Research Progress on Ebola Hemorrhagic Fever Vaccine
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ABSTRACT

Ebola hemorrhagic fever is a potent infectious disease by Ebola virus caused 90% mortality rate. Ebola virus was first isolated in 1976 by, for single-stranded negative segment, non-segmented, enveloped RNA viruses belonging to filamentous virus family. Ebola virus can be divided into five different subtypes. Vaccination is the most conventional and effective prevention and infection control methods in recent years. It has made great progress in the study on the vaccine for Ebola virus. In this paper, research progress Ebola hemorrhagic fever vaccine was reviewed.

Background

Ebola virus (EBOV) has the virus envelope, non-segmented genome and single stranded negative strand RNA. EBOV belongs to a single negative strand mesh filamentous virus genus, which including Marburg virus and Ebola virus. According to the occurrence and antigenic characteristics of EBOV were divided into 5 subtypes: Zaire Ebola virus, Sultan Ebola virus, Ebola virus in Ivory Coast, Reston Ebola virus and Bundibugyo Ebola virus [1,2].

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The first epidemic of EHF break out in the Zaire (now the Democratic Republic of the Congo) and Sultan in 1976. However, it is well known from 1995, due to the outbreak in the Democratic Republic of Congo. As the epidemic is only confined to Africa, sporadic and with short duration, people did not pay unprecedented attention to that until the 2014 outbreak in West Africa. The incubation period of Ebola hemorrhagic fever was 21 Days, thus the infected patient may not be detected before the symptoms appear, and the epidemic gradually spread out to other countries. WHO has listed EBOV as one of the most serious hazards virus to human.

Vaccination for the prevention and control of infectious diseases of conventional means, but there is no vaccine for Eboha virus. The reason is that the disease is rare and subject to geographical limitations, lack of economic value to develop a vaccine caused by vaccine research and development enterprises. But more than in previous research laboratory has made remarkable progress, especially in nonhuman primates can obtain rapid and effective protection. At present in the vaccine according to classification can be divided into traditional virus vaccine (inactivated and attenuated), virus vector vaccine (adenovirus vector vaccine, replication defective virus vaccine), DNA vaccine, virus like particles (VLPs) and other four categories are summarized as follows.

**Progress in vaccine research**

1. Traditional virus vaccine

Inactivated vaccine is currently the most widely used vaccine, and it is the first attempt of research on Ebola virus vaccine in early days. Lupton et al, who from the U.S. Army Medical Research Institute of infectious diseases, investigate the methods that using heat inactivated and formalin inactivated to prepare inactivated vaccine of Zaire Ebola virus in 1980. The vaccine can make the guinea pigs fully protected, but the control group had only 30.8% death ration, so it is not a good method to evaluate the effectiveness of the vaccine [3].

Several EBOV inactivated vaccine have been reported, but the vaccine failed to activate the protective immunity [4]. Changing of the adjuvant and route of administration, elongation of immune time, and protection experiments are also found to be not effective [5]. Besides, Attenuated vaccines have reported that had potential to atavism. Some attenuated strain EBOV vaccine had been reported fit the mice and guinea pigs, but pathogenicity of NHPs still retained [6].

Using genetic engineering methods may find safe and effective candidates of EBOV vaccine. Recently, it was reported that mice and guinea pigs that were vaccinated by two dose of replicated defective EBOV (VP30 deletion) and
protein preparations were able to be protected [7]. Therefore, it is necessary to develop safe and effective vaccines for prevention and treatment, anti-bioterrorism, and protection of medical personnel and laboratory personnel.

2. Virus vector vaccine

The virus can act as a vaccine vector. Viral vectors can be type of replicated or defective replicated. Such vaccine will works by insertion of EBOV antigen gene into the virus vector for expression. Replicated type of viral vector vaccine can cause strong and lasting immune response, but the carrier is not suitable for immunocompromised individuals. Replicated defective vectors are relatively safe, but it is need large dose to achieve the best immunogenicity.

At present, the study of EBOV vaccine mainly focused on various recombinant vector that can express EBOV gene protein, including vaccinia virus, Venezuelan equine encephalitis virus (VRP) vaccine, particle replication adenovirus vector vaccine (AD), recombinant vesicular stomatitis virus (VSV) vaccine, influenza vaccine.

2.1 Adenovirus vector vaccine

Adenovirus vector vaccine due to its low toxicity, high expression of exogenous gene, easy induction of specific humoral and cellular immune response, and availability of immunization conduction through muscle and mucosal etc [8], is widely used in many research of pathogen vaccines, and there are a number of vaccines have already been in the phase I or II clinical trial to counter various virus including Ebola [9], HIV [10,11] malaria and tuberculosis [12] influenza [13-14].

Human adenovirus type 5 (AdHu5) is the most commonly used adenovirus vaccine carrier, because the recombinant adenovirus can cause severe immune reaction [15]. AdHu5 vector can be identified easily; it can cause mild respiratory disease, gastroenteritis and conjunctivitis. The recombinant AdHu5 vector lose E1 gene in the early stage, which can stop the replication of the virus, the exogenous genes are usually inserted into the site to express. Further deletion of E3 and E4, can increase the carrying ability of AdHu5 virus vector to 8kb. The recombinant DNA vaccine ZEBOV GP+ZEBOV NP+SEBOV GP + ICEBOV GP were used as the initial immunization, then the recombinant AdHu5-ZGP were applied to strengthen immunity. Such method did not give good immunization results, but it still laid the foundation for the advanced investigation on immunization strategy of the adenovirus vector vaccine [16]. Recently, it was reported that the mixed injection of AdHu5-GP and AdHu5-NP achieved better immune effect, in which AdHu5-GP vaccines was improved by optimizing the GP codon, replacing by strong promoter. Applied the vaccine 30 minutes prior to the mice were exposed to the virus, it will give the mice
fully protection (100%)\textsuperscript{[17]}.

However, adenovirus vector vaccine still has some defects, which is mainly due to preexisting immunity (PEI) phenomenon in the humans, which human body contains antibodies against the human adenovirus. According to the statistic data, the antibody positive rate of China’s type 5 adenovirus (Ad5) of healthy population in Guangzhou area was 77.34\textsuperscript{[18]}, and the positive rate of Ad5 antibody of American reached 35\%, additionally, Ad5 antibody positive rate in South Africa even reached 90\%\textsuperscript{[19]}, immune effect of pre-existing immunity phenomenon will directly weaken the adenovirus vector vaccine. A major cause of failure of HIV adenovirus vaccine in phase II clinical trial is the PEI, so the PEI is the most important issue need the researchers to focus.

Currently people explored other ways to avoid the PEI. For example, some not-popular- serotypes of virus were attempt to use, including AdHu12, AdHu35 and AdHu6, which was reported that had better immune effect in NHPs; On the other hand, various source of adenovirus from different species were tested as well, such as monkey, cow or pig adenovirus vector vaccine has been investigated. It was shown that when the mice, which carried AdHu5-neutralizing antibody, was injected with bovine adenovirus type 3 (Bad3), the mice can be protected under the deadly bird flu attack \textsuperscript{[20]}. Some other researches showed that changing the immune pathway, such as nasal, oral, and especially sublingual introduction of vaccine, can protect mice and guinea pigs \textsuperscript{[21]}.

2.2 Replication defective vaccine

Halfmann et al, replaced the VP30 gene of EBOV by neomycin gene via reverse genetic technology. VP30 is an essential transcription factor for viral replication, thus the modified virus lost normal replication ability, and it can only be replicated in the cell groups that can steadily express the VP30. The virus morphology did not change after the transformation \textsuperscript{[22]}. The replication defective virus that obtained from such research is more secure than the wild type virus. It is not necessary to run the experiment in level IV biosafety laboratory. So it can be used for screening anti Ebola drug or vaccine research. The research team further evaluates the safety and immune protection of such replication defective virus vaccine. They inoculated the STAT-1 knockout mice with 2X10\textsubscript{6} FFU replication defective Ebola viruses, and the mice did not show any symptoms. The interferon signaling pathway of STAT-1 knockout mice were cut, which the mice did not have natural immune function, so the virus was highly susceptible. The experimental results showed that the safety of the replication defective Ebola virus vaccine is reliable. In addition, they gave the mice and guinea pigs vaccine two times, and exposed them
to the virus. All the animals were completely protected, which proved that the vaccine has good immune protection. At the same time, they were also observed that specific antibody against Ebola virus GP protein and NP protein in CD8+T cell was immune activated after vaccination induced \[23\]. The research results proved that although the vaccine has good safety in the laboratory, the vaccine only removed the VP30 gene, if recombinant replication allows the vaccine recovery ability in nature; the consequences will be disastrous, so the biological safety of the vaccine still need to be further evaluated.

2.3 Other Ebola virus vector vaccine

Studies have shown that vaccinia virus vaccine, which was able to express EBOV-GP, can protect guinea pigs to avoid the occurrence of EHF. However, it is not effective in the case of macaques and rhesus monkey \[24\].

Vesicular stomatitis virus (VSV) vector vaccine, replicating VSV vaccine both had high titer growth, can cause very strong humoral and cellular immunity \[25\]. The recombinant virus expressing VSV ZEBOVGp (VSV-GP), through the muscles, abdominal and mucosal support immunity to mice, after enhanced immunization, and the mice can be fully protected. Immune VSV-GP before NHPs challenged ZEBOV can get 100% protection \[26\]. More importantly, VSV vaccine has been proved to be an effective therapeutic agent after exposure. Immunization of VSV-GP, after 24h of infection of mice and guinea pigs, will give protection of 100% and 50% respectively. After the infection of BHPs of ZEBOV 30min, VSV-GP can produce a 50% immune protection rate \[27\].

Rabies virus vector vaccine, which was replaced ZEBOV GP with G protein of SADB19 strain of rabies virus, was a false virus without virulence. The false virus expresses ZEBOV GPs, and after the enhanced immunization, it can fully protect the mice from attacks from RABV and EBOV, by way of muscle introduction \[28\].

Recombinant human parainfluenza virus type 3 (HPIV-3) vector vaccine express ZEBOV GP (HPIV-3-GP) or ZEBOV-GP + NP (HPIV-3-GP+NP), and it can induce strong systemic immune response by intranasal immunization to guinea pigs; can produce 100% safe and effective protection. Preliminary data showed that the vaccine of NHPs could also produce protection \[29\]. HPIV-3 recombinant vector vaccine can activate the local and systemic immune effective. But the virus has the ability to replicate, and adult prevalence of HPIV-3 antibody, is the unfavorable factors of potential vaccine candidates.

2.3 DNA vaccine

DNA vaccine is the first nucleic acid vector vaccine that been investigated, because of the abilities that can induce humoral and cellular immune responses.
simultaneously, and provide immune protection during growing, moreover DNA vaccine is easy to prepare. It has been widely used to study the viral, bacterial and parasitical disease vaccine, there are a number of vaccines in clinical trials, and in 2005 the first successful attempting of using DNA vaccine (named West Nile-Innovator) to prevent horse West-Nile virus infection was approved. However, DNA vaccines still have some problems, such that the efficiency of plasmid insertion the host cell is not high, the immune effect is not strong, and complication of inoculation, which all limited its application.

Ling Xu et al. [30] proved the DNA vaccine of EBOV was effective in guinea pigs. First of all, guinea pigs were inoculated with plasmid DNA that encoding EBOV-GP, after that, they were exposed to EBOV environment; the results showed that the survival rate is closely related to the antibody titers, while in the control group such phenomenon was not presented. In a mouse model research of EBOV-Z, researchers inserted 2 dose (about 5 g) of the plasmid DNA that expressing GP or NP gene to mice every 4 weeks into the mice by gene gun bombardment, and it was found that EBOV-Z could protect mice from infection, two vaccines can produce antibodies and cytotoxic T lymphocyte response [31].

Because of low efficiency of insertion of the DNA to a host cell, immune efficiency was limited, so no independent report on DNA vaccine of Ebola virus in nonhuman primates, but it can be matched with other vaccines used to form long-term immune protection [32].

2.4 Virus like particles

Virus like particle (VLPs) is a shell structure, without viral nucleic acid. Many viral structural proteins have the ability of automatically assembling and releasing the VLPs. VLPs is similar to the natural virus particles in the size and morphology structure. It has strong immunogenicity and biological activity. Since the VLPs does not contain the viral genetic material, it is not contagious, and the repeat injection will improve the individual immunity level; and it is allow the exogenous gene or gene fragment inserted to format mosaic VLPs and displayed exogenous antigens on its surface; In addition, the number of VLPs also has the ability of carrying viral nucleic acid or other small molecules, and can be used as carrier of drug and gene; in mammalian or insect cell lines it can be multiplied much. In view of these characteristics, many research groups applied the VLPs to the study of Ebola vaccine. Because of the VLPs vaccine is safer and more effective than traditional inactivated and live attenuated vaccine, so it has been used to study a variety of diseases, including influenza vaccine, HIV, hepatitis C virus, enterovirus and parvovirus etc. At present, two papilloma virus VLPs vaccine were listed [33], for the prevention of the virus...
infection.

VP40 and EBOV-GP containing VLPs successfully inoculated in a rodent model\textsuperscript{[30, 31]}. Whether adjuvant contains or not, the VLPs vaccine gave protection to BALB/c and C57BL/6 mice after inoculating at a dose of 10⁻¹ 000 PFU or 300⁻³ 000 LD\textsubscript{50}\textsuperscript{[34-36]}. By adding saponin from Quillaja saponin (QS-21) or saponin that contains RIBI adjuvant, VLPs vaccine dose can be reduced, and can completely protect mice and guinea pigs from EBOV infection, even just inoculated a single dose\textsuperscript{[37]}.

For the truly practical application of the Ebola VLPs vaccine, the production problems need to be solved, because the VLPs used in previous studies were made by 293T, and it cannot meet the requirements of mass production. At present, Baculovirus insect cell expression system has been used in a lot of viral VLPs production. Ye et al from Emory University in U.S. attempted to express Ebola VLPs with this system, and they successfully used the system to achieve a high level of EBOV VLPs expression and assembling. The VLPs they produced can induce the generation of antibody of Ebola and block the pseudotyped Ebola virus infection; moreover it can stimulate secretion of dendritic cell cytokine\textsuperscript{[38]}. Warfield from American Army Medical Research Institute of infectious diseases also successfully assembled VLPs by the GP, NP and VP40 of Ebola virus by using such system, and proved that the VLPs can mature human myeloid dendritic cells, mice immunized with 293T cells induced by VLPs derived from the same cell immunity and humoral immunity, and protects mice from a lethal dose of Ebola virus attacks. The protection effect was dose dependent. Ebola VLPs vaccine vector vaccine is safer, if its immunogenicity can be further improved, and the immune period can be shortened, it is expected to become the most potential Ebola vaccine.

Discussion

Typically, attenuated vaccines, which are easier to produce and stimulate natural or acquired immune response, have better immune response than replication-defective vaccines. However, it’s not the case with Ebola virus, due to the difficulty to ensure the safety of the vaccines. Attenuated vaccines are synthesized based on weak viruses, such as vesicular stomatitis virus, parainfluenza virus, etc. Though showing good results from the animal experiments, including those on immunodeficient animals\textsuperscript{[39]}, security risks still exist. For adenovirus, human parainfluenza virus may cause pre-existing autoimmune problems\textsuperscript{[40]}. From recent studies on antibody response, T cell proliferation, and T cell cytotoxic have shown that cell memories between antibodies and T helper cells are necessary in mediated protective immune response. As a comparison, cell-mediated immune
response is not necessary, though important. The preclinical evaluations on these vaccines are in progress. A vaccine supported by the NIH is waiting for the FDA approval to enter clinical trials, which is expected to enter Phase I clinical trials in fall 2014. An adenoviral vector vaccine, which contains two pieces of Ebola virus glycoprotein genes, has been tested on chimpanzees. One of two other vaccines that contain vesicular stomatitis pseudo virus has been chosen as candidate for clinical trials, and may be carried out in early 2015.

Currently, several proposals to fight Ebola virus including the adenovirus vector vaccine, VSV vector vaccine, HPIV3 vector vaccine, the rabies virus vector vaccine, VEEV vector vaccines and VLPs vaccines, have been proved to be providing effective protecting towards non-human primates. Among them, the adenoviral vector vaccines and the VSV vector vaccines are the most probable ones to be applied to control the Ebola virus in the recent. The US and Canada stand in the lead position in the studies in this area. Due to the lack of level 4 biosafety laboratories, China has its difficulty in testing the vaccine by performing animal experiments. However, with the putting to use of these laboratories, China has entered a new period in this kind of research.

References


