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牛泡沫病毒中国株感染兔的研究*

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摘要：将牛泡沫病毒（BFV3026）感染的细胞经耳缘静脉注射兔子，并以正常细胞注射的兔为对照。1年后处死，病毒挽救实验及PCR检测显示：兔经一次注射即可被BFV3026感染，病毒广泛分布于感染兔的多种脏器中，通过共培养可从感染兔血、肝、脾、肺、肾中拯救出相应感染性病毒颗粒，并在脑、骨髓、心、胰、肠系膜中检到高拷贝BFV原病毒DNA存在。同时，血清学检测表明：感染兔在接受注射一个月后即产生高滴度抗病毒蛋白抗体，并维持该滴度水平直至实验终止，兔未表现任何可观病变。

关键词：牛泡沫病毒3026；兔子感染

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The Study on the Infection of Rabbits with Bovine Foamy Virus 3026*

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Abstract: Rabbits were injected with a single intravenation of *Bovine foamy virus* 3026 (BFV3026)-infected cells in the ear and monitored for sera-conversion serologically up to 1 year. Results of the serological and virus-rescue assays indicated that all animals were persistently infected with BFV3026 and showed sustained BFV3026-specific humoral immune response. BFV3026 was rescued by co-cultivation with fetal bovine lung (FBL) cells from the spleen, kidney, lung, liver and peripheral blood leukocytes of the infected animals. Virus sequence in *pol* was recovered and amplified from the tissues where the virus was rescued from, such as from the heart, brain, bone marrow, mesentery and the pancreas. In addition, BFV transcripts could be detected in PBL by RT-PCR. This animal model might be useful for studying the interaction between BFV and BIV.

Key words: *Bovine foamy virus* 3026; Rabbit infection

Foamy viruses (FVs) are endemic in many vertebrates, including cows, cats, horses and all primates examined other than humans[1, 2]. It is remarkable that the FV distribution mirrors that of the lentiviruses. All groups that are naturally infected with a lentivirus are also infected with an FV[3].

It is still not known whether FV infections could lead to any kind of diseases. However, there are some evidences suggesting that their presence in host may contribute toward diseases caused by other pathogens[4~6]. Additionally, FV infection was recently shown to be lethal in nude mice[7]. In our previous study, we found that *Bovine foamy virus* (BFV) Tas could activate the bovine immunodeficiency virus (BIV) promoter and induce high viral production[8]. Presumably, their co-infection may lead to the death of the cow, but these have not been identified yet.

For characterizing the relationships between the virus and the host immune system, there were several investigations of primate FV infection *in vivo*[9~13].

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However, some of the published data were contradictory, and a clear picture of the tropism and status of viral expression did not emerge.

BFV is widely prevalent in every country and has high rates of occurrence in farm animals, in which ten percent of the animals are co-infected with BIV. Very little is known about the tissue-specific tropism of BFV in animal and the role of BFV in causing bovine disease as well as the host immune response to BFV infection. We are interested in developing a BFV-small-animal model that may be useful in studying the mechanisms of persistent BFV infection and its pathology.

1 Materials and methods

1.1 Animals

Out-bred, specific-pathogen-free, barrier-raised male Chinese White Rabbits were obtained shortly after weaning and maintained in a barrier facility throughout the experiment. Three of them were injected with fetal bovine lung (FBL) cells infected with BFV3026 that was originally recovered by us from a BIV-positive cow named number 3026, and two control animals received uninfected FBL cells. All five animals were given a single intravenous injection of 1×10^7 cells in ear and used for serologic, biologic and molecular analyses.

1.2 Virus stocks

BFV3026 virus stock was propagated in FBL cells and used to inoculate the rabbits. The FBL cells were cultured in DMEM medium containing 10% fetal bovine serum and antibiotics. After infected with BFV3026, they were monitored visually for syncytium production until more than 50% of the cell monolayer showed cytopathic effects (CPE) of the virus. Infected and uninfected cells (1×10^7 cells) were typsinized, washed three times with sterile 1× phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS), diluted in 0.5mL PBS and used respectively for inoculation in animals at once.

1.3 Serological test

All rabbits were bled once by ear venipuncture prior to inoculation and monthly thereafter until euthanized. Blood samples were used for detection of BFV antibodies by enzyme-linked immunosorbent assay (ELISA) and Western- blot in sera..

1.4 Virus detection

The presence of BFV-induced specific cytopathic effect of virus particles after co-culture experiments with permissive FBL cells were studied by light and electron microscopy.

1.5 Gene amplification by PCR and RT-PCR

DNA or RNA from infected or uninfected rabbit peripheral blood leukocytes (PBLs) or organs and tissues was extracted as described by Joseph[14]. Enzymatic amplification of the BFV sequences was performed using oligonucleotide ('aac cag gtc gga cat c and acc agc tgt gga cag t) complementary to RT conserved regions in the BFV *pol* genes.

1.6 Electron microscopy

Co-cultures between rabbit organs and permissive FBL cells were fixed in 2.5% glutaraldehyde, post-fixed in OsO4, embedded in epoxy resin, sectioned and stained with uranyl acetate and lead citrate. They were observed under a Philips 301 electron microscope.

2 Results

2.1 Serological response

Serum samples were tested for positivity against antibodies specific for the BFV3026 proteins via ELISA. Positive samples were reactive toward the antibodies against various BFV proteins, as shown by the Western immunoblot (Fig.1). We found that all BFV3026-infected rabbits became seropositive within one month and their immunoglobulin G (IgG) antibody titers were in the range of 1:1000, and the same approximal level was kept until they were euthanized (Fig.2). Sera from the control rabbits were negative for BFV3026-specific IgG in all assays.

2.2 Rescue of BFV from rabbit peripheral blood cells

Whole bloods were plated onto subconfluent FBL cells in DMEM medium supplemented as described above. After 24 hours, the culture medium was removed and replaced with the fresh. The cultures were carried for five passages and visually monitored daily for syncytium production. From the first bleed to the last, all blood samples from the inoculated rabbits could induce the syncytia formation, whereas the

control group could not.

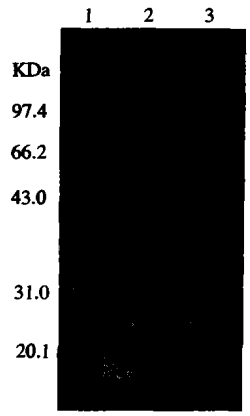

Fig. 1 Reactivity of sera from experimentally infected rabbits

Uninfected fetal bovine lung cells (line 1), fetal bovine lung cells infected with BFV3026 virus (line 2) or transfected with BFV3026 DNA (line 3), respectively. The cell proteins were separated by 10% SDS-PAGE gel and transferred to nitrocellulose membrane. After blocked by 5% nonfat milk, the membrane was first incubated with rabbit anti-whole BFV3026 serum at 1:100 for 2hr, then incubated with goat anti-rabbit IgG-HRP at 1:50 dilution for another 2hr, and last visualized by 4-chloro-1-Napthol (Amresco).

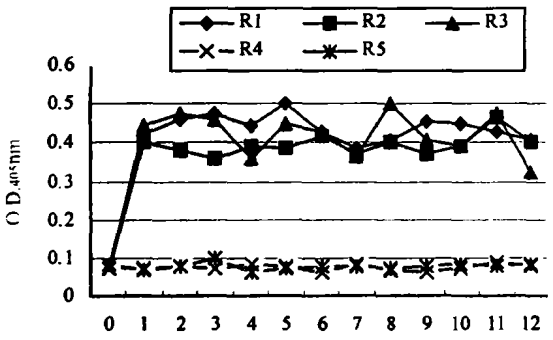

Fig. 2 Serologic responses of rabbits to infection with BFV 3026

Sera from rabbits inoculated with 10^7 BFV3026-infected fetal bovine lung cells (R1, R2, R3) and with 10^7 uninfected fetal bovine lung cells (R4, R5)were tested.

2.3 Detection of BFV provirus by PCR and viral transcripts by RT-PCR

Total PBMC were centrifuged at 2500 r/min for 10 min, and the white layer between the erythrocytes and the sera was collected. After lysis of remaining erythrocytes with lysis buffer (15mmol/L NH_4Cl, 40mmol/L NH_4HCO_3, 100mmol/L EDTA [pH 7.4]), the PBLs were collected and processed for genomic DNA and total cellular RNA, PCR on DNA templates and RT-PCR on RNA templates were performed. Amplified products were detected on 1% agarose gel, and all samples (except the ones from the control group) showed band in the correct and predicted size of 596bp.

2.4 Distribution of BFV in different organs

All animals were euthanized by perfusion with sterile PBS 12 months post-inoculation. Organs and tissues including liver, lung, brain, spleen, kidney, bone marrow, heart, mesentery and the pancreas were taken under sterile conditions and homogenized for virus isolation and gene amplification, which were done according to procedures described above for blood samples. In all cases, BFV was successfully rescued from the spleens, livers, lungs, or the kidneys of the BFV-infected rabbits by co-cultivation with FBL cells (Fig. 3A). BFV from the various organs was identified by detecting syncytia formation in the co-cultured medium, and positive identification of the viral particles was done through electron microscopy, in Fig. 3B, we can see that viral particle has the typical FV appearance, enveloped particles surrounded by spikes and a clear central core. Through PCR analysis, BFV DNA sequences were detected in all perfused organs and tissues taken (Fig. 4), whereas virus particles was not rescued from other organs apart from spleens, livers, lungs, or the kidneys, and the corresponding parts from the control animals were negative for both syncytia formation and BFV DNA sequences.

Fig. 3 Virus rescued from BFV-infected rabbits

A, Fetal bovine lung (FBL) cells which have been cocultivated with the lung of a BFV-infected rabbit; syncytia are present. B, Electron micrograph of a thin section of the BFV-infected fetal bovine lung cell culture shown in A.,The viral particle has the typical FV appearance, enveloped particles surrounded by spikes and a clear central core

2.5 Quantification of BFV DNA in different organs

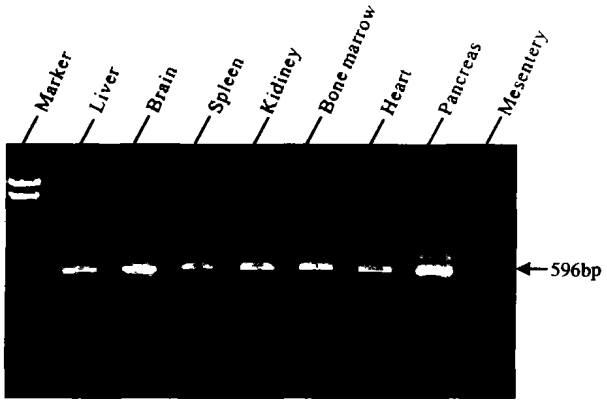

Fig. 4 DNA from selected tissues of infected rabbits were analyzed by PCR

To semi-quantify the amount of proviral DNA present in the selected tissues, ten-fold serial dilution of DNA extracted from brain, heart, bone marrow, pancreas and mesentery were used for amplification of 596bp segment from the *pol* region. Considering the sensitivity of this PCR not as high as that of *pol* nested PCR, which has the sensitivity of about 5 copies of viral DNA[15], the viral copy number per nanogram of cellular DNA present in each sample was calculated. The result showed that tissues including brain, mesentery, and heart harbored at least 10 copies per nanogram of cellular DNA, bone marrow had about 100 copies and pancreas only had about 5 copies (Fig.5). This result is unexpected, as recent report suggested that there is only 1 copy per nanogram of cellular DNA in most organs of infected African Green Monkeys[6].

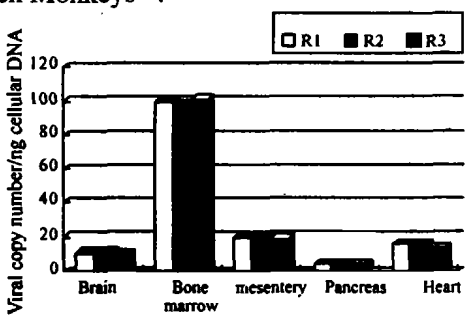

Fig. 5 Semiquantitative analysis of proviral load in some positive organs of infected rabbits.

Bars represent the copy number of viral DNA per nanogram of cellular DNA. R1, R2, R3 are three rabbits infected with BFV3026

2.6 Pathology

All rabbits experimentally infected with BFV3026 appeared normal throughout the experiment and their differential and total leukocyte counts remained within normal ranges for both infected and uninfected animals (data not shown).

3 Discussion

Animal models for primate foamy virus have been developed in different species including rabbits and mice[13]. However, there is no report studied the persistent infection of animals by non-primate FVs just like BFV. This could be important for thoroughly understanding of the host response to FV infection. In this study, the infection of rabbits with 1×10^7 BFV3026-infected FBL cells was accomplished by single ear venipuncture. The infected rabbits showed a high-titered humoral immune response within one month of inoculation and kept within this titer range thereafter until euthanized. Virus was rescued not only from PBLs but also from other tissues including the spleen, liver, kidney and lung. Moreover, viral transcript was detected in PBLs by RT-PCR, this result suggested that BFV LTR promoter can function efficiently in leukocytes, which was not consistent with that of the SFV[15].

By PCR analysis, BFV sequence was also detected in the heart, brain, bone marrow, mesentery and the pancreas, in addition to other tissues where the live virus was rescued. These results indicated that the BFV3026 virus could infect various tissue types within the rabbit body and this infection could not be overcome by the rabbits' immune system. On the other hand, the infected rabbits lacked the manifestation of disease symptoms and their organ tissues did not show any significant lesions. The bone marrow had the largest DNA copies revealing that it had the highest cell tropism, and bone marrow could be served as the target organ for gene therapy.

Although more information is needed to completely comprehend the BFV infection process in rabbits, our model offers an opportunity to study the co-infection of BIV and BFV and to search for possible therapeutic agents against HIV as well as the opportunity to study the mechanisms of foamy virus persistence and replication.

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