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Isolation of genuine human monoclonal antibodies from a Chikungunya virus (CHIKV)-infected patient: Characterization of CHIK (OPY1, OPY4, OPY6), Ross River and O'nyong-nyong viruses neutralizing antibodies.

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Introduction

Chikungunya virus (CHIKV) was first isolated from the blood of a febrile patient in Tanzania in 1953, and has since been cited as the cause of numerous human epidemics in many areas of Africa and Asia and most recently in limited areas of Europe. Chikungunya virus is a member of the genus *Alphavirus*, belonging to the *Togaviridae* family, transmitted to humans by the bite of infected mosquitoes. Chikungunya fever is diagnosed based on symptoms, physical findings (e.g., joint swelling), laboratory testing, and the possibility of exposure to infected mosquitoes. In the absence of specific treatment for Chikungunya fever, care is mainly based on symptoms.

Objective

In order to generate biological tools that will help to better understand the interactions between human and CHIKV proteins, we attempted to isolate genuine anti-CHIKV antibodies-secreting B lymphocytes from a patient who has recovered from CHIKV infection.

Methods

PBMC were isolated from 50ml of peripheral blood and immortalized through a highly efficient strategy that combines CD40 activation and EBV transformation.

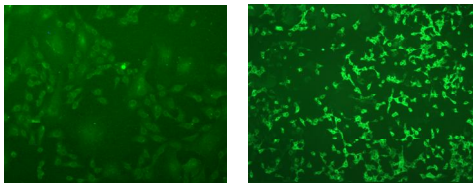
Results

More than 2000 wells were found positive during the screening by immunofluorescent staining of CHIKV-infected cells.
70 clones specifically recognizing CHIKV-infected cells were obtained among which 30 were selected for further characterization.
11 of them displayed neutralizing activities against several CHIKV strains and/or against 2 related viruses (Ross River and O'nyong-nyong).
Representative results obtained from specificity, neutralization and cross-reactivity assays are shown.

a-Specificity assay

CHIKV specific recognition was revealed by comparative immunofluorescent staining of mock *versus* CHIKV-infected VERO cells with the monoclonal antibody to be tested.

VERO cells



control cells ***CHIKV-OPY1 infected cells***

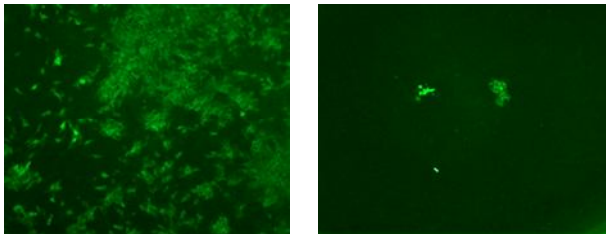
These assays allowed the identification of 2000 wells reacting positively with monoclonal antibodies CHIKV-infected cells.

b-Seroneutralization assay

After a cloning step, 70 monoclonal antibodies were tested in seroneutralization assays. Briefly, CHIKV suspension was pre-incubated either with isotype control antibody, or with the monoclonal

antibody to be tested. After infection, immunofluorescent staining with an anti-CHIKV monoclonal antibody was performed.

CHIKV-infected VERO cells



Isotype control ***mAb anti-CHIKV***

These experiments allowed the identification of 11 monoclonal antibodies able to neutralize VERO cells infection by CHIKV.

c-Cross-reactivity assays

We have isolated antibody-secreting B cell clones from a patient infected by the OPY1 strain of the CHIKV. To further characterize their neutralizing properties, the monoclonal antibodies were tested against primary isolates of other CHIKV strains.

Seroneutralization of Chikungunya strains

mAb dilution	OPY1					OPY4					OPY6				
	+++	++	++	+++	+++	++	++	++	++	+++	++	+	++	++	+++
	++	+	+	++	+++	++	+	++	+	+++	+	+	-/+	+	+++
	++	+	+	+	+++	+	+	+	+	+++	+	-	-	-	+++
	+	-	-	-	+++	+	-	-	+	+++	+	-	-	-	++
	-	-	-	-	++	-	-	-	-	+++	-	-	-	-	++
	-	-	-	-	+	-	-	-	-	++	-	-	-	-	+
	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	E26.B4.01	E26.F6.01	E26.C4.01	E26.B6.01	E26.D9.02	E26.B4.01	E26.F6.01	E26.C4.01	E26.B6.01	E26.D9.02	E26.B4.01	E26.F6.01	E26.C4.01	E26.B6.01	E26.D9.02

+++: 100% neutralization

These assays clearly demonstrated the ability of the antibodies to neutralize the 3 CHIKV strains, with a higher capacity for the E26.D9.02 clone.

Both Ross River and O'Nyong Nyong viruses being described as closely related to CHIKV, we evaluated whether these anti-CHIKV antibodies were able to neutralize the infection of VERO cells by RRV or ONNV.

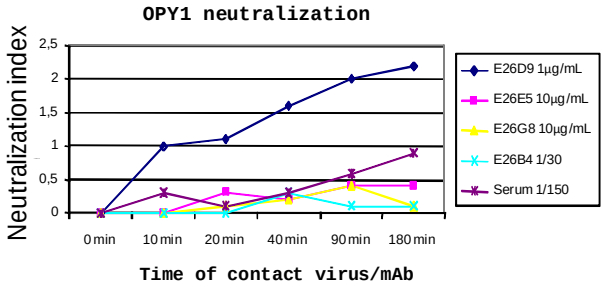
Seroneutralization of related virus by anti-CHIKV antibodies

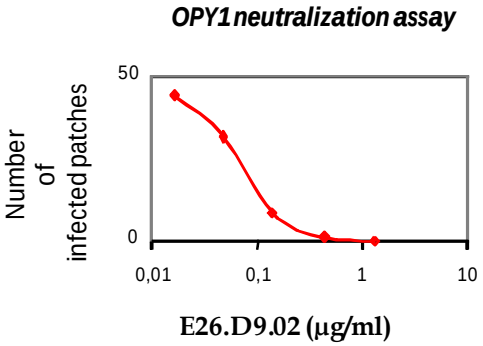
mAb dilution	ROSS RIVER VIRUS					O'NYONG NYONG VIRUS				
	++	+	+	++	+++	+++	+	++	+++	+++
	++	+	+	+	+++	++	+	+	++	+++
	+	-	-	+	+++	+	-	-	+	++
	-	-	-	-	++	-	-	-	-	-
	-	-	-	-	+	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-
	E26.B4.01	E26.F6.01	E26.C4.01	E26.B6.01	E26.D9.02	E26.B4.01	E26.F6.01	E26.C4.01	E26.B6.01	E26.D9.02

+++: 100% neutralization

As expected, several cross-reactivities were observed, the E26.D9.02 clone displaying the higher capacity to cross-neutralize both RRV and ONNV infections.

The interesting properties of the E26.D9.02 antibody led us to further study its neutralization capacity. Comparative neutralization studies of the OPY1 strain which infected this patient between several clones and the patient serum were performed.





These studies demonstrated that E26.D9.02 is a very high quality and performing antibody:

-a very short incubation time (<10min) with the virus is sufficient to induce a significant neutralization index

-very low concentrations of E26.D9.02 are required to neutralize infection by OPY1 CHIKV strain.

Summary

In summary, 30 monoclonal antibodies recognizing CHIKV were identified. Eleven of them displayed neutralizing activities against 3 CHIKV strains as well as against other related viruses, Ross River virus and O'Nyong Nyong virus, the E26D9.02 clone being the most efficient one.
The neutralizing properties of representative monoclonal antibodies are summarized in the table below:

Seroneutralization assay

		CHIKV			OTHERS		Isotypes	Reference
		OPY1	OPY4	OPY6	RRV	ONNV		
Monoclonal antibodies anti-CHIKV	E04C1.02	-	+		-	-		
	E18E6.01	-	-	+	-	-		
	E26B4.01	+	+	+	+	+		
	E26B6.01	+	+	+	+	+	IgG1	
	E26C4.01	+	+	+	+	+	IgG1	DDX9102
	E26C9.01	-	+	-	-	-		
	E26D3.01	+	+	+	+	-		
	E26D9.02	+	+	+	+	+	IgG1	DDX9100
	E26E5.03	+	+	+	+	-		
	E26F6.01	+	+	+	+	+	IgM	
	E26G8.05	-	+	+	+	-		DDX9103

+: neutralization

Conclusion

A set of genuine human anti CHIKV monoclonal antibodies have been developed to provide dispensable tools for scientists interested in a better understanding of the CHIKV infection. These monoclonal antibodies are suitable for various applications such as seroneutralization, detection of infected cells by immunofluorescent staining, detection of viral particles in biological fluids by ELISA.

The E26.D9.02 monoclonal antibody displayed very promising neutralizing properties (9 CHIKV primary isolates, all CHIKV laboratory strains and Ross River and O'nyong-nyong viruses).