

# Alphaviruses

Current Biology

Edited by

Suresh Mahalingam

Lara J. Herrero

Institute for Glycomics

Griffith University

Gold Coast, QLD

Australia

and

Belinda L. Herring

School of Biomedical Sciences, Faculty of Health

Queensland University of Technology

Brisbane, QLD

Australia



Copyright © 2016

Caister Academic Press  
Norfolk, UK

[www.caister.com](http://www.caister.com)

British Library Cataloguing-in-Publication Data  
A catalogue record for this book is available from the British Library

ISBN: 978-1-910190-15-9 (hardback)  
ISBN: 978-1-910190-16-6 (ebook)

Description or mention of instrumentation, software, or other products in this book does not imply endorsement by the author or publisher. The author and publisher do not assume responsibility for the validity of any products or procedures mentioned or described in this book or for the consequences of their use.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publisher. No claim to original U.S. Government works.

Cover images courtesy of Weiqiang Chen: chikungunya virus infection of bone cells in murine proximal tibial epiphysis.

### **Ebooks**

Ebooks supplied to individuals are single-user only and must not be reproduced, copied, stored in a retrieval system, or distributed by any means, electronic, mechanical, photocopying, email, internet or otherwise.

Ebooks supplied to academic libraries, corporations, government organizations, public libraries, and school libraries are subject to the terms and conditions specified by the supplier.

# Contents

	<b>Contributors</b>	v
	<b>Preface</b>	ix
1	<b>Alphavirus Genome Structure and Replication</b> Belinda L. Herring, Lara J. Herrero, Weiqiang Chen, Kuo-Ching Sheng, Nestor E. Rulli and Suresh Mahalingam	1
2	<b>Alphavirus Evolution</b> Lark L. Coffey, Scott C. Weaver and Naomi L. Forrester	21
3	<b>Laboratory Diagnosis and Detection of Alphaviruses</b> Linda Hueston	37
4	<b>The Interaction of Alphaviruses with the Interferon System</b> William B. Klimstra and Kate D. Ryman	49
5	<b>Alphaviruses and their Role in Elucidating Antiviral Responses in Mosquitoes</b> Melanie McFarlane and Alain Kohl	77
6	<b>Animal Models of Alphavirus-induced Inflammatory Disease</b> Lara J. Herrero, Adam Taylor, Pierre Roques, Brett A. Lidbury and Suresh Mahalingam	89
7	<b>Clinical Manifestations of Arthritogenic Alphaviruses</b> Lara J. Herrero, Adam Taylor, Suan Sin Foo, Lynden Roberts, Natkunam Ketheesan and Suresh Mahalingam	125
8	<b>Encephalitic Alphaviruses</b> Clive S. McKimmie and John K. Fazakerley	139
9	<b>Application of Alphavirus Vectors for Gene Therapy</b> Kenneth Lundstrom	157
10	<b>Case Study: Chikungunya Virus</b> Lisa F.P. Ng	169
	<b>Index</b>	181

# Preface

Alphaviruses are small, enveloped, single-stranded positive-sense RNA viruses that are typically transmitted by arthropods, especially mosquitoes. A number of alphaviruses are known as clinically important human pathogens (e.g. Venezuelan equine encephalitis virus, chikungunya virus). Key areas of alphavirus research are focused on treatment and prevention of disease, but there is also considerable potential for harnessing alphaviruses as vaccine vectors and in gene therapy (e.g. Semliki Forest virus). In addition, alphaviruses have also been very valuable model systems for a wide range of basic studies in diverse areas such as cell biology, virology and immunology.

The book presents the current status of alphavirus research across a wide range of disciplines, but has a particular focus on molecular biology, pathogenesis and host interactions. The book, which is the first of its kind to focus specifically on alphaviruses, has been written in response to a

clear need within the alphavirus research community for a comprehensive and up-to-date overview of the field.

Each chapter has been written by leaders in that particular research field. Chapter topics covered include genome structure and replication; viral evolution; laboratory diagnosis and detection; interaction with the interferon system; antiviral responses in mosquitoes; animal models of alphavirus-induced inflammatory disease; clinical manifestations of arthritogenic alphaviruses; encephalitic alphaviruses; the application of alphavirus vectors for gene therapy; and chikungunya virus pathogenesis and the development of control strategies.

Finally, we thank all contributors for their hard work in providing chapters describing the latest developments in their fields of expertise and for their patience while waiting for the book to be published.

Professor Suresh Mahalingam

6K protein 5–6

## A

ADE 66, 89

Alphavirus assembly and egress 10–13  
 interactions between capsid and glycoproteins 12–13  
 nucleocapsid processing and assembly 10  
 structural protein processing and assembly 11  
 transport of viral components 12

Alphavirus expression vectors 158–164

cancer gene therapy 160  
 cancer vaccines 160–161  
 clinical trials 163–164  
 CNS therapy 162–163  
 oncolytic vectors 162  
 tumour targeting 162

Alphavirus genomic organization 1

Alphavirus replication 6–13  
 assembly and egress 10–13  
 attachment and entry 6–8  
 replication 8–10

Alphavirus structure 1–6  
 non-structural region 2–4  
 structural region 4–6

Animal models 89–115

avian models 115  
 guinea pig and hamster models 114–115  
 horse models 114  
 mouse models 89–109  
 non-human primate models 110–114

Antibodies 41, 42–45, 52, 66, 103, 104–105, 110, 143, 149, 173–175

Antibody-dependent enhancement *see* ADE

Apoptosis

encephalitis 145–146  
 in mosquitoes 84–85

## B

BFV 22, 24, 128–130

clinical manifestations 128–130  
 phylogeny 24

B lymphocytes 52, 93, 149, 173–175

Barmah Forest virus *see* BFV

## C

Capsid protein 4–5, 8–13

Chikungunya virus *see* CHIKV

CHIKV 22–24, 27–28, 30, 31, 38, 52, 95–101, 110–113, 131–133, 140–141, 169–175

clinical manifestations and pathology 131–133, 171–172

encephalitis 140–141

epidemiology 170–171

innate immunity and interferons 172

interferons 52

molecular biology 170

mouse models 95–101

non-human primate models 110–113

phylogeny 22–24

role of antibodies 173

role of T cells 173–174

therapeutic antibodies 175

therapeutics 174

vaccines 174–175

Cholesterol, requirement in budding 13

## D

DC-SIGN and L-SIGN 6, 65–66

Demyelinating disease 150–151

Detection and diagnosis 37–44

cell culture 40

complement fixation 44

ELISA 41, 44–45

haemagglutination inhibition 43

immunofluorescence 41, 44

laboratory animals 38

mosquito cell culture 39

mosquito inoculation 38–39

neutralization tests 42–43

PCR 41–42

vertebrate cell culture 39

virus isolation 38

## E

Eastern equine encephalitis virus *see* EEEV

EEEV 24–25, 29–30, 51, 56–57, 65–66, 106–107, 113–115, 141, 143–144

encephalitis 141, 143–144

guinea pig and hamster models 114–115  
 horse model 114  
 interferons 51, 56–57, 65–66  
 microevolution 29–30  
 mouse models 106–107  
 non-human primate models 113–114  
 phylogeny 24–25  
 Encephalitis 100–109, 140–151  
 age-related virulence 145  
 antibodies 149  
 CHIKV 100–101, 140–141  
 CNS pathology 146–147  
 dissemination to CNS 142–143  
 EEEV 107, 141  
 immune response 147–151  
 neural cellular targets 144  
 neuroinvasion via blood-brain barrier 143  
 neuroinvasion via olfactory tract 143  
 neuronal cell death 145–146  
 SFV 101–105, 140  
 Sindbis virus 105, 140  
 VEEV 109, 141  
 WEEV 107–108, 141–142  
 Envelope glycoproteins 5  
 Evolution, alphaviral 26–32

**I**

IFN-inducible antiviral effectors 60–63  
 cGAMP synthase 63  
 IFIT protein family 61  
 ISG15 61  
 ISG20 61–62  
 PKR and eIF2 $\alpha$  pathways 60–61  
 UNC93b1 62–63  
 viperin 62  
 zinc finger antiviral protein 62  
 IMD 82–84  
 Interferons 49–66  
 antagonism by viruses 63–66  
 pathogenesis 49–52  
 induction by infection 52–59  
 induction by infection, myeloid cells 56–57  
 induction by infection, non-myeloid cells 54–56  
 induction of antiviral effectors 57–59

**J**

JAK/STAT pathway 57–58, 65, 82–84, 172

**M**

Macrophages 56–57, 66, 91–94, 98, 112  
 Mayaro virus 24, 131  
 Mouse models  
 CHIKV 95–101  
 EEEV 106–107  
 RRV 89–95  
 Semliki Forest virus 101–105  
 Sindbis virus 105–106  
 VEEV 108–109  
 WEEV 107–108

**N**

NK cells 52, 93  
 Non-human primate models  
 CHIKV 110–113  
 RRV 113  
 VEEV, WEEV and EEEV 113–114  
 Non-structural region 2  
 nsP1 2, 4, 8–9, 63  
 nsP2 2–4, 8–9, 63–65, 172  
 nsP3 2, 4, 8–9, 12, 84, 144  
 nsP4 4, 8–9

**O**

O'nyong-nyong virus 130

**P**

Phylogeny of alphaviruses 21–26  
 arthralgic 22–24  
 encephalitic 24–26

**R**

RIG-I/MDA5 53–55, 60, 62  
 RNA interference, in mosquitoes 78–82  
 Ross River virus *see* RRV  
 RRV 66, 89–95, 113, 128  
 ADE 66  
 clinical manifestations 128  
 mouse model 89–95  
 non-human primate model 113  
 phylogeny 24

**S**

Semliki Forest virus *see* SFV  
 SFV 51–52, 101–105, 133–134, 140, 144, 146, 162, 163–164  
 clinical manifestations 133–134  
 clinical trials 163–164  
 encephalitis 140, 144, 146  
 gene therapy 160–161  
 interferons 51–52  
 mouse model 101–105  
 tumour targeting 162  
 Sindbis virus *see* SINV  
 SINV 25–26, 52, 105–106, 133, 140, 160–161, 162  
 clinical manifestations 133  
 encephalitis 140  
 gene therapy 160–161  
 interferons 52  
 mouse model 105–106  
 phylogeny 25–26  
 tumour targeting 162  
 Structural region 4

**T**

T lymphocytes 52, 93, 150  
 Toll-like receptors 53–54, 82–84, 148

**V**

VEEV 25, 31–32, 50, 56–57, 108–109, 113–114, 141, 143–144, 160–161, 163  
 clinical trials 163

- encephalitis 141, 143–144
- envelope gene mutations 31–32
- gene therapy 160–161
- horse model 114
- interferons 50, 56–57
- mouse models 108–109
- non-human primate models 113–114
- phylogeny 25

Venezuelan equine encephalitis virus *see* VEEV

**W**

WEEV 25, 51, 107–108, 113–115, 141–142

- avian models 115
- encephalitis 141–142
- guinea pig and hamster models 114–115
- horse models 114
- interferons 51
- mouse models 107–108
- non-human primate models 113–114
- phylogeny 25

Western equine encephalitis virus *see* WEEV