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Neutralizing Antibodies from Convalescent Chikungunya Virus Patients Can Cross-Neutralize Mayaro and Una Viruses

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Abstract. Most alphaviruses are mosquito-borne and can cause severe disease in domesticated animals and humans. The most notable recent outbreak in the Americas was the 2014 chikungunya virus (CHIKV) outbreak affecting millions and producing disease highlighted by rash and arthralgia. Chikungunya virus is a member of the Semliki Forest (SF) serocomplex, and before its arrival in the Americas, two other member of the SF complex, Una (UNAV) and Mayaro (MAYV) viruses, were circulating in Central and South America. This study examined whether antibodies from convalescent CHIKV patients could cross-neutralize UNAV and MAYV. Considerable cross-neutralization of both viruses was observed, suggesting that exposure to CHIKV can produce antibodies that may mitigate infection with UNAV or MAYV. Understanding the impact of CHIKV exposure on population susceptibility to other emerging viruses may help predict outbreaks; moreover, identification of cross-reactive immune responses among alphaviruses may lead to the development of vaccines targeting multiple viruses.

The genus *Alphavirus* in the family *Togaviridae* comprises of small, spherical, enveloped viruses with genomes consisting of a single-stranded, positive-sense RNA ~11–12 kb in length.[1] Alphaviruses comprise 31 recognized species classified into 11 complexes based on antigenic and/or genetic similarities.[1,2] Most alphaviruses are mosquito-borne and infect diverse vertebrate hosts, including equids, birds, humans, and nonhuman primates.[1] The ability to infect both mosquitoes and vertebrates enables the maintenance of alphaviruses in natural endemic transmission cycles that occasionally spillover into the human population and cause disease. Old World viruses cause human disease highlighted by arthralgia, whereas New World viruses can cause fatal encephalitis.

Most alphaviruses capable of causing debilitating arthralgia are members of the Semliki Forest (SF) complex.[1] The complex consists of both Old World (chikungunya [CHIKV], o'nyong'nyong [ONNV], Ross River [RRV], Semliki Forest [SFV], Getah [GETV], Sagiyama [SAGV], Bebaru [BEBV]), and New World [Mayaro (MAYV), Una (UNAV)] viruses. With the exception of GETV, BEBV, and UNAV, for which limited human data are available, all other viruses within the complex can cause human disease highlighted by arthralgia.[1]

In the last two decades, CHIKV has emerged and caused multiple outbreaks in Africa, Asia, and more recently in the Americas, producing millions of human cases.[3] Human infections with CHIKV are rarely fatal, and produce febrile illness characterized by high fever, rash, and severe joint pain.[1,3] Following acute infection, a large proportion of patients, up to 50%, have reported chronic arthritis termed chronic CHIKV-induced arthralgia.[3]

In contrast to CHIKV, much less is known about the only two New World members of the SF complex, MAYV and UNAV. Mayaro virus was first isolated in 1954 from human sera recovered from febrile patients in Trinidad.[4] Sporadic MAYV outbreaks have been reported in several countries in Central and South America; however, infections are likely underdiagnosed/misdiagnosed because of confusion with dengue.[5,6] The detection of MAYV infection in Haiti, international travelers, as well as evidence of continuous transmission in Brazil has sparked a renewal of interest in this emerging arbovirus.[5–8] Similar to CHIKV, human infections with MAYV are highlighted by rash and debilitating arthralgia that can last for weeks or years.[9] Una virus was first isolated in 1959 from *Psorophora* and *Aedes* spp.[10] Limited data are available on the biology and epidemiology of UNAV. The virus can infect humans; however, no known disease is associated with infection.[11]

The arrival of CHIKV in the Americas may have consequences for transmission cycles for the two New World members of the SF complex. In this report, we assessed whether neutralizing antibodies from natural CHIKV infection could potentially cross-neutralize MAYV and UNAV. Sera were collected from subjects in Northwest Colombia (Atlantico and Bolivar) in 2014–2015; samples from CHIKV-infected subjects were collected at least 1 year after acute CHIKV infection.[12,13] History of clinical CHIKV infection was confirmed by serological analysis, as described in the following paragraphs; a subset of the patients had reported chronic arthralgia for at least 3 months after diagnosis of CHIKV infection, as described previously.[12,13] The study protocol was approved by the George Washington University Institutional Review Board (IRB) (protocol 041612), the Universidad El Bosque IRB (UB 387-2015), and the U.S. Army Medical Research Institute of Infectious Diseases Human Research Protections Office (FY15-32).

The UNAV strain v76 and MAYV strain BeH407 were obtained from Dr. Michael Diamond's laboratory at Washington University School of Medicine. Chikungunya virus strain 99659 was obtained from the World Reference Center for Emerging Viruses and Arboviruses at the University of Texas Medical Branch. The viruses were amplified on Vero (CCL-81) from American Tissue Culture Collection at an multiplicity of infection of 1. Cell culture, plaque assay, and plaque reduction

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neutralizationtiter ($PRNT_{80}$) assay were performed as published previously.[18] All samples were heat-inactivated for 30 minutes at 56°C.

Fifty human serum samples were used for the study. Thirty-five subjects were clinically diagnosed and laboratory confirmed to be convalescent CHIKV-infected subjects; 15 were negative controls. Laboratory confirmation used the InBios CHIKjj Detect ELISAs for IgG and IgM (CHIKG-R and CHKM-R). Of the 35 samples that tested positive for anti–CHIKV IgG antibodies, one was positive for anti–CHIKV IgM antibodies (Table 1). All samples were then used in $PRNT_{80}$ assays to determine $PRNT_{80}$ against CHIKV as well as cross-reactivity with MAYV and UNAV. Chikungunya virus–neutralizing antibodies were detected in 34 of 35 CHIKV-positive subjects, with $PRNT_{80}$ titers ranging from 160 to 5,120 (Table 1). Surprisingly, 17 of the 35 samples (49%) and 22 of the 35 (63%) samples could also neutralize MAYV and UNAV, respectively (Table 1). The $PRNT_{80}$ titers ranged from 20 to 80 and 20 to 640 for MAYV and UNAV, respectively (Table 1). Similar cross-neutralizing responses have been reported in previous animal model studies of SF complex members.[14–17]

TABLE 1
Serological analysis of convalescent CHIKV human serum samples

	ELISA		$PRNT_{80}$ titer		
Sample number	CHIKV IgM	CHIKV IgG	CHIKV	MAYV	UNAV
EB3	Neg	Pos	**1,280**	**80**	**640**
EB4	Neg	Pos	**160**	*< 20*	*< 20*
EB5	Neg	Pos	**640**	*< 20*	*< 20*
EB7	Neg	Pos	**1,280**	**40**	*< 20*
EB9	Neg	Pos	*< 20*	*< 20*	*< 20*
EB13	Neg	Pos	**1,280**	*< 20*	*< 20*
EB14	Neg	Pos	**2,560**	*< 20*	*< 20*
EB15	Neg	Pos	**640**	*< 20*	**160**
EB16	Neg	Pos	**320**	*< 20*	*< 20*
EB18	Neg	Pos	**320**	*< 20*	**40**
EB19	Neg	Pos	**160**	*< 20*	**80**
EB20	Neg	Pos	**320**	*< 20*	**80**
EB21	Neg	Pos	**320**	*< 20*	*< 20*
EB22	Neg	Pos	**640**	*< 20*	**80**
EB23	Neg	Pos	**1,280**	**20**	**80**
EB25	Neg	Pos	**320**	*< 20*	*< 20*
EB26	Neg	Pos	**160**	*< 20*	**40**
EB27	Neg	Pos	**1,280**	**40**	**640**
EB28	Neg	Pos	**640**	**40**	**80**
EB29	Neg	Pos	**320**	*< 20*	**40**
EB30	Neg	Pos	**1,280**	*< 20*	**40**
EB31	Neg	Pos	**320**	**80**	*< 20*
EB32	Neg	Pos	**2,560**	**40**	**80**
EB33	Neg	Pos	**5,120**	*< 20*	**40**
EB34	Pos	Pos	**1,280**	**20**	*< 20*
EB35	Neg	Pos	**2,560**	**40**	*< 20*
EB36	Neg	Pos	**640**	**20**	**20**
EB37	Neg	Pos	**1,280**	*< 20*	**20**
EB38	Neg	Pos	**640**	**40**	**20**
EB39	Neg	Pos	**2,560**	**20**	**40**
EB40	Neg	Pos	**640**	**80**	*< 20*
EB41	Neg	Pos	**320**	**20**	**40**
EB42	Neg	Pos	**1,280**	**40**	**80**
EB43	Neg	Pos	**640**	**40**	**20**
EB44	Neg	Pos	**1,280**	**80**	**40**
EB1	Neg	Neg	*< 20*	*< 20*	*< 20*
EB2	Neg	Neg	*< 20*	*< 20*	*< 20*
EB6	Neg	Neg	*< 20*	*< 20*	*< 20*
EB8	Neg	Neg	*< 20*	*< 20*	*< 20*
EB10	Neg	Neg	*< 20*	*< 20*	*< 20*
EB11	Neg	Neg	*< 20*	*< 20*	*< 20*
EB12	Neg	Neg	*< 20*	*< 20*	*< 20*
EB17	Neg	Neg	*< 20*	*< 20*	*< 20*
EB24	Neg	Neg	*< 20*	*< 20*	*< 20*
EB45	Neg	Neg	*< 20*	*< 20*	*< 20*
EB46	Neg	Neg	*< 20*	*< 20*	*< 20*
EB47	Neg	Neg	*< 20*	*< 20*	*< 20*
EB48	Neg	Neg	*< 20*	*< 20*	*< 20*
EB49	Neg	Neg	*< 20*	*< 20*	*< 20*
EB50	Neg	Neg	*< 20*	*< 20*	*< 20*

CHIKV = chikungunya virus; MAYV = Mayaro virus; UNAV = Una virus; $PRNT_{80}$ = plaque reduction neutralization titer. Samples were tested for anti-CHIKV IgM and IgG antibodies. Neutralizing antibody response to CHIKV, MAYV, and UNAV was determined via $PRNT_{80}$ assay. Limit of detection was a titer of 1:20 and all negative samples were assigned a titer of < 1:20. All positive samples are indicated in bold and negative samples are indicated in italics.

There are several potential explanations for the high cross-reactivity of anti-CHIKV neutralizing antibodies with UNAV and MAYV. First, the cross-reactivity may be due to prior exposure. Both viruses have been isolated in southern/south eastern parts of Colombia with sporadic human exposure reported for MAYV, thus it possible that this cohort had prior exposure to UNAV and MAYV.[5] However, there are no published reports on human exposure and/or virus isolation in the extreme northwest of Colombia, from which our cohort was derived. In addition, cross-protection studies in murine and NHP models with SF complex members have shown that wild-type viruses or vaccine candidates comprising full-length viruses can provide protection against heterologous viruses.[14,18] Infection of rhesus macaques with MAYV or CHIKV provided protection against clinical disease from heterologous MAYV or CHIKV challenge.[14] Furthermore, data from human clinical trials with alphavirus vaccine candidates comprising full-length viruses (VEEV TC83 and CHIKV 181/25) demonstrated that prior vaccination with either candidate blunted immune responses to heterologous vaccine administration suggesting potential protection against distantly related viruses from different serocomplexes.[19] Consequently, a prior exposure to viruses from the same serocomplex, UNAV and MAYV, should have provided some protection against CHIKV infection; however, all of the subjects in this study had significant overt disease post–CHIKV infection. The lack of evidence for circulation/human exposure of viruses in northwest Colombia and the detection of overt human CHIKV disease in our cohort does not support the prior exposure hypothesis with UNAV and MAYV.

A second explanation for the cross-reactivity may be because of higher conservation of E2 glycoprotein, the main target of neutralizing antibodies, among UNAV, MAYV, and CHIKV.[1] A genetic analysis of E2 protein was performed with members of SF complex. CHIKV E2 displayed the highest amino acid identity and similarity scores of 82% and 91% with ONNV, respectively (Table 2). Identity and similarity scores with all other members were comparable and ranged from 54% to 57% and 69% to 72%, respectively (Table 2). However, when analysis was performed with domains A and B, the main targets of neutralizing antibodies on E2 glycoprotein, both MAYV and UNAV had higher identity and similarity scores to CHIKV than most of the other members.[1] UNAV domain A comparison displayed 56% identity and 70% similarity scores with CHIKV (Table 2). The identity score was 3–8% higher than that of all other members except RRV and ONNV. The similarity score was also higher than those of all others except MAYV and ONNV. Mayaro virus displayed a 52% identity score, which was higher than those of SAGV, GETV, and BEBV. The Mayaro virus similarity score of 72% was higher than those of all others except ONNV (Table 2). The analysis of domain B yielded similar results. Mayaro virus displayed higher identity and similarity scores than all other members

TABLE 2
Amino acid comparison of Semliki Forest complex members based on E2 glycoprotein

	E2								
	CHIKV	**ONNV**	**SAGV**	**GETV**	**SFV**	**RRV**	**BEBV**	**MAYV**	**UNAV**
CHIKV	-	82	54	54	57	57	56	***55***	***55***
ONNV	*91*	-	54	54	57	56	55	56	55
SAGV	*70*	*71*	-	98	68	80	66	61	63
GETV	*70*	*71*	*98*	-	68	80	66	61	63
SFV	*72*	*72*	*82*	*82*	-	69	69	65	65
RRV	*70*	*70*	*90*	*91*	*83*	-	67	61	64
BEBV	*71*	*71*	*80*	*80*	*82*	*79*	-	62	62
MAYV	***70***	*72*	*76*	*76*	*80*	*76*	*76*	-	64
UNAV	***69***	*70*	*78*	*78*	*81*	*78*	*78*	*79*	-
	Domain A								
	CHIKV	**ONNV**	**SAGV**	**GETV**	**SFV**	**RRV**	**BEBV**	**MAYV**	**UNAV**
CHIKV	-	87	49	48	57	53	51	***52***	***56***
ONNV	*93*	-	48	48	57	53	49	55	56
SAGV	*69*	*70*	-	97	59	74	57	51	56
GETV	*68*	*69*	*97*	-	58	75	58	52	56
SFV	*66*	*69*	*76*	*75*	-	59	65	64	61
RRV	*67*	*68*	*86*	*88*	*78*	-	59	51	57
BEBV	*67*	*67*	*75*	*75*	*79*	*74*	-	59	59
MAYV	***72***	*74*	*74*	*74*	*81*	*73*	*76*	-	63
UNAV	***70***	*70*	*75*	*76*	*82*	*74*	*82*	*84*	-
	Domain B								
	CHIKV	**ONNV**	**SAGV**	**GETV**	**SFV**	**RRV**	**BEBV**	**MAYV**	**UNAV**
CHIKV	-	80	63	64	61	66	66	***68***	***63***
ONNV	*86*	-	58	59	56	61	58	56	61
SAGV	*76*	*73*	-	98	75	83	73	66	70
GETV	*78*	*75*	*98*	-	76	85	71	68	71
SFV	*80*	*75*	*83*	*85*	-	71	70	61	63
RRV	*81*	*76*	*90*	*92*	*83*	-	70	68	70
BEBV	*78*	*75*	*81*	*80*	*81*	*81*	-	63	56
MAYV	***80***	*73*	*85*	*86*	*85*	*86*	*76*	-	66
UNAV	***75***	*71*	*76*	*78*	*75*	*81*	*71*	*81*	-

BEBV = Bebaru virus; CHIKV = chikungunya virus; GETV = Getah virus; MAYV = Mayaro virus; ONNV = o'nyong'nyong virus; RRV = Ross River virus; SAGV = Sagiyama virus; SFV = Semliki Forest virus; Una = UNAV virus. Percent amino acid identity is shown above the diagonal in black. Percent amino acid similarity is shown below the diagonal in grey.

except ONNV, 68% and 80%, respectively (Table 2). The Una virus identity and similarity scores of 63% and 75% were comparable with those of SAGV, GETV, and SFV but were lower than those of other members (Table 2). The higher conservation between MAYV, UNAV, and CHIKV domains A and B may explain the cross-neutralization.

Neutralizing antibodies are considered reliable correlates of protection against alphavirus infection.[1,20] Accordingly, the primary endpoint of human Investigational New Drug vaccines against eastern, western, and Venezuelan equine encephalitis virus is to achieve a $PRNT_{80}$ titer of $\geq$ 1:20, which has been associated with protection against clinical disease.[19,20] Our study demonstrates that prior CHIKV infection could achieve $PRNT_{80}$ titers of similar or high magnitude against UNAV and MAYV. In addition, the cross-protection studies in animal models have demonstrated protection against challenges with heterologous SF complex viruses.[14,18] Taken together, these data suggest that cross-neutralizing antibody responses generated because of either vaccine administration or natural infection may provide some protection against infection with a closely related heterologous virus.

The observation of cross-reactive neutralizing antibody in convalescent CHIKV subjects may have implications for transmission cycles and vaccine development, as well as the epidemiology of emerging arboviruses. From an epidemiological standpoint, herd immunity—even low-level immunity—against closely related viruses may protect a population from a new and emerging outbreak. Following changes in population antibody responses to circulating pathogens may be a tool to predict incidence and spread of emerging arboviruses. From a medical countermeasures standpoint, identifying the epitopes or domains of CHIKV that elicit cross-reactive NAb may permit rational design of CHIKV vaccines with efficacy against closely related alphaviruses such as UNAV and MAYV.

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