

Summary

Distal body endoplasmic reticulum (ER) is a specialized compartment of the ER that is enriched in B cells. It is associated with the production of antibodies and is a site of viral replication. The ER is a site of protein synthesis and folding, and is involved in the regulation of gene expression. The ER is a site of protein synthesis and folding, and is involved in the regulation of gene expression.

Rishi R. Gellera, Satsuki A. Akita, Mark P. Dijk, Matthew A. J. Hill, Patrick O. K. Sigurdson, Philip Hicks, Weibo He, Aadi R. S. Saah, Diger, Kedala. Lidgee, Letcia K. -Centes, Shao Adalin, Anna Hick, Scott K. Deek, A. Obridge, Amy. Bar, Joseph R. Giles, Madeline Weirck, Christopher M. Altier, Jean D. Ghery, Sheela L. K. D'Adena, Jacob T. Harib, Michael R. Bes, Elin T. Ling Pak Palu Bats, Scott E. Heby, Alan R. Geepat, E. John Weyer *

*Corresponding author. Email: wey@pedu

Published Online: 15 April 2021, ScienceDirect

DOI: 10.1016/j.abi.2021.04.001

10.11

This PDF file includes:

Suppl Fig 1. Colocalization of ER markers

Suppl Fig 2. Identification of antigen-specific B cells

Suppl Fig 3. Characterization of antigen-specific B cell subsets that

express ER-associated proteins

Suppl Fig 4. B cell protein expression profiles

Suppl Fig 5. Relationship between body endoplasmic reticulum RNA expression

and protein synthesis

Suppl Table 1. Flow cytometry analysis of antigen-specific B cell subsets

Suppl Table 2. Receptor expression profiles of antigen-specific B cell subsets

Suppl Table 3. Analysis of J[4] expression profiles of antigen-specific B cell subsets

(available at www.sciencedirect.com/journal/1016/j.abi)

Suppl Table 4:

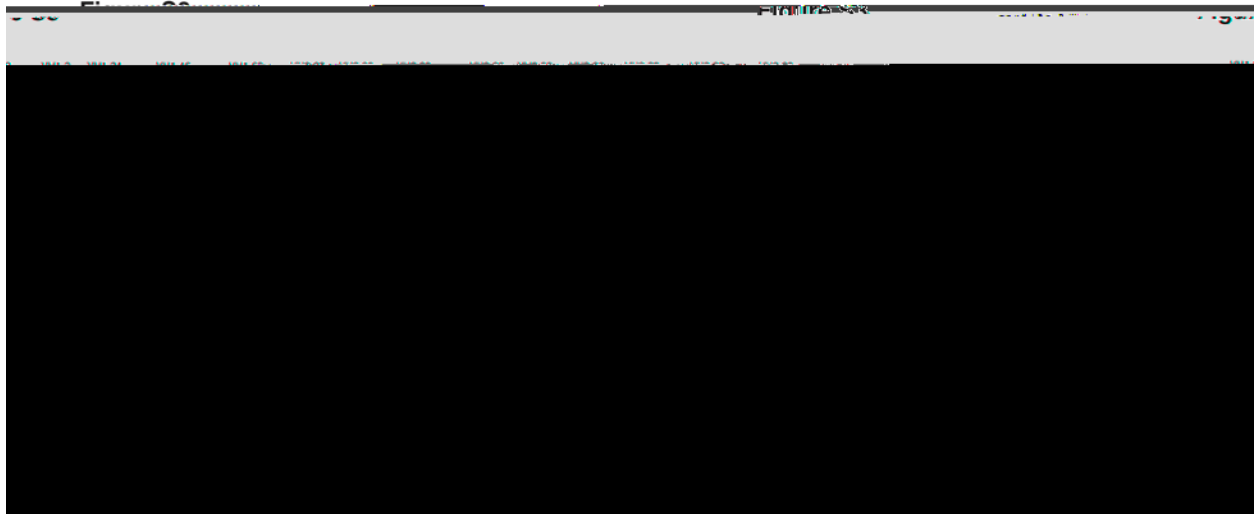
Raw data files

Supplemental Figure 1. Cohort design and summary statistics. A) Demographic and clinical

Figure S2



Supplemental Figure 2. Identification of antigen-specific B cells. A) Lymphocytes were gated by FSC vs. SSC. Doublets were then excluded by FSC-A vs. FSC-H and FSC-A vs. FSC-W. Live cells were identified as Ghost 510⁻ and total B cells were identified as CD3⁻ CD19⁺. Naïve B cells were then identified as IgD⁺ CD27⁻ and excluded with a boolean not gate. Memory B cells were identified as CD20⁺ CD38^{lo/int} non-naïve B cells. A decoy SA-BV711 probe was used to gate out cells that non-specifically bind streptavidin. Spike- and hemagglutinin-specific B cells were then identified based on their binding to fluorescent probes. Spike⁺ cells were further analyzed for binding to fluorescent RBD probe. Both spike⁺ and spike⁺/RBD⁺ cells were analyzed for IgG vs. IgM expression. **B)** Frequencies of total naïve, non-naïve, and memory B cells over time in vaccinated individuals.



Supplemental Figure 3. Clonal evolution of spike-binding memory B cell lineages that were present prior to vaccination in a recovered individual. Analysis was restricted to subject 20, who had the largest number of clones sampled. For lineage trees, numbers refer to mutations compared to the preceding vertical node. Colors indicate the timepoint and black dots indicate inferred nodes. Node size is proportional to sequence copy number. GL = germline sequence.

Supplemental Figure 4. B cell populations in sex and age subgroups. Frequencies of **A)** naïve B, **B)** non-naïve B, and **C)** total memory B cell populations compared with sex and age in SARS-CoV-2 naïve

Fluorophore	Target	Source	Clone	Catalog #	Dilution
BUV 395	CD27	BD	L128	563815	1:200
BUV 563	CD3	BD	UCHT1	748569	1:200
BUV 661	CD38	BD	HIT2	612969	1:200
BUV 805	CD20	BD	2H7	612905	1:500
BV421	Streptavidin	Biolegend	--	405226	--
BV480	IgD	BD	IA6-2	566138	1:50
BV510	L/D	Tonbo	--	13-0870-T100	1:600
BV711	Streptavidin	Biolegend	--	405241	--
BV750	CD19	Biolegend	HIB19	302262	1:100
PerCP/Cy5.5	IgM	Biolegend	MHM-88	314512	1:200
PE	Streptavidin	Biolegend	--	405203	--
PE-Cy7	IgG	Biolegend	M1310G05	410722	1:400
APC	Streptavidin	Biolegend	--	405207	--
APC-H7	CD71	BD	M-A712	563671	1:50
--	Fc Block	Biolegend	--	422302	1:200

Supplemental Table 1. Flow cytometry panel used for antigen-specific B cell assays.

Protein	Source	Catalog #
Recombinant SARS-CoV-2 Spike (Trimer) His Biotin	R&D Systems	BT10549-050
Recombinant SARS-CoV-2 Spike RBD His Biotin	R&D Systems	BT10500-050
HA(TM)(A/Brisbane/02/2018)(H1N1)	Immune Tech	IT-003-00110 TMp
HA(TM)(B/Colorado/06/2017)	Immune Tech	IT-003-B21 TMp

Supplemental Table 2. Recombinant proteins used for antigen-specific B cell assays.

Sample ID	# Replicates	Input DNA Per Replicate (ng)	Cell Count	# Valid Sequences	# Clones	CDR3 Length (nt)	Average VH Identity
IHCV2020.005 - Spike+ T1	2	1.224	348	122612	172	51.45	0.9439
IHCV2020.005 - Spike+ T2	2	0.69	1598	113423	213	54.04	0.9406
IHCV2020.005 - Spike+ T4	2	0.564	820	92657	137	47.93	0.9433
IHCV2020.019 - Spike+ T1	2	0.87	613	47538	125	48.94	0.9552
IHCV2020.019 - Spike+ T2	2	0.492	1665	91424	99	45.73	0.9558
IHCV2020.019 - Spike+ T4	2	-	858	59281	34	52.06	0.9506
IHCV2020.020 - Spike+ T1	2	1.26	743	75801	320	50.01	0.9404
IHCV2020.020 - Spike+ T2	2	1.846	4915	66491	504	52.29	0.9393
IHCV2020.020 - Spike+ T4	2	8.385	8051	242672	2374	52.65	0.9354
IHCV2020.022 - Spike+ T1	2	0.312	906	141334	88	47.05	0.9689
IHCV2020.022 - Spike+ T2	2	0.534	1685	109535	83	50.96	0.9595
IHCV2020.022 - Spike+ T4	2	0.606	656	99896	94	50.01	0.9598
IHCV2020.029 - Spike+ T1	2	-	370	2	1	66.00	0.9718
IHCV2020.029 - Spike+ T2	2	0.3	3166	21479	20	44.55	0.9720
IHCV2020.029 - Spike+ T4	2	2.838	1416	37433	75	48.84	0.9595

Supplemental Table 3. B cell receptor sequencing metadata. Sample ID indicates subject, T = time point; Two independent PCR amplifications (biological replicates) were performed for each sample; Input DNA per replicate; Sorted cell count (gated as dump⁻ CD19⁺; CD27⁺ CD38^{lo/int}; HA⁻ Spike⁺ by flow cytometry); Number of valid sequence copies (passing length and other QC filters, see methods); Clones are defined as sequences that share the same VH, JH, CDR3 length and are at least 85% identical in the third complementarity determining region (CDR3) amino acid sequence; Clones with only 1 copy at the subject level are excluded; CDR3 length in nucleotides (nt) and Average VH identity compared to the nearest germline VH gene (average identity was calculated for each clone and then averaged across clones with each clone counted once per sample). Productive rearrangements only.