

**B-CELL RECEPTOR CHARACTERIZATION OF
PLASMABLASTS AND MEMORY B CELLS IN
HUMAN DENGUE NATURAL INFECTIONS**

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**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES,
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MEMORY B CELLS IN HUMAN DENGUE NATURAL INFECTIONS**

by

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&



**ICGEB-Emory Vaccine Center
International Center for Genetic Engineering and Biotechnology
(ICGEB), New Delhi**

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Dedicated to the women who shaped my journey.

यत्र नार्यस्तु पूज्यन्ते रमन्ते तत्र देवताः।

Yatra nāryastu pūjyante ramante tatra devatāḥ.

Where women are honored, there the gods rejoice.

CERTIFICATE

This is to certify that the thesis entitled “**B-cell receptor characterization of plasmablasts and memory B cells in human dengue natural infections**” being submitted by **Mr. Elluri Seetharami Reddy** to the **Kusuma School of Biological Sciences**, Indian Institute of Technology Delhi, New Delhi for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research work carried out by him, prepared under my supervision, in conformity with the rules and regulations of the ‘Indian Institute of Technology Delhi’. The research report and the results presented in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

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Place: New Delhi

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A PhD journey made me realize it is not just a chase for degrees or research papers—It's the kind of adventure that changes you forever, shaping not just your mind but your very life, opening doors to endless possibilities. This thesis might carry my name on its cover, but the real story behind it is a saga of many hearts and hands—mentors who believed, friends who stood by, companions who cheered, and family who never let me walk alone.

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immense wisdom become a burden to those around him. Rather, he inspires through calm assurance. In a world that screams “do or die,” he quietly teaches you how to ‘do’. His presence is reassuring, his words simple yet deeply transformative.

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Cinema: A magical world

I am deeply grateful to the world of cinema—for giving me the chance to embark on countless journeys beyond the ordinary, into realms where imagination knows no bounds. Films have never been just stories on a screen; they are portals to dreams, allowing me to escape, to explore, and to believe in realities far richer than the one around me. They offer a space where the world unfolds

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Shoulders of Seniors

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Matti Vaasana (మట్టి వాసన): The People Who Smell Like Rain

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People Who Change Your Trajectory Forever

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Vamshi — *go home or go big*. A god-gifted powerhouse of unconventional brilliance, with a curiosity that stretches across disciplines and a passion that refuses to be boxed in. He carries the willpower of *Sati Savitri*, the one who argued with *Yama* to bring her husband back from death. I

root for his success with desperation—because I know when he rises, he lifts all of us with him. He never leaves his friends behind.

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Timelines & Twists

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Daivam Manushya Rupena

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“दुःखेष्वनुद्विग्नमनाः सुखेषु विगतस्पृहः।

वीतरागभयक्रोधः स्थितधीरमुनिरुच्यते ॥”

— भगवद्गीता 2.56

duḥkheṣv-anudvigna-manāḥ: sukheṣu vigata-spr̥haḥ

vīta-rāga-bhaya-krodhaḥ: sthita-dhīr munir uchyate

-Bhagavad Gita 2.56

"One whose mind remains undisturbed in sorrow,

who does not crave for pleasures,

who is free from attachment, fear, and anger —

such a person is called a sage of steady wisdom (sthita-dhīr munih̥)."

ABSTRACT

B-cell responses play a central role in protecting against viral infections. Upon activation, naïve B cells proliferate and differentiate into short-lived antibody-secreting effector cells, known as plasmablasts. These antibodies engage in various immune effector functions, including the neutralization of the infecting virus. A subset of these primed B cells develop into long-lived memory B cells, which can mount rapid and robust recall antibody responses if the host re-encounters the same pathogen.

Research over the past several decades has revealed that as the B cells transition from naïve to effector to memory recall response states, their B cell receptors (BCRs) undergo evolution, enabling the immune system to mount increasingly precise and effective responses. This capacity to tailor responses to specific antigens is a hallmark of adaptive immunity, primarily driven by somatic hypermutation, which leads to affinity maturation of the BCRs. Despite these critical insights, our understanding of B cell responses during natural viral infections in humans, particularly those infections that occur naturally for the second time, remains limited. A deeper understanding of these is essential for gaining novel insights on immunological memory, pathogen recognition, and the evolution of adaptive immunity during repetitive natural infections.

Dengue virus (DENV) is a valuable model for studying immune responses during natural viral infections in humans. In endemic regions, such as India, four antigenically related dengue virus serotypes (DENV1-4) co-circulate. A primary infection with any of the four serotypes induces long-term immunity to that serotype but not to others, making reinfections with heterologous serotypes common. This natural pattern of sequential infections enables studies to investigate how

the effector and memory B cell responses generated in response to a secondary natural infection differ from those induced during a primary infection.

In this study, we analyzed human B cell response at a single-cell level by generating and characterizing monoclonal antibodies from sorted plasmablasts and memory B cells derived from primary and secondary dengue infections. We find that B cell responses following primary infection are dominated by low-affinity, poorly neutralizing antibodies with minimal somatic hypermutation, whereas secondary infections induce a markedly enhanced response characterized by higher affinity, broader, and more potently neutralizing antibodies. This maturation of the B cell repertoire reinforces classical immunological paradigms of affinity maturation and highlights the functional evolution of humoral immunity in natural viral infections.

वायरल संक्रमण से बचाव में बी-कोशिका प्रतिक्रियाएँ केंद्रीय भूमिका निभाती हैं। सक्रिय होने पर, नैव बी कोशिकाएँ बढ़ती हैं और अल्पकालिक एंटीबॉडी-स्रावी प्रभावकारी कोशिकाओं में विभेदित हो जाती हैं, जिन्हें प्लाज़्माब्लास्ट कहा जाता है। ये एंटीबॉडी विभिन्न प्रतिरक्षा प्रभावकारी कार्यों में संलग्न होती हैं, जिनमें संक्रमित वायरस को बेअसर करना शामिल है। इन प्राइमर्ड बी कोशिकाओं का एक उपसमूह लंबे समय तक रहने वाली मेमोरी बी कोशिकाओं में परिवर्तित हो जाता है, जो मेज़बान द्वारा उसी रोगजनक का फिर से सामना किए जाने पर तेज़ और मज़बूत रिकॉल एंटीबॉडी प्रतिक्रियाएँ उत्पन्न कर सकता है। पिछले कई दशकों के शोध से पता चला है कि जैसे-जैसे बी कोशिकाएँ नैव से प्रभावकारी और मेमोरी रिकॉल प्रतिक्रिया अवस्थाओं में परिवर्तित होती हैं, उनके बी सेल रिसेप्टर्स (बीसीआर) विकास से गुजरते हैं, जिससे प्रतिरक्षा प्रणाली अधिक सटीक और प्रभावी प्रतिक्रियाएँ देने में सक्षम होती है। विशिष्ट प्रतिजनों के प्रति प्रतिक्रियाओं को अनुकूलित करने की यह क्षमता अनुकूली प्रतिरक्षा की एक पहचान है, जो मुख्य रूप से दैहिक अतिउत्परिवर्तन द्वारा संचालित होती है, जो बीसीआर की आत्मीयता परिपक्वता की ओर ले जाती है। इन महत्वपूर्ण जानकारी के बावजूद, मनुष्यों में प्राकृतिक वायरल संक्रमणों के दौरान बी सेल प्रतिक्रियाओं की हमारी समझ, विशेष रूप से वे संक्रमण जो स्वाभाविक रूप से दूसरी बार होते हैं, सीमित बनी हुई है। इनके बारे में गहन समझ प्रतिरक्षात्मक स्मृति, रोगजनक पहचान और बार-बार होने वाले प्राकृतिक संक्रमणों के दौरान अनुकूली प्रतिरक्षा के विकास पर नई अंतर्दृष्टि प्राप्त करने के लिए आवश्यक है।

डेंगू वायरस (DENV) मनुष्यों में प्राकृतिक वायरल संक्रमणों के दौरान प्रतिरक्षा प्रतिक्रियाओं का अध्ययन करने के लिए एक मूल्यवान मॉडल है। भारत जैसे स्थानिक क्षेत्रों में, चार एंटीजेनिक रूप से संबंधित डेंगू वायरस सीरोटाइप (DENV1-4) सह-परिसंचारित होते हैं। चारों सीरोटाइप में से

किसी एक के साथ एक प्राथमिक संक्रमण उस सीरोटाइप के लिए दीर्घकालिक प्रतिरक्षा उत्पन्न करता है, लेकिन अन्य के लिए नहीं, जिससे विषम सीरोटाइप के साथ पुनः संक्रमण आम हो जाता है। अनुक्रमिक संक्रमणों का यह प्राकृतिक पैटर्न अध्ययनों को यह जांचने में सक्षम बनाता है कि द्वितीयक प्राकृतिक संक्रमण की प्रतिक्रिया में उत्पन्न प्रभावकारी और स्मृति बी सेल प्रतिक्रियाएँ प्राथमिक संक्रमण के दौरान प्रेरित प्रतिक्रियाओं से कैसे भिन्न होती हैं।

इस अध्ययन में, हमने प्राथमिक और द्वितीयक डेंगू संक्रमणों से प्राप्त सॉर्ट किए गए प्लाज़्माब्लास्ट और मेमोरी बी कोशिकाओं से मोनोक्लोनल एंटीबॉडी उत्पन्न कर और उनकी विशेषताएँ बताकर एकल-कोशिका स्तर पर मानव बी सेल प्रतिक्रिया का विश्लेषण किया। हम पाते हैं कि प्राथमिक संक्रमण के बाद बी सेल प्रतिक्रियाओं में कम आत्मीयता, न्यूनतम दैनिक अतिउत्परिवर्तन के साथ, कमजोर निष्क्रिय करने वाले एंटीबॉडी का प्रभुत्व होता है; जबकि द्वितीयक संक्रमण उच्च आत्मीयता, व्यापक और अधिक शक्तिशाली रूप से निष्क्रिय करने वाले एंटीबॉडी की विशेषता वाली एक उल्लेखनीय रूप से बढ़ी हुई प्रतिक्रिया को प्रेरित करते हैं। बी सेल प्रदर्शनों की यह परिपक्वता आत्मीयता परिपक्वता के शास्त्रीय प्रतिरक्षात्मक प्रतिमानों को पुष्ट करती है और प्राकृतिक वायरल संक्रमणों में ह्यूमरल प्रतिरक्षा के कार्यात्मक विकास को उजागर करती है।

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ABBREVIATIONS AND SYMBOLS

Abbreviation	Full Form
°C	Degrees Celsius
%	Percent
A260	Absorbance at 260 nm
A280	Absorbance at 280 nm
ADE	Antibody-dependent enhancement
AIIMS	All India Institute of Medical Sciences
AID	Activation-induced cytidine deaminase
APCs	Antigen-presenting cells
ATCC	American Type Culture Collection
BCR	B cell receptor
BAFF	B cell-activating factor
BSA	Bovine serum albumin
bp	Base pair
Bcl6	B-cell lymphoma 6 protein
BD	Becton Dickinson
BLIMP-1	B lymphocyte-induced maturation protein 1
CD	Cluster of differentiation
CDRs	Complementarity-determining regions
C	Capsid protein
cDNA	Complementary DNA
C6/36	Aedes albopictus cell line
CMV	Cytomegalovirus
CO ₂	Carbon dioxide

CSR	Class-switch recombination
DAMPs	Damage-associated molecular patterns
DENV	Dengue virus
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
EC ₅₀	Effective concentration 50%
EDTA	Ethylenediaminetetraacetic acid
E	Envelope protein
E2A	E-protein transcription factor A
EBF1	Early B cell factor 1
ELISA	Enzyme-linked immunosorbent assay
EDE	Envelope dimer epitope
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FFU	Focus-forming units
FDCs	Follicular dendritic cells
FITC	Fluorescein isothiocyanate
Foxo1	Forkhead box protein O1
FRNT	Focus reduction neutralization test
GC	Germinal center
HCl	Hydrochloric acid
HEK	Human embryonic kidney
HF	High fidelity
HSCs	Hematopoietic stem cells

HRP	Horseradish peroxidase
HTS	High-throughput sequencing
IC ₅₀	Inhibitory concentration 50%
IFN- γ	Interferon gamma
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgH	Immunoglobulin heavy chain
IgK	Immunoglobulin kappa chain
IgL	Immunoglobulin lambda chain
IgM	Immunoglobulin M
IL-21	Interleukin-21
IL-4	Interleukin-4
	International ImMunoGeneTics information
IMGT	system
IRF4	Interferon regulatory factor 4
J	Joining gene segment
kb	Kilobase
kDa	Kilodalton
LB	Luria-Bertani medium
M	Marker (molecular weight standard)
MBC	Memory B cell
MFI	Mean fluorescence intensity
MG	Milligram
min	Minutes

ml	Milliliter
MOI	Multiplicity of infection
MWCO	Molecular weight cut-off
nm	Nanometer
	4-hydroxy-3-nitrophenylacetyl conjugated to
NP-KLH	keyhole limpet hemocyanin
NS1	Non-structural protein 1
NS2A	Non-structural protein 2A
NS2B	Non-structural protein 2B
NS3	Non-structural protein 3
NS4A	Non-structural protein 4A
NS4B	Non-structural protein 4B
NS5	Non-structural protein 5
OD	Optical density
OPD	o-Phenylenediamine dihydrochloride
PAGE	Polyacrylamide gel electrophoresis
PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PES	Polyethersulfone
PIPE	Polymerase incomplete primer extension
PBMCs	Peripheral blood mononuclear cells
PRRs	Pattern recognition receptors
prM	Premembrane protein
RAG1	Recombination activating gene 1

RAG2	Recombination activating gene 2
RBC	Red blood cell
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Reverse transcription
	Reverse transcription polymerase chain
RT-PCR	reaction
	Severe acute respiratory syndrome
SARS-CoV-2	coronavirus 2
	Sodium dodecyl sulfate–polyacrylamide gel
SDS-PAGE	electrophoresis
SHM	Somatic hypermutation
	Super optimal broth with catabolite
SOC	repression
TCR	T cell receptor
Tfh	T follicular helper cell
TGF- β	Transforming growth factor beta
TMB	Tetramethylbenzidine
UV	Ultraviolet
V	Variable gene segment
	Woodchuck hepatitis virus posttranscriptional
WPRE	regulatory element
WHO	World Health Organization