purification.

Analysis of the rGCR in the supernatant of the cultures under microcarrier conditions demonstrated significantly more glucocerebrosidase in the culture media than would have been predicted from production levels observed in the initial cell lines. In addition, there was a significant decrease in the pH of the culture media. The pH dependence for rGCR activity in the production medium was experimentally confirmed. The lower the pH, the more rGCR was remaining. Experiments with cell culture showed a significant decrease in cell viability below pH 6.7, so setting manufacturing conditions below that level was not possible. But even at pH 6.2, there was a significant loss of rGCR activity.

This led to an examination of the components in the production media. Extensive experiments indicated that there were components in the medium which were involved in the inactivation of rGCR. The addition of bovine serum albumin to the medium had a protective effect, but would have been costly and excessively difficult to purify away from the rGCR.

Therefore, a large series of agents were examined to determine their effect on rGCR degradation. Of the compounds examined, a series of agents were found which were effective. They stabilized rGCR activity in media in both a dose and pH dependent manner. The lower the pH or the higher the agent concentration, the more rGCR was recovered.

Even when these changes to stabilize the recombinant protein are coupled with improvements in the manufacturing system, expression of rGCR is

Table 1
Made by genetic engineering, no. 11

Product	Recombinant glucocerebrosidase	Lyme disease vaccine
Principal trade names	Cerezyme®	LYMErix
Principal uses	Treatment of Gaucher's disease Type I	Prevention of Lyme disease
Manufacturers	Genzyme Corporation, USA	SB Biologicals, Belgium
Donor organism	Fetal human lung fibroblasts	<i>B. burgdorferi</i>
Host organism	CHO (Chinese hamster ovary)	<i>Escherichia coli</i>
Advantages	Highly clinically effective for treatment of Gaucher's disease Safe and unlimited supply Near absence of any side effects	Produced in complete animal-free medium Highly protective against Lyme disease Unlimited supply

only 10–20% of that achieved for other recombinant therapeutic proteins. The reason for this is not known. Despite these challenges, recombinant production of the enzyme provides a clearly safe and sufficient supply of enzyme for Gaucher patients in need of this therapy.

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2. An effective vaccine against Lyme disease

Lyme disease (LD) or Lyme borreliosis was first recognized as a distinct clinical entity in 1975 in Lyme, Connecticut. LD is a multisystem inflammatory disorder with dermatologic, neurologic, rheumatic and cardiac manifestations. The etiologic agent of this disease is the spirochete *Borrelia burgdorferi*, transmitted to human mostly by Ixodes ticks.

Reports of symptoms compatible with Lyme disease are found in the medical literature as early as in 1883. Alfred Buchwald from Breslau, Germany, described then a 'diffuse idiopathic skin atrophy' which is the earliest description of acrodermatitis chronica atrophicans, a late manifestation of Lyme borreliosis (cited in Herxheimer and Hartmann, 1902, Arch. Dermatol. Syph. 61, 57–76; 255–300).

The causative agent of LD was isolated by Willy Burgdorfer in 1982 from the midgut of an infected deer tick, on an island close to Long Island, NY. Since then 100 000 cases were recorded by the US Centers for Disease Control and Prevention, most of them (80%) occurring in five states (Connecticut, New Jersey, NY, Pennsylvania and Rhode Island).

LD is also widespread in Europe (e.g. Germany, Austria, Switzerland, France, Belgium, Sweden) and correlates with the geographic ranges of Ixodes ticks and the percentage of ticks infected. In Belgium, at least 200 cases are recorded yearly. *B. burgdorferi* is sensitive to antibiotics which are the basis of the treatment of the disease.

In 1990, SB Bio has initiated the development of a vaccine against Lyme disease (Table 1). At that time, OspA, a lipoprotein present on the surface of the bacteria, had been identified as capable to induce a protective immunity against *B. burgdorferi*.

The culture of *Borrelia* strains is quite fastidious. Not only does it require a complex medium containing several products of animal origin, but it also grows slowly, and only to low cell densities. Because of these difficulties, the production of OspA is made recombinantly in *Escherichia coli*. This bacterium grows rapidly to high cell densities and fully synthetic, controlled media could be used. Further, it expresses OspA at high level, and processes it into a lipoprotein identical to the molecule found in *B. burgdorferi*.

This post-translational addition of lipids at the N-terminus of OspA is critical for its immunologic properties. The unlipidated form of the protein is indeed poorly immunogenic: when injected to mice, it induced the production of antibodies at levels at least 10-fold lower than its lipidated counterpart.

The protective efficacy of OspA in humans has been demonstrated in a large scale trial involving more than 10 000 volunteers on the East coast of the United States. Half of these volunteers have received three doses of 30 µg of OspA, at months 0, 1 and 12, and have been followed for 2 years in 1995 and 1996. The results of this trial showed that after three injections, 85% of the vaccinees were protected against confirmed Lyme disease, and 100% of them were protected against asymptomatic infection, as determined by the seroconversion to multiple *Borrelia* antigens during the follow-up period. SmithKline Beecham Biologicals has received in December 1998 a license from FDA to sell the new vaccine called LYMERix. It is currently marketed in the United States.

The mechanism of protection of this vaccine is unusual. The understanding of the interactions between *Borrelia* and its vector, the Ixodes tick, is critical: when a *Borrelia*-infected tick bites a mammal, the bacteria are present only in its midgut and cannot be transmitted directly into the mammal's blood directly; *Borrelia* must first migrate to the tick's salivary glands, from where it is injected into the host skin with the saliva. This whole transmission process takes more than 24 h. Interestingly enough, *Borrelia* expresses OspA at high level when located in the midgut of the tick. Upon migration of the spirochete in the salivary glands of the tick, or after its transmission into the skin and blood of the mammalian host, the expression of OspA is dramatically reduced, to undetectable level.

Because of this strict differential expression of OspA by *Borrelia*, the anti-OspA antibodies are thought to kill *Borrelia* in the tick midgut, before the transmission to the mammalian host takes place. This has been confirmed in preclinical experiments, where the *Borrelia* content of infected ticks was analyzed after completion of their blood meal: when ticks feed on control animals, *Borrelia* are recovered from their midgut after the blood meal; when they feed on OspA-vaccinated animals, how-

ever, no or, unfrequently, very low number of spirochetes are recovered, indicating that they have been destroyed by the anti-OspA antibodies.

If in the United States only one species of *Borrelia* (*B. burgdorferi sensu stricto*) is responsible for Lyme disease, three species of *Borrelia* have been found in humans suffering of this illness in Europe: *B. burgdorferi sensu stricto*, the species present in the United States, *B. garinii* and *B. afzelii*. The sequence divergences of OspA between the three species do not allow a single molecule to protect against all three species. SmithKline Beecham Biologicals is currently developing for the European market a trivalent vaccine containing one OspA derived from each *Borrelia* species.

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The products published in this series so far are summarised in Table 2.

Table 2
Summary of products published in the series MBGE

Product	Author/manufacturer	Reference
Lipase	B. Diderichsen, Novo Nordisk, Denmark	MBGE No. 1, Biotech. Forum Europe 8, 246-247, 1991.
Hepatitis B vaccine	P. Crooy, SmithKline Beecham Biologicals, Belgium	
Human insulin	E. Rasmussen, Novo Nordisk, Denmark	MBGE No. 2, Biotech. Forum Europe 9, 144-145, 1992.
Human growth hormone Protein G	L. Fryklund, KabiPharmacia, Sweden R. Hjorth, Pharmacia LKB Biotechnology, Sweden	MBGE No. 3, Biotech. Forum Europe 9, 641-642, 1992.
Interferon alfa-2a	S. Ryser, F. Hoffmann-La Roche AG, Switzerland	
AIDS test	E. Baumann, F. Hoffmann-La Roche AG, Switzerland	MBGE No. 4, J. Biotechnol. 38, 193-197, 1995.
α -Amylase	B. Diderichsen, Novo Nordisk, Denmark	

Table 2 (Continued)

Product	Author/manufacturer	Reference
Erythropoietin	C. Kionka, Boehringer Mannheim, Germany	MBGE No. 5, J. Biotechnol. 43, 73–77, 1995.
Interferon beta-1b	T. Petri, Schering AG, Germany	
Interferon gamma	E. Falkner and I. Maurer-Fogy, Bender & Co GesmbH/Boehringer Ingelheim Vienna, Austria	MBGE No. 6, J. Biotechnol. 46, 155–159, 1996.
Rabies vaccine	J. Terré, G. Chappuis, M. Lombard and P. Desmettre, Rhone Mérieux, France	
Tissue plasminogen activator (rt-PA)	W. Werz and R.G. Werner, Dr Karl Thomae GmbH/Boehringer Ingelheim Pharma, Germany	MBGE No. 7, J. Biotechnol. 61, 157–161, 1998.
Granulocyte-macrophage colony-stimulating factor (GM-CSF)	R. Till, Novartis/Schering Plough International, Switzerland	
Human coagulation factor VII	U. Hedner and T. Lund-Hansen, Novo Nordisk A/S, Denmark	MBGE No. 8, J. Biotechnol. 61, 231–236, 1998.
Folicle stimulating hormone (FSH)	J.C. Heikoop and W. Olijve, N.V. Organon, Akzo Nobel, Oss, The Netherlands	
Alzheimer tau test	E. Vanmechelen and H. Vanderstichele, Innogenetics N.V., Belgium	MBGE No. 9, J. Biotechnol. 66, 229–233, 1998.
Detergent cellulase	B. Jones and W. Quax, Genencor International, The Netherlands	
Chymosin	P.W.M. van Dijck, Royal Gist-brocades, The Netherlands	MBGE No. 10, J. Biotechnol. 67, 77–80, 1998.
Phytase	P.W.M. van Dijck, Royal Gist-brocades, The Netherlands	
Recombinant glucocerebrosidase	H. Hoppe, Genzyme Corporation, USA	MBGE No. 11, J. Biotechnol. 259–263, 2000.
Lyme disease vaccine	P. Crooy and Y. Lobet, SB Biologicals, Rixensart, Belgium	