DISCLOSURES

Maurizio Federico, direttore del Centro Nazionale per la Salute Globale
Istituto Superiore di Sanità

Analisi, opinioni e conclusioni relativi a questa presentazione sono del tutto personali e non riflettono in alcun modo le posizioni ufficiali sull'argomento dell'Istituto presso cui presto servizio.

Nessun conflitto di interesse da dichiarare.

CellPress

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Perspective

Rethinking next-generation vaccines for coronaviruses, influenzaviruses, and other respiratory viruses

David M. Morens,[1] Jeffery K. Taubenberger,[2,*] and Anthony S. Fauci[1]

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https://doi.org/10.1016/j.chom.2022.11.016

Table 1. Epidemiologic and immunologic parameters of selected human respiratory viruses and vaccines used to control them

Virus	Incubation period[a]	Marked viremia	Infection elicits long-term protective immunity	Re-infections are rare	Vaccines elicit long-term protective immunity	Vaccine type
Measles (to prodrome)	≈10 days	yes	yes	yes	yes	replicating
Mumps	≈16 days	yes	yes	yes	yes	replicating
Rubella	≈16 days	yes	yes	yes	yes	replicating
Smallpox[b]	≈12 days	yes	yes	yes	yes	replicating
VZV[c]	≈14 days	yes	yes	yes	yes	replicating
Endemic coronaviruses	≈5 days	no	no	no	no	none
Influenza virus	≈2 days	no	no	no	no	replicating, other
Parainfluenzaviruses	≈4 days	no	no	no	no	none
RSV	≈5 days	no	no	no	no	none
SARS-CoV-2	≈4 days	no[d]	no	no	no	non-replicating

respiratory, lower respiratory tract, and systemic vaccination[44,76,134–141]; or optimized combinations of these. Attempting to control mucosal respiratory viruses with systemically administered non-replicating vaccines has thus far been largely unsuccessful, indicating that new approaches are needed. For example, respiratory disease often reflects host genetic susceptibility factors.[16,51,146,147]

PUBLIC HEALTH CONSIDERATIONS RELATING TO NEXT-GENERATION RESPIRATORY VACCINES MUST

Lack of neutralizing immunity in nasal swabs from COVID-19 mRNA vaccinees

nature medicine

ARTICLES

https://doi.org/10.1038/s41591-021-01318-5

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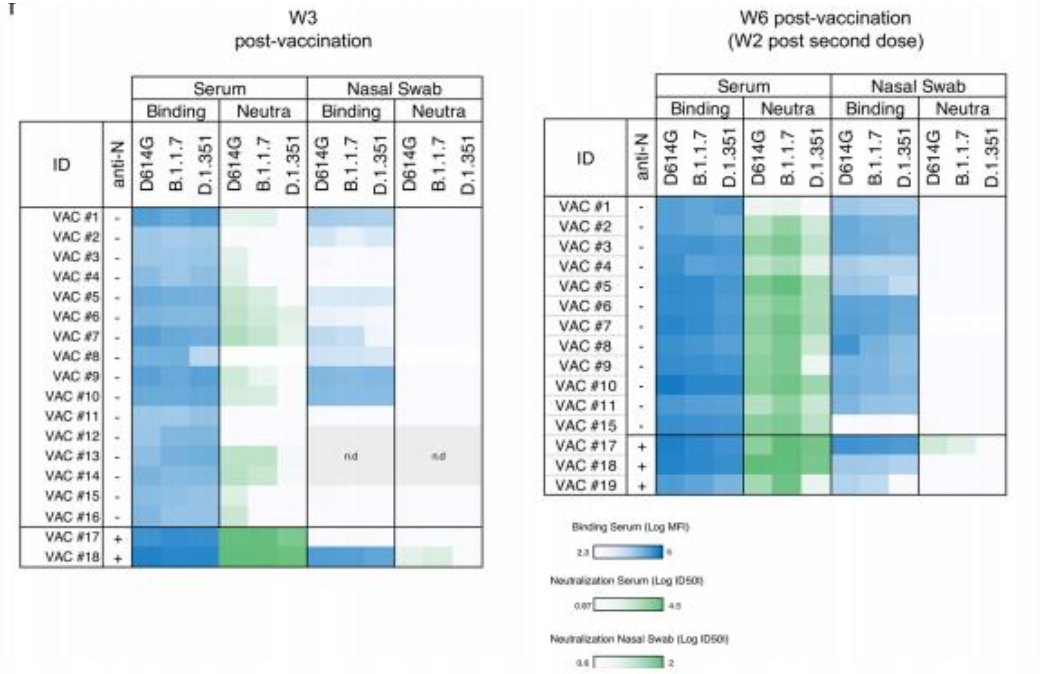
Sensitivity of infectious SARS-CoV-2 B.1.1.7 and B.1.351 variants to neutralizing antibodies

Delphine Planas[1,2,3,22], Timothée Bruel[1,2,3,22], Ludivine Grzelak[1,2,3,4], Florence Guivel-Benhassine[1,2,3], Isabelle Staropoli[1,2,3], Françoise Porrot[1,2,3], Cyril Planchais[5], Julian Buchrieser[1,2,3], Maaran Michael Rajah[1,2,3,4], Elodie Bishop[1,2,3,4], Mélanie Albert[6,7], Flora Donati[6,7], Matthieu Prot[8], Sylvie Behillil[6,7], Vincent Enouf[6,7], Marianne Maquart[9], Mounira Smati-Lafarge[10], Emmanuelle Varon[10], Frédérique Schortgen[11], Layla Yahyaoui[12], Maria Gonzalez[13], Jérôme De Sèze[14,15], Hélène Péré[16], David Veyer[16,17], Aymeric Sève[18], Etienne Simon-Lorière[8], Samira Fafi-Kremer[19,20], Karl Stefic[9,21], Hugo Mouquet[5], Laurent Hocqueloux[18], Sylvie van der Werf[6,7,23], Thierry Prazuck[18,23] and Olivier Schwartz[1,2,3,23] ✉



Either low or absent anti-Spike immunity in lungs of vaccinees

July 19, 2022

Science Immunology

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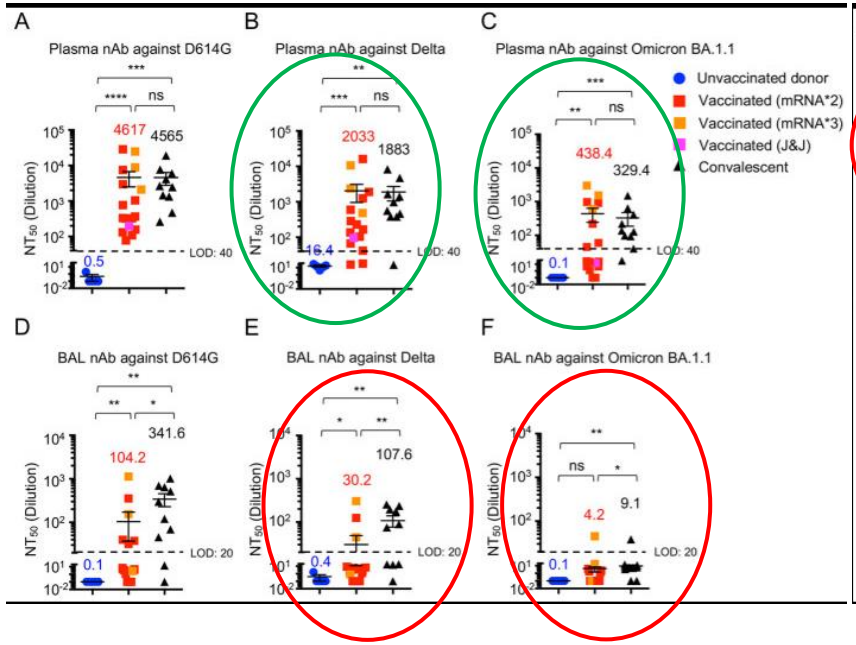
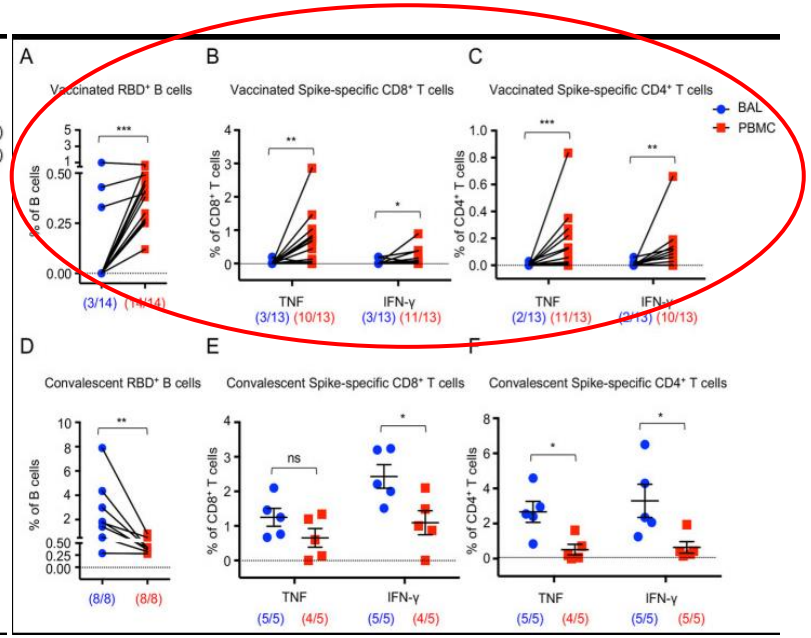
REPORTS | CORONAVIRUS

Respiratory mucosal immunity against SARS-CoV-2 following mRNA vaccination

JINYI TANG, CONG ZENG, THOMAS M. COX, CHAOFAN LI, YOUNG MIN SON, IN SU CHEON, YUE WU, SUPRIYA BEHL, JUSTIN J. TAYLOR, [...] JIE SUN +18 authors Authors Info & Affiliations

SCIENCE IMMUNOLOGY · 19 Jul 2022 · First Release · DOI: 10.1126/sciimmunol.add4853





Vaccines designed to elicit respiratory immunity must deliver antigen to the lungs

nature immunology

ARTICLES

https://doi.org/10.1038/s41590-018-0260-6

The establishment of resident memory B cells in the lung requires local antigen encounter

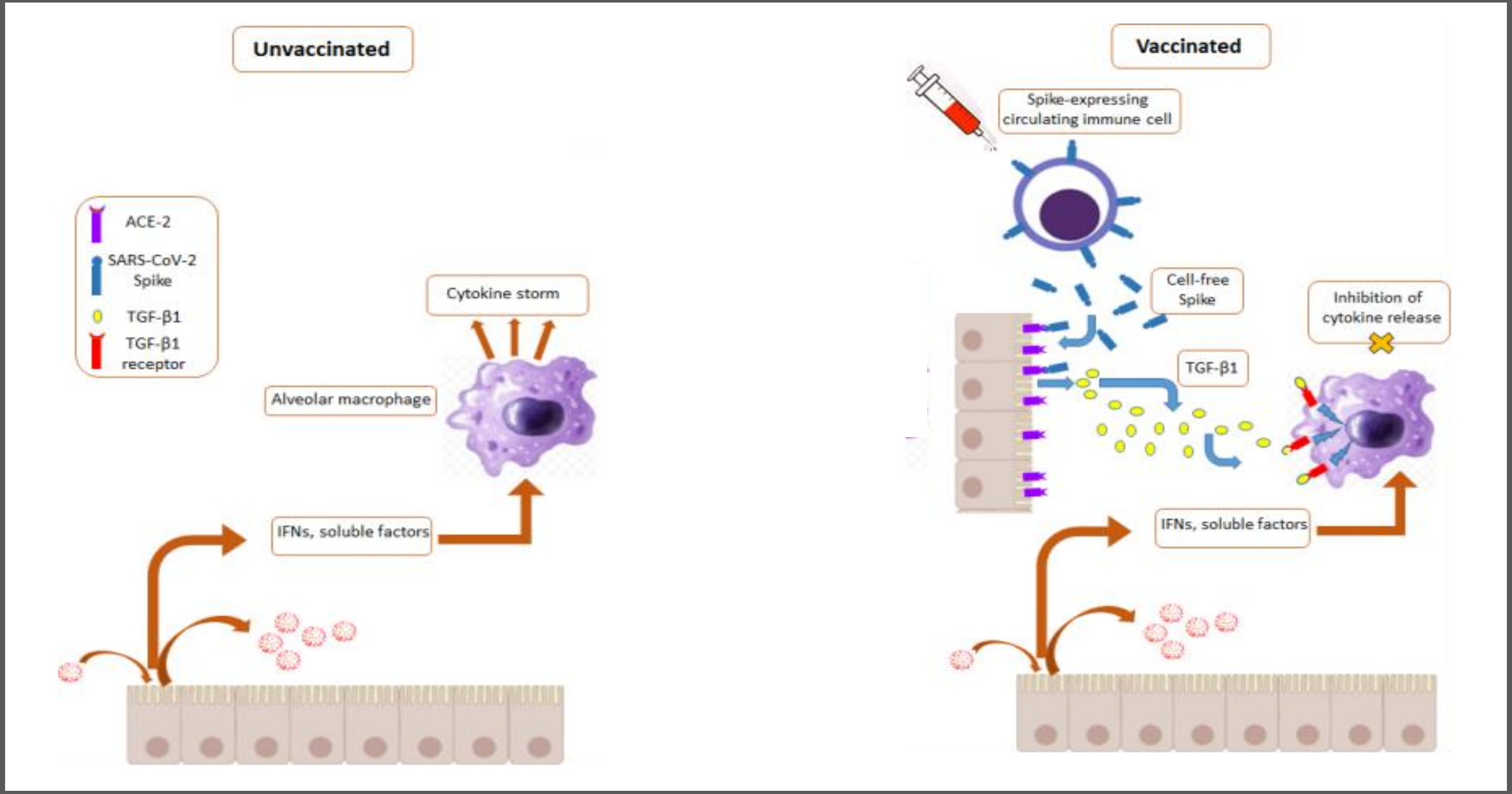
S. Rameeza Allie[1], John E. Bradley[1], Uma Mudunuru[1], Michael D. Schultz[2], Beth A. Graf[2], Frances E. Lund[2] and Troy D. Randall[1*]

Memory B cells are found in lymphoid and non-lymphoid tissues, suggesting that some may be tissue-resident cells. Here we show that pulmonary influenza infection elicited lung-resident memory B cells (BRM cells) that were phenotypically and functionally distinct from their systemic counterparts. BRM cells were established in the lung early after infection, in part because their placement required local antigen encounter. Lung BRM cells, but not systemic memory B cells, contributed to early plasmablast responses following challenge infection. Following secondary infection, antigen-specific BRM cells differentiated in situ, whereas antigen-non-specific BRM cells were maintained as memory cells. These data demonstrate that BRM cells are an important component of immunity to respiratory viruses such as influenza virus and suggest that vaccines designed to elicit BRM cells must deliver antigen to the lungs.

- The development of lung immune memory is largely not influenced by events occurring in both peripheral circulation and lymphoid organs;

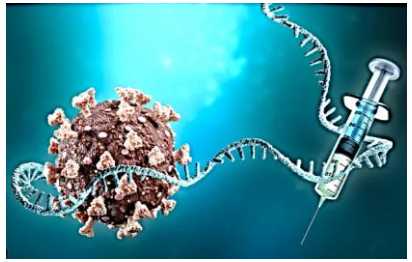
- Lymphocytes in lungs are maintained independently of the pool of circulating lymphocytes, and their continuous loss through intraepithelial migration towards airways is constantly replenished by homeostatic proliferation

How does the anti-COVID-19 vaccine work?

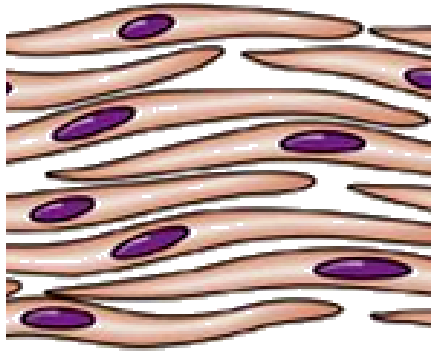


Autoimmunity generated by the anti-COVID-19 vaccine: anti-idiotype antibodies

mRNA-LNP



Muscle cells



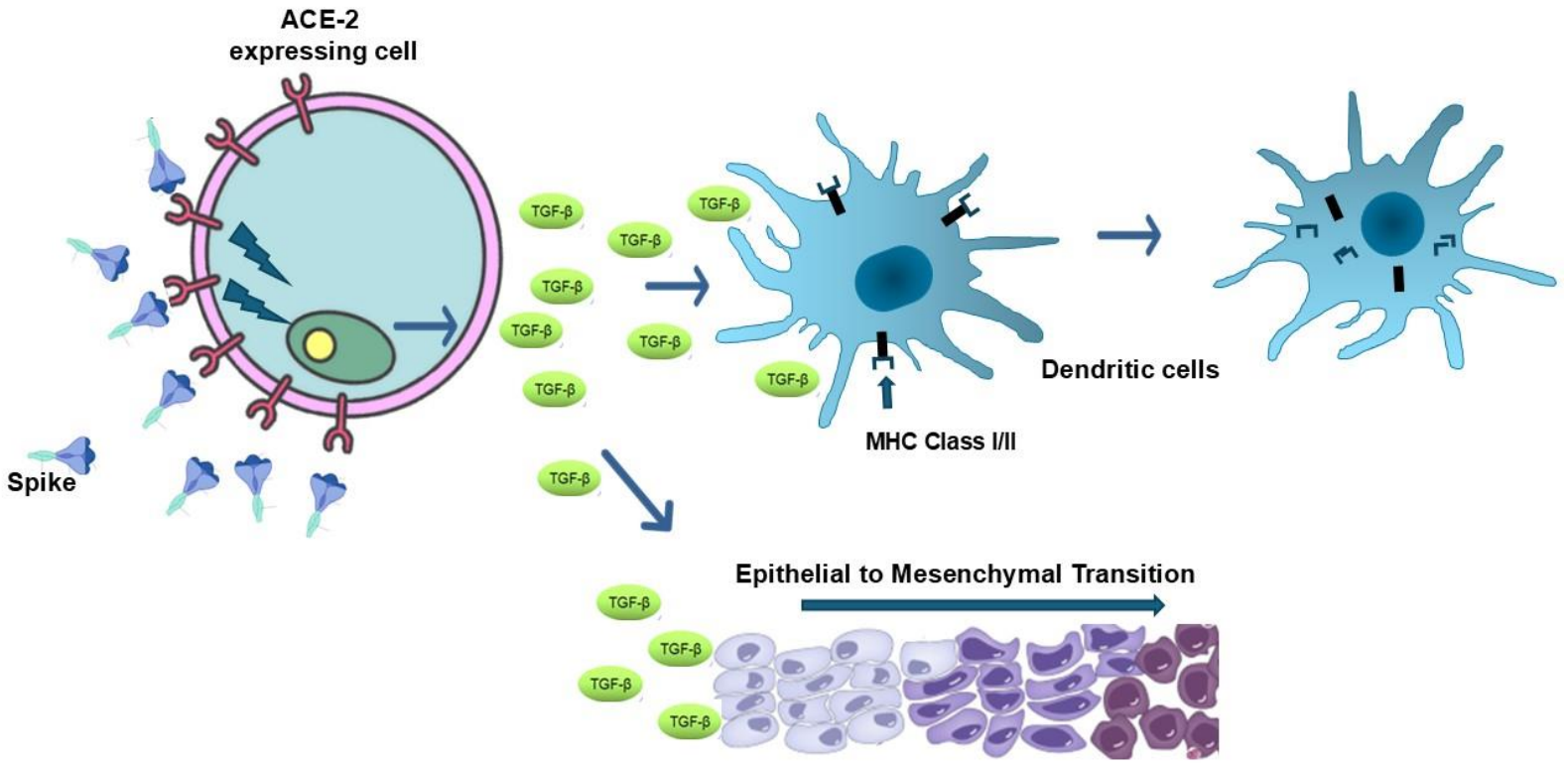
Spike

ACE-2
expressing cells

Anti-idiotype,
ACE-2 binding antibodies

Anti-Spike antibodies

- ***Bystander effects of the Spike/ACE-2 binding***



COVID-19 vaccine-induced autoimmunity: auto-antibodies

frontiers | Frontiers in Immunology

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PUBLISHED 02 August 2024
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High serum prevalence of autoreactive IgG antibodies against peripheral nerve structures in patients with neurological post-COVID-19 vaccination syndrome

Friederike A. Arlt[1,2†], Ameli Breuer[1†], Elli Trampenau[1,2], Fabian Boesl[1], Marieluise Kirchner[3], Philipp Mertins[3], Elisa Sánchez-Sendín[1,2], Mahoor Nasouti[1,2], Marie Mayrhofer[4], Martin Blüthner[4], Matthias Endres[1,2,5,6,7], Harald Prüss[1,2†] and Christiana Franke[1*†]

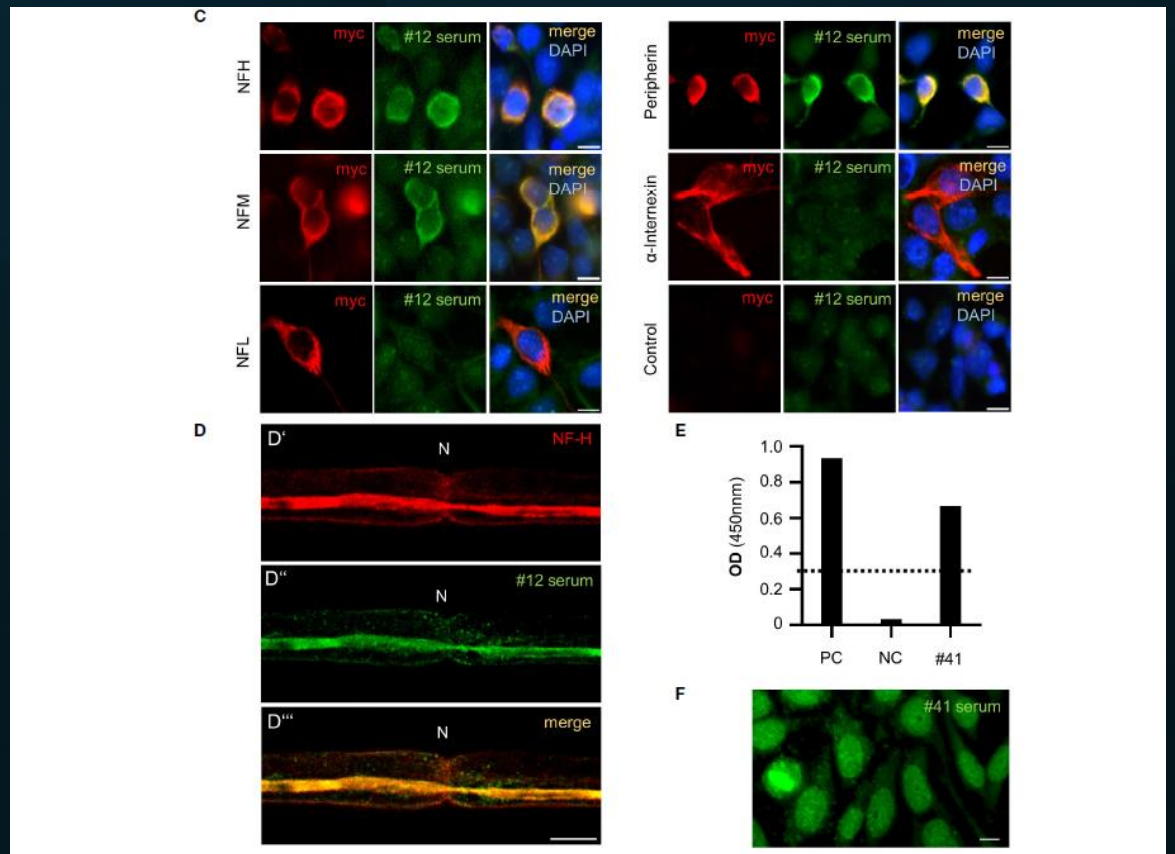


FIGURE 2
Antibody target identification and confirmation in PCVS sera. Volcano plot representing significantly enriched proteins (labeled in red) in patient #12 IgG IP **(A)** and patient #41 IgG IP **(B)** compared to a negative control; in A-B: the x-axis displays the log2-transformed fold change, and the y-axis represents the -log10-transformed p value. **(C)** Cell-based assays with patient #12 serum testing IgG reactivity against neurofilament subunits and control HEK293 cells. **(D)** Costaining of sciatic nerve teased fibers with a commercial NF-H antibody (D') and patient #12 serum (D'') showing clear signal overlap (D'''). **(E)** ELISA analysis of DFS-70 and patient #41 serum. PC: positive control serum. NC: negative control serum. The standard reference serum OD was 0.278 (dotted line). OD: optical density. **(F)** Hep2 staining of patient #41 serum resembling fine speckled nuclear staining typical of DFS-70 IgGs.

Alphaviruses: the biological basis of self-amplifying RNAs

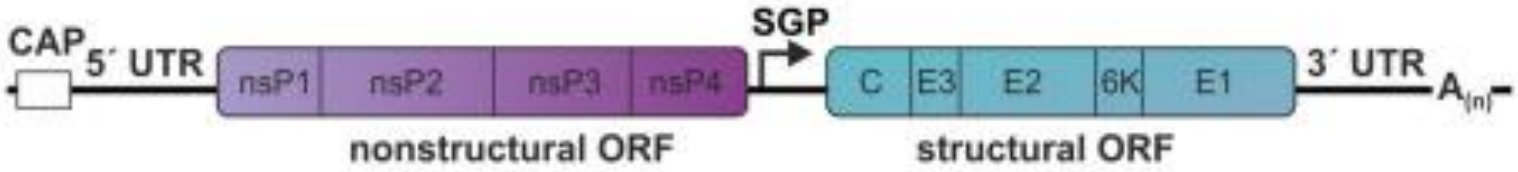
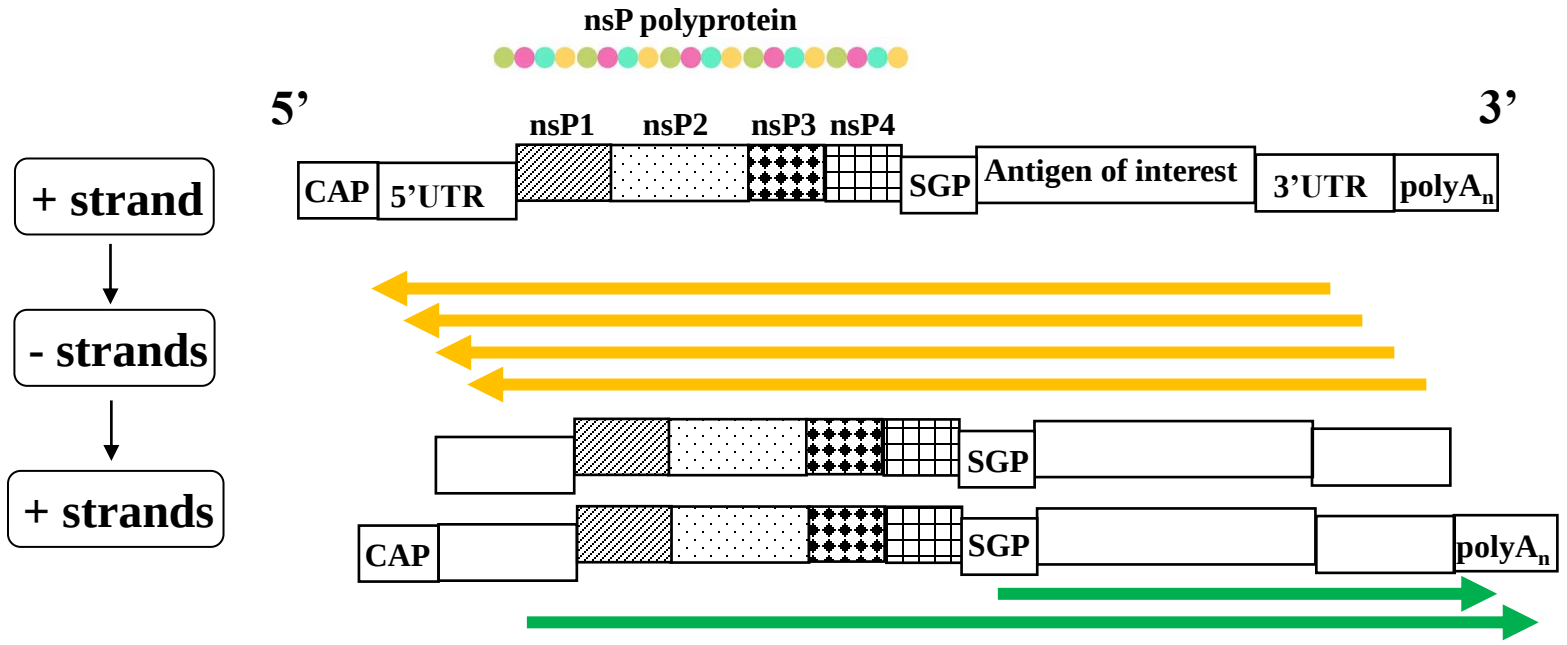


Fig. 1. Alphavirus genome structure. The positive-sense RNA genome is about 11.5 kilobases in length and contains two open reading frames; first encoding for the nonstructural proteins (nsPs) 1–4 and second for structural proteins (C, capsid; E1/2/3, envelope glycoproteins and 6 K, a 6 kDa protein). UTR, untranslated region; SGP, subgenomic promoter; A(n), polyA.



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REVIEW ARTICLE OPEN

Regulation of cargo selection in exosome biogenesis and its biomedical applications in cancer

Yu Jin Lee[1,2✉], Kyeong Jin Shin[1] and Young Chan Chae[1✉]

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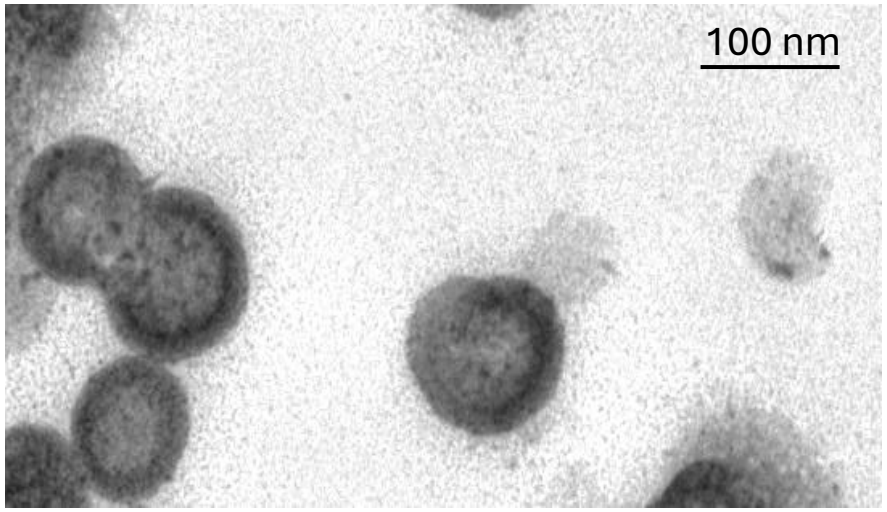
Exosomes as RNA carriers

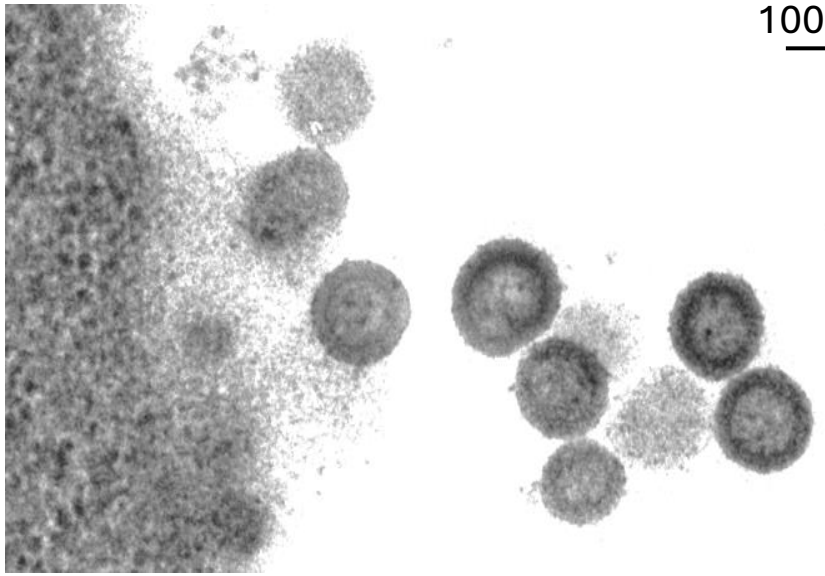
Despite the well-established process of cargo selection for transmembrane receptors through ubiquitylation and recognition by ESCRT components in exosome biogenesis, the mechanisms that govern the incorporation of cytoplasmic cargoes, including RNAs and RNA-binding proteins (RBPs), into exosomes have not been fully elucidated. Multiple reports using next-generation sequencing and microarray technologies to characterize RNA content in exosomes sourced from cell cultures, tissues, or biological fluids have revealed the enrichment of specific RNAs. These included mRNA fragments (≤1 kb in length), miRNAs, snRNAs, tRNA fragments, snoRNAs, mitochondrial RNAs (mtRNAs), piRNAs, vault RNAs (vtRNAs), and Y RNAs. Circular RNAs (circRNAs), rRNA fragments, and long non-coding RNAs (lncRNAs) have also been identified in exosomes[58,59].

For the protein cargo, the current literature indicates that the RNA composition of exosomes varies depending on the cellular context. Emerging evidence suggests that RBPs play a pivotal role in orchestrating the selective sorting of various small RNAs, including miRNAs, tRNAs, Y RNAs, vault RNAs, and others, into exosomes. RBPs are primarily localized to sites of exosome biogenesis and function as adaptors between RNA cargo and exosome biogenesis machinery[14,60] (Table 1). Generally, sorting mechanisms are classified as active RNA-loading processes[61]. In these processes, specific regulatory mechanisms are involved in actively selecting and incorporating certain RNAs into exosomes. Passive loading, on the other hand, is primarily driven by the intracellular concentration of a specific RNA, and the presence of a specific RNA in exosomes is largely dependent on the abundance of that RNA within the source cell[62].

saRNA and exosomes

Exosomes at EM



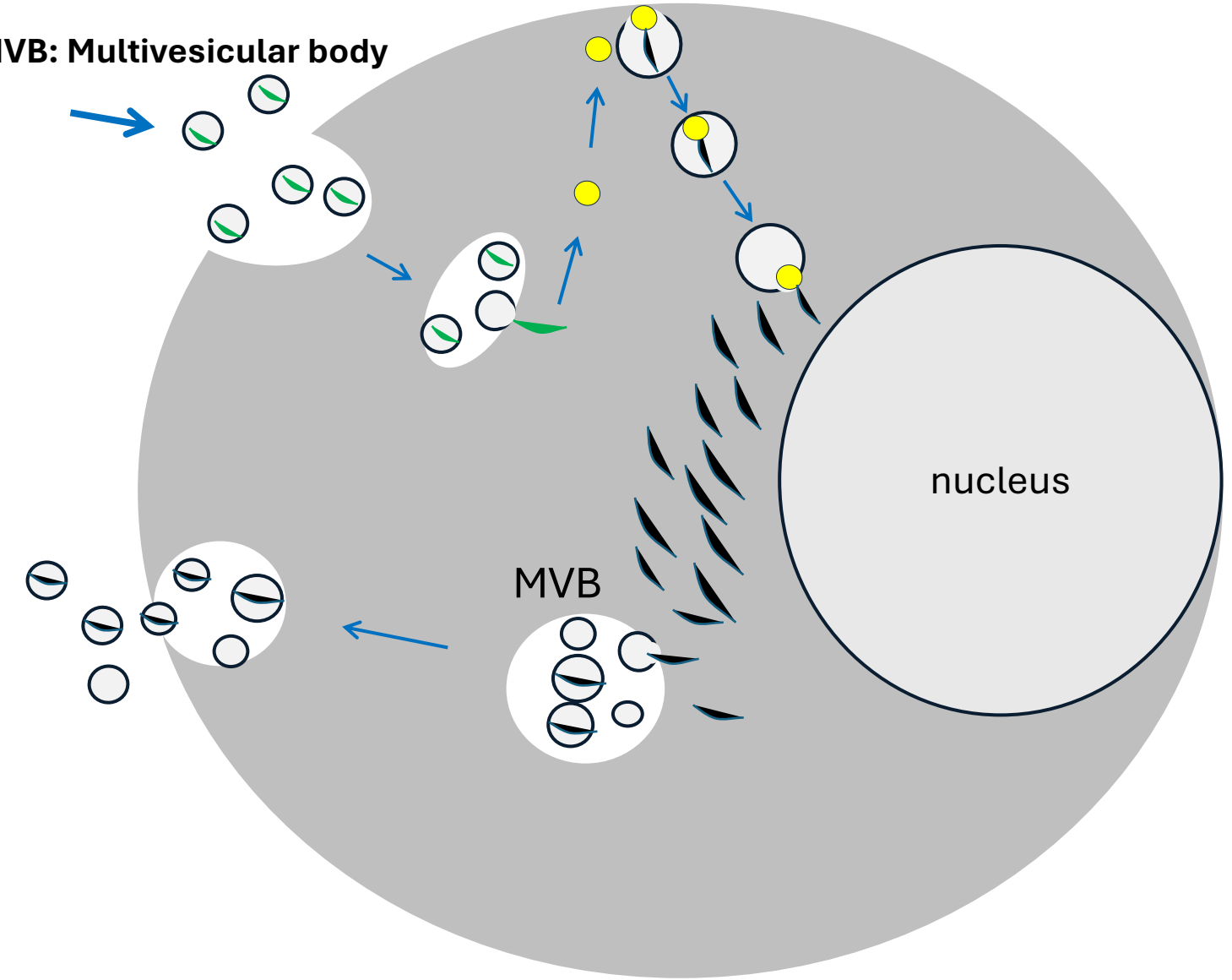


Synthetic RNA

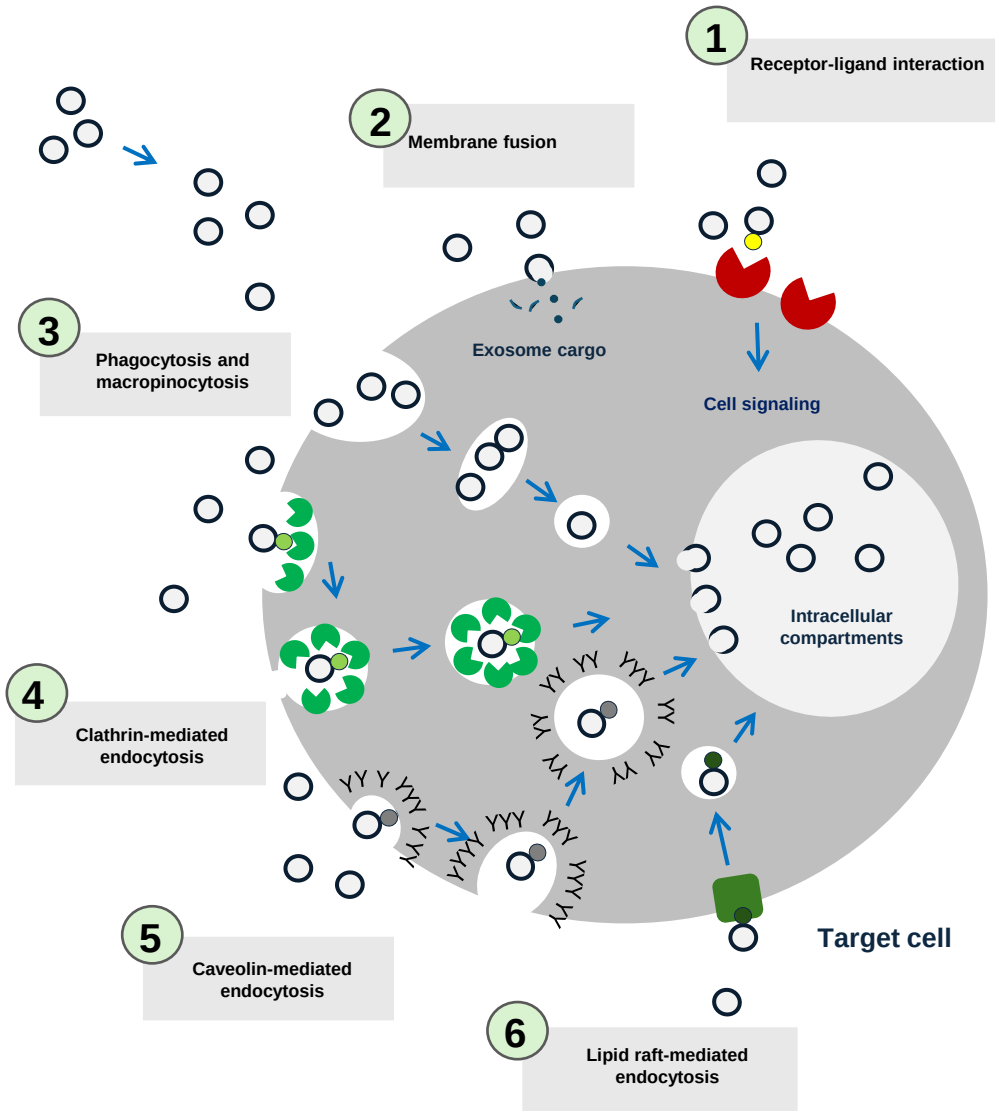
nsP1-4 complex

Neo-synthesized RNA

MVB: Multivesicular body



The multiple mechanisms of cell entry of exosomes



The Trojan exosome hypothesis

Stephen J. Gould*†, Amy M. Booth*, and James E. K. Hildreth‡

*Departments of *Biological Chemistry and ‡Pharmacology and Molecular Sciences, The Johns Hopkins University School of Medicine, Baltimore, MD 21205*

ing pathway (9–12). The Trojan exosome hypothesis states that retroviruses use the preexisting, nonviral exosome biogenesis pathway for the formation of infectious particles, and the preexisting, nonviral pathway of exosome uptake for a receptor-independent, Env-independent mode of infection. The following presents a portion of the empirical support for this hypothesis and its major implications for the

Exosome biodistribution

Time after exosome i.v. injection

Ref	Exo tracking	Exo source	5’	10’	20’	30’	40’	1h	2h	3h	4h	6h	8h	24h	48h	72h
Takahashi et al. 2013	Biolumin.	Texo		Liv **Lu**		Liv **Lu**		Liv **Lu**			Sp **Lu**					
Lai et al. 2014	Biolumin./ biotin	Texo				Liv-**Lu** Sp-Ki		Liv-**Lu** Sp-Ki	Liv-**Lu** Sp			Liv-**Lu** Sp				
Smyth et al. 2015	Fluorescence	Texo			Liv Sp			Liv Sp	Liv Sp				Liv Sp	Liv Sp-Ki		
Bala et al. 2015	miR	Texo		Liv Ad												
Wiklander et al. 2015	Fluorescence	Texo	Liv **Lu**-Sp			Liv Sp		Liv Sp		Liv Sp				Liv Sp-Gi	Liv Pa	
Wiklander et al. 2015	Fluorescence	Dexo												Liv-**Lu** Sp-Gi		
Gangadaran et al. 2017	Biolumin./ fluorescence	Texo		Liv-**Lu** Sp		Liv-**Lu** Sp								Liv-**Lu**-Sp	Liv **Lu**	Liv **Lu**
Zhang et al. 2018	Fluorescence	Texo												Liv-Sp Bm		

Lu: lungs

J Allergy Clin Immunol. 2013 Jul;132(1):219-22. doi: 10.1016/j.jaci.2013.03.035. Epub 2013 May 14.

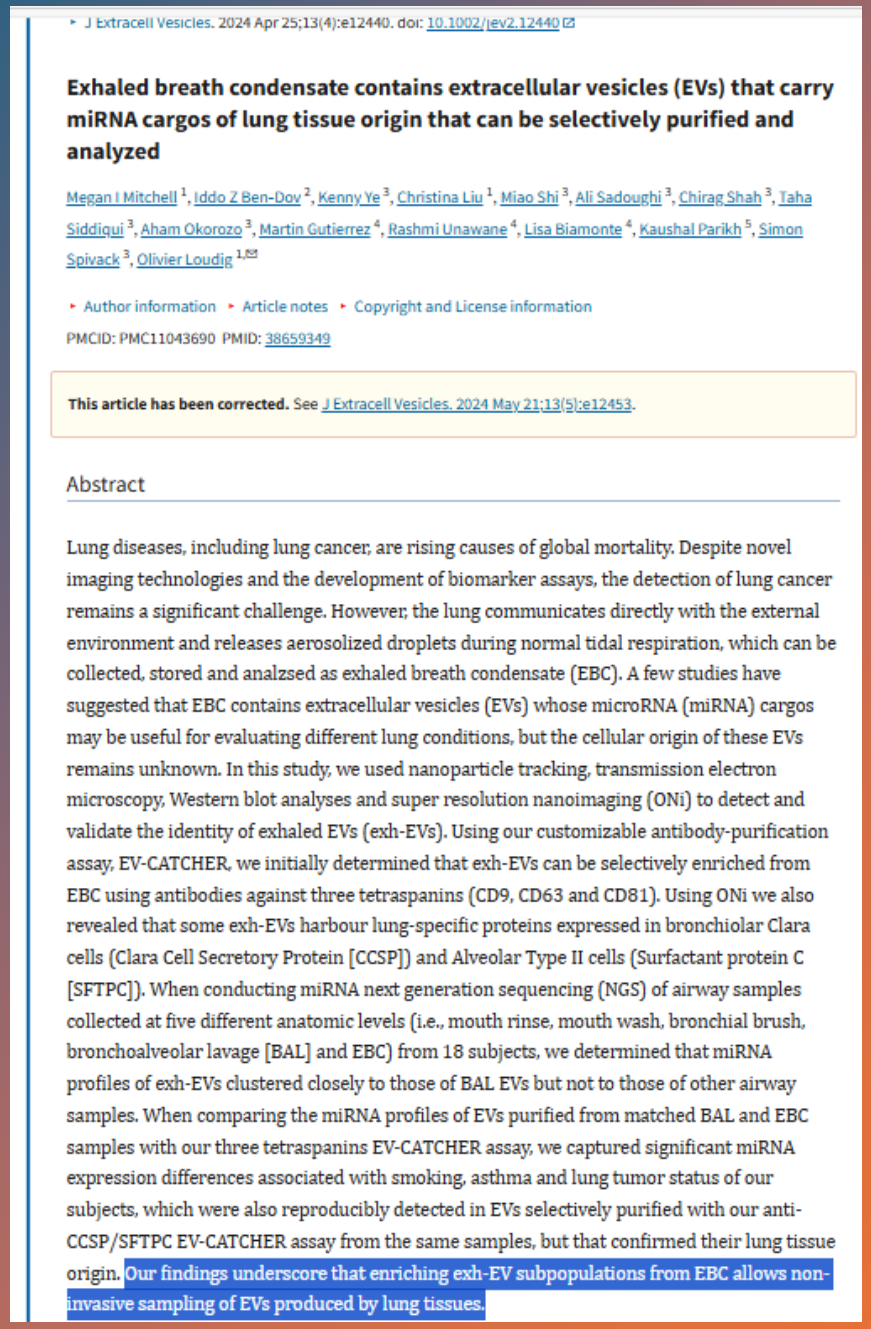
Exosome-enclosed microRNAs in exhaled breath hold potential for biomarker discovery in patients with pulmonary diseases

Anirban Sinha, Amit Kumar Yadav, Samarpana Chakraborty, S K Kabra, R Lodha, Manish Kumar, Ankur Kulshreshtha, Tavpritesh Sethi, Rajesh Pandey, Gaurav Malik, Saurabh Laddha, Arijit Mukhopadhyay, Debasis Dash, Balaram Ghosh, Anurag Agrawal

PMID: 23683467 DOI: 10.1016/j.jaci.2013.03.035

In summary, we found that miRNAs are present in EBC, mostly in a stable membrane-enclosed form. We also provide proof of principle that lung disease can lead to alteration in the EBC miRNome and that the study of miRNAs in EBC is a fertile ground for clinical biomarker discovery, as well as understanding disease.

In conclusione, abbiamo dimostrato che molecole di RNA sono rilevabili nelle esalazioni respiratorie associate a strutture stabili lipidiche.



J Extracell Vesicles. 2024 Apr 25;13(4):e12440. doi: 10.1002/jev2.12440

Exhaled breath condensate contains extracellular vesicles (EVs) that carry miRNA cargos of lung tissue origin that can be selectively purified and analyzed

Megan I Mitchell [1], Iddo Z Ben-Dov [2], Kenny Ye [3], Christina Liu [1], Miao Shi [3], Ali Sadoughi [3], Chirag Shah [3], Taha Siddiqui [3], Aham Okorozo [3], Martin Gutierrez [4], Rashmi Unawane [4], Lisa Biamonte [4], Kaushal Parikh [5], Simon Spivack [3], Olivier Loudig [1,✉]

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PMCID: PMC11043690 PMID: 38659349

This article has been corrected. See J Extracell Vesicles. 2024 May 21;13(5):e12453.

Abstract

Lung diseases, including lung cancer, are rising causes of global mortality. Despite novel imaging technologies and the development of biomarker assays, the detection of lung cancer remains a significant challenge. However, the lung communicates directly with the external environment and releases aerosolized droplets during normal tidal respiration, which can be collected, stored and analzsed as exhaled breath condensate (EBC). A few studies have suggested that EBC contains extracellular vesicles (EVs) whose microRNA (miRNA) cargos may be useful for evaluating different lung conditions, but the cellular origin of these EVs remains unknown. In this study, we used nanoparticle tracking, transmission electron microscopy, Western blot analyses and super resolution nanoimaging (ONi) to detect and validate the identity of exhaled EVs (exh-EVs). Using our customizable antibody-purification assay, EV-CATCHER, we initially determined that exh-EVs can be selectively enriched from EBC using antibodies against three tetraspanins (CD9, CD63 and CD81). Using ONi we also revealed that some exh-EVs harbour lung-specific proteins expressed in bronchiolar Clara cells (Clara Cell Secretory Protein [CCSP]) and Alveolar Type II cells (Surfactant protein C [SFTPC]). When conducting miRNA next generation sequencing (NGS) of airway samples collected at five different anatomic levels (i.e., mouth rinse, mouth wash, bronchial brush, bronchoalveolar lavage [BAL] and EBC) from 18 subjects, we determined that miRNA profiles of exh-EVs clustered closely to those of BAL EVs but not to those of other airway samples. When comparing the miRNA profiles of EVs purified from matched BAL and EBC samples with our three tetraspanins EV-CATCHER assay, we captured significant miRNA expression differences associated with smoking, asthma and lung tumor status of our subjects, which were also reproducibly detected in EVs selectively purified with our anti-CCSP/SFTPC EV-CATCHER assay from the same samples, but that confirmed their lung tissue origin. Our findings underscore that enriching exh-EV subpopulations from EBC allows non-invasive sampling of EVs produced by lung tissues.

I nostri risultati sottolineano che l'analisi delle sottopopolazioni di esosomi/vescicole extracellulari provenienti dalle esalazioni respiratorie consente l'identificazione non invasiva degli esosomi/vescicole prodotti dai tessuti polmonari.

J Breath Res. 2024 Jul 11;18(4). doi: 10.1088/1752-7163/ad5eae.

Extraction and characterization of exosomes from the exhaled breath condensate and sputum of lung cancer patients and vulnerable tobacco consumers–potential noninvasive diagnostic biomarker source

Afsareen Bano [1], Pooja Yadav [1], Megha Sharma [1], Deepika Verma [2], Ravina Vats [1], Dhruva Chaudhry [3], Pawan Kumar [3], Rashmi Bhardwaj [1]

Affiliations + expand

PMID: 38988301 DOI: 10.1088/1752-7163/ad5eae

Abstract

Noninvasive sample sources of exosomes, such as exhaled breath and sputum, which are in close proximity to the tumor microenvironment and may contain biomarkers indicative of lung cancer, ar far more permissive than invasive sample sources for biomarker screening. Standardized exosome extraction and characterization approaches for low-volume noninvasive samples are critically neede We isolated and characterized exhaled breath condensate (EBC) and sputum exosomes from health nonsmokers (n= 30), tobacco smokers (n= 30), and lung cancer patients (n= 40) and correlated the findings with invasive sample sources. EBC samples were collected by using commercially available Tubes. To collect sputum samples the participants were directed to take deep breaths, hold their breath, and cough in a collection container. Dynamic light scattering, nanoparticle tracking analysis, and transmission electron microscopy were used to evaluate the exosome morphology. Protein isolation, western blotting, exosome quantification via EXOCET, and Fourier transform infrared spectroscopy were performed for molecular characterization. Exosomes were successfully isolated from EBC and sputum samples, and their yields were adequate and sufficiently pure for subsequent downstream processing and characterization. The exosomes were confirmed based on their size, shape, and surface marker expression. Remarkably, cancer exosomes were the largest in size not on in the plasma subgroups, but also in the EBC (p < 0.05) and sputum (p= 0.0036) subgroups, accordi to our findings. A significant difference in exosome concentrations were observed between the control sub-groups (p < 0.05). Our research confirmed that exosomes can be extracted from noninvasive sources, such as EBC and sputum, to investigate lung cancer diagnostic biomarkers for research, clinical, and early detection in smokers.

La nostra ricerca conferma che gli esosomi possono essere isolati da fonti facilmente accessibili quali le esalazioni respiratorie e la saliva.

Istituto Superiore di Sanità

National Center for the Global Health

Un grazie e un saluto a tutti