Finally, there have been some reports of recombinant rabies viruses lacking an entire gene in the genome, in order to completely attenuate the virus. Shoji et al. [43] established a recombinant virus lacking the P gene, of which the product, P protein, is known as a cofactor of viral RNA polymerase. Although the genome did not contain the P gene, the P gene-deficient (def-P) virus could be recovered from the genome plasmid with supplementation of the P protein from helper plasmid. They showed that the def-P virus propagated in cell lines stably expressing P protein. On the other hand, the def-P virus did not effectively grow in normal cells that did not express the protein. Because of this property, the def-P virus was completely apathogenic for adult and suckling mice even when inoculated intracranically. It was also shown that the def-P virus was capable of primary transcription by virion-associated P protein and, consequently, transient expression of viral proteins other than P protein from the viral genome. Immunization of mice with the def-P virus resulted in high-level induction of virus-neutralizing antibody (VNA) and conferment of protective immunity to inoculated mice against a lethal rabies virus infection. Interestingly, it has recently been reported that the P protein blocks both type I interferon production [44] and the signaling pathway [45], thereby inhibiting host innate immunity. The loss of this function in the def-P virus may contribute to the high immunogenicity of the virus.

We reported generation of an M gene-deficient rabies virus (RC-HLΔM strain) as a vaccine strain [47] using the gene manipulation system of the RC-HL strain [32,46], which is a highly attenuated strain used for production of animal rabies vaccine in Japan. The gene product, M protein, is known to be necessary for viral assembly and budding [48] (Fig. 4). Therefore, it is expected that the RC-HLΔM strain infects cells and synthesizes viral proteins other than M protein in infected cells, thus eliciting protective immunity more effectively than an inactivated vaccine. However, no particle formation of the progeny virus can occur, because the M protein is not expressed in infected cells due to deletion of the coding gene in the genome. Hence, it is thought that this property greatly reduces the risk of vaccine-caused rabies in inoculated animals.

In the presence of M protein supplied from an additional helper plasmid, the RC-HLΔM strain was recovered from the genomic plasmid (Fig. 4). Infection of the RC-HLΔM strain in cultured cells was confined to single cells, in contrast to that of the parental RC-HL strain, which extensively spread in a few days after infection. The infectious progeny virus was not detected in a culture supernatant from RC-HLΔM strain-infected cells, whereas the infectious virus was effectively produced in the supernatant from RC-HL strain-infected cells. A baby hamster kidney cell line expressing M protein of the RC-HL strain has been established as a supporting cell line for propagation of the RC-HLΔM strain.

The body weights of adult mice intracerebrally inoculated with RC-HL strain transiently decreased, whereas those of mice inoculated with RC-HLΔM strain continued to increase without any clinical signs of rabies. RC-HLΔM strain also did not cause lethal infection in the mice in contrast to RC-HL strain, which killed suckling mice by intracerebral inoculation. These findings showed that RC-HLΔM strain is completely apathogenic for both adult and suckling mice. The RC-HLΔM

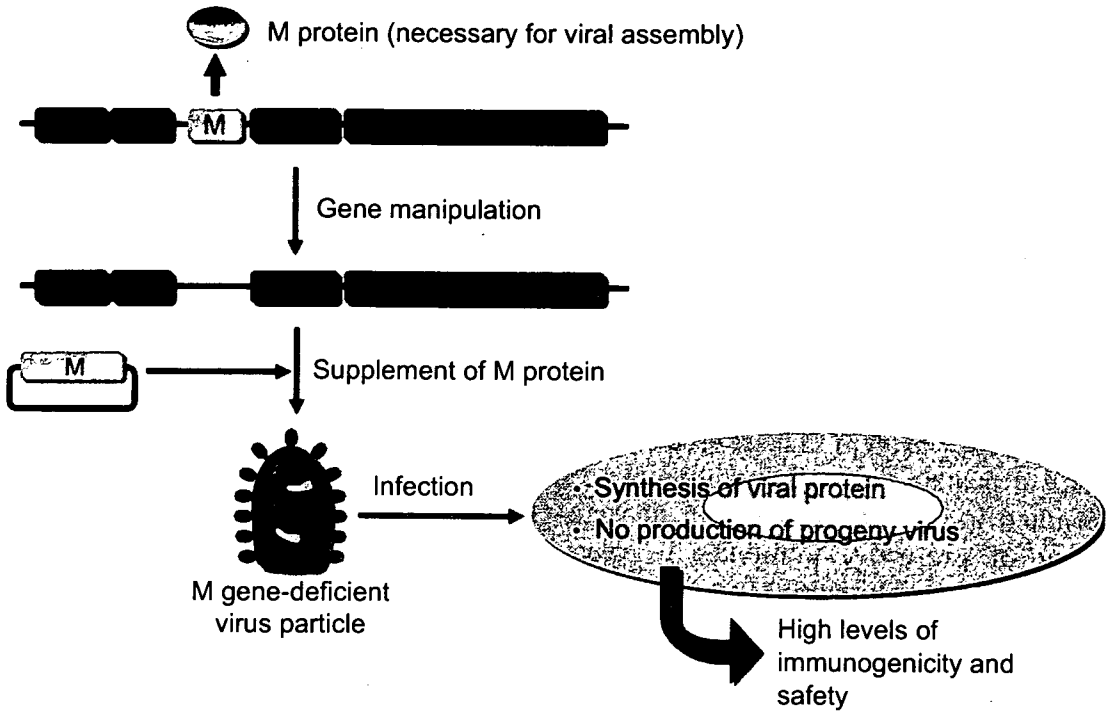

Fig. 4. Rescue of an M gene-deficient rabies virus particle from cloned cDNA and characteristics as a vaccine strain.

strain induced effective VNA in adult mice by intramuscular inoculation, whereas the UV-inactivated RC-HL strain failed to induce it. It was also shown that intranasal inoculation with RC-HLΔM strain much more efficiently induced anti-rabies virus antibody than did that of the UV-inactivated RC-HL strain, indicating the possibility that the intranasal route is useful for inoculation of RC-HLΔM strain as a needle-free delivery method. We clearly showed that the RC-HLΔM strain is a good candidate for new-generation rabies vaccine. Further studies are needed to determine whether immunization with the RC-HLΔM strain protects inoculated mice against challenge with lethal rabies virus infection.

As mentioned above, the gene manipulation system of rabies virus has opened up the possibility of development of new-generation rabies vaccines. On the other hand, we need to carefully investigate whether these genetically modified rabies viruses have sufficient aptitude for the vaccine. In this aspect, the viruses mentioned above still have problems to be resolved. For example, safety of a recombinant virus with a foreign gene has to be cautiously examined since we cannot exclude the possibility that expression of the foreign gene will cause serious side-effects in inoculated animals and humans. Furthermore, in the case of a recombinant virus with a foreign gene or an attenuation-related mutation, there is a possibility that the virus will become pathogenic during viral propagation in the body of the inoculated animal due to a certain mutation hindering expression of the foreign gene or the attenuation property of virus. This possibility might be ruled out by development of a virus that has multiple attenuation-related mutations in the genome. We believe that

accumulation of fundamental information about the molecular function of rabies virus is important not only for solutions of these problems, but also for further development of attenuated live rabies vaccines using the gene manipulation system of the virus.

References

[1] Knobel DL, Cleaveland S, Coleman PG, Fevre EM, Meltzer MI, Miranda ME, et al. Re-evaluating the burden of rabies in Africa and Asia. Bull World Health Organ 2005;83:360–8.
[2] Kim JH, Hwang EK, Sohn HJ, Kim DY, So BJ, Jean YH. Epidemiological characteristics of rabies in South Korea from 1993 to 2001. Vet Rec 2005;157:3–56.
[3] Windiyaningsih C, Wilde H, Meslin FX, Suroso T, Widarso HS. The rabies epidemic on Flores Island, Indonesia (1998–2003). J Med Assoc Thai 2004;87:1389–93.
[4] Gould AR, Hyatt AD, Lunt R, Kattenbelt JA, Hengstberger S, Blacksell SD. Characterisation of a novel lyssavirus isolated from Pteropid bats in Australia. Virus Res 1998;54:165–87.
[5] Smith JS, Orciari LA, Yager PA, Seidel HD, Warner CK. Epidemiologic and historical relationships among 87 rabies virus isolates as determined by limited sequence analysis. J Infect Dis 1992;166:296–307.
[6] Kissi B, Bourhy H, Tordo N. Genetic polymorphism in the rabies virus nucleoprotein gene. Virology 1995;209:526–37.
[7] Ito N, Sugiyama M, Oraveerakul K, Piyaviriyakul P, Lumlertdacha B, Arai YT, et al. Molecular epidemiology of rabies in Thailand. Microbiol Immunol 1999;43:551–9.
[8] David D, Yakobson B, Smith JS, Stram Y. Molecular epidemiology of rabies virus isolates from Israel and other middle and near eastern countries. J Clin Microbiol 2000;38:755–62.
[9] Arai YT, Takahashi H, Kameoka Y, Shiino T, Wimalaratne O, Lodmell DL. Characterization of Sri Lanka rabies virus isolates using nucleotide sequence analysis of nucleoprotein gene. Acta Virol 2001;45:327–33.
[10] Nishizono A, Mannen K, Elio-Villa LP, Tanaka S, Li KS, Mifune K, et al. Genetic analysis of rabies virus isolates in the Philippines. Microbiol Immunol 2002;46:413–7.
[11] Susetya H, Sugiyama M, Inagaki A, Ito N, Oraveerakul K, Traiwanatham N, et al. Genetic characterization of rabies field isolates from Thailand. Microbiol Immunol 2003;47:653–9.
[12] Nadin-Davis SA, Simani S, Armstrong J, Fayaz A, Wandeler AI. Molecular and antigenic characterization of rabies viruses from Iran identifies variants with distinct epidemiological origins. Epidemiol Infect 2003;131:777–90.
[13] Kuzmin IV, Botvinkin AD, McElhinney LM, Smith JS, Orciari LA, Hughes GJ, et al. Molecular epidemiology of terrestrial rabies in the former Soviet Union. J Wildl Dis 2004;40:617–31.
[14] Hyun BH, Lee KK, Kim IJ, Lee KW, Park HJ, Lee OS, et al. Molecular epidemiology of rabies virus isolates from South Korea. Virus Res 2005;114:113–25.
[15] Denduangboripant J, Wacharapluesadee S, Lumlertdacha B, Ruankaew N, Hoonsuwan W, Puanghat A, et al. Transmission dynamics of rabies virus in Thailand: implications for disease control. BMC Infect Dis 2005;5:52.
[16] Nagarajan T, Mohanasubramanian B, Seshagiri EV, Nagendrakumar SB, Saseendranath MR, Satyanarayana ML, et al. Molecular epidemiology of rabies virus isolates in India. J Clin Microbiol 2006;44:3218–24.
[17] Smith JS, Fishbein DB, Rupprecht CE, Clerk K. Unexplained rabies in three immigrants in the United States. A virologic investigation. N Engl J Med 1991;324:204–11.
[18] Toro G, Vergara I, Rom G. Neuroparalytic accidents of antirabies vaccination with suckling mouse brain vaccine. Clinical and pathologic study of 21 cases. Arch Neurol 1977;34:694–700.
[19] Javier RS, Kunishita T, Koike F, Tabira T. Semple rabies vaccine: presence of myelin basic protein and proteolipid protein and its activity in experimental allergic encephalomyelitis. J Neurol Sci 1989;93:221–30.

[20] Morimoto K, McGettigan JP, Foley HD, Hooper DC, Dietzschold B, Schnell MJ. Genetic engineering of live rabies vaccines. Vaccine 2001;19:3543–51.
[21] Faber M, Pulmanausahakul R, Hodawadekar SS, Spitsin S, McGettigan JP, Schnell MJ, et al. Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. J Virol 2002;76:3374–81.
[22] Shoji Y, Inoue S, Nakamichi K, Kurane I, Sakai T, Morimoto K. Generation and characterization of P gene-deficient rabies virus. Virology 2004;318:295–305.
[23] Ito N, Sugiyama M, Yamada K, Shimizu K, Takayama-Ito M, Hosokawa J, et al. Characterization of M gene-deficient rabies virus with advantages of effective immunization and safety as a vaccine starain. Microbiol Immunol 2005;49:971–9.
[24] Mansfield KL, Racloz V, McElhinney LM, Marston DA, Johnson N, Ronsholt L, et al. Molecular epidemiological study of Arctic rabies virus isolates from Greenland and comparison with isolates from throughout the Arctic and Baltic regions. Virus Res 2006;116:1–10.
[25] Winkler WG, Baer GM. Rabies immunization of red foxes (Vulpes fulva) with vaccine in sausage baits. Am J Epidemiol 1976;103:408–15.
[26] Black JG, Lawson KF. The safety and efficacy of immunizing foxes (Vulpes vulpes) using bait containing attenuated rabies virus vaccine. Can J Comp Med 1980;44:169–76.
[27] Schumacher CL, Coulon P, Lafay F, Bnjean J, Aubert MF, Barrat J, et al. SAG-2 oral rabies vaccine. Onderstepoort J Vet Res 1993;60:459–62.
[28] Esh JB, Cunningham JG, Wiktor TJ. Vaccine-induced rabies in four cats. J Am Vet Med Assoc 1982;180:1336–9.
[29] Whetstone CA, Bunn TO, Emmons RW, Wiktor TJ. Use of monoclonal antibodies to confirm vaccine-induced rabies in ten dogs, two cats, and one fox. J Am Vet Med Assoc 1984;185:285–8.
[30] Schnell MJ, Mebatsion T, Conzelmann KK. Infectious rabies viruses from cloned cDNA. EMBO J 1994;13:4195–203.
[31] Faber M, Pulmanausahakul R, Nagao K, Prosniak M, Rice AB, Koprowski H, et al. Identification of viral genomic elements responsible for rabies virus neuroinvasiveness. Proc Natl Acad Sci USA 2004;101:16328–32.
[32] Ito N, Takayama M, Yamada K, Sugiyama M, Minamoto N. Rescue of rabies virus from cloned cDNA and identification of the pathogenicity-related gene: glycoprotein gene is associated with virulence for adult mice. J Virol 2001;75:9121–8.
[33] Inoue K, Shoji Y, Kurane I, Iijima T, Sakai T, Morimoto K. An improved method for recovering rabies virus from cloned cDNA. J Virol Methods 2003;107:229–36.
[34] Yamada K, Ito N, Takayama-Ito M, Sugiyama M, Minamoto N. Multigenic relation to the attenuation of rabies virus. Microbiol Immunol 2006;50:25–32.
[35] Shimizu K, Ito N, Mita T, Yamada K, Hosokawa-Muto J, Sugiyama M, et al. Involvement of nucleoprotein, phosphoprotein, and matrix protein genes of rabies virus in virulence for adult mice. Virus Res 2006, published on line.
[36] Pulmanausahakul R, Faber M, Morimoto K, Spitsin S, Weihe E, Hooper DC, et al. Overexpression of cytochrome C by a recombinant rabies virus attenuates pathogenicity and enhances antiviral immunity. J Virol 2001;75:10800–7.
[37] Faber M, Bette M, Preuss MA, Pulmanausahakul R, Rehnelt J, Schnell MJ, et al. Overexpression of tumor necrosis factor alpha by a recombinant rabies virus attenuates replication in neurons and prevents lethal infection in mice. J Virol 2005;79:15405–16.
[38] Dietzschold B, Wunner WH, Wiktor TJ, Lopes AD, Lafon M, Smith CL, et al. Characterization of an antigenic determinant of the glycoprotein that correlates with pathogenicity of rabies virus. Proc Natl Acad Sci USA 1983;80:70–4.
[39] Seif I, Coulon P, Rollin PE, Flamand A. Rabies virulence: effect on pathogenicity and sequence characterization of rabies virus mutations affecting antigenic site III of the glycoprotein. J Virol 1985;53:926–34.
[40] Tuffereau C, Leblois H, Bnjean J, Coulon P, Lafay F, Flamand A. Arginine or lysine in position 333 of ERA and CVS glycoprotein is necessary for rabies virulence in adult mice. Virology 1989;172:206–12.

[41] Mebatsion T. Extensive attenuation of rabies virus by simultaneously modifying the dynein light chain binding site in the P protein and replacing Arg333 in the G protein. J Virol 2001;75:11496–502.
[42] Faber M, Pulmanausahakul R, Hodawadekar SS, Spitsin S, McGettigan JP, Schnell MJ, et al. Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. J Virol 2002;76:3374–81.
[43] Shoji Y, Inoue S, Nakamichi K, Kurane I, Sakai T, Morimoto K. Generation and characterization of P gene-deficient rabies virus. Virology 2004;318:295–305.
[44] Brzzka K, Finke S, Conzelmann KK. Identification of the rabies virus alpha/beta interferon antagonist: phosphoprotein P interferes with phosphorylation of interferon regulatory factor 3. J Virol 2005;79:7673–81.
[45] Vidy A, Chelbi-Alix M, Blondel D. Rabies virus P protein interacts with STAT1 and inhibits interferon signal transduction pathways. J Virol 2005;79:14411–20.
[46] Ito N, Takayama-Ito M, Yamada K, Hosokawa J, Sugiyama M, Minamoto N. Improved recovery of rabies virus from cloned cDNA using a vaccinia virus-free reverse genetics system. Microbiol Immunol 2003;47:613–7.
[47] Ito N, Sugiyama M, Yamada K, Shimizu K, Takayama-Ito M, Hosokawa J, et al. Characterization of M gene-deficient rabies virus with advantages of effective immunization and safety as a vaccine strain. Microbiol Immunol 2005;49:971–9.
[48] Mebatsion T, Weiland F, Conzelmann KK. Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. J Virol 1999;73:242–50.