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Rabies Vaccines

A Review of Progress Towards Improved Efficacy and Safety

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Contents

Abstract

Over the years, the technology for producing human rabies vaccines has undergone many improvements. These improvements consist in the use of tissue cultures for the production of viral antigens, replacing the former nervous tissue substrate vaccines. The low virus yields in tissue cultures led to the development

of the concentration and purification of virus supernatants. Another technical improvement was obtained by using microcarriers for virus production in VERO cell suspension cultures. This technique permits commercial-scale production of rabies vaccine, lowering production costs and thus extending the availability of the vaccine to a broader population in developing countries.

Besides improvements in rabies vaccine production technology, the use of various vaccination regimens and routes of administration in field trials has resulted in considerable gains in our experience of postexposure treatment (PET) of this disease. The standard WHO recommended regimen for PET using concentrated and purified tissue culture vaccines consists of a 5-dose course of intramuscular injections at days 0, 3, 7, 14 and 28. Reduced vaccination regimens such as the 2-1-1 have been proven to be efficient in raising protective antibody responses. Reduction in the total volume of rabies vaccine is also possible by using the intradermal route of injection, provided the vaccine is administered at several sites. The overall consequence is a progressive shift in the worldwide use of rabies vaccines from those of nervous tissue origin to the contemporary tissue culture vaccines.

Unlike most other infectious viral diseases, rabies is the only one for which postexposure treatment (PET) based on vaccination can be performed. For all the other viral infections, only preventive vaccinations can be applied. The unique situation with rabies occurs because the contamination occurs mainly through a well identified event: a bite from a potentially rabid animal. This is not the case for most other viral diseases (such as influenza, polio or measles). PET for rabies is thus a race between the spread of the virus from the site of the virus entry (a wound) to the final target cells (neurons in the CNS), and the elicitation of an immune response able to eliminate the virus from the organism, preventing the disease from occurring.

During the past century, since the discovery of a rabies vaccine by Louis Pasteur, continuous efforts have been made to produce more potent vaccines containing fewer exogenous biological proteins, specifically by reducing and ultimately eliminating nervous tissues present in the vaccines produced in brain tissues. Another aim is also of importance: the production of more economical vaccines for use in tropical regions and in developing countries.

Rabies vaccination is also the only vaccination procedure that involves a series of inoculations. Although the number of injections has been significantly reduced by using tissue culture instead of nervous tissue vaccines, clinical trials have established the limits of the reduction possible without impairment of the protective antibody response.

1. World Rabies Situation

World wide, the rabies situation is continually evolving. In some areas, difficulties in collecting information are a major drawback to the attainment of comprehensive data from different continents. Human rabies has a low incidence in industrialised countries (North America, Europe) and is not found in island countries such as Australia, Great Britain, Japan, New Zealand, etc. The incidence is also relatively low in Latin America, but the data for Africa are incomplete. Cases of human rabies are mostly concentrated in Asia, and mainly on the Indian subcontinent (India, Pakistan, Bangladesh, Nepal); in other Asian countries, the incidence is lower. Estimates of the number of cases of human rabies infection during the last decades range from 35 000 to 45 000 annually (table I). It is interesting to note that after elimination of the risk of transmission of dog rabies in industrialised countries (North America and Europe), the disease reservoir and vectors

Table I. Estimated incidence of human rabies cases

Total	**35 000-45 000**
Europe	10-20
North America	4-8
Latin America	200-400
Africa	500-5000
Asia	35 000-45 000
(India	30 000-40 000)

have shifted to wildlife, whereas in Africa and Asia the dog remains the major vector for transmission of the disease.

1.1 North and South America

From the 1996 report on rabies surveillance in the US,[1] it appears that the situation is extremely complex. A total of 7877 nonhuman cases were reported in 1995, from which a majority (92%) were wild animals (raccoons, 3964; skunks, 1774; foxes, coyotes, etc., are also involved). Several species of bats were among the animals found to be rabid (787 cases), with an increase of 24.7% over the 1994 figures. It must be noted that bats have been involved in half of the cases of rabies which have occurred during the past decade, most of the patients presenting without a history of exposure.

From the 1995 report of the rabies situation in the Americas,[2] the annual number of cases in humans in Latin America has been reported to be about 200 (table II), although unreported infections might occur.

1.2 Europe

The history of rabies in Europe has been one of continual change during the past century. After the elimination of dog rabies by the vaccination of domestic animals and massive elimination of stray dogs, several Western European countries were rabies-free for various periods of time. Following the Second World War, adaptation of the rabies virus to the red fox allowed the disease to spread to most European countries, with the exception of those bordering the Mediterranean countries. The attempt to eliminate fox rabies by hunting and poisoning did not prevent the spread of the disease, and it was only after the use of oral vaccines in animal baits that a continuous reduction in the global incidence of rabies could be seen (table III).

Human rabies is rare in Europe. Most reported cases are from dog bites in the former Soviet Union (an average of 10 or fewer cases annually), or imported cases of patients who were bitten by dogs in countries where the disease is still endemic (Asia, Africa).[3]

1.3 Asia

The main vector in Asia is the dog. The Asian continent accounts for the majority of human rabies cases.[4] There is a general consensus that the approximate number of deaths is 40 000 annually; India accounts for the great majority. Several countries have established national programmes for rabies prevention and prophylaxis, significantly reducing the number of human cases (China, Indonesia, Malaysia, Thailand), while the situation remains serious in others (India, Bangladesh, Nepal, Sri Lanka, Myanmar, Laos).

1.4 Africa

The dog remains the major cause of human rabies in Africa (average of 90% of cases). Over 4000 animal rabies cases have been reported per year, of which 2600 have been confirmed by laboratory diagnosis.[5] It is also likely that a high proportion of rabies cases are not reported, especially in remote areas. Human rabies (141 cases in 1994)[5] is mainly reported as having been clinically diagnosed as fewer than 10% of cases are diagnosed by

Table II. Annual number of cases of rabies in North and South America

1995	154
1994	150
1993	216
1992	225
1991	215
1990	252
1980-1989	293 (mean value)
1970-1979	255 (mean value)

Table III. Annual number of reported cases of rabies in Europe

	Wildlife	Domestic	Human
1996	5 385	2673	8
1995	5 816	2750	10
1994	6 698	2284	9
1993	6 914	2088	3
1992	8 330	2685	6
1991	12 585	2894	0
1990	14 765	4791	10
1989	16 975	3762	4
1988	13 142	2933	0

laboratory techniques. Again, there is a high probability that the number of human cases is underestimated.

2. History of Rabies Virus Vaccine

2.1 The Original Pasteur Vaccine

It is well documented that Galtier performed the first attempts to prevent rabies by experimental immunisation of dogs with intravenous inoculation of infectious virus.[6] However, it was Louis Pasteur who designed the concept of vaccination using attenuation of virus infectivity for the immunisation of naïve organisms. The attenuation of viral infectivity was directly performed on the virus-infected rabbit spinal cord. The virulence was reduced by serial passages of the virus in the nervous tissues of rabbits and by desiccation at room temperature. After testing the vaccine in dogs, the rabies virus was applied to humans for the first time in 1885.[7]

2.2 The Improved Brain Tissue Vaccines

Pasteur's original vaccine consisted of daily injection of spinal cord preparations. The vaccines contained high concentrations of nervous tissues and residual infectious virus particles. Progressive dessication of spinal cords was performed to attenuate the virulence of the vaccine. During the first days of the treatment, the patients were innoculated with highly attentuated preparations and subsequently with nervous tissues containing increasing amounts of infectious virus. There were 3 main drawbacks for the original vaccine as prepared by L. Pasteur: difficulty in maintenance of spinal cords at different times of dessication, occurrence of cases of vaccine recipients developing rabies and accidental paralysis because of the presence of high concentration of myelin.

Major modifications were introduced by Fermi,[8] and Semple[9] who used sheep brain homogenates, increasing the reproducibility of vaccine preparations. Chemicals were used for attenuating the virulence of the rabies virus (phenol), or for total inactivation of the vaccine (β-propiolactone). These vaccines are still prepared in several countries with either sheep or goat brain.

The use of younger animals significantly decreased the content of myelin, thus reducing the percentage of vaccination adverse effects such as neuroparalytic accidents. In this regard, the production of inactivated rabies vaccine in suckling mouse brain has been an important improvement for developing countries where the technology for tissue culture on a commercial scale is not yet available.[10] This vaccine is still widely used in Latin America, Asia and Africa.

2.3 The Avian Embryo Vaccine

A chick embryo rabies vaccine was first developed by Koprowski and Cox in 1948,[11] after attenuation of virulence by passaging the Flury virus strain. In 1959, Powell and Culbertson[12] developed a human vaccine in duck embryos. This vaccine consisted of a 10% duck embryo suspension inactivated by β-propiolactone. Although it elicits poor antibody response and gives rise to frequent allergic reactions, it has been extensively used in the US. This vaccine has been improved through concentration and purification by gradient density centrifugation.[13,14] The result is the production of an immunogenic vaccine of excellent quality, very similar to the standards of those obtained with tissue cultures.

3. Tissue Culture Vaccines

The use of tissue culture vaccines has been a considerable breakthrough in the improvement of rabies vaccines, since these compounds totally

eliminate the nervous tissue. The first attempts to use cell culture for the replication of rabies virus were performed in primary hamster kidney cells in 1958,[15] and in human diploid cells (HDC) in 1964.[16] The first studies to use virus supernatants for the development of experimental vaccine were performed as far back as 1963 by Kissling and Reese with hamster kidney cells,[17] and 1965 by Wiktor and Koprowski with HDC.[18]

However, commercial production of rabies vaccines was hampered by difficulty in obtaining high-titre virus yields from infected cell culture supernatants. The alternative is, of course, to concentrate the infected supernatants. However, this step of vaccine production would also concentrate non-viral proteins, capable of inducing unwanted reactions. Furthermore, it would also increase the amount of cellular DNA material in the final product. As a consequence, rabies tissue culture vaccines are relatively expensive in comparison with other viral vaccines prepared on similar cellular substrates. However, recent vaccine technology has permitted production on a larger scale, significantly increasing the total amount of vaccine produced in recent years (table IV).

3.1 Primary Hamster Kidney Cell Vaccine

Rabies vaccine prepared on primary hamster kidney cell cultures was the earliest vaccine to be produced on a large scale. Although other cells (canine, bovine, etc.) have been used for producing rabies vaccine, none has been produced commercially over a long period. The hamster kidney cell vaccine was originally evaluated in animals in Canada.[19] Using different rabies virus strains, the former USSR[20] and China[21] are producing these inactivated vaccines on a commercial scale. In both cases, the virus supernatant is inactivated. The primary Syrian hamster kidney cell vaccine using the Vnukovo-32 virus strain has been developed at the Institute of Poliomyelitis and Encephalitic Diseases in Moscow. This vaccine is inactivated by ultraviolet irradiation and has been tested in non-concentrated and concentrated form. A similar vaccine using the Beijing strain has been developed in China. It is produced on a large scale in national vaccine institutes and is widely used (6 million doses per year).[22]

3.2 Human Diploid Cell Vaccine

The use of primary cultures for the production of vaccines is limited by the scale of the production, and the lack of reproducibility of vaccine potency in each batch. In addition, the risk of oncogenicity of transformed cell lines has limited their use to animal rabies vaccine production. A rabies vaccine was prepared on HDC at the Wistar Institute in Philadelphia, using the Pittman Moore (PM) strain.[18] This vaccine was the first to be produced commercially on a large scale by the Institut Mérieux, France. Clinical trials and laboratory experiments have established that HDC rabies vaccine elicited neutralising antibodies at higher levels than those obtained with nerve tissue vaccines.[23,24] This vaccine was successfully used in Iran for PET,[25] and has been extensively used in the US. Today, the HDC rabies vaccine is prepared on MRC-5 HDC using the Pitman-Moore L503 3M strain. The infected supernatant from cell cultures is inactivated by β-propiolactone and concentrated by ultrafiltration (Pasteur Mérieux Sérums et Vaccins, France).

Rabies vaccine prepared in these cells has been used in different countries. In Canada, the ERA virus strain has been used (Connaught Laboratories). In Germany, the vaccine is concentrated and purified by zonal centrifugation (Behring Institute, Germany). A similar HDC rabies vaccine is also produced in Japan on a small scale.

Table IV. Estimate of the annual production of purified concentrated vaccines (1996)

Vaccine (manufacturer)	No. of doses
PVRV (PM-Connaught)	3 000 000
HDCV (PM-Connaught)	600 000
PDEV (Berna)	500 000
PCEC (Behring)	3 500 000
Estimated total production	7 600 000

HDCV = human diploid cell vaccine; **PCEC** = purified chick embryo cell; **PDEV** = purified duck embryo vaccine; **PVRV** = purified VERO rabies vaccine.

This type of HDC vaccine, of which we now have more than 20 years' experience, has demonstrated its reliability and efficacy together with low levels of reactions. However, due to low virus yields and the necessity of expensive concentration procedures, the high cost of this vaccine militates against its widespread use in developing countries.

3.3 VERO Cell Vaccine

A rabies vaccine using cell line as a substrate for replication of rabies virus was developed in the 1980s. This vaccine took advantage of 2 elements. The first was the development of cell cultures on microcarrier as described originally by Van Wezel in 1967.[26] This made possible the large-scale production of vaccines on microcarrier-adherent cells in suspension. The second was the use of a cell line established from a primary culture of kidney cells prepared from the vervet monkey (*Cercopithecus aethiops*).[27] This cell line was used for the preparation of both polio and rabies vaccines.[28] The absence of potential tumourigenicity of the VERO cell line was assessed and it was established that this cell line should be used within the 142nd passage level with regard to the passage number described by the American Type Culture Collection (ATCC).[29] The vaccine consists of ultrafiltration-concentration of infected supernatants. After purification by sucrose-gradient, the virus suspension is inactivated by β-propiolactone, supplemented with 5% human serum albumin and distributed as 1.0ml aliqouts and freeze-dried.[30]

Preparation of a large scale suspension of VERO cells on microcarriers permitted the production of a potent vaccine with a minimum value of 2.5IU per dose, which can be widely used at a lower cost than that of the HDC vaccine.[31,32] Clinical studies demonstrated its potency in eliciting neutralising antibodies[33] and in PET of patients exposed to canine rabies contamination in Thailand.[34]

3.4 Purified Chick Embryo Cell Vaccine

Production of rabies vaccine from chick embryo cells had started as early as 1974[35] using the Flury HEP rabies strain. This strain had previously been adapted to grow in chick embryos in 1948.[36] However, adverse allergic reactions prompted the development of a purified chick embryo cell vaccine using the Flury LEP rabies virus strain.[37]

The purified chick embryo cell vaccine is currently produced by Behringwerke, Marburg, Germany. This vaccine has been shown to be efficacious in postexposure protection in experimental rabies infection and in clinical trials.[38] Preparation requires specific pathogen-free eggs. This vaccine is prepared by infection of primary chicken fibroblasts using the Flury LEP-C25 strain. The viral supernatant is concentrated, purified and delivered as a freeze-dried β-propiolactone–inactivated virus preparation.[39] A similar vaccine produced on chick embryo cells is also distributed in Japan.[40]

Most of the tissue vaccines produced today were licensed more than 10 years ago. Thus, there is extensive experience with these vaccines which have been used in both developing and industrial countries.

3.5 Adsorbed Rabies Vaccine

An adsorbed rabies vaccine has been developed and distributed by the Michigan Department of Public Health in the US. This vaccine is prepared from the CVS (Kissling strain) rabies virus which had been adapted to fetal rhesus lung diploid cells.[41,42]

4. Rabies Vaccination

4.1 History

The historical immunisation of L. Meister on 6th July 1885 by Louis Pasteur was performed with a regimen of daily inoculation of spinal cord homogenate, for 10 days; the homogenate had from 14 days to 1 day of inactivation.[7] Subsequently, several regimens ranging from 15 to 25 daily inoculations were applied. Naturally, PET failures and adverse effects were recorded.[43] With the introduction of suckling mouse vaccine, which contained higher virus concentrations, better protec-

tive effects were obtained and the number of inoculations reduced. Again, the real breakthrough came when commercially prepared tissue culture vaccines became available.

4.2 World Situation of Rabies Vaccination

Because rabies is mainly prevalent in tropical countries where the disease is still common in humans, PET is often performed using brain tissue rabies vaccines. With some exceptions, it is the general case in Africa, Asia and Latin America. In most Western European countries and in North America, rabies vaccinations are exclusively performed with purified and concentrated tissue culture–derived vaccines, with the exception of a duck embryo purified rabies vaccine. Rabies PET of humans occurs on all continents, whether the country is free from rabies or the disease is endemic.

4.3 Recommended Vaccination Regimens

Despite recommendations by the WHO[44] that tissue culture vaccines be used in place of brain tissue vaccines, the majority of antirabies treatments are still performed either with suckling mouse brain or sheep brain vaccines. This is the case for most of the developing countries which cannot afford the high cost of tissue culture vaccines. The vaccination regimens recommended by the WHO are mainly those established for tissue culture vaccines which are used in industrialised countries, whereas local vaccination regimens for brain tissue vaccines vary from one country to another, and even from one institute to another in the same country. Thus, the regimens vary according to the type and origin of the vaccines. Their application also depends on the level of exposure according to the WHO recommendations (table V). The vaccines can be divided into 3 main groups: those utilising brain tissue; those prepared from unconcentrated and/or unpurified tissue cultures; and those prepared from concentrated and purified virions, whether they are obtained from avian embryos or from tissue cultures.

4.3.1 Brain Tissue Vaccines

The Semple vaccine is inoculated as 2- to 5ml doses of a 5% sheep brain homogenate, β-propiolactone– or phenol-inactivated. The vaccination regimen usually consists of 10 daily injections. In cases where the bite is category III (table V), the patient is given immunoglobulin with boosters given at days 17 and 30. This vaccine is produced and utilised in India, Pakistan and several African countries.

The vaccine used most often is the suckling mouse brain inactivated vaccine, which is widely produced and used in Latin America and several countries in Asia.

4.3.2 The Essen Vaccination Regimen

The PET regimen (also known as the Essen vaccination regimen) for rabies tissue culture vaccines has been defined in the guidelines of the WHO.[44] It consists of 5 to 6 intramuscular inoculations on days 0, 3, 7, 14, 28 and on day 90 (optional). This regimen has been shown to elicit high virus-neutralising antibody titres in humans and is widely applied. The 5-dose regimen is highly recommended in countries where rabies is endemic and where human deaths from the disease are often reported. It is to be used especially in cases where the treatment is associated with antirabies serum.

5. Reduced Vaccination Regimens

5.1 The 2-1-1 Regimen

Several modifications of the original Essen vaccination regimen have been reported. The most commonly used is the 2-1-1 regimen. Tested in Europe and also in countries where rabies is endemic, this regimen has proved to be efficient and to have the great advantage that the number of treatment visits is reduced (3 instead of 5).[45] It consists of 2 doses of vaccine applied one on each side of the deltoid region on day 0 and subsequent vaccination with a single dose on days 7 and 21 (hence the designation 2-1-1). This regimen has been shown to induce early neutralising antibody response and has been effectively tested with several concentrated and purified vaccines.[45,46]

5.2 Intradermal Inoculation of Vaccines

In order to use less antigen and thus reduce the cost of rabies treatment, intradermal inoculation of concentrated and purified rabies vaccine has been extensively investigated. Much of the data come from clinical trials in Thailand using HDC strain vaccine.[47] However, there are several drawbacks to its more general use. One is the need for well trained medical personnel in order to avoid failure in performing the intradermal inoculation; incorrect inoculation could lead the vaccinee to receive only one-fifth to one-tenth of the dose. This failure can result in insufficient protection. The second drawback is the use of vaccine in clinics where the full amount is not used immediately, and the remainder might be used after having been kept under inadequate conditions of conservation. To prevent this, it is recommended that the intradermal vaccination is applied only in institutions where large numbers of patients are treated every day. Finally, the WHO recommends that the intradermal route be used under the responsibility of the national health authorities.[48] Other intradermal regimens of vaccination have been described, such as the multiple site, where the vaccine is injected at 8 different sites on day 0 instead of the 2-site regimen.[49]

5.3 The Pre-Exposure Vaccination

The pre-exposure vaccination of individuals with a high risk of contamination with rabies is recommended to be performed using a purified tissue culture vaccine.[44] The vaccination consists of 3 doses of vaccine, with a minimum antigenic value of 2.5IU per dose, by the intramuscular route, administered on days 0, 7 and 28. The efficiency of the immunisation is monitored by titration of rabies-neutralising antibodies 1 to 3 weeks after the last dose.

5.4 Post-Exposure Treatment with Antirabies Serum

The third parameter for the efficacy of rabies vaccine for PET is the association of active immunisation with serotherapy. The combination of rabies vaccine and antirabies sera for the treatment of severely bitten patients has been a source of debate for several decades. Habel and Koprowski[50] reported data supporting the use of antirabies serum both in the laboratory and in wolf-bitten patients. A field study conducted in Iran definitively established the synergy of antirabies serum used concomitantly with rabies vaccine.[51] From one group of 13 patients (12 of 13 survived), one died from rabies after treatment with the serum-vaccine combination whereas only 2 of 5 patients survived from a second group treated with the vaccine only. Experimentally-induced rabies in dogs and mice also gave clear arguments for the use of serum in combination with the postexposure vaccination.[52,53]

Table V. Guide for postexposure treatment [after World Health Organization (WHO) recommendations][44]

Category	Type of contact[a]	Recommended treatment
I	Touching or feeding of animals Licks on intact skin	None if reliable case history is available
II	Nibbling of uncovered skin Minor scratches or abrasion without bleeding Licks on broken skin	Administer vaccine immediately. Stop treatment if animal remains healthy throughout an observation period of 10 days or if animal is killed humanely and found to be negative for rabies by appropriate laboratory techniques
III	Single or multiple transdermal bites or scratches Contamination of mucous membrane with saliva (i.e. licks)	Administer rabies immunoglobulin and vaccine immediately. Stop treatment if animal remains healthy throughout an observation period of 10 days or if animal is killed humanely and found to be negative for rabies by appropriate laboratory techniques

a Defined as contact with a suspected or confirmed rabid domestic or wild animal, or animal unavailable for observation.

6. Research and Development in Rabies Vaccines

6.1 Subunit Vaccines

Experimental subunit vaccines have been developed in several laboratories using solubilised rabies glycoprotein from purified virions.[54-56] A soluble form of rabies glycoprotein is produced by proteolytic cleavage of the glycoprotein. This protein, containing 447 amino acids, consists of the extracellular domain of the full length glycoprotein and lacks the cytoplasmic tail and part of the transmembrane domain, and is antigenic.[57] Expression of a soluble form of glycoprotein in transfected Chinese ovary fibroblasts can assemble in homodimers and homotrimers and has exhibited antigenic and immunogenic properties.[58]

6.2 Polypeptides

The alternative of using synthetic peptides has been tested in laboratory animals. The resulting antibodies have been shown to react with the corresponding peptides and to the epitopes of viral antigens. However, neither neutralising antibody production nor protective activity at a significant level could be obtained for practical use. The possibility of using anti-idiotype antibodies as a vaccine has not yet been demonstrated to be applicable for rabies protection.[59]

6.3 Immunosomes, ISCOMS and Microspheres

In order to increase the antigenicity of subunit vaccines, insertion of virus glycoprotein has been tested in various systems, virosomes[60] and Immune Stimulating Complex (ISCOMS).[61] Although experimental data indicate enhanced antibody production for some preparations, the overall results do not suggest a practical use for these systems.

More recently, rabies nucleoprotein peptides have been incorporated into different poly (DL-lactide-co-glycolide) formulations to modify the release rate into the organism.[62] More data are needed before we can consider the use of microencapsulation a promising means of delivering rabies virus antigens into the organism.

6.4 Genetic Engineering

The prospect of new generations of rabies vaccines based on the use of genetic engineering has triggered research into the development of a series of experimental vaccines. The first recombinant virus expressing the rabies glycoprotein was constructed using the ERA virus strain glycoprotein mRNA. Here, double-stranded cDNA copy was inserted into the *Bam* HI site downstream of the vaccinia gene promoter, in the thymidine kinase gene.[63,64] The vaccinia-expressed rabies glycoprotein was identified as similar to the ERA rabies virus glycoprotein by radioimmunoassay experiments using monoclonal antibodies against the virus glycoprotein epitopes.[65] Vaccination using the V-RG recombinant virus produced protective neutralising antibodies, provided the antigen was glycosylated.[66,67] Resistance to street rabies virus challenge was demonstrated in laboratory animals[65] as well as in domestic and wildlife species.[68]

Expression of the rabies glycoprotein has been achieved using other systems: in M13 (a phage vector for genes) and in *Escherichia coli*,[67] baculovirus,[69] and also in yeast using an expression vector which contains an inducible promoter from the copper metallothienein gene.[70] Human adenovirus type 5 has been used for the construction of a recombinant virus (AdRG1).[71] Use of canary pox vectors has also triggered development research for expression of rabies vaccines.[72,73]

There is no doubt that during the past few years genetic engineering techniques and concepts have raised new hopes for the development of new generations of vaccines. The most surprising and exciting results came from the finding that intramuscular inoculation of a simple 'naked' DNA molecule coding for a viral antigen could elicit a significant immune response. Experimental inoculation of purified plasmid vectors leads to *in vivo* transfection of cells and expression of correspond-

ing proteins. In the case of rabies, inoculation of a plasmid vector expressing the full-length rabies virus G protein under the control of the SV4O early promoter results in the elicitation of T cells and a neutralising antibody response.[74,75]

However, although the experimental data are promising, much remains to be investigated before such vaccines can be used in clinical conditions. The potential risk of integration of viral sequences into the host cell genome cannot be neglected at present. In addition, the slow kinetics of neutralising antibody production by genetic immunisation are not compatible with the necessity of an early immune response in postexposure human vaccination.[76]

There are new trends towards the preparation of future vaccines using plants for the expression of virus proteins. This conceptually promising approach, however, still needs much research to be done to allow for the commercial production of immunogenic and protective virus antigens.

7. Conclusions

Looking back over the series of improvements in designing rabies vaccines and their application, we can see that tremendous progress has been made. In addition to technical improvements leading to better and safer vaccines, considerable experience has been accumulated with vaccination regimens. This has led to well tolerated PET when WHO recommendations are applied. The recent WHO recommendations[48] for intradermal administration of vaccine are also steps towards reducing the cost of the PET while preserving the efficacy of the vaccination. There is also a worldwide consensus that application of rabies serotherapy for severe risk of rabies contamination is compulsory for the PET.

However, it is also remarkable that these improvements have not been sufficient to eradicate human rabies worldwide. What are the limiting factors for reaching this goal? The first is economic: PET and rabies serotherapy are both expensive. They are not always affordable for every potentially rabies-contaminated patient in developing countries. The second is geographical: people bitten in remote areas may not have ready access to rabies treatment. Finally, there is a need for information to be provided to populations at risk and targeted at the most exposed population, which is the children.

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