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ORIGINAL ARTICLE

Construction, Immunogenicity Study. Recombinant Ad5 Vaccine Expressing HIV-1 CRF01_ AE Gag Gene

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Abstract: Objective To construct recombine AD5 Vaccine Encoding codon-optimized HIV-1 crf01_ AE gag gene and evaluate Its Immunity in Mice.Methods The HIV-1 crf01_ AE gag gene was optimized in the previous phase of our laboratory,A combined AD5 vaccine carrying the gag gene,RAd5-HIV aegag,Was connected.After confirmation that the combined AD5 vaccine ould express Gag protein,The immune effect of recombine vaccine rAd5-Hivaegag was evaluated in BALB/c mice.: Recombinant Ad5 vaccine expressed Gag protein efficiently, induced high level. humoral, cellular immune responses. immunized BALB/c mice.Conclusion. recombinant Ad5 Vaccine Encoding HIV-1 CRF01 of AE gag gene. constructed successfully.

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1.1 Carrier and reagentAdEasy^{TM}Adenovirus Packaging System

Cells, which were screened by laboratoryCrf01 _ AE gagSpecific polypeptideP24Stimulating mouse spleen lymphocytes.FollowELISPOTKit manual detection, after the end of the experimentAid ELISPOT ReaderRead the number of spots,GraphpadSoftwareNewman-Keuls multiple comparison testComparison of experimental data between groups.

2. Knot Guo

2..1.RestructuringAD5VaccineRAd5-HivaegagGene Level IdentificationTaking ProteaseKDigested recombinant adenovirusRAd5-HIV aegagProduct as a templatePdc316-HIV aegagPlasmid as an positive control samples DesignGagSpecific PrimersPCRReaction (Figure1)1. 2%Agarose gel electrophoresis run glue displayRAd5-

HIV aegagAnd positive control samples were can amplification the size about

1500bpOf specific bands (GagGene has been successfully insert to recombinant adenovirus of genome in.

2.2 RecombinantAd5VaccineRAd5-HivaegagProtein Level of IdentificationThe recombinant adenovirusRAd5-HIV aegagCertain dose Infection293Cells stay cells completely lesions after collection cellsWestern BlotMethods The detection objective protein of expression(Figure2)RAd5-HIV aegagInfection293Cells samples in can see relative molecular quality140kDAbout the Strip normal293Cells samples only37kDAboutGAPDHControl strip (recombinant adenovirusRAd5-HIV aegagCan effective to expression objective protein Gag.

2.3 RecombinantAd5Vaccine separate Immune Effect In0Weeks immune In Rise state peak were there in immune after the first4Weeks; to the first8

Times the first1,2,3,4,6And8Weeks respectively the mice eye venous plexus take Weeks when mice of humoral

immune level has fallen to a low

Blood and separation serumELISAMethod detection Recombinant Vaccine Immune after Level.

Mice humoral immune reaction strength of experimental results the statistical credit While the sterile conditions under anatomy mice remove the spleen and separation small

Analysis (Figure3)RAd5-HIV aegagVaccine can induced mice Production Rat spleen lymphocytes cells with laboratory early screening theCRF01 of AE

StudentsGagSpecific humoral immune reaction in the first4Weeks when1 × $10^{7/}$ SubtypeGagSpecific polypeptideP24Stimulation Mice Spleen Lymphocytes cells Mining

$TCID_{50}$/Only and1 × $10^{8}TCID_{50}$/Only2A dose group mice were was With-Mouse IFN-γELISPOTKit DetectionGagSpecific Vaccine induced by the average up3 500More than of antibody drop degree;2A is Cells immune response reaction.Immune after the first1,2,3,4,6And8Weeks when

High dose group of mice and2A low dose group of mice of humoral-free Mice in vivo of cells immune response strength compare the (figure4) In recombinant Disease effect compared difference has statistical significance ($P<0.05$);In the first4 VaccineRAd5-HIV aegagImmune after of mice can be detection

Weeks with the time of increase,4A dose group of immune reaction strength were HIV-1 gagSpecific cells immune response reaction the first4Weeks when 1× $10^{8}TCID_{50}$/Only dose group mice were vaccine induced by the average high 25 284OfIFN-γNumber of spots(SFC)/10^{6}Spleen Lymphocytes fine

Cell;AndPBSControl groupHIV-1 gagSpecific immune reaction for negative results;In the first4Weeks with the time of increase,4A dose group of specific cells immune response reaction were in rise state in the first

4. Weeks when mice of cells Immune Response Level to peak then reaction strength decreased until the first8Weeks when mice in still keep is high cells immune response strength can the Construction of Recombinant Vaccine the induced by the specific cells immune response reaction is compare the strong and compare

Lasting;Immune after the first4Weeks mice in vivo of cells immune response strength (Figure5)Can see,2A is high dose group of mice and2A low dose group of mice induced byELISPOTNumber of spots compared difference has statistical significance ($P<0.05$);2A is high dose group of mice induced byELISPOTNumber of spots between difference no statistical significance ($P>0.05$).Comprehensive on the according to save vaccine and need to induced by the compare the high immune response reaction strength of Principle,1 × $10^{7/}$

$TCID_{50}$/Only of dose can as an the experimental of best vaccine immune dose.

3. Please On

Laboratory early the of based onB'OrCSubtypeHIVTreatment of vaccine of multi-carrier vaccine order sequential and repeat immune strategy in Animal Model in can induced by the continuous time is long,Reaction strength is high[10]

Specific cells immune reaction in Early Research of based on successful construction the expressionHIV aegagGene of recombinant adenovirus vector vaccine experimental results can see the vaccine can effective to expression objective gene protein the vaccine can in mice in vivo can INDUCED BY THE STABILITY,Continuous time long and reaction HIGH STRENGTHHIV gagSpecific humoral and cell immune reactionHIVVaccine of immune Treatment Strategy provide the candidate vaccine.At present expression the gene of other carrier vaccine also basic construction complete, future will further evaluation these vaccine of multi-carrier order sequential and repeat immune of effect and the sub style vaccine and other subtype carrier Vaccine Combined with application of effect for late of clinical study provide experimental data.

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