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Monoclonal Antibodies in Rabies Therapy

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Summary

Virus-neutralising antibodies play a major role in immunological protection against rabies. A combination of anti-rabies immunoglobulin and vaccine has become the standard World Health Organization treatment for humans with severe exposure to rabies virus. Equine anti-rabies serum (ERIG) and human rabies immunoglobulin (HRIG) are currently used for rabies post-exposure serotherapy. These products are either extremely expensive or their use is associated with adverse effects, as in the case of ERIG. Therefore, it is desirable to replace ERIG and HRIG by more cost effective and safer products.

Recent studies have shown that passive immunisation of rodents with murine or human monoclonal antibodies (mAbs) specific for the G protein of rabies virus protects from the disease in a post-exposure situation. Such treatment can also abrogate a lethal rabies virus infection after the virus has entered the central nervous system. These findings indicate the great potential of antiviral mAbs as effective therapeutics against rabies. Because of their highly specific protective activity and the lack of major risks and adverse effects, these mAbs have a major advantage over hyperimmune globulins.

However, there still exist inherent problems in the clinical application of mAbs. The high immunogenicity of murine mAbs in humans and the risk of virus contamination of human mAb preparations represent major obstacles for the use of mAbs. Recombinant DNA technology can be used to humanise murine mAbs. This increases the biological half-life and minimises the possibility of adverse effects. The problem of possible virus contamination of mAb preparations can be overcome by the expression of the mAb in a heterologous system.

Rabies remains a significant human health problem throughout the world. A survey of rabies in humans revealed that nearly half a million people receive post-exposure prophylaxis annually.[1] It is likely that the actual number of human rabies cases worldwide exceeds the statistic compiled by the World Health Organization (WHO). The post-exposure treatment recommended by WHO includes the prompt administration of rabies vaccine together with the use of anti-rabies immunoglobulin. There are 2 major types of rabies immunoglobulins that are currently used for post-exposure serotherapy: human rabies immunoglobulin (HRIG) and equine rabies immunoglobulin (ERIG). ERIG

is used in many parts of the world because of its relatively low cost. However, the use of ERIG is often associated with adverse effects, ranging from local reactions at the injection site to anaphylaxis.[2] The drawbacks of ERIG can be partially prevented by using HRIG. Unfortunately, there is often insufficient HRIG available to initiate the recommended post-exposure treatment. Also, in countries where rabies prophylaxis is most needed, HRIG is too costly for extensive use. Furthermore, since HRIG is derived from pooled hyperimmune sera, there is a potential risk of transferring blood-borne biological contaminants such as human immunodeficiency virus and hepatitis B virus.

For these reasons, it is desirable to replace HRIG and ERIG in rabies post-exposure treatment by more cost effective and safer products. The recent development of murine and human monoclonal antibodies (mAbs) directed against rabies virus antigens offers a promising alternative to the existing therapeutic modalities. Studies have shown that rabies virus neutralising mAbs are extremely efficacious in protecting animals from a lethal rabies virus infection. The possible application of murine and human rabies virus–specific mAbs for post-exposure treatment of humans is discussed herein.

1. The Role of Rabies Virus–Specific Antibody in the Immune Defence Against Rabies

Rabies virus neutralising antibodies, which are directed solely against the rabies virus glycoprotein (G), play a major role in immunoprotection against rabies.[3] Virus-neutralising antibodies may offer protection, especially in the early critical phase following exposure to rabies virus before it reaches the central nervous system (CNS). The possible mechanisms whereby virus-neutralising antibodies exert their anti-viral effect include:

- neutralisation of extracellular rabies virus;
- complement-mediated lysis of rabies-infected cells; and
- antibody-dependent cytotoxicity.[4]

Data obtained with different rabies virus–neutralising antibodies imply that antibody-mediated neutralisation is the result of several different mechanisms, including:

- inhibition of virus attachment;
- release of bound virus; and
- inhibition of the intra-endosomal acid-catalysed fusion step that leads to virus uncoating.[3]

Antibodies directed against the rabies virus G and N proteins have also been shown to play an important role in potentiating the antigen-induced proliferative response of rabies virus–specific T lymphocytes and their production of interferon-γ, which is involved in the protection against lethal rabies virus infection.[5]

Recently, a novel function for antibodies in protection against rabies virus CNS infection was discovered.[6,7] It was shown that rabies virus–neutralising antibodies can mediate the complete clearance of rabies virus from the CNS. This antibody-mediated viral clearance appears to be facilitated by a novel mechanism that is distinct from the classical viral clearance process. Interestingly, the protective activity *in vivo* of a particular antibody does not correlate with its virus-neutralising ability *in vitro*. Instead, it correlates with the ability to prevent virus spread from cell to cell and to inhibit transcription of viral RNA.[6,7] The finding that the antibody can exert its inhibitory activity even after the virus has entered the CNS indicates the great potential of anti–rabies virus antibodies as an effective therapeutic modality against rabies virus infection.

2. Generation and Selection of Anti-Rabies Virus Monoclonal Antibodies for Post-Exposure Prophylaxis

Hybridomas that secrete murine mAbs specific for rabies virus antigens can be produced relatively easily by fusion of mouse myeloma cells with splenocytes of Balb/c mice immunised with rabies virus or rabies virus antigens.[8] In contrast, the generation of human hybridomas by somatic cell hybridisation of B lymphocytes of individuals im-

Table I. Biological activities of some monoclonal antibodies used for post-exposure prophylaxis of rabies

mAb	Origin	Isotype	Antigen and epitope specificity	Virus strain specificity	Specific virus-neutralising activity[a] (IU/mg)	ED_{50} mouse-protective dose[b] (IU)	Spread inhibition activity[c] (IU/ml)
509-6	Mouse	γ2a	G, site I	All strains of serotype 1	1667	2.0	6.0
1112-1	Mouse	γ1	G, site IIc	All strains of serotypes 1 and 4	61	0.03	0.04
523-11	Mouse	γ2a	G, site III	All strains of serotype 1 and Mokola	8242	2.36	6.0
57	Human	γ2	Unclassified	All strains of serotype 1	13 000	0.80	ND

a IU were determined by using the National Institute of Health reference serum (lot R-3) as a standard.

b ED_{50} is the amount of mAb (IU) necessary to protect 50% of mice against an intramuscular challenge infection with the CVS-24 strain of rabies virus.

c Spread inhibition activity is the concentration of mAb that results in protection of 50% of the cells from infection with CVS-11 virus at 72 hours post-infection.

Abbreviations: IU = international unit; mAb = monoclonal antibody; ND = not determined.

munised with rabies vaccine[9] is considerably more problematical. Human mAbs are frequently not of the appropriate isotype or do not possess the desired specificity and biological activity. Although several laboratories have been able to produce numerous mouse anti-rabies mAbs, only one laboratory has succeeded in generating a human anti-rabies mAb (mAb 57) that has potential for use in rabies therapy.[10]

Not all anti-rabies mAbs are suitable for post-exposure rabies prophylaxis. mAbs selected for therapeutic intervention should meet the following criteria: (a) the mAb should neutralise a broad spectrum of rabies and rabies-related strains *in vitro*; (b) the mAb should effectively inhibit virus spread from cell to cell;[6] (c) the mAb must be of the IgG isotype, as the IgM and IgA isotypes are not effective *in vivo*;[6,11,12] (d) the mAb should passively protect laboratory rodents against intramuscular challenge infection with rabies virus; and (e) the mAb should be efficacious in the post-exposure treatment of rabies in rodents and primates.

It must also be taken into consideration that a single mAb may be insufficient to replace HRIG or ERIG in the post-exposure treatment of human rabies, since a single mAb may not be effective against all antigenically diverse strains of rabies virus. Furthermore, the potential risk of the emergence of neutralisation-resistant escape variants must be considered.[12] Therefore, it is necessary to use a mixture of several mAbs that differ in their epitope specificicity.

Table I summarises the biological activities of 3 mouse mAbs and 1 human mAb. These mAbs fulfil most of the criteria necessary for clinical application. They not only have a broad range of virus-neutralising activity, but are also very effective in prophylactic treatment of laboratory mice against lethal rabies virus infection.

3. Efficacy of Monoclonal Antibodies in the Post-Exposure Treatment of Rabies

Hamsters treated 3 hours after virus inoculation with a mixture of mouse mAbs 509-6, 1112-1 and 523-11 were completely protected from lethal rabies virus infection. When the mAb mixture was given 36 hours after virus challenge, 80% of the animals survived (table II).[7] In addition, treatment of hamsters with the human mAb 57 also prevented a lethal rabies virus infection in a post-exposure situation.[10] As shown in table II, 100% of hamsters receiving mAb 57 24 hours after challenge infection survived, while 80% of the untreated control animals succumbed to rabies virus infection.

Table II. Responses of Syrian hamsters to post-exposure rabies treatment with mouse and human monoclonal antibodies

Time of mAb treatment (hours post-infection)	Survival[a]	
	mouse mAb mixture[b] (1550 IU/kg)	human mAb 57 (500 IU/kg)
3	5/5	ND
24	ND	5/5
36	4/5	ND
72	ND	3/5
Control	1/5	1/5

a Hamsters were challenged intramuscularly with a suspension of dog salivary gland infected with rabies virus.

b The mixture contained mAb 509-6, mAb 1112-1 and mAb 523-11.

Abbreviations: IU = international unit; mAb = monoclonal antibody; ND = not determined.

Recently, we have shown that mAb not only protects animals from lethal rabies encephalomyelitis, but can also completely clear rabies virus from the CNS.[6,7] Viral RNA can be detected in the brain of rats 24 hours after intranasal infection with rabies virus. However, when the animals were treated with the mouse mAb 1112-1 24 hours post-infection, 4 out of 5 rat brains showed no evidence of viral RNA 24 hours afterwards.[7] Furthermore, 80% of the animals that were treated 24 hours post-infection with mAb 1112-1 remained clinically normal with no signs of rabies virus infection 30 days later, and viral RNA could not be detected in the surviving animals.[6] These experiments demonstrate that mAb can prevent death from a lethal rabies virus encephalitis by mediating the clearance of rabies virus from neuronal cells of the CNS.

4. Obstacles to the Clinical Application of Monoclonal Antibodies

Even though the results obtained with several mouse and human anti-rabies antibodies indicate their great potential for antibody therapy in humans, there are inherent problems that render clinical application of these mAbs difficult. Since they are foreign proteins, murine mAbs are highly immunogenic in humans and their use carries the risk of possible adverse effects such as anaphylactic reactions and hypersensitisation. However, non-immunosuppressed cancer patients tolerated multiple applications of mouse mAbs without adverse effects.[13,14] Therefore, the adverse effects of mouse anti-rabies mAbs can be assumed to be low, especially since only a single dose of antibody is administered during the post-exposure treatment of rabies. In the case of second exposure to the virus, administration of antirabies antibody would not be necessary since treatment of the first exposure would include active immunization with rabies vaccine.

A more serious problem associated with mouse mAbs that might significantly affect the efficacy of these agents is their short biological half-life in humans. This problem could be circumvented by humanising the murine mAbs by introducing the 6 hypervariable regions from the heavy and light chains of a murine antibody into a human framework sequence that could then be combined with human constant regions.[15] Such humanised antibodies retain the immunological specificity of the murine antibodies.[16]

With respect to the clinical application of human monoclonal antibodies, there also exist inherent problems such as the instability of the human hybridoma cell lines, especially after cryopreservation. Furthermore, the use of Epstein-Barr virus to transform B cells in hybridoma cloning introduces the risk of viral contamination of the mAb preparation. One possibility to overcome the virus contamination and stability problems is to express the immunoglobulin in a heterologous expression system. For example, high level production of intact immunoglobulin can be achieved in a baculovirus expression system.[17] We have recently produced the Fab domain of the human anti-rabies mAb 57 in a bacterial expression system and demonstrated the virus-neutralising activity of this Fab domain.[18]

5. Conclusion

There is a great need for the development of safer and more cost effective products for post-exposure prophylaxis of human rabies. Much evi-

dence indicates that HRIG and ERIG, which are either expensive or unsafe, can be replaced by mAbs. Hybridoma technology for production of mAbs is, with the exception of the generation of human mAbs, relatively easy and straightforward. Furthermore, reliable *in vitro* assays, such as virus spread inhibition tests,[6] and relevant animal models are available to screen and select mAbs for rabies therapy.

The main advantages of mAbs over immunoglobulin preparations are: (a) high specific protective activity; (b) invariability of biological activity; (c) lack of major risks and adverse effects; and (d) possibly greater cost effectiveness. However, problems remain such as the relatively high immunogenicity of murine mAbs in humans and the potential risk of virus contamination in human mAb preparations, which makes the clinical application of mAbs difficult. One approach to overcome these problems is to take advantage of recombinant DNA technology to humanise murine mAbs, and to express human or humanised murine mAbs in a heterologous expression system. This approach may prove useful for the development of mAbs that can be applied for rabies post-exposure prophylaxis in humans.

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