Vetenskaplig artikel

Kunskapsprov för läkare utbildade utanför EU/EES

Vetenskaplig artikel

Vetenskaplig artikel är en del av det teoretiska kunskapsprovet. För att kunna svara på frågorna under tidspress och trötttheten i slutet av provet behöver man att kunna snabb navigering i en artikel så att hitta svar på frågor så fort som möjligt utan att behöva läsa hela artikeln. Alltså är det viktigt att tänka på artikelstruktur. Allmänt består de flesta artiklarna av nedanstående struktur.

Tänk på olika delar av artikeln!

- ☞Abstract
- ☞Introduction
- ☞Methods
- ☞Results
- ☞Discussion

- Du skulle få ett utskrift av artikeln, men i examprogrammet högst upp till höger du kan också hitta en digital version av detta. Tyvärr är det inte sökbar. Du får instruktion på detta under provet.

- Läs först alla frågor sedan börja hitta svar på dem.
- Läs **noggrant** abstract.
- Gå genom titlar och rubriker och bilder och diagrmen i artikeln snabbt.

Nu går vi igenom frågor!

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- A lipid-encapsulated mRNA encoding a potently neutralizing human monoclonal antibody protects against chikungunya infection
- BY NURGUN KOSE, JULIE M. FOX, GOPAL SAPPARAPU, ROBIN BOMBARDI, RASHIKA N. TENNEKOON, A. DHARSHAN DE SILVA, SAYDA M. ELBASHIR, MATTHEW A. THEISEN, ELISABETH HUMPHRIS-NARAYANAN, GIUSEPPE CIARAMELLA, SUNNY HIMANSU, MICHAEL S. DIAMOND, JAMES E. CROWE, JR.
- SCIENCE IMMUNOLOGY17 MAY 2019

#1

Vad är det vetenskapliga målet för denna studie?

A. Att behandla patienter med humana antikroppar mot virusinfektion
B. Att jämföra mRNA-vaccin med mRNA som kodar för humana antikroppar
C. Att visa att mRNA kan användas som ett virusvaccin
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1- Titel

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3- Sista stycket av Introduction

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SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

EMERGING INFECTIONS

A lipid-encapsulated mRNA encoding a potently neutralizing human monoclonal antibody protects against chikungunya infection

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Infection with chikungunya virus (CHIKV) causes an acute illness characterized by fever, rash, and arthralgia. However, CHIKV infection can sometimes progress to chronic arthritis or even lethal disease. CHIKV continues to cause substantial morbidity worldwide as its vector mosquitoes expand and spread. There are currently no approved vaccines or antiviral drugs available for the prevention or treatment of CHIKV. Although antibody therapy has shown promise in the prevention or treatment of CHIKV disease in preclinical models, challenges remain for implementing such therapies. Here, from the B cells of a survivor of natural CHIKV infection, we isolated ultrapotent neutralizing human monoclonal antibodies (mAbs) and encoded their sequences into mRNA molecules delivered by infusion. One human mAb, CHKV-24, was expressed to biologically significant levels in vivo after infusion of mRNAs in lipid nanoparticles in mice. We evaluated the protective capacity of CHKV-24 mAb immunoglobulin G protein or mRNA in mouse models of CHIKV infection. Treatment with CHKV-24 mRNA protected mice from arthritis, musculoskeletal tissue infection, and lethality and reduced viremia to undetectable levels at 2 days after inoculation. Infusion of macaques with CHKV-24 mRNA achieved a mean maximal mAb concentration of 10.1 to 35.9 micrograms per milliliter, with a half-life of 23 days, a level well above what is needed for protection in mice. Studies with CHKV-24 mRNA in macaques demonstrated a dose-response effect after the first dose of mRNA and maintained levels after second dose. These preclinical data with CHKV-24 mRNA suggest that it might be useful to prevent human disease.

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Vilka är de karaktäristiska symptomen vid chikungunyavirus-infektion?

A. Feber, hepatit och diarré
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Vilka är de karaktäristiska symptomen vid studerade sjukdomen?

Sådana bakgrund kan hittas på:

1- Abstract

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attributed to assay and study variability and the outbred population of animals. Furthermore, the serum half-life of this antibody in macaques was found to be 23 days after a single infusion. These studies provide a rational basis for use of similar RNA LNP formulations in humans and point the way toward defining the human dose of mRNA needed to accomplish biologically meaningful expression of human IgGs in vivo.

Treatment with CHKV-24 mRNA or mAb significantly protected mice from lethality in a dose-dependent manner. Viremia was reduced to the limit of detection on 2 dpi, further supporting the efficacy of CHKV-24 in this mouse model. Protection was mediated by systemic levels of 10 μg/ml of CHKV-24 mAb, which has an in vitro neutralization IC_{50} value of 4 ng/ml. The higher concentration needed for effect in vivo may be explained, in part, by the stringency of the testing in immunocompromised AG129 mice, which lack interferon-α/β and interferon-γ responses and the expected antigen excess, which effectively shifts the neutralization to a requirement for greater antibody concentrations (*41*). Determining the ratio of effective in vitro and in vivo concentrations for antiviral antibodies is complex and often requires combined experimental-mathematical approaches that include precise estimates of virion-antibody interaction stoichiometry, tissue distribution, and half-life (*42*). Postexposure treatment in WT mice reduced viremia, diminished infection in the ipsilateral foot, prevented sp[illegible]d to the contralateral foot, and protected against foot swelling. A[illegible] tration of CHKV-24 mRNA, when given as a single or two 6[illegible] intravenous infusions, was well tolerated in monkeys at all dose le[illegible]ls tested. Human IgG1 antibodies were detectable through day 83 when

mRNA encoding CHKV-24. Antibody concentrations after day 8 were calculated only for the highest dose level (3 mg/kg). At 24 hours after dosing with 3.0 mg/kg mRNA, the maximum CHKV-24 IgG serum concentration was 16.2 or 28.8 μg/ml for dose 1 or dose 2, respectively. The mean values are indicated, and error bars show the SD.

dosed once with CHKV-24 mRNA at 0.5 mg/kg and through day 100 after two doses of CHKV-24 mRNA at 3 mg/kg.

These studies suggest that passive immunization or treatment of humans by administration of LNP formulations containing mRNAs encoding for an anti-CHIKV antibody may be feasible. The ability to deliver sufficient protective levels of antibodies in humans using such LNP RNA formulations can only be determined in human clinical studies. On the basis of our results, the CHKV-24 mRNA has been selected as a development candidate for testing in humans. The high levels of mAb expression achieved here with CHKV-24 mRNA in mice and NHPs, and the complete protection of mice against lethal disease or arthritis, suggest that additional studies are warranted to determine the promise of this approach for prevention or treatment of CHIKV disease. Prophylaxis with this antibody treatment could be considered for travelers to affected areas, and clinical testing of therapy of infected patients could be evaluated to

a platform for rapid development and deployment of mRNA-encoded mAbs for many other emerging infectious diseases. Although there are

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#3

I studien behövde forskarna använda neutraliserande antikroppar mot CHIKV.

Hur valde man ut dessa antikroppar?

A. Plasma från 44 möss screenades för neutraliserande antikroppar
B. Plasma från 44 chikungunyavirus screenades för neutraliserande antikroppar
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E. Plasma från 44 lymfoblastoida cellinjer screenades för neutraliserande antikroppar

Gissa var i artikeln skulle finnas svar?

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RESULTS

Donor selection

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#4

I en del av studien användes humana monoklonala antikroppar.

Varför valdes just CHKV-24?

A. Den hade bäst V-D-J rekombination
B. Den hade bäst neutraliserande effekt
C. Den hade bäst IgG-subtyp
D. Den hade bäst vaccineffekt
E. Den hade bäst mRNA-sekvens

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#5

I den första studien användes möss som saknar interferonreceptorer.

Varför användes immunsupprimerade möss?

A. De producerar makrofager som tar upp antikroppar
B. De får svullna tassar
C. De dör efter infektion av chikungunyavirus
D. De producerar antikroppar mot mRNA
E. De överlever efter infektion av chikungunyavirus

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#6

Monoklonala antikroppar kan teoretiskt ha flera effekter.

Vad var den huvudsakliga verkningsmekanismen för de monoklonala antikropparna som användes i studien?

A. De stimulerar hudcellernas antivirala egenskaper
B. De neutraliserar immunceller
C. De neutraliserar infektion av chikungunyavirus
D. De nedreglerar immunsvaret mot chikungunyavirus
E. De uppreglerar det medfödda immunförsvaret

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Var hittar du infromation om fall/material/index/test användes i studien?

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We recently showed that some human mAbs to CHIKV prepared as immunoglobulin G (IgG) proteins have potent neutralizing activity (*29*–*31*) and confer protective effects against virus replication in mice (*29*, *32*) and nonhuman primates (NHPs) (*33*). Here, we developed a distinct panel of ultrapotent human mAbs to CHIKV and used mRNA encoding the antibodies to treat and protect against CHIKV. The experiments revealed that delivery of optimized mRNA molecules encoding a potent human antibody resulted in expression at biologically significant levels in the serum of both mice (14.9 μg/ml) and NHPs (10.1 to 35.9 μg/ml) and elicited protection against arthritis, musculoskeletal disease, and lethal challenge in mouse models.

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Donor selection

We screened plasma samples from 44 subjects for the presence for neutralizing antibodies to CHIKV using virus replicon particles based on the Sri Lankan strain SL15649 (*34*). Most (40 of 44) donors had endpoint plasma neutralizing titers of >40, and 10 subjects had titers of >5000. The highest titer observed was a remarkable value of 31,766. We used the peripheral blood mononuclear cells (PBMCs) from this donor for mAb discovery experiments.

Generation of CHIKV-specific mAbs

After Epstein-Barr virus (EBV) transformation, we generated 59 lymphoblastoid cell lines with supernatants containing CHIKV-reactive antibodies that bound to CHIKV particles [181/25 vaccine strain (35)] and exhibited 66% or greater neutralizing activity. We fused the

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potent inhibitory antibody (CHKV-24, an IgG1 with an IC_{50} of 4 ng/ml) for further study in the mRNA delivery experiments.

Protection of mice by delivery of CHKV-24 mAb

AG129 mice that lack receptors for interferon-α/β and interferon-γ are highly vulnerable to infection with CHIKV (*36*) and thus provide a stringent model for testing antiviral compounds (*37*–*39*) or the protective efficacy of CHKV-24 mAb. Mice were treated by the intravenous route with a single administration of purified IgG for CHKV-24 mAb at doses of 10, 2, or 0.4 mg/kg. A dose-dependent concentration of human IgG in mouse serum was observed, as expected (Fig. 1A). At 24 hours, mice were challenged by subcutaneous injection in the footpad and hock of the right leg with a total volume of 0.1 ml of the diluted virus (0.05 ml each site) with a lethal dose of CHIKV [$10^{2.5}$ $TCID_{50}$ (50% tissue culture infectious doses)]. All mice survived after previous infusion of the CHKV-24 mAb with the dose of 10 or 2 mg/kg (Fig. 1B). Half (50%) of animals treated with 0.4 mg/kg mAb survived (Fig. 1B). All animals treated with a control mAb against influenza A

Monoklonala antikroppar kan teoretiskt ha flera effekter.

Vad var den huvudsakliga verkningsmekanismen för de monoklonala antikropparna som användes i studien?

A. De stimulerar hudcellernas antivirala egenskaper
B. De neutraliserar immunceller
✓C. De neutraliserar infektion av chikungunyavirus
D. De nedreglerar immunsvaret mot chikungunyavirus
E. De uppreglerar det medfödda immunförsvaret

#7

För att göra det mer troligt att den givna behandlingen har den specifika effekt som eftersträvas så behövde man jämföra resultaten från kontrollexperiment med andra behandlingar.

Vad användes i denna studie som främsta kontroll vid immunoglobulinbehandling?

A. IgG mot andra virus
B. Ingen antikropp
C. IgG mot chikungunyavirus
D. IgM mot andra virus
E. IgM mot chikungunyavirus

Gissa var i artikeln skulle finnas svar?

Vem/vad är kontroll grupp?

Sådant kan hittas på:

1- Abstract

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Protection of immunocompromised mice against lethal challenge by delivery of CHKV-24 mRNA

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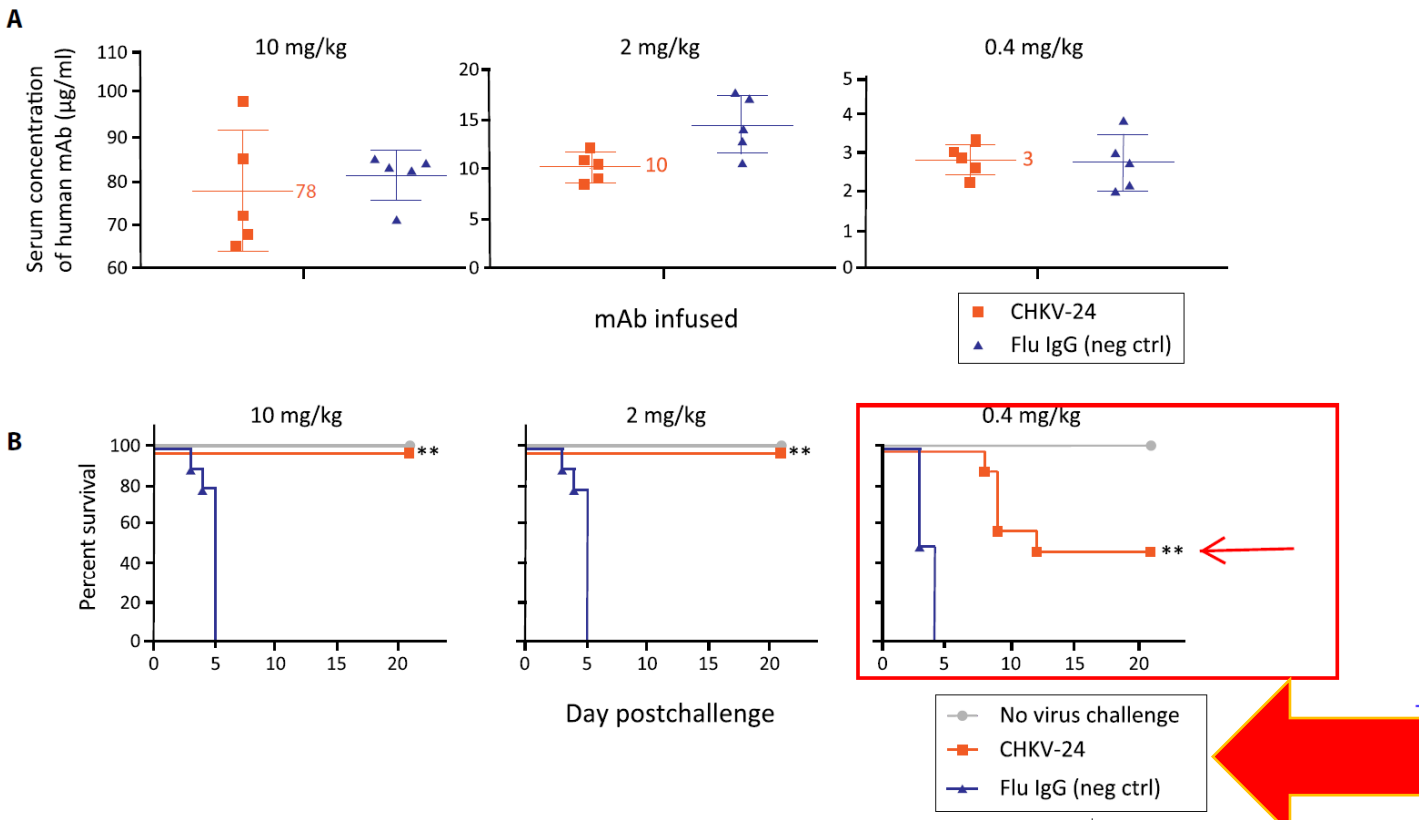

Fig. 1. Prophylactic efficacy of CHKV-24 IgG protein. (**A**) Concentration of human IgG in AG129 mouse serum after CHKV-24 IgG protein infusion. Total human IgG levels were measured 24 hours after infusion of purified human mAb IgG protein for CHIKV-24 (orange) or an irrelevant control mAb to influenza (flu; blue). Animals re-

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✓A. IgG mot andra virus
B. Ingen antikropp
C. IgG mot chikungunyavirus
D. IgM mot andra virus
E. IgM mot chikungunyavirus

#8

Möss som överlevde CHIKV-infektion hade behandlats med antikroppar (mAb CHKV-24 IgG protein).

Vid vilken koncentration av antikroppar överlevde hälften av mössen?

A. 10 mg/kg
B. 40 mg/kg
C. 1 mg/kg
D. 0,4 mg/kg
E. 2 mg/kg

Gissa var i artikeln skulle finnas svar?

Var finns resultat!!?

Sådant kan hittas på:

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2- Results

3- Figures, Tables, etc

Vid vilken koncentration av antikroppar överlevde hälften av mössen?

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Protection of immunocompromised mice against lethal challenge by delivery of CHKV-24 mRNA

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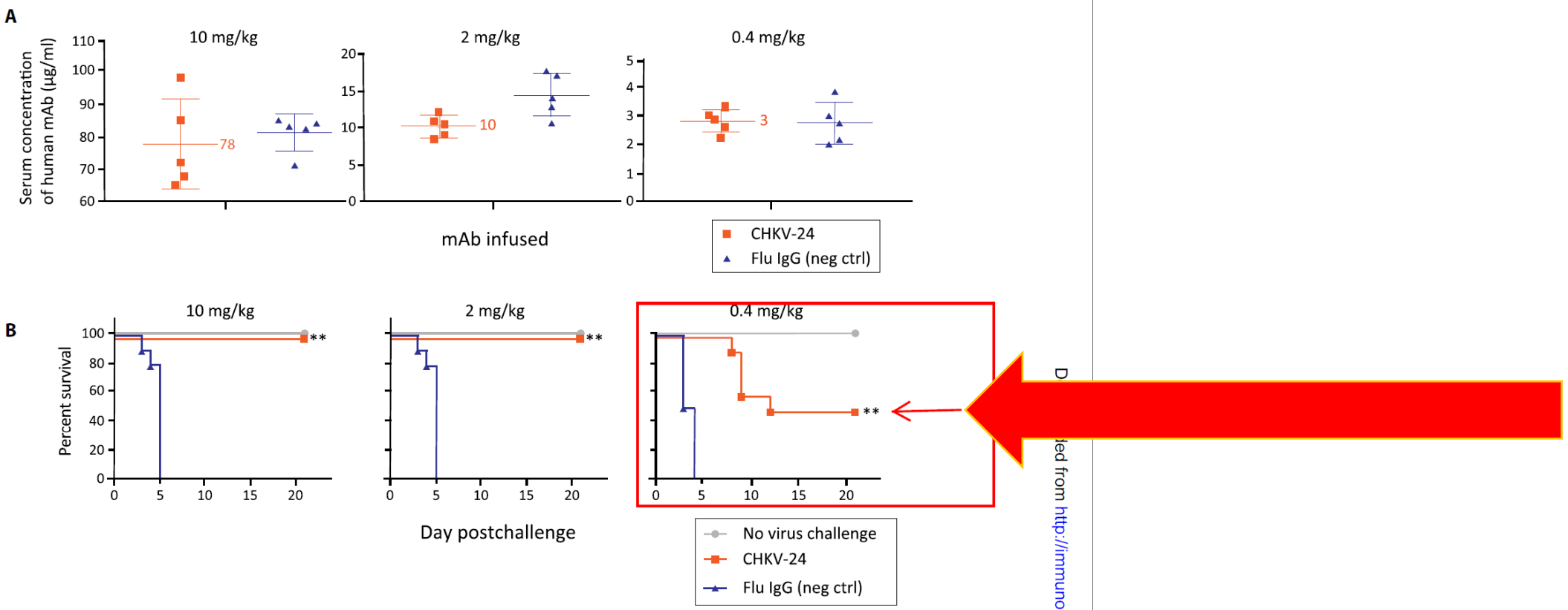

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Möss som överlevde CHIKV-infektion hade behandlats med antikroppar (mAb CHKV-24 IgG protein).

Vid vilken koncentration av antikroppar överlevde hälften av mössen?

A. 10 mg/kg
B. 40 mg/kg
C. 1 mg/kg
✓D. 0,4 mg/kg
E. 2 mg/kg

#9

Möss som överlevde CHIKV-infektioner hade behandlats med mRNA som uttrycker antikroppar (CHKV-24 mRNA).

Vid vilken koncentration av mRNA överlevde 100 % av mössen?

A. 0,02 mg/kg
B. 0,05 mg/kg
C. 0,5 mg/kg
D. 5 mg/kg
E. 0,1 mg/kg

Gissa var i artikeln skulle finnas svar?

Var finns resultat!!?

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Vid vilken koncentration av mRNA överlevde 100 % av mössen?

formulated in a LNP and stored at 4°C until use. AG129 mice were treated by the intravenous route with a single administration of CHKV-24–encoding mRNA at doses of 0.5, 0.1, or 0.02 mg/kg. The mRNA infusion resulted in expression of human antibody in vivo, with a dose-dependent concentration of human IgG detected in mouse serum 24 hours after infusion (Fig. 2A). The mean peak serum concentration of the 0.5 mg/kg–treated group was 14.9 μg/ml. Complete survival of mice (100%) was observed after treatment with the highest dose of 0.5 mg/kg of CHKV-24 mRNA (Fig. 2B). Forty percent of the animals survived after treatment with 0.1 mg/kg mRNA, whereas survival was not observed at the lowest dose of 0.02 mg/kg mRNA (Fig. 2B). Despite the lower level of protection at the two lower doses of mRNA, the survival curves were improved ($P < 0.01$) compared with placebo treatment, demonstrating a benefit of the CHKV-24 mRNA treatment even at the lower doses tested. We compared the level of serum human IgG levels achieved by mRNA infusion in a parallel group of nonchallenged animals receiving 0.5 or 0.1 mg/kg IgG (Fig. 2A) with the results of the survival experiments (Fig. 2B). The comparison suggested that the CHKV-24 mRNA treatment could completely protect AG129 mice in the lethal challenge model when a concentration of 10 μg/ml of systemic CHKV-24 was achieved, while at least half of the animals were protected at CHKV-24 serum levels of about 3 μg/ml.

Virus titer in serum 2 days after challenge was reduced below the level of detection in all mice treated with CHKV-24 mRNA, as compared with an average of 4.6 $\log_{10}$ $TCID_{50}$ in placebo-treated controls (Fig. 2C). Although virus was not observed in the serum in the low-dose treatment group, virus likely replicated in other tissues because

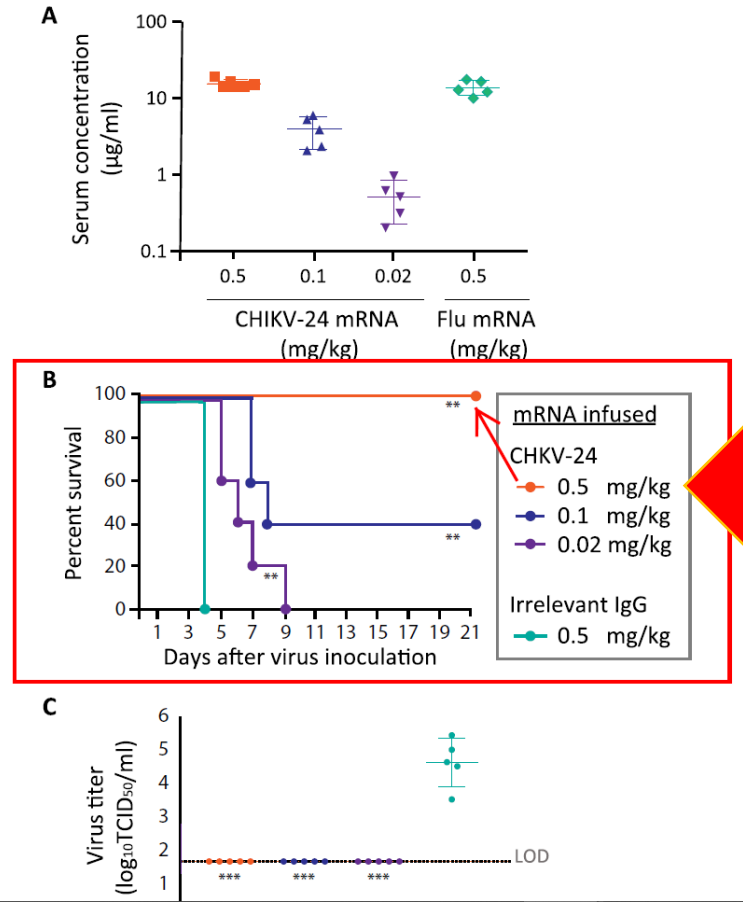
Protection of immunocompetent mice against arthritis and musculoskeletal disease by delivery of CHKV-24 mRNA

While immunocompromised mice provide a stringent protection model, CHIKV infection is rarely fatal in humans but instead causes severe, acute, and chronic polyarthralgia and polyarthritis. Accordingly, we evaluated whether postexposure treatment with CHKV-24 mRNA LNP could protect in the immunocompetent mouse model of CHIKV-induced arthritis and musculoskeletal disease, where subcutaneous infection results in a biphasic swelling of the infected foot peaking at 3 and 7 days post-infection (dpi) (*34*, *40*). When CHKV-24 mRNA was administered 4 hours after infection, wild-type

February 13, 2021

Äls

Vid vilken koncentration av mRNA överlevde 100 % av mössen?



CHKV-24 mRNA had titers at the limit of detection, whereas high levels of viremia were observed in the control-treated mice (Fig. 3B). At 7 dpi, CHKV-24 mRNA–treated mice had an 80-fold reduction in viral RNA in the ipsilateral ankle, with no spread to the contralateral ankle compared with the control mRNA–treated mice (Fig. 3C). Histological analysis of the ipsilateral foot at 7 dpi showed large cellular infiltration into the joint space of the control mRNA–treated mice, whereas this finding was absent in the CHKV-24 mRNA–treated group (Fig. 3D). Slides from two of five mice administered CHKV-24 mRNA showed minimal cellular infiltration in the midfoot (Fig. 3D, right), although the remainder had detectable cellular infiltration in the soft tissue (Fig. 3D, middle). However, the extent of immune cells and edema in the midfoot was reduced markedly compared with the control mRNA–treated mice

CHIKV arthritis.

CHKV-24 expression from modified RNA in cynomolgus macaques

We next tested whether infusion of CHKV-24 mRNA LNPs could induce expression of human IgG in the serum of monkeys that corresponds to the protective concentrations observed in mice. A group of four macaques was infused by the intravenous route with mRNA encoding CHKV-24 at 0.5 mg/kg. This study was repeated with six macaques per group. There were no test article–related clinical signs, changes in body weight, or changes in food consumption during the study. Human IgG1 expression peaked at 24 hours

Möss som överlevde CHIKV-infektioner hade behandlats med mRNA som uttrycker antikroppar (CHKV-24 mRNA).

Vid vilken koncentration av mRNA överlevde 100 % av mössen?

A. 0,02 mg/kg
B. 0,05 mg/kg
✓C. 0,5 mg/kg
D. 5 mg/kg
E. 0,1 mg/kg

#10

För att studera den terapeutiska effekten av olika behandlingar användes möss som efter infektion med CHIKV får svullna tassar. De behandlades 4 timmar efter infektion med CHKV-24 mRNA.

Vad hände med de behandlade mössen jämfört med de obehandlade?

A. De fick svullna tassar
B. De fick svullna tassar och feber
C. De fick feber
D. De fick inga svullna tassar
E. De dog

Gissa var i artikeln skulle finnas svar?

Var finns resultat!!?

Sådant kan hittas på:

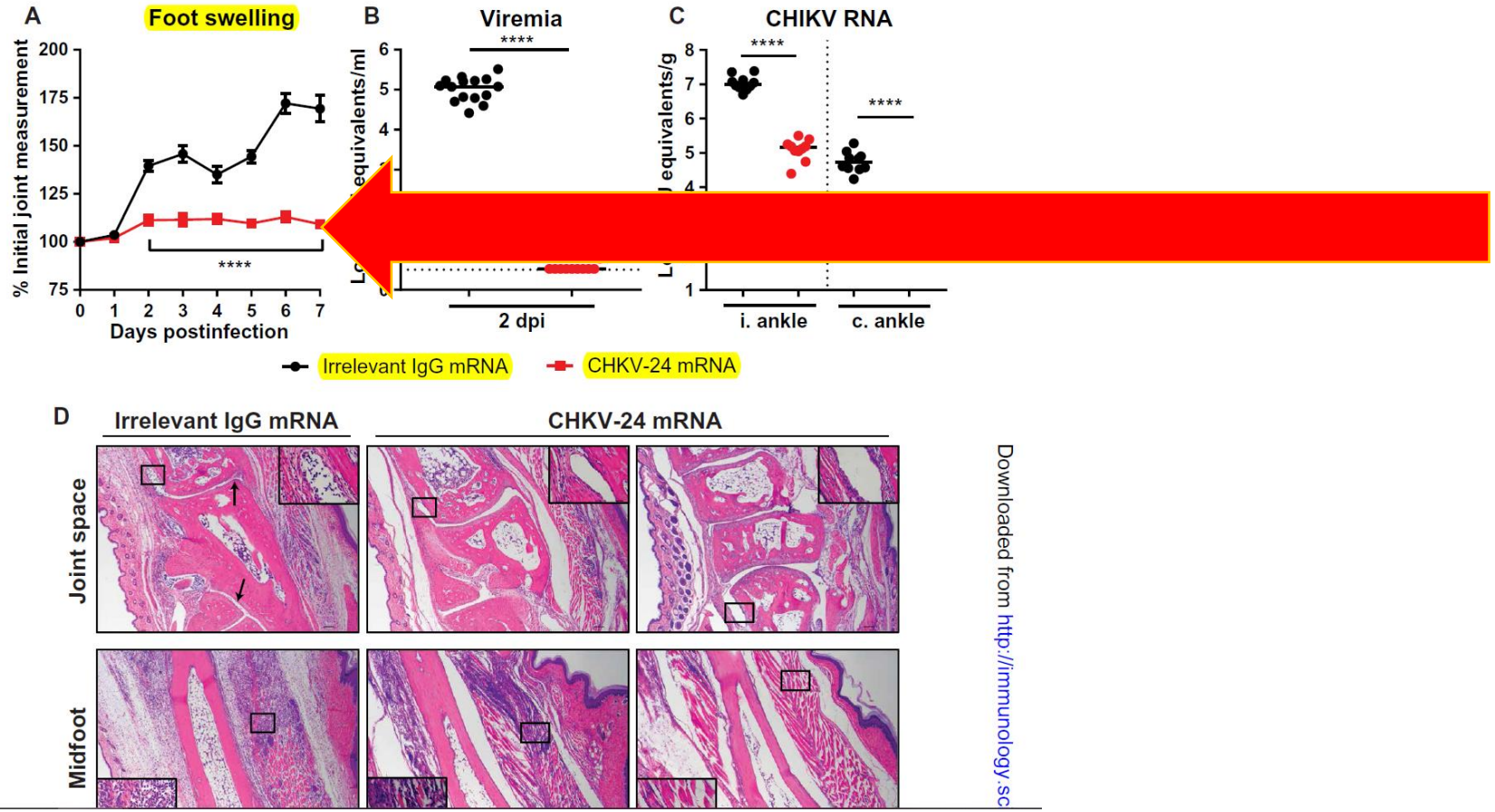
1- Abstract

2- Results

3- Figures, Tables, etc

Vad hände med de behandlade mössen jämfört med de obehandlade?

Fig. 3. Therapeutic administration of CHKV-24 mRNA reduces clinical disease and viral titer in WT mice. C57BL/6 mice received human IgG mRNA (10 mg/kg) by intravenous injection 4 hours after inoculation with CHIKV-LR06. (**A**) Foot swelling was monitored by digital calipers [n =15 per group, two experiments, two-way analysis of variance (ANOVA) with Sidak's posttest]. Line indicates significance between the groups at each time point. Error bars indicate SEM. (**B**) Serum was collected at 2 dpi or (**C**) ipsilateral (i.) and contralateral (c.) ankles were harvested on 7 dpi, and viral RNA was quantified by qRT-PCR (serum: n = 15 per group, two experiments; ankles: n = 10 per group, two experiments, Mann-Whitney test for each tissue). Bars indicate median values. Dotted lines indicate the limit of detection. (**D**) Ipsilateral feet were collected at 7 dpi, fixed in PFA, decalcified, paraffin-embedded, sectioned, and stained with H&E. Images show low magnification (scale bar, 100 μm) with a high-magnification inset (scale bar, 10 μm). Top and bottom panels are representative images of the joint space and midfoot, respectively (n = 5 per group, two experiments). Arrows indicate cellular infiltrate in joint space.



Vad hände med de behandlade mössen jämfört med de obehandlade?

(influenza; flu) mAb mRNA. The CHKV-24 mRNA was administered to mice at a dose of 0.5 mg/kg (orange), 0.1 mg/kg (blue), or 0.02 mg/kg (purple) or the influenza mRNA at a dose of 0.5 mg/kg (cyan), by intravenous tail vein injection. Animals were bled at 24 hours after infusion to measure systemic levels of IgG. Each group had five animals. An additional group of 10 animals was infused with these mRNAs and doses at the same time and challenged with virus 24 hours after infusion [results shown in (B) and (C)]. The mean values are indicated, and error bars show the SD. (**B**) Protection against lethal CHIKV infection mediated by human mAb expressed from mRNA. CHKV-24 mRNA was administered to mice as a prophylaxis at 0.5 mg/kg (orange), 0.1 mg/kg (blue), or 0.02 mg/kg (purple) by intravenous tail vein injection. An irrelevant IgG mRNA was used at 0.5 mg/kg as a control (cyan). Each group of animals was challenged 24 hours after infusion with CHIKV strain LR06 and monitored for mortality. The number of animals in each group was 10. $^{**}P < 0.01$, which indicates that the survival differed significantly from that of the group treated with 0.5 mg/kg of the irrelevant IgG (Wilcoxon log-rank survival test). (**C**) Titer of CHIKV in AG129 mice treated with mRNA encoding mAb CHKV-24 IgG or an mRNA encoding an irrelevant control mAb. Serum samples obtained 2 days after virus challenge were assayed on Vero cell monolayer cultures to determine virus titer ($log_{10}TCID_{50}$/ml). The limit of detection (LOD) was 1.7. The mean values are indicated, and error bars show the SD. Comparisons were made by Kruskal-Wallis test with Dunn's posttest. $^{***}P < 0.0003$, as compared with control IgG. The number of animals in each group was five.

(WT) C57BL/6 mice did not develop foot swelling compared with the mice that received an mRNA LNP encoding an irrelevant IgG control (Fig. 3A). At 2 dpi, serum from the majority of mice receiving

time point serum samples from the studies shown in Fig. 4 were tested for anti-CHIKV activity. Antibody function was assessed by a 50% focus reduction neutralization test ($FRNT_{50}$) and ELISA; a standard curve for concentration versus activity in each assay was generated using dilution curves of purified recombinant CHKV-24 at defined concentrations (Fig. 5). These analyses suggested that the mRNA-expressed antibody was fully functional.

CHKV-24 expression from modified RNA in cynomolgus macaques after multiple doses

After a single-dose study in NHPs using the CHKV-24 mRNA, we tested expression of CHKV-24 IgG after multiple mRNA doses in a NHP study under good laboratory practice (GLP) conditions. Macaques were administered two intravenous doses [phosphate-buffered saline (PBS) control or 0.3, 1.0, or 3.0 mg/kg of CHKV-24 mRNA)] 1 week apart on days 0 and 7, followed by a necropsy on main study animals on day 8 or after a 12-week treatment-free recovery period (day 98). The study design contained the following endpoints: clinical observations, body weights, food consumption, hematology, coagulation, clinical chemistry, cytokine analysis, C3a and Bb complement analysis, toxicokinetics analysis, human IgG protein ex-

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C. De fick feber
✓D. De fick inga svullna tassar
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#11

I studien behandlade man också apor (makaker) med CHKV-24 mRNA. Dessa apor infekterades aldrig med chikungunyavirus.

Vad studerade man i stället?

A. Om antikropparna som producerades i aporna neutraliserade mRNA i laboratoriet
B. Om mRNA neutraliserade chikungunyavirus i laboratoriet
C. Om chikungunyavirus som producerades i aporna infekterade celler i laboratoriet
D. Om antikropparna som producerades i aporna neutraliserade chikungunyavirus i laboratoriet
E. Om aporna uttryckte chikungunyavirus som neutraliserade mRNA i laboratoriet

Gissa var i artikeln skulle finnas svar?

Var finns resultat!!?

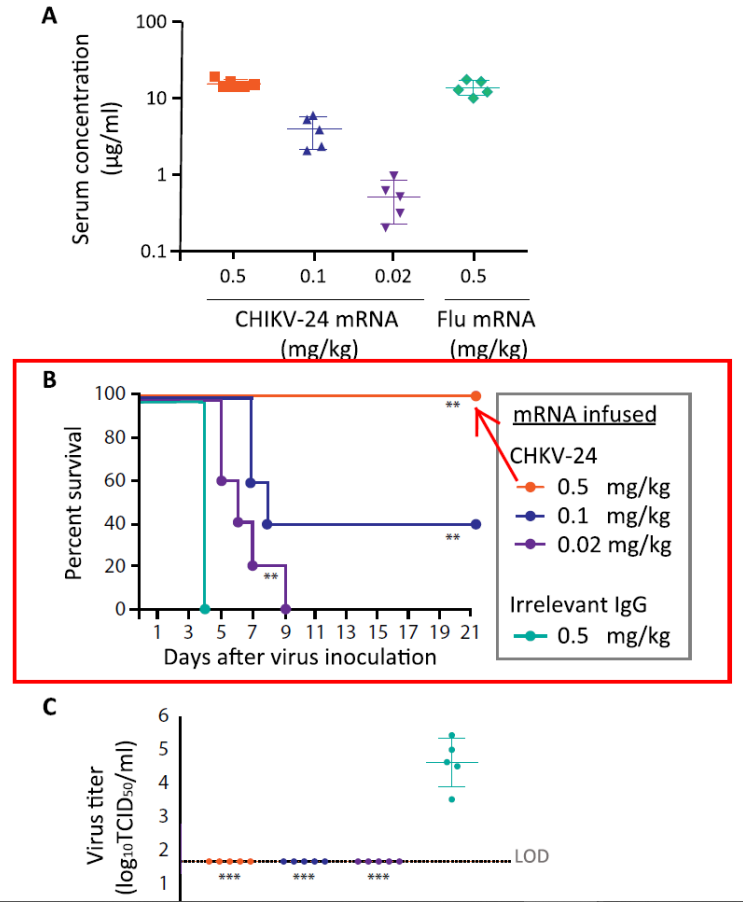
Sådant kan hittas på:

1- Methods

2- Results

3- Figures, Tables, etc

Vad studerade man i stället?



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We next tested whether infusion of CHKV-24 mRNA LNPs could induce expression of human IgG in the serum of monkeys that corresponds to the protective concentrations observed in mice. A group of four macaques was infused by the intravenous route with mRNA encoding CHKV-24 at 0.5 mg/kg. This study was repeated with six macaques per group. There were no test article–related clinical signs, changes in body weight, or changes in food consumption during the study. Human IgG1 expression peaked at 24 hours

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✓D. Om antikropparna som producerades i aporna neutraliserade chikungunyavirus i laboratoriet
E. Om aporna uttryckte chikungunyavirus som neutraliserade mRNA i laboratoriet

#12

I studien undersöks effektiviteten av monoklonala antikroppar.

Vad skyddar CHKV-24 monoklonala antikroppar mot?

A. Ledsvullnad och feber
B. CHKV-24
C. Alla virus
D. mRNA
E. Chikungunyavirus infektion

Gissa var i artikeln skulle finnas svar?

Var hittar du infromation om fall/material/index/test användes i studien?

Sådana bakgrund kan hittas på:

1- Abstract

2- Introduction

3- Methods

4- Results

Vad skyddar CHKV-24 monoklonala antikroppar mot?

SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

EMERGING INFECTIONS

A lipid-encapsulated mRNA encoding a potently neutralizing human monoclonal antibody protects against chikungunya infection

Nurgun Kose[1], Julie M. Fox[2], Gopal Sapparapu[1,3], Robin Bombardi[1], Rashika N. Tennekoon[4], A. Dharshan de Silva[4,5], Sayda M. Elbashir[6], Matthew A. Theisen[6], Elisabeth Humphris-Narayanan[6], Giuseppe Ciaramella[6], Sunny Himansu[6], Michael S. Diamond[2,7], James E. Crowe Jr.[1,3,8]*

Infection with chikungunya virus (CHIKV) causes an acute illness characterized by fever, rash, and arthralgia. However, CHIKV infection can sometimes progress to chronic arthritis or even lethal disease. CHIKV continues to cause substantial morbidity worldwide as its vector mosquitoes expand and spread. There are currently no approved vaccines or antiviral drugs available for the prevention or treatment of CHIKV. Although antibody therapy has shown promise in the prevention or treatment of CHIKV disease in preclinical models, challenges remain for implementing such therapies. Here, from the B cells of a survivor of natural CHIKV infection, we isolated ultrapotent neutralizing human monoclonal antibodies (mAbs) and encoded their sequences into mRNA molecules delivered by infusion. One human mAb, CHKV-24, was expressed to biologically significant levels in vivo after infusion of mRNAs in lipid nanoparticles in mice. We evaluated the protective capacity of CHKV-24 mAb immunoglobulin G protein or mRNA in mouse models of CHIKV infection. Treatment with CHKV-24 mRNA protected mice from arthritis, musculoskeletal tissue infection, and lethality and reduced viremia to undetectable levels at 2 days after inoculation. Infusion of macaques with CHKV-24 mRNA achieved a mean maximal mAb concentration of 10.1 to 35.9 micrograms per milliliter, with a half-life of 23 days, a level well above what is needed for protection in mice. Studies with CHKV-24 mRNA in macaques demonstrated a dose-response effect after the first dose of mRNA and maintained levels after second dose. These preclinical data with CHKV-24 mRNA suggest that it might be useful to prevent human disease.

I studien undersöks effektiviteten av monoklonala antikroppar.

Vad skyddar CHKV-24 monoklonala antikroppar mot?

A. Ledsvullnad och feber
B. CHKV-24
C. Alla virus
D. mRNA
✓E. Chikungunyavirus infektion

#13

För att ge effektivt skydd behöver den IgG-produktion som initierats av behandling kvarstå åtminstone en viss tid.

Hur många dagar efter behandling med CHKV-24 mRNA i apor kunde man påvisa CHIKV IgG i plasma?

A. 90 dagar
B. 98 dagar
C. 24 dagar
D. 7 dagar
E. 70 dagar

Gissa var i artikeln skulle finnas svar?

Var finns resultat!!?

Sådant kan hittas på:

1- Abstract

2- Results

3- Figures, Tables, etc

Hur många dagar efter behandling med CHKV-24 mRNA i apor kunde man påvisa CHIKV IgG i plasma?

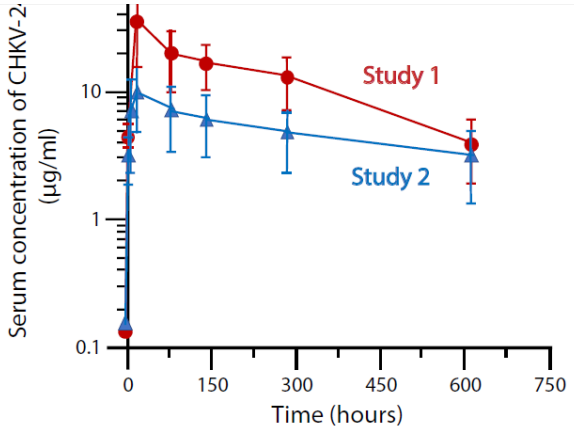

Fig. 4. Pharmacodynamics of CHK-24 mRNA in cynomolgus monkeys. Data show the total human IgG1 concentrations from two NHP studies in which animals were treated with 0.5 mg/kg of CHKV-24 mRNA by intravenous infusion. CHKV-24 mRNA was delivered over 60 min, in a volume of 5 ml/kg and dose concentration of 0.02 mg/ml. Four or six animals were tested in each group for study 1 or 2, respectively. The mean values are indicated, and error bars show the SD. Maximum concentration of 35.9 and 10.1 µg/ml was observed at 24 hours after infusion for study 1 (red curve) and study 2 (blue curve), respectively.

after the second dose (day 8), which was not observed in the recovery animals (day 98) and (ii) three of five male macaques in the

ples tested. Multiple serum samples were collected throughout the duration of the study to measure expression after multiple doses.

CHKV-24 IgG was detected in macaque plasma samples from all animals after mRNA administration. Increasing CHKV-24 IgG concentrations were observed with increasing doses of mRNA. At 24 hours after dosing, maximum CHKV-24 IgG concentrations of 16.2 µg/ml (after dose 1) or 28.8 µg/ml (after dose 2) were observed for animals administered the high dose of 3.0 mg/kg mRNA (Fig. 6). Sex-based differences were not detected in CHKV-24 IgG plasma levels. In animals in the group treated with the highest dose (3.0 mg/kg), CHKV-24 IgG plasma levels were detected 90 days after the second dose, with an average serum concentration of 2.9 µg/ml (Fig. 6).

DISCUSSION

Here, we show that an mRNA-encoded antibody with virus neutralizing activity has potency at equivalent levels as observed with the corresponding purified IgG form of the mAb. We showed that infusion of mRNA encoding a potent virus neutralizing antibody can induce concentrations of human IgG in the serum that protect immunocompromised and immunocompetent mice against lethal challenge and arthritis, respectively. The same mRNA infusions achieved protective concentrations of CHKV-24 in macaques with peak con-

För att ge effektivt skydd behöver den IgG-produktion som initierats av behandling kvarstå åtminstone en viss tid.

Hur många dagar efter behandling med CHKV-24 mRNA i apor kunde man påvisa CHIKV IgG i plasma?

✓A. 90 dagar
B. 98 dagar
C. 24 dagar
D. 7 dagar
E. 70 dagar

#14

Författarna beskriver verkningsmekanismen bakom den skyddande effekten av CHKV-24 mRNA, dvs vad som uttrycktes av CHKV-24 mRNA.

Vad var det som uttrycktes?

A. Vaccin mot chikungunyavirus som skyddar mot infektion
B. Neutraliserande antikroppar mot chikungunyavirus men som inte skyddar mot infektion
C. DNA mot chikungunyavirus som skyddar mot infektion
D. Neutraliserande antikroppar mot chikungunyavirus som skyddar mot infektion
E. Neutraliserande antikroppar mot virus som skyddar mot alla infektionstyper

Gissa var i artikeln skulle finnas svar?

Var hittar du infromation om fall/material/index/test användes i studien?

Sådana bakgrund kan hittas på:

1- Abstract

2- Introduction

3- Methods

Vad var det som uttrycktes?

We recently showed that some human mAbs to CHIKV prepared as immunoglobulin G (IgG) proteins have potent neutralizing activity (*29*–*31*) and confer protective effects against virus replication in mice (*29*, *32*) and nonhuman primates (NHPs) (*33*). Here, we developed a distinct panel of ultrapotent human mAbs to CHIKV and used mRNA encoding the antibodies to treat and protect against CHIKV. The experiments revealed that delivery of optimized mRNA molecules encoding a potent human antibody resulted in expression at biologically significant levels in the serum of both mice (14.9 μg/ml) and NHPs (10.1 to 35.9 μg/ml) and elicited protection against arthritis, musculoskeletal disease, and lethal challenge in mouse models.

RESULTS

Donor selection

We screened plasma samples from 44 subjects for the presence for neutralizing antibodies to CHIKV using virus replicon particles based on the Sri Lankan strain SL15649 (*34*). Most (40 of 44) donors had endpoint plasma neutralizing titers of >40, and 10 subjects had titers of >5000. The highest titer observed was a remarkable value of 31,766. We used the peripheral blood mononuclear cells (PBMCs) from this donor for mAb discovery experiments.

Generation of CHIKV-specific mAbs

After Epstein-Barr virus (EBV) transformation, we generated 59 lymphoblastoid cell lines with supernatants containing CHIKV-reactive antibodies that bound to CHIKV particles [181/25 vaccine strain (*35*)] and exhibited 66% or greater neutralizing activity. We fused the

potent inhibitory antibody (CHKV-24, an IgG1 with an IC_{50} of 4 ng/ml) for further study in the mRNA delivery experiments.

Protection of mice by delivery of CHKV-24 mAb

AG129 mice that lack receptors for interferon-α/β and interferon-γ are highly vulnerable to infection with CHIKV (*36*) and thus provide a stringent model for testing antiviral compounds (*37*–*39*) or the protective efficacy of CHKV-24 mAb. Mice were treated by the intravenous route with a single administration of purified IgG for CHKV-24 mAb at doses of 10, 2, or 0.4 mg/kg. A dose-dependent concentration of human IgG in mouse serum was observed, as expected (Fig. 1A). At 24 hours, mice were challenged by subcutaneous injection in the footpad and hock of the right leg with a total volume of 0.1 ml of the diluted virus (0.05 ml each site) with a lethal dose of CHIKV [$10^{2.5}$ $TCID_{50}$ (50% tissue culture infectious doses)]. All mice survived after previous infusion of the CHKV-24 mAb with the dose of 10 or 2 mg/kg (Fig. 1B). Half (50%) of animals treated with 0.4 mg/kg mAb survived (Fig. 1B). All animals treated with a control mAb against influenza A

Författarna beskriver verkningsmekanismen bakom den skyddande effekten av CHKV-24 mRNA, dvs vad som uttrycktes av CHKV-24 mRNA.

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✓D. Neutraliserande antikroppar mot chikungunyavirus som skyddar mot infektion
E. Neutraliserande antikroppar mot virus som skyddar mot alla infektionstyper

#15

Det går att från studien dra slutsatser om metodens möjliga användbarhet i samband med CHIKV-epidemier.

Hur beskrivs bäst metodens användningsområde(n)?

A. Varken profylaktisk eller terapeutisk behandling mot CHIKV
B. Som vaccin mot CHIKV
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D. Enbart profylaktisk behandling mot CHIKV
E. Enbart terapeutisk behandling mot CHIKV

Gissa var i artikeln skulle finnas svar?

Var hittar du infromation om användningsområde(n)?

Sådana bakgrund kan hittas på:

1- Abstract

2- Introduction

3- Discussion / Conclusion

Hur beskrivs bäst metodens användningsområde(n)?

SCIENCE IMMUNOLOGY | RESEARCH ARTICLE

EMERGING INFECTIONS

A lipid-encapsulated mRNA encoding a potently neutralizing human monoclonal antibody protects against chikungunya infection

Nurgun Kose[1], Julie M. Fox[2], Gopal Sapparapu[1,3], Robin Bombardi[1], Rashika N. Tennekoon[4], A. Dharshan de Silva[4,5], Sayda M. Elbashir[6], Matthew A. Theisen[6], Elisabeth Humphris-Narayanan[6], Giuseppe Ciaramella[6], Sunny Himansu[6], Michael S. Diamond[2,7], James E. Crowe Jr.[1,3,8]*

Infection with chikungunya virus (CHIKV) causes an acute illness characterized by fever, rash, and arthralgia. However, CHIKV infection can sometimes progress to chronic arthritis or even lethal disease. CHIKV continues to cause substantial morbidity worldwide as its vector mosquitoes expand and spread. There are currently no approved vaccines or antiviral drugs available for the prevention or treatment of CHIKV. Although antibody therapy has shown promise in the prevention or treatment of CHIKV disease in preclinical models, challenges remain for implementing such therapies. Here, from the B cells of a survivor of natural CHIKV infection, we isolated ultrapotent neutralizing human monoclonal antibodies (mAbs) and encoded their sequences into mRNA molecules delivered by infusion. One human mAb, CHKV-24, was expressed to biologically significant levels in vivo after infusion of mRNAs in lipid nanoparticles in mice. We evaluated the protective capacity of CHKV-24 mAb immunoglobulin G protein or mRNA in mouse models of CHIKV infection. Treatment with CHKV-24 mRNA protected mice from arthritis, musculoskeletal tissue infection, and lethality and reduced viremia to undetectable levels at 2 days after inoculation. Infusion of macaques with CHKV-24 mRNA achieved a mean maximal mAb concentration of 10.1 to 35.9 micrograms per milliliter, with a half-life of 23 days, a level well above what is needed for protection in mice. Studies with CHKV-24 mRNA in macaques demonstrated a dose-response effect after the first dose of mRNA and maintained levels after second dose. These preclinical data with CHKV-24 mRNA suggest that it might be useful to prevent human disease.

Hur beskrivs bäst metodens användningsområde(n)?

We recently showed that some human mAbs to CHIKV prepared as immunoglobulin G (IgG) proteins have potent neutralizing activity (*29*–*31*) and confer protective effects against virus replication in mice (*29*, *32*) and nonhuman primates (NHPs) (*33*). Here, we developed a distinct panel of ultrapotent human mAbs to CHIKV and used mRNA encoding the antibodies to treat and protect against CHIKV. The experiments revealed that delivery of optimized mRNA molecules encoding a potent human antibody resulted in expression at biologically significant levels in the serum of both mice (14.9 μg/ml) and NHPs (10.1 to 35.9 μg/ml) and elicited protection against arthritis, musculoskeletal disease, and lethal challenge in mouse models.

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1	>
4	>
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Hur beskrivs bäst metodens användningsområde(n)?

Treatment with CHKV-24 mRNA or mAb significantly protected mice from lethality in a dose-dependent manner. Viremia was reduced to the limit of detection on 2 dpi, further supporting the efficacy of CHKV-24 in this mouse model. Protection was mediated by systemic levels of 10 μg/ml of CHKV-24 mAb, which has an in vitro neutralization IC_{50} value of 4 ng/ml. The higher concentration needed for effect in vivo may be explained, in part, by the stringency of the testing in immunocompromised AG129 mice, which lack interferon-α/β and interferon-γ responses and the expected antigen excess, which effectively shifts the neutralization to a requirement for greater antibody concentrations (*41*). Determining the ratio of effective in vitro and in vivo concentrations for antiviral antibodies is complex and often requires combined experimental-mathematical approaches that include precise estimates of virion-antibody interaction stoichiometry, tissue distribution, and half-life (*42*). Postexposure treatment in WT mice reduced viremia, diminished infection in the ipsilateral foot, prevented spread to the contralateral foot, and protected against foot swelling. Administration of CHKV-24 mRNA, when given as a single or two 60-min intravenous infusions, was well tolerated in monkeys at all dose levels tested. Human IgG1 antibodies were detectable through day 83 when dosed once with CHKV-24 mRNA at 0.5 mg/kg and through day 100 after two doses of CHKV-24 mRNA at 3 mg/kg.

These studies suggest that passive immunization or treatment of humans by administration of LNP formulations containing mRNAs encoding for an anti-CHIKV antibody may be feasible. The ability to deliver sufficient protective levels of antibodies in humans using such LNP RNA formulations can only be determined in human clinical studies. On the basis of our results, the CHKV-24 mRNA has been selected as a development candidate for testing in humans. The high levels of mAb expression achieved here with CHKV-24 mRNA in mice and NHPs, and the complete protection of mice against lethal disease or arthritis, suggest that additional studies are warranted to determine the promise of this approach for prevention or treatment of CHIKV disease. Prophylaxis with this antibody treatment could be considered for travelers to affected areas, and clinical testing of therapy of infected patients could be evaluated to see whether reductions of virus load prevent the development of acute and/or chronic arthritis. If successful, such studies could suggest a platform for rapid development and deployment of mRNA-encoded mAbs for many other emerging infectious diseases. Although there are

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Källor:

- https://www.umu.se/globalassets/centralwebb/utbildningswebben/_centralt-innehall/dokument/kunskapsprov-lakare/200910/delprov-vetenskaplig-artikel-2020-09-10---med-svar.pdf
- A lipid-encapsulated mRNA encoding a potently neutralizing human monoclonal antibody protects against chikungunya infection
- BY NURGUN KOSE, JULIE M. FOX, GOPAL SAPPARAPU, ROBIN BOMBARDI, RASHIKA N. TENNEKOON, A. DHARSHAN DE SILVA, SAYDA M. ELBASHIR, MATTHEW A. THEISEN, ELISABETH HUMPHRIS-NARAYANAN, GIUSEPPE CIARAMELLA, SUNNY HIMANSU, MICHAEL S. DIAMOND, JAMES E. CROWE, JR.
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