Università degli Studi di Padova

CCHFV: meccanismi di interazione virus-vettore-ospite vertebrato

Cristiano Salata

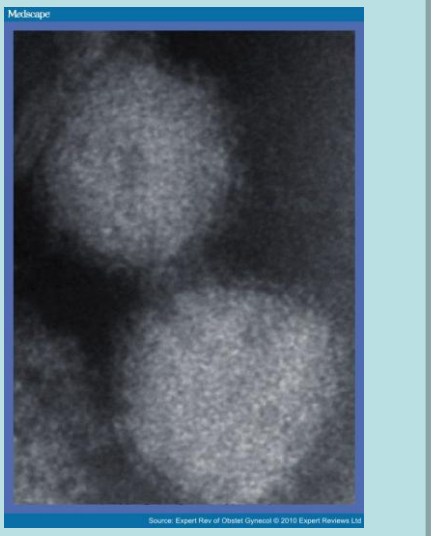
DMM Dipartimento di Medicina Molecolare Università di Padova

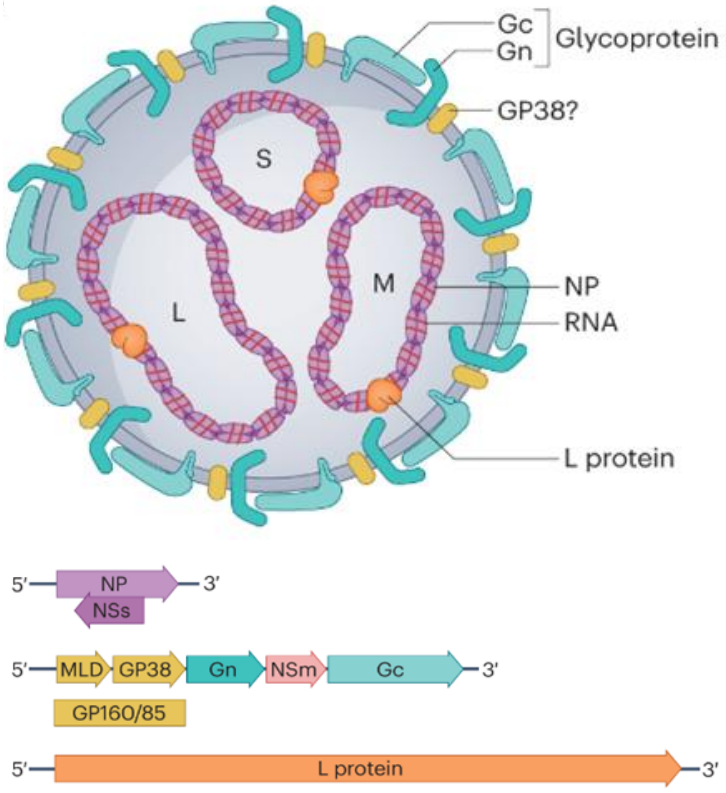
Virus della Febbre Emorragica della Crimea e Congo

Membro del genere *Orthonairovirus,* famiglia *Nairoviridae,* che comprende virus con RNA a filamento singolo segmentato, circolare, con senso negativo.

CCHFV deve essere maneggiato in BSL-4

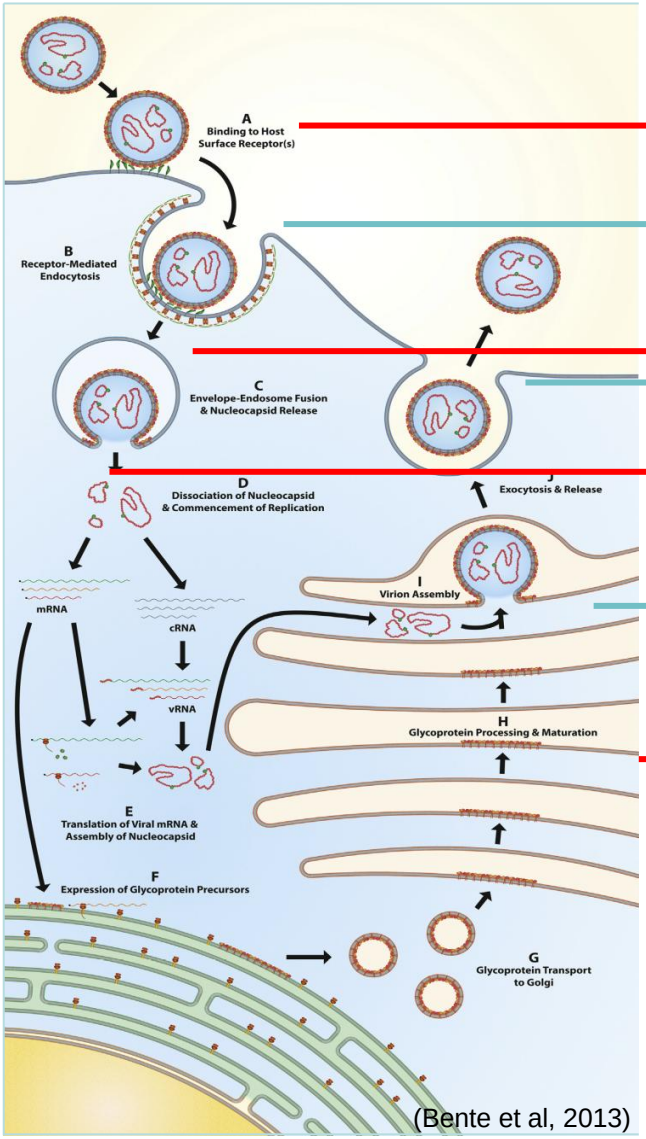
TEM of CCHFV





(modificato da Hawman and Feldmann, 2023)

Ciclo replicativo del CCHFV



(Bente et al, 2013)

Legame a LDLR (Monteil et al 2024, Xu et al 2024, Ritter et al 2024)

Ingresso tramite endocitosi mediata da clatrina, influenzata dal colesterolo *(Simon et al 2009)*.

I processi di internalizzazione, assemblaggio e uscita del virus dipendono dal funzionamento dei microtubuli *(Simon et al 2009)*.

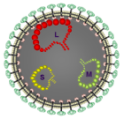
Fusione dipendente dal pH, avviene a livello dei MVB *(Simon et al 2009; Shtanko et al 2014)*.

L'assemblaggio e gemmazione delle particelle virali mature avvengono a livello del Golgi *(Bertolotti-Ciarlet et al 2005; Haferkamp et al 2005)*.

La glicoproteina è processata da endoproteasi SKI-1/S1P *(Bergeron et al 2007)*.

Infezione e conseguenze dell'infezione

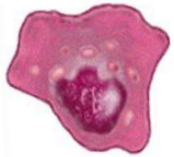
Ingresso del virus nel corpo



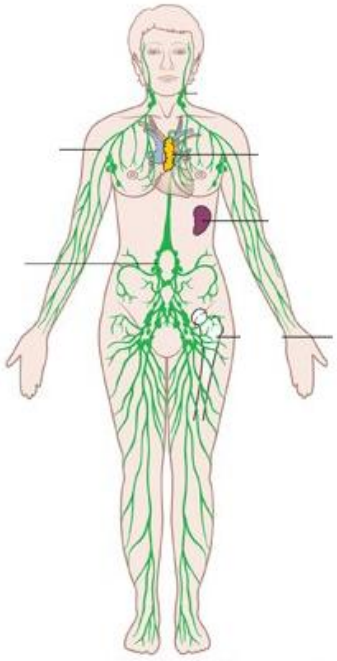
Infezione e amplificazione

Apoptosi delle cellule infettate

C'è una soppressione dell'apoptosi?

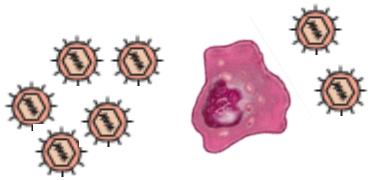


Fagociti mononucleati **(cellule mobili)**

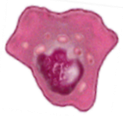


I macrofagi trasportano il virus ai linfonodi regionali

Diffusione dei virioni liberi e associati ai monociti attraverso i canali linfatici e il sangue



TNF-α



Aumento di macrofagi e cellule dendritiche in linfonodi, milza, fegato e altri tessuti.

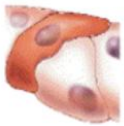
Down regolazione dell'immunità?

Infezione delle cellule parenchimali circostanti (epatociti, corteccia surrenale)

Down regulation delle integrine?

Espansione dei focolai di necrosi

Alterazione vascolare legata alle cellule endoteliali?



(Salata, Roma 27.02.2025)

CCHFV e apoptosi

OPEN ACCESS Freely available online

PLoS one

Crimean-Congo Hemorrhagic Fever Virus-Infected Hepatocytes Induce ER-Stress and Apoptosis Crosstalk

Raquel Rodrigues[1], Gláucia Paranhos-Baccalà[1]*, Guy Vernet[1], Christophe N. Peyrefitte[1,2]

1 Emerging Pathogens Laboratory, Fondation Mérieux, Lyon, France, 2 Unité de Virologie, Institut de Recherche Biomédicale des Armées, La Tronche, France

CCHFV induce

- ***ER-stress***
- ***Attivazione dei mediatori infiammatori***
- ***Modulazione dei pathways apoptotici mitocondriale e «death receptor»***

Virus Research 179 (2014) 187–203

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

Hepatocyte pathway alterations in response to *in vitro* Crimean Congo hemorrhagic fever virus infection

Christophe Fraisier[a,b,1], Raquel Rodrigues[c,1], Vinh Vu Hai[a,b], Maya Belghazi[e], Stéphanie Bourdon[a], Glaucia Paranhos-Baccala[c], Luc Camoin[f,g], Lionel Almeras[a,b,1], Christophe Nicolas Peyrefitte[c,d,h,*,1]

Coinvolti pathway cellulari legati all'infiammazione, allo stress ossidativo e apoptosi, all'ubiquitinazione e sumoilazione, alla regolazione del trasporto nucleocitoplasmatico e nell'ingresso del virus.

CCHFV e apoptosi

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 286, NO. 5, pp. 3227–3234, February 4, 2011

Induction of Caspase Activation and Cleavage of the Viral Nucleocapsid Protein in Different Cell Types during Crimean-Congo Hemorrhagic Fever Virus Infection*

Received for publication, May 28, 2010, and in revised form, November 29, 2010 Published, JBC Papers in Press, December 1, 2010, DOI 10.1074/jbc.M110.149369

Helen Karlberg[‡§], **Yee-Joo Tan**[¶], **and Ali Mirazimi**[‡§1]

From the [‡]*Swedish Institute for Infectious Disease control, SE-171 82 Solna, Sweden, the* [§]*Institution of Microbiology, Tumor, and Cell Biology/Karolinska Institute, SE-177 72 Stockholm Sweden, and the* [¶]*Department of Microbiology, Yong Loo Lin School of Medicine, National University Health System, National University of Singapore, Singapore 117597, Singapore*

I risultati suggeriscono che il CCHFV sopprime l'attivazione della caspasi nelle fasi iniziali del ciclo di replicazione virale, il che forse favorisce l'instaurarsi dell'infezione. Inoltre, la risposta cellulare dell'ospite nelle fasi avanzate post-infezione induce molecole pro-apoptotiche cellulari dell'ospite attraverso il percorso del «death receptor»

Journal of General Virology (2015), **96**, 538–546 DOI 10.1099/vir.0.000011

Crimean–Congo haemorrhagic fever replication interplays with regulation mechanisms of apoptosis

Helen Karlberg,[1,2] Yee-Joo Tan[3,4] and Ali Mirazimi[1,2,5]

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[1]Public Health Agency of Sweden, SE-171 82, Sweden
[2]Karolinska Institute, Stockholm SE-171 77, Sweden
[3]Department of Microbiology, Yong Loo Lin School of Medicine, National University Health System (NUHS), National University of Singapore, Singapore
[4]Institute of Molecular and Cell Biology, A*STAR (Agency for Science, Technology and Research), Singapore
[5]National Veterinary Institute, Uppsala SE-756 51, Sweden

CCHFV e apoptosi

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(*Pediatr Infect Dis J* 2015;34:208–213)

PATHOGENESIS AND HOST RESPONSE

Evaluation of Serum Perforin, Caspase-3, sFasL and M-30 Levels as Apoptotic Markers in Children With Crimean-Congo Hemorrhagic Fever

Ahmet S. Güven, MD, Enver Sancakdar, MD,† Elif B. Uysal, MD,‡ Ali Kaya, MD,* Mehmet B. Oflaz, MD,* Hekim Karapınar, MD,§ Fatih Bolat, MD,* Nevin Tuzcu, PhD,¶ Köksal Deveci, MD,† Ömer Cevit, MD,* and Füsun D. İcagasioglu, MD**

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PLOS ONE

RESEARCH ARTICLE

Apoptosis-Related Gene Expression in an Adult Cohort with Crimean-Congo Hemorrhagic Fever

Nil Guler[1]*, Cafer Eroglu[2], Hava Yilmaz[2], Adil Karadag[3], Hasan Alacam[4], Mustafa Sunbul[2], Tom E. Fletcher[2,5], Hakan Leblebicioglu[2]

1 Department of Hematology, School of Medicine, Ondokuz Mayis University, Samsun, Turkey, **2** Department of Clinical Microbiology and Infectious Diseases, School of Medicine, Ondokuz Mayis University, Samsun, Turkey, **3** Department of Medical Microbiology, School of Medicine, Ondokuz Mayis University, Samsun, Turkey, **4** Department of Medical Biochemistry, School of Medicine, Ondokuz Mayis University, Samsun, Turkey, **5** Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom

PLOS ONE | DOI:10.1371/journal.pone.0157247 June 15, 2016

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Received: 25 January 2019 | Revised: 19 March 2019 | Accepted: 20 March 2019

DOI: 10.1002/jmv.25467

RESEARCH ARTICLE

WILEY JOURNAL OF MEDICAL VIROLOGY

Apoptosis and its relation with clinical course in patients with Crimean-Congo hemorrhagic fever

Aynur Engin[1] | Huseyin Aydin[2] | Ziynet Cinar[3] | Seyit Ali Buyuktuna[1] | Mehmet Bakir[1]

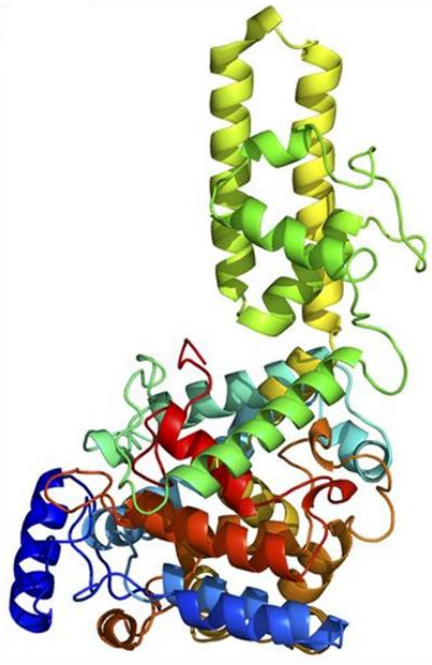
CCHFV induce apoptosi sia in vitro che in vivo e biomarker dell'apoptosi sono sovra-regolati in pazienti con CCHF, sebbene non vi sia correlazione con la severità della malattia.

Il processo apoptotico è coinvolto nella patogenesi dell'infezione

Nucleoproteina

Proteina multifunzionale che:

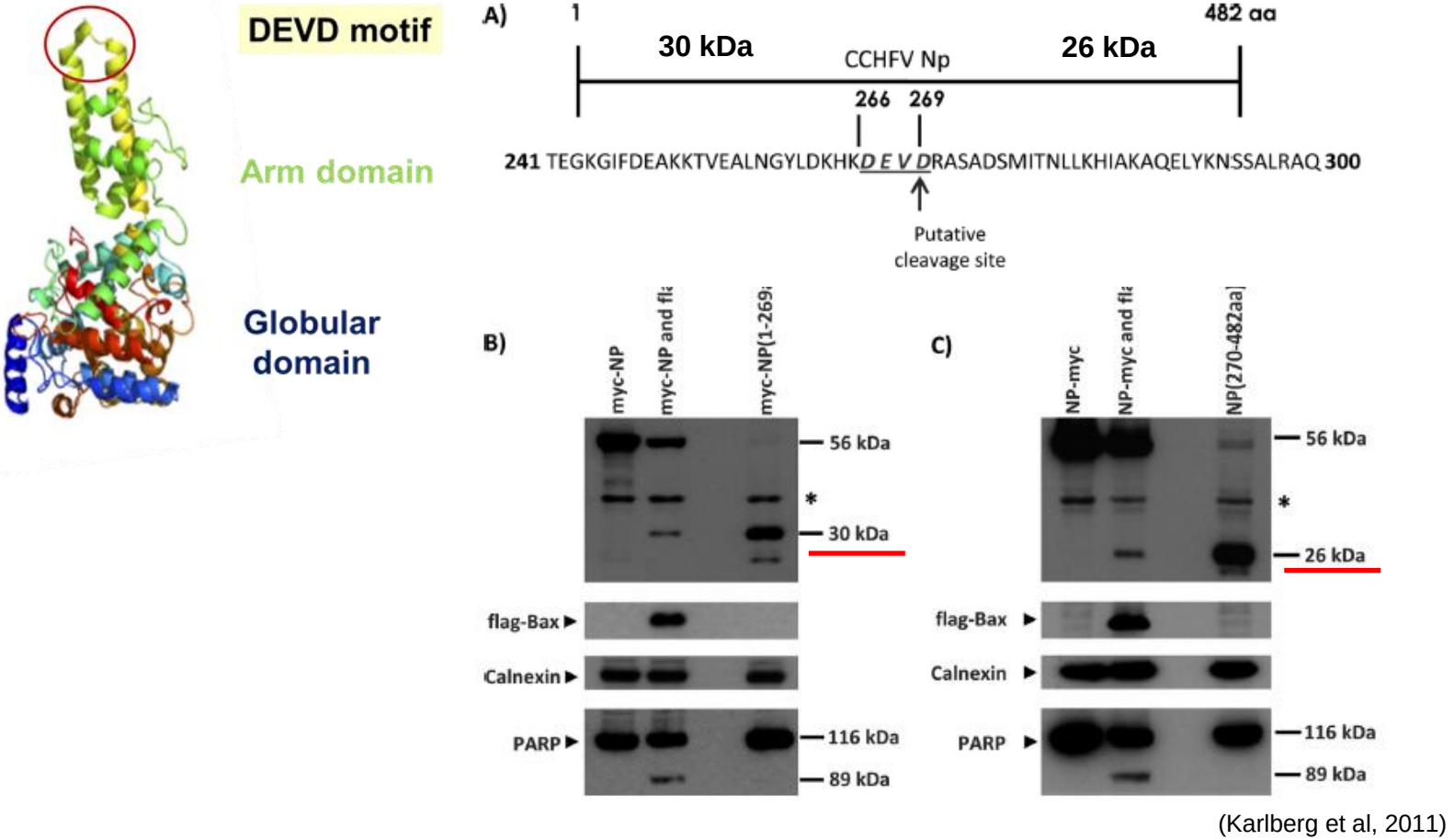
- Interagisce con l'RNA genomico virale (complessi RNP)
- Interagisce con RdRp e le glicoproteine virali
- Interagisce con l'actina cellulare e le proteine Hsp70



(modificato da Wang et al, 2012)

- Aumenta la traduzione degli mRNA
- Ha una forte attività endonucleasica del DNA metallo-dipendente
- **Ritarda l'induzione dell'apoptosi**
- **Ha un motivo di scissione «DEVD» specifico per la caspasi-3 che è molto conservato**

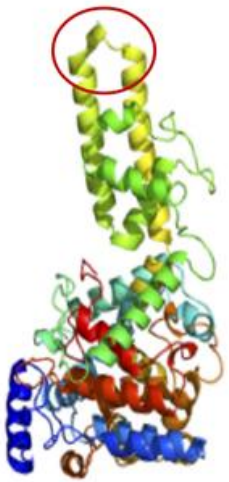
Nucleoproteina e apoptosi



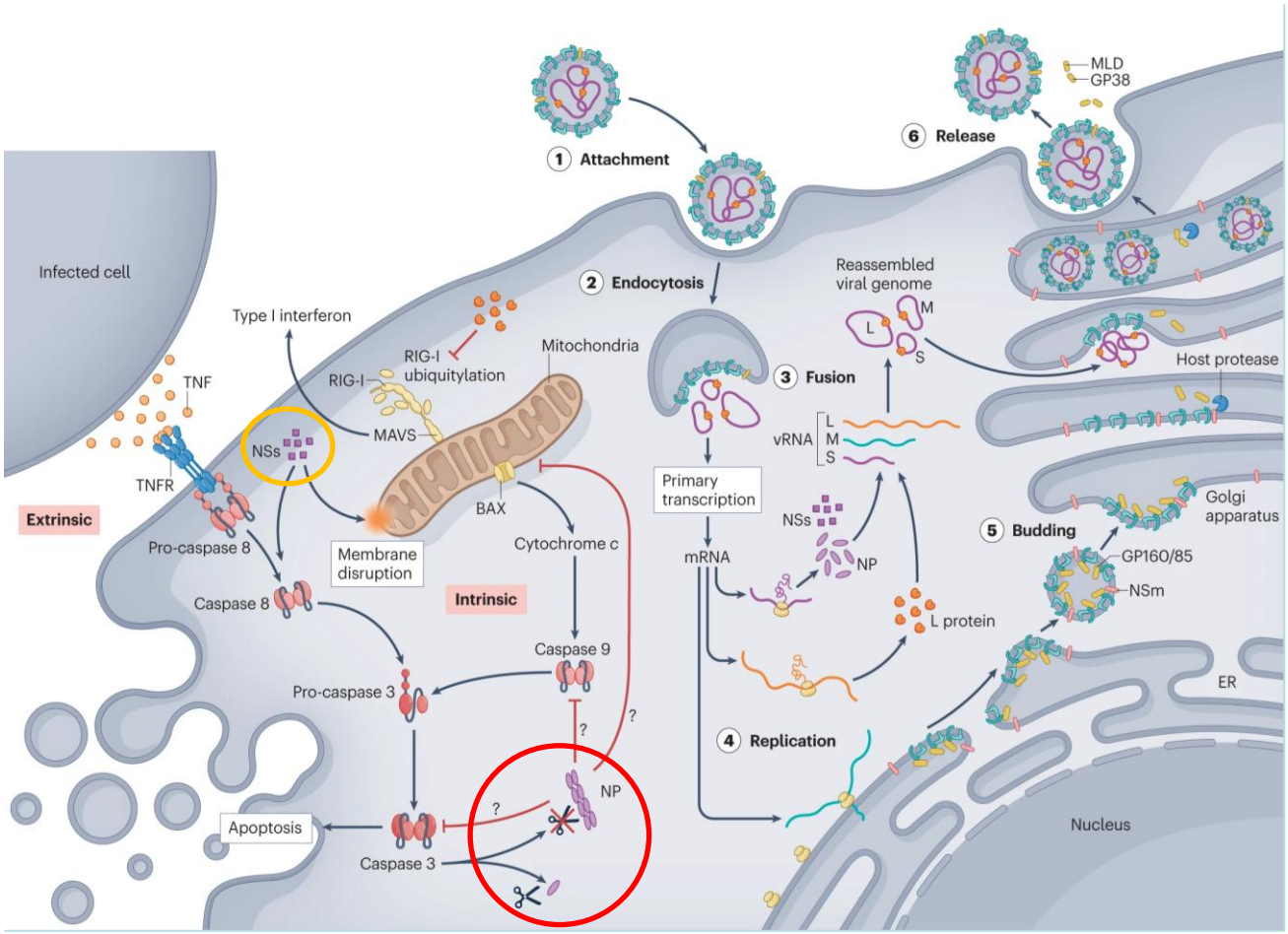
(Karlberg et al, 2011)

L'inibizione dell'apoptosi nelle cellule dei mammiferi induce un aumento di ~1 log del titolo virale

Nucleoproteina e apoptosi



N ritarda l'apoptosi agendo sulle Caspasi 3 e 9 e sui mitocondri



(modificato da Hawman and Feldmann, 2023)

CCHFV e risposta immunitaria

Il CCHFV è sensibile agli interferoni di tipo I (IFN-α e IFN-β), è inibito dagli IFN naturali e sintetici e da mediatori come l'ossido nitrico e la proteina MxA (Andersson et al 2004, 2006; Simon et al 2006; Karlberg et al 2010).

Tuttavia, la replicazione del CCHFV è insensibile al successivo trattamento con IFN-α, suggerendo un meccanismo di evasione immunitaria dell'ospite.

Journal of Medical Virology 80:1397–1404 (2008)

Crimean-Congo Hemorrhagic Fever Virus Delays Activation of the Innate Immune Response

Ida Andersson,[1,2] Helen Karlberg,[1,2] Mehrdad Mousavi-Jazi,[1] Luis Martínez-Sobrido,[3] Friedemann Weber,[4] and Ali Mirazimi[1,2]*

[1]*KCB/Swedish Institute for Infectious Disease control, Solna, Sweden*
[2]*MTC, Karolinska Institutet, Stockholm, Sweden*
[3]*Department of Microbiology, Mount Sinai School of Medicine, New York, New York*
[4]*Department of Virology, University of Freiburg, Freiburg, Germany*

E' stato dimostrato che la replicazione di CCHFV ritarda la risposta dell'IFN, probabilmente interferendo con la via di attivazione dell'IRF-3

RdRp e inibizione dell'interferone

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

CELLULAR RESPONSE TO INFECTION
15 October 2015 Volume 89 Issue 20
https://doi.org/10.1128/jvi.01643-15

RIG-I Mediates an Antiviral Response to Crimean-Congo Hemorrhagic Fever Virus

Jessica R. Spengler[a], Jenish R. Patel[b,c], Ayan K. Chakrabarti[a], Marko Zivcec[a], Adolfo García-Sastre[b,c,d], Christina F. Spiropoulou[a], Éric Bergeron [a]

Crimean-Congo Hemorrhagic Fever Virus Suppresses Innate Immune Responses via a Ubiquitin and ISG15 Specific Protease

Florine E.M. Scholte,[1] Marko Zivcec,[1] John V. Dzimianski,[2] Michelle K. Deaton,[2] Jessica R. Spengler,[1] Stephen R. Welch, Stuart T. Nichol,[1] Scott D. Pegan,[2] Christina F. Spiropoulou,[1] and Éric Bergeron[1,3,*]
[1]Viral Special Pathogens Branch, Division of High-Consequence Pathogens and Pathology, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA 30333, USA
[2]Department of Pharmaceutical and Biomedical Sciences, University of Georgia, Athens, GA 30602, USA
[3]Lead Contact
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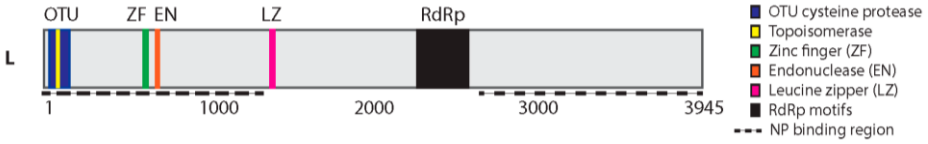
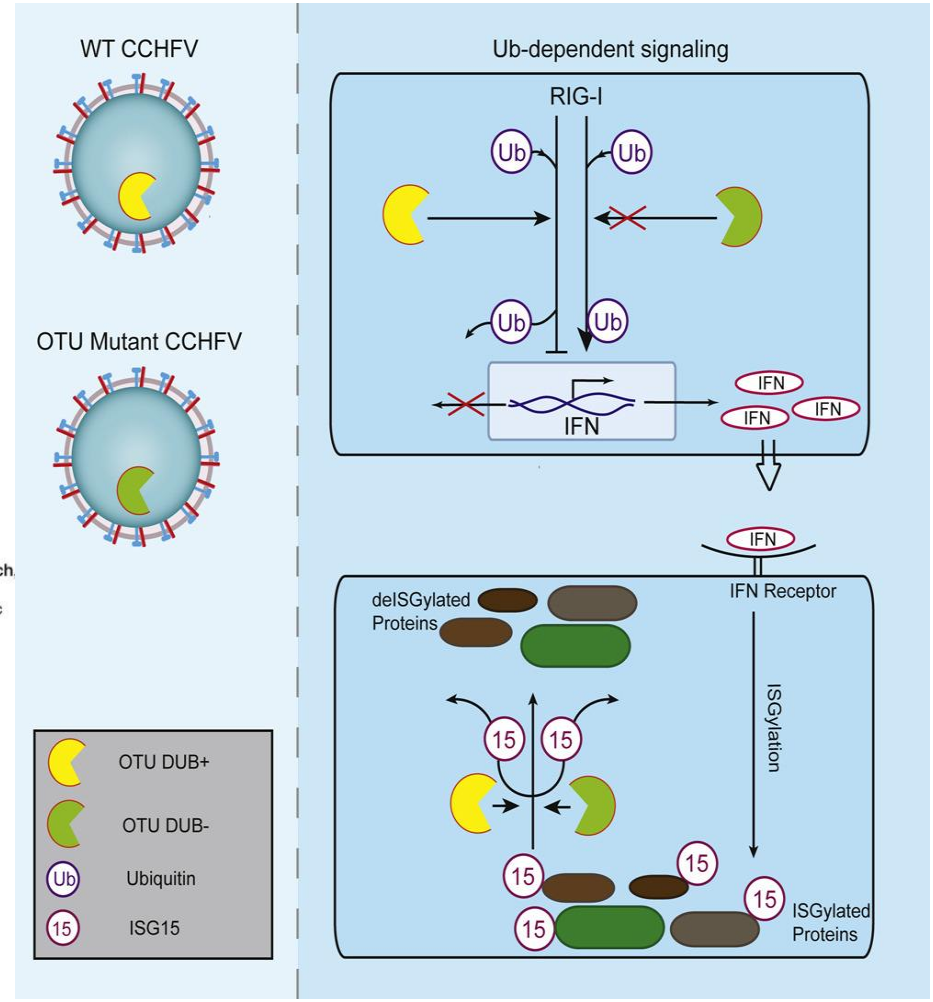
http://dx.doi.org/10.1016/j.celrep.2017.08.040

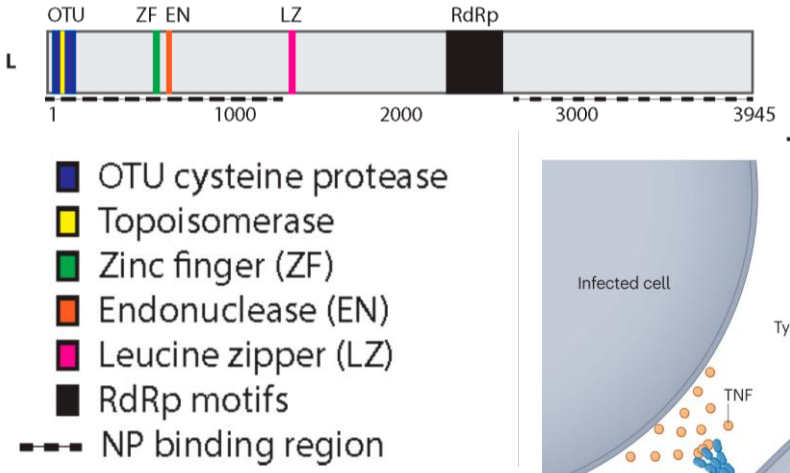


(modificato da Zivcec et al, 2016)



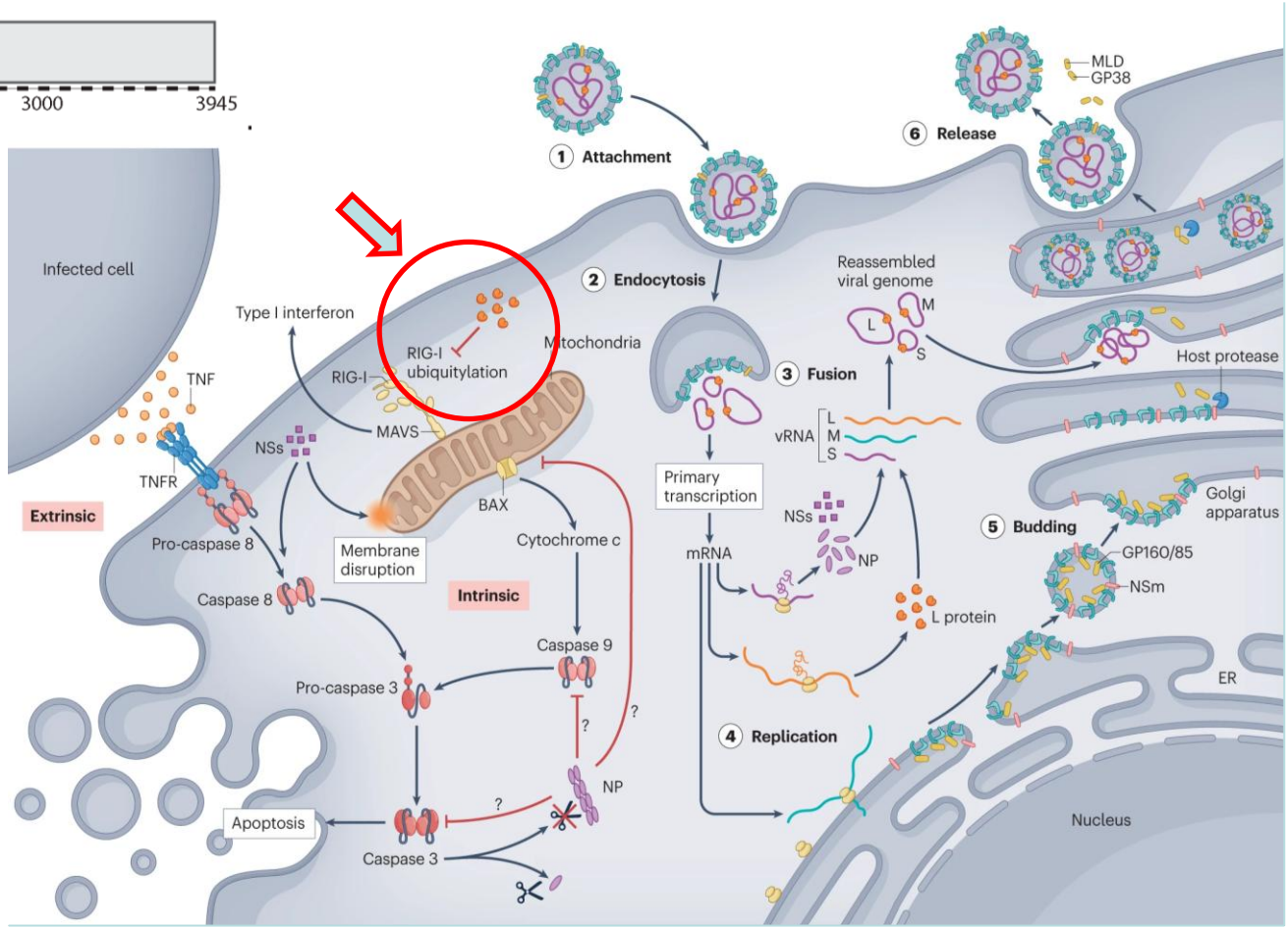
(Scholte et al, 2016)

RdRp e inibizione dell'interferone



(modificato da Zivcec et al, 2016)

La RdRp (L protein) inibisce la via di attivazione dell'interferone



(modificato da Hawman and Feldmann, 2023)

CCHFV e sensibilità all'interferone

Crimean–Congo hemorrhagic fever virus infection is lethal for adult type I interferon receptor-knockout mice

Sándor Bereczky,[1] Gunnel Lindegren,[1] Helen Karlberg,[1] Sara Åkerström,[1] Jonas Klingström[1,2] and Ali Mirazimi[1,2]

[1]KCB/Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden

[2]MTC/Karolinska Institutet, SE-171 77 Stockholm, Sweden

Journal of General Virology (2010), **91**, 1473–1477

Pathogenesis and Immune Response of Crimean-Congo Hemorrhagic Fever Virus in a STAT-1 Knockout Mouse Model[▽]†

Dennis A. Bente,[1]* Judie B. Alimonti,[1,3] Wun-Ju Shieh,[4] Gaëlle Camus,[1,2] Ute Ströher,[1,3] Sherif Zaki,[4] and Steven M. Jones[1,2,3]

Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba, Canada[1]; Department of Immunology, University of Manitoba, Winnipeg, Manitoba, Canada[2]; Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada[3]; and Infectious Disease Pathology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia[4]

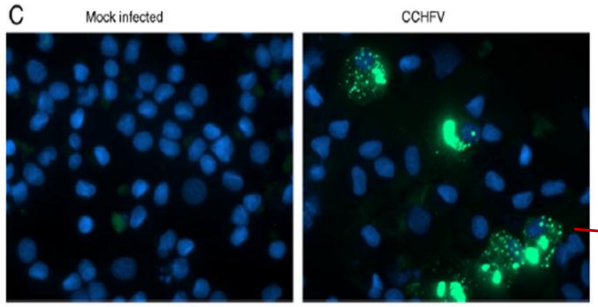
JOURNAL OF VIROLOGY, Nov. 2010, p. 11089–11100

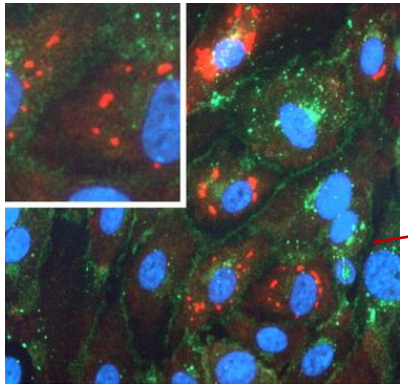
Lethal Crimean-Congo Hemorrhagic Fever Virus Infection in Interferon α/β Receptor Knockout Mice Is Associated With High Viral Loads, Proinflammatory Responses, and Coagulopathy

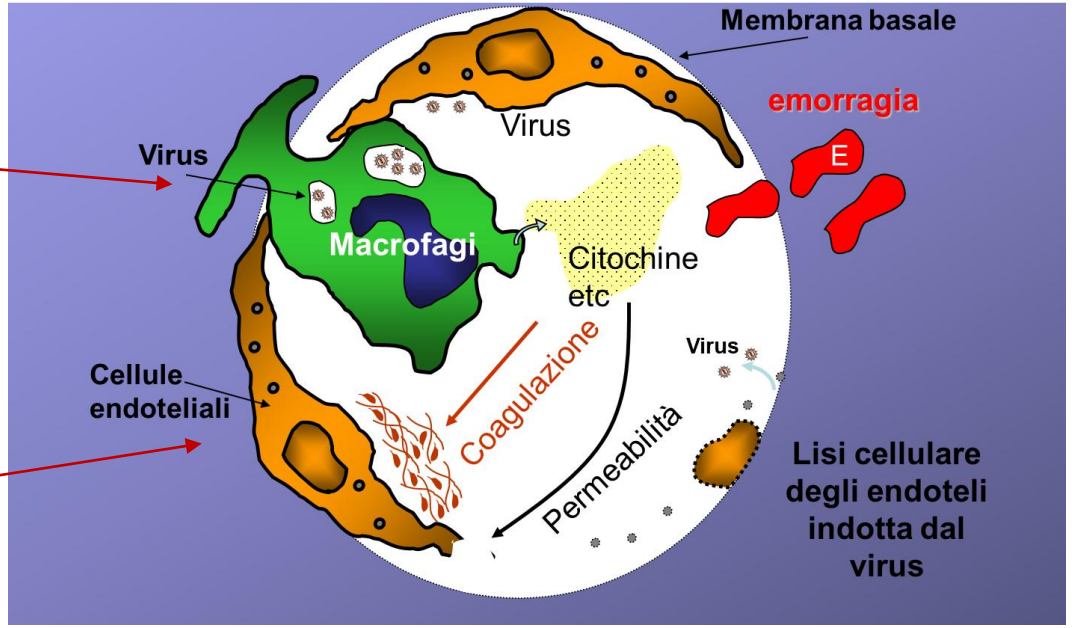
Marko Zivcec,[1,3] David Safronetz,[1] Dana Scott,[2] Shelly Robertson,[1] Hideki Ebihara,[1] and Heinz Feldmann[1,3]

JID 2013:207 (15 June) • 1909

CCHFV infezione e danno vascolare







- Macrofagi producono TNF-alfa che attiva le cellule endoteliali
- Cellule endoteliali infettate da CCHFV presentano marcatori di attivazione
- Il virus provoca lisi degli endoteli e tramite le citochine la coaugulazione
- Danno diretto da proteine virali?

(Salata, Roma 27.02.2025)

CCHFV infezione e danno vascolare

Golden *et al.*, *Sci. Adv.* 2019; **5** : eaaw9535 10 July 2019

SCIENCE ADVANCES | RESEARCH ARTICLE

HEALTH AND MEDICINE

GP38-targeting monoclonal antibodies protect adult mice against lethal Crimean-Congo hemorrhagic fever virus infection

Joseph W. Golden[1]*, Charles J. Shoemaker[2], Michael E. Lindquist[1], Xiankun Zeng[3], Sharon P. Daye[3], Janice A. Williams[3], Jun Liu[3], Kayla M. Coffin[3], Scott Olschner[2], Olivier Flusin[2†], Louis A. Altamura[2‡], Kathleen A. Kuehl[3], Collin J. Fitzpatrick[1], Connie S. Schmaljohn[4], Aura R. Garrison[1]*

GP38:

- ✓ Induce disfunzione della barriera endoteliale in vitro interrompendo lo strato di glicocalice innescando iperpermeabilità.
- ✓ Causa perdite vascolari in un modello murino.
- ✓ Incrementa i danni vascolari in vivo nei topi infettati, facilitando la disseminazione di CCHFV nei tessuti bersaglio come il fegato.

Anticorpi protettivi contro GP38 inibiscono l'iperpermeabilità endoteliale in vitro e la perdita vascolare in vivo durante l'infezione da CCHFV.

GP38 si dimostra essere un fattore coinvolto nella patogenesi

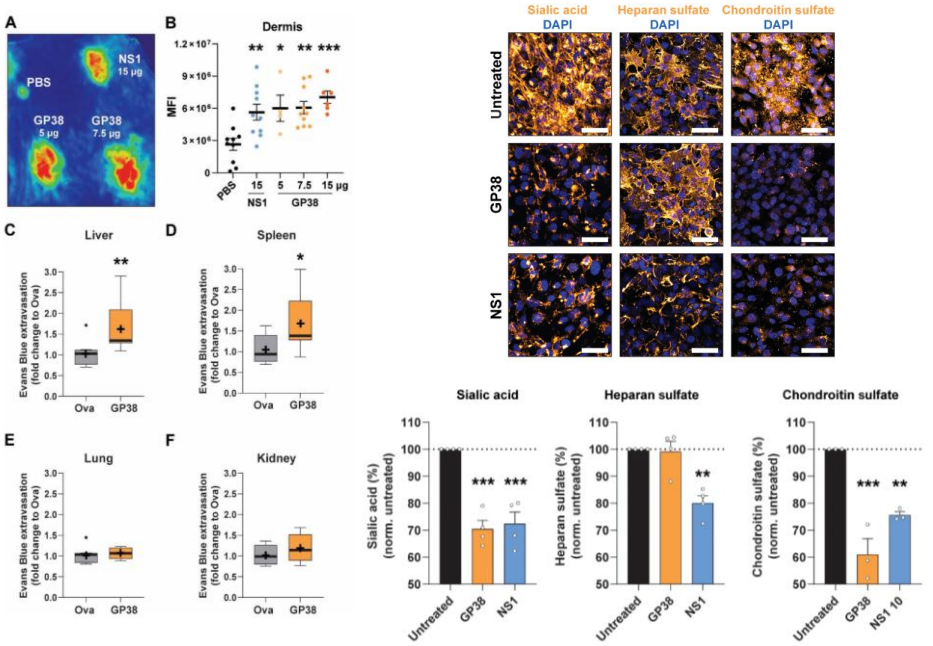
Pahmeier *et al.*, *Sci. Transl. Med.* **17**, eadq5928 (2025) 19 February 2025

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

INFECTIOUS DISEASES

Antibodies targeting Crimean-Congo hemorrhagic fever virus GP38 limit vascular leak and viral spread

Felix Pahmeier[1,2]†, Stephanie R. Monticelli[3,4]†‡, Xinyi Feng[1], Christy K. Hjorth[5], Albert Wang[6], Ana I. Kuehne[3], Russell R. Bakken[3], Thomas G. Batchelor[3,7], Saeyoung E. Lee[1], Marissa Middlecamp[8], Lauren Stuart[8], Amaro N. Duarte-Neto[9], Dafna M. Abelson[8], Jason S. McLellan[5], Scott B. Biering[1,10], Andrew S. Herbert[3]*, Kartik Chandran[6]*, Eva Harris[1,11]*



(Salata, Roma 27.02.2025)

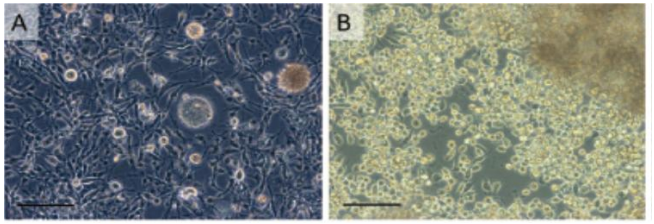
Modelli di studio delle interazioni virus-vettore

Laboratorio BSL-4

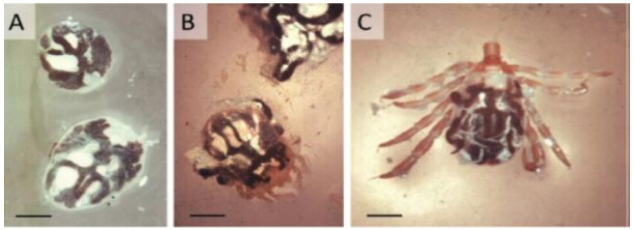
- In vivo

(Xia et al. 2016; Thangamani and Bente, 2014)

- In vitro



(Salata et al., 2018; Bell-Sakyi et al., 2012)



(Salata et al., 2021)

Studio delle interazioni virus-vettore in vivo

SCIENTIFIC REPORTS

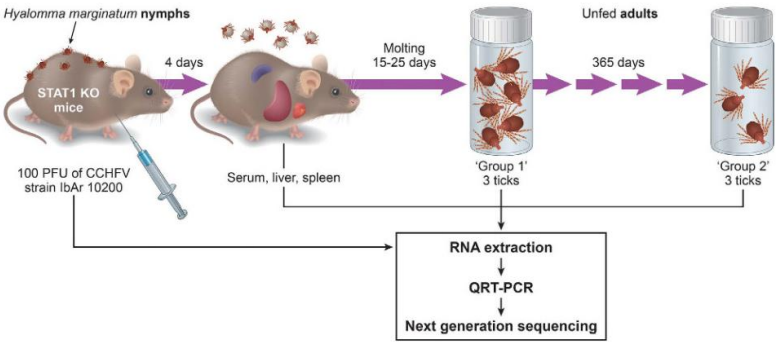
OPEN

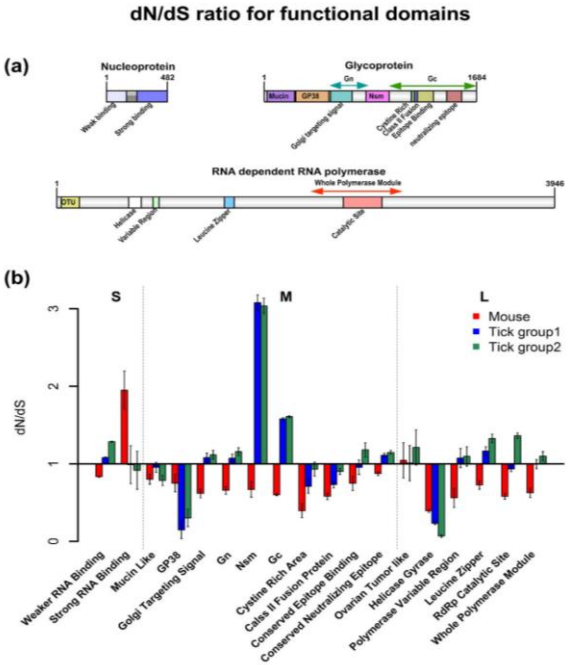
Received: 04 May 2016
Accepted: 04 October 2016
Published: 24 October 2016

Transstadial Transmission and Long-term Association of Crimean-Congo Hemorrhagic Fever Virus in Ticks Shapes Genome Plasticity

Han Xia[1,2,3], Andrew S. Beck[4], Aysen Gargili[5], Naomi Forrester[4], Alan D. T. Barrett[4] & Dennis A. Bente[1,2]

The trade-off hypothesis, the current paradigm of arbovirus evolution, proposes that cycling between vertebrate and invertebrate hosts presents significant constraints on genetic change of arboviruses. Studying these constraints in mosquito-borne viruses has led to a new understanding of epizootics. The trade-off hypothesis is assumed to be applicable to tick-borne viruses too, although studies are lacking. Tick-borne Crimean-Congo hemorrhagic fever virus (CCHFV), a member of the family *Bunyaviridae*, is a major cause of severe human disease worldwide and shows an extraordinary amount of genetic diversity compared to other arboviruses, which has been linked to increased virulence and emergence in new environments. Using a transmission model for CCHFV, utilizing the main vector tick species and mice plus next generation sequencing, we detected a substantial number of consensus-level mutations in CCHFV recovered from ticks after only a single transstadial transmission, whereas none were detected in CCHFV obtained from the mammalian host. Furthermore, greater viral intra-host diversity was detected in the tick compared to the vertebrate host. Long-term association of CCHFV with its tick host for 1 year demonstrated mutations in the viral genome become fixed over time. These findings suggest that the trade-off hypothesis may not be accurate for all arboviruses.

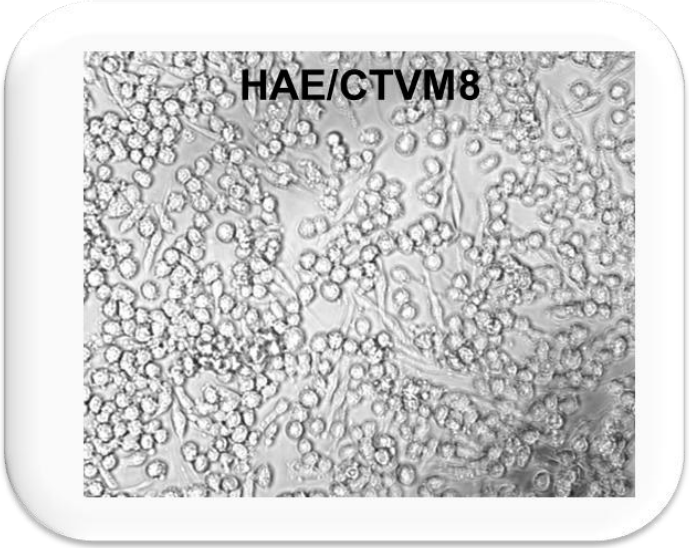


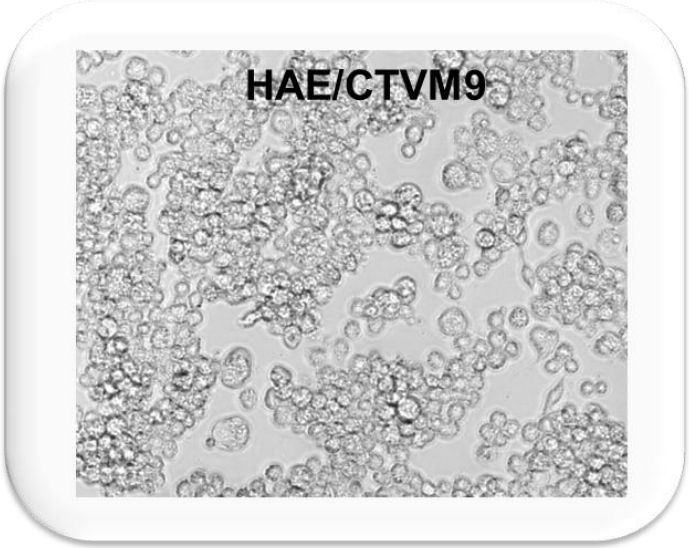


I risultati suggeriscono l'esistenza di una pressione selettiva unica per CCHFV nella zecca e l'inadeguatezza dell'ipotesi «trade-off» per i virus trasmessi dalle zecche.

Studio delle interazioni virus-vettore in vitro

HAE/CTVM8 e HAE/CTVM9: linee cellulari stabilizzate da tessuti embrionali di zecche *Hyalomma anatolicum anatolicum*

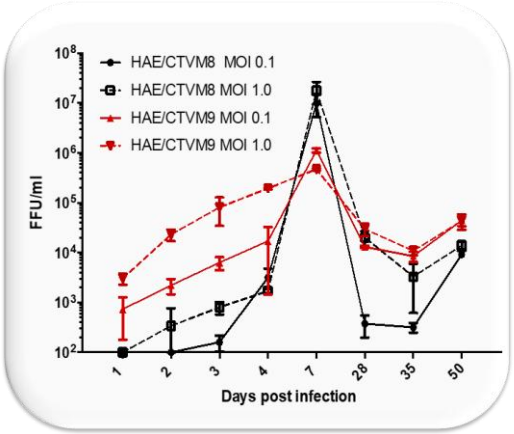


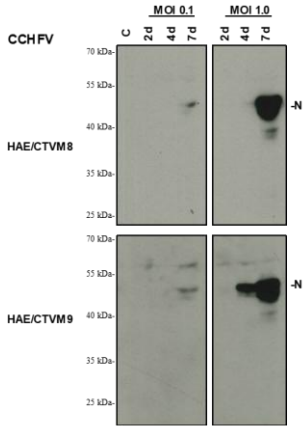


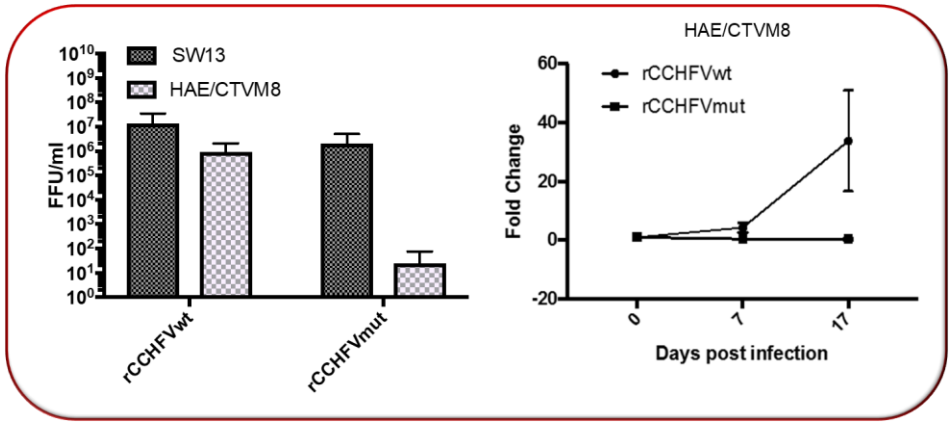
HAE/CTVM8 e HAE/CTVM9 possono essere infettate con CCHFV e non mostrano effetti citopatici

Bell-Sakyi L et al,2012

Valutazione dell'infezione di CCHFV in linee cellulari di zecca



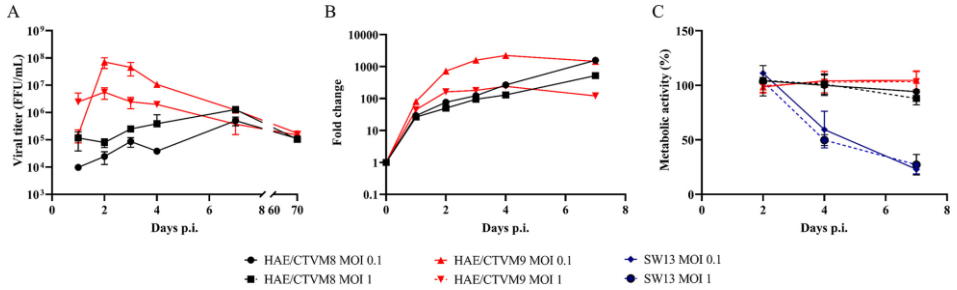


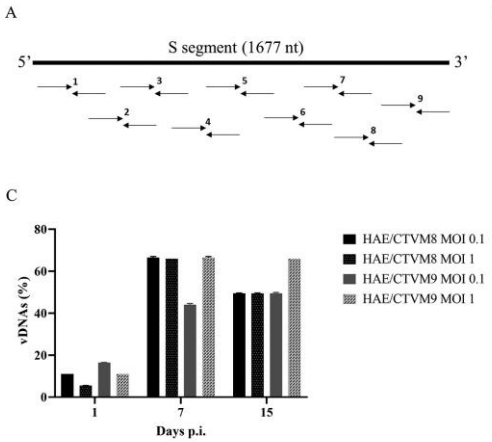


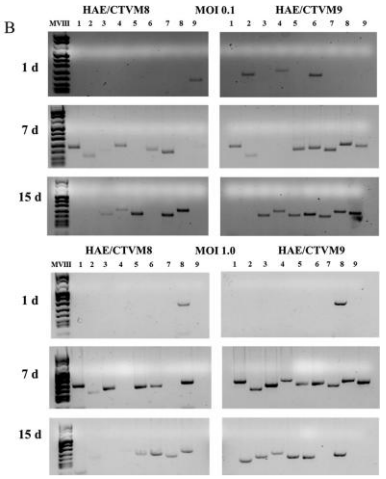
Salata et al, 2018

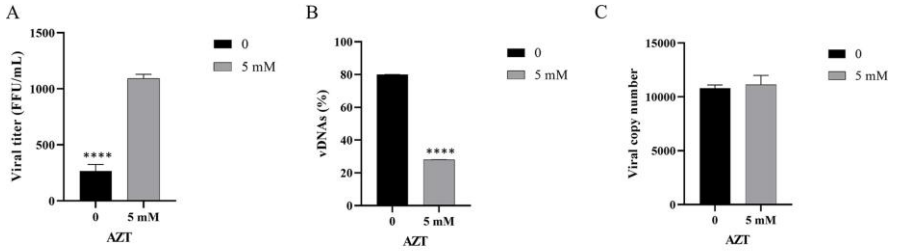
- CCHFV infetta le cellule delle zecche senza evidenti effetti deleteri
- CCHFV stabilisce un'infezione persistente che è stata monitorata per 282 giorni
- Nessun segno di scissione nucleoproteica
- rCCHFVmut (DEVD->AEVA) ha mostrato una forte compromissione della replicazione dell'RNA (>99%) rispetto a rCCHFVwt nelle cellule di zecca
- Ruolo del motivo DEVD nelle zecche?

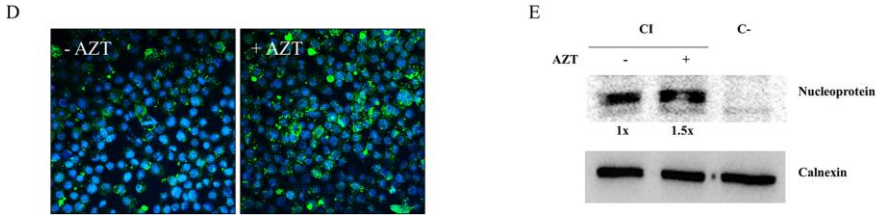
vDNA e modulazione dell'infezione nelle cellule di zecca

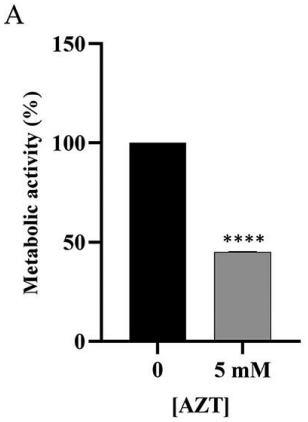


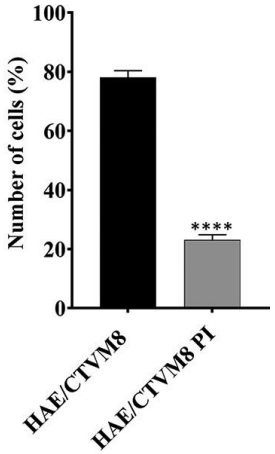








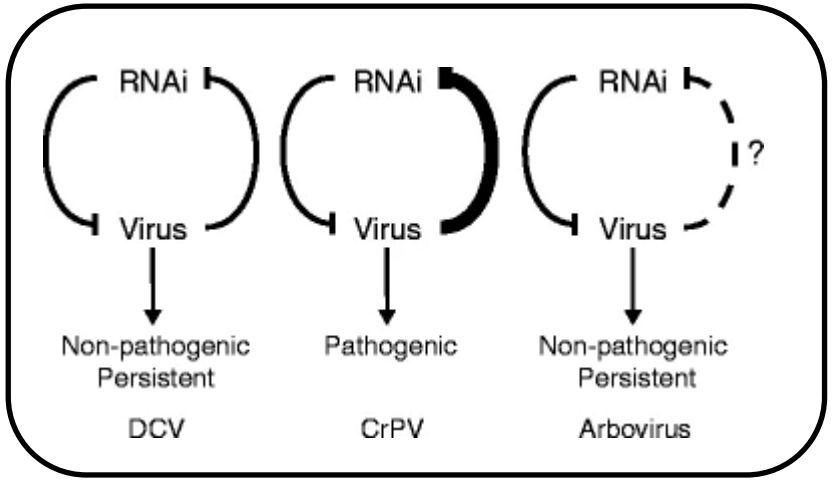




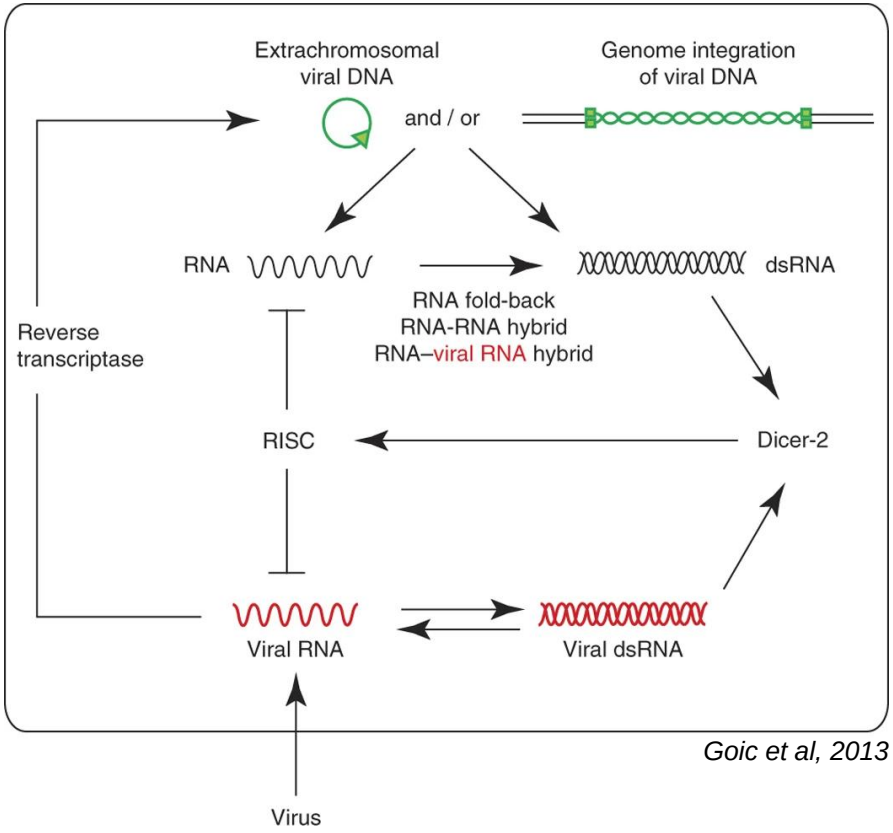
La presenza di vDNA è associata a riduzione della replicazione virale e alla sopravvivenza delle cellule infettate

Salvati et al, 2021

vDNA: mediatore della risposta antivirale



Le vDNA potrebbero contribuire alla definizione della competenza vettoriale



Goic et al, 2013

Conclusioni

- Le interazioni CCHFV-ospite vertebrato ed invertebrato sono ancora largamente sconosciute
- E’ importante caratterizzare in dettaglio la biologia del virus e la patogenesi della CCHF per trovare target terapeutici
- Lo studio dell’interazione virus-vettore può portare a nuovi approcci di controllo della diffusione del virus

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E tutti voi per l'attenzione!

(Salata, Roma 27.02.2025)