Journal Club

PREPRINT WATCH

SEEING SARS-COV-2 VARIANTS THROUGH THE EYES OF T CELLS

The recent emergence of new SARS-CoV-2 variants of concern, including the B.1.1.7 variant in the UK, B.1.351 variant in South Africa, P.1 variant in Brazil and B.1.427 variant in California, USA, has important implications for future responses to the pandemic. Whereas the effects of mutations in the viral spike (S) protein on antibody binding and neutralization have been addressed in several reports, the impact of SARS-CoV-2 variant mutations on T cell reactivity remains poorly understood.

In this non-peer-reviewed preprint, Tarke et al. applied an integrated approach to assess T cell responses to SARS-CoV-2 variants from 11 COVID-19 convalescent individuals and 19 recipients of the Moderna (mRNA-1273) or Pfizer/BioNTech (BNT162b2) vaccines. Different methodologies were used to detect T cells with a range of functionalities and specific cytokine activity in response to overlapping peptide pools spanning the S protein of the original SARS-CoV-2 sequence and each of the corresponding variants.

Both $CD4^+$ and $CD8^+$ T cells from COVID-19 convalescent donors were found to recognize the ancestral reference strain and the variant proteome-wide sequences with similar efficiency. In mRNA vaccine recipients also, $CD4^+$ and $CD8^+$ T cell responses to the ancestral and variant peptide pools were similar, with the exception of the B.1.351 variant, for which mildly decreased T cell reactivity to S protein peptides was observed. Analysis of defined T cell epitopes showed that 93% of $CD4^+$ T cell epitopes and 97% of $CD8^+$ T cell epitopes are conserved in the analysed variants. Single point mutations in the T cell epitopes were predicted to have no negative effect on HLA binding capacity, which provides a molecular basis for the marginal impact of the mutations on T cell responses in the study group.

Together, these findings suggest a negligible impact of the SARS-CoV-2 variant mutations on global $CD4^+$ and $CD8^+$ T cell responses in COVID-19 convalescent donors and COVID-19 mRNA vaccine recipients, and have important implications for the design of vaccines inducing broader immunity against variants of concern. The use of overlapping peptide pools in this study does not exclude the possibility that mutations described in the variants could interfere with antigen processing, thereby affecting T cell activation and function. It remains to be determined whether T cell responses following infection with circulating variants can efficiently cross-recognize the ancestral sequence in approved vaccines.

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ORIGINAL ARTICLE Tarke, A. et al. Negligible impact of SARS-CoV-2 variants on $CD4^+$ and $CD8^+$ T cell reactivity in COVID-19 exposed donors and vaccinees. Preprint at *bioRxiv* https://doi.org/10.1101/2021.02.27.433180 (2021)
RELATED ARTICLE Alrubayyi, A. Oxford–Mount Sinai (OxMS) Preprint Journal Club. *OxMS* https://www.preprintclub.com/2021-mar-tarke (2021)

TUMOUR IMMUNOLOGY

Mechanosurveillance of tumour metastasis

Immunosurveillance of cancer cells by cytotoxic lymphocytes depends on the receptor recognition of cell-surface ligands indicative of stress and/or transformation, resulting in the formation of a cytotoxic synapse. Several of these receptors are further activated by the mechanical forces exerted by the synapse (known as mechanotransduction), and indeed increased rigidity of the target surface correlates with increased lymphocyte activation. Given that tumour metastasis involves marked cytoskeletal remodelling, Tello-Lafoz et al. set out to investigate whether these biophysical changes also affect lymphocyte activation and killing.

Metastasis involves the activation of myocardin-related transcription factor A (MRTFA) and MRTFB by cancer cells. As expected, therefore, suppression of MRTF expression reduced lung colonization by melanoma and breast cancer cell lines in a mouse model of metastasis. Surprisingly, however, overexpression of MRTFB also reduced lung colonization. The metastatic activity of MRTF-overexpressing cells could be rescued by depletion of cytotoxic lymphocytes, which suggests that the metastatic potential conferred by MRTF activation might be partially restricted by the immune system.

Further studies showed that MRTF-overexpressing cancer cells are more susceptible to cytotoxicity, with synapse formation more likely to result in cancer cell death and increased speed of killing. MRTF-overexpressing cells induced stronger degranulation of cytotoxic lymphocytes than control cells, which is consistent with MRTF overexpression inducing stronger lymphocyte activation.

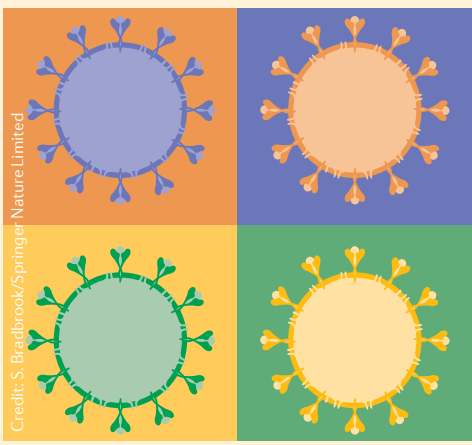
COVID-19

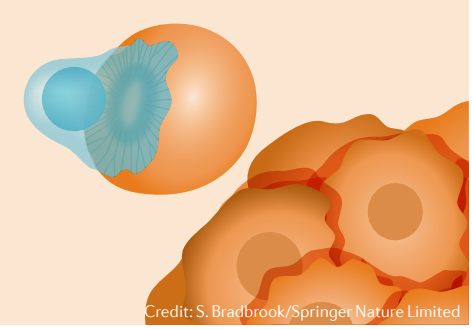
Variant constraint by mRNA vaccines

Several vaccines against SARS-CoV-2 are now licensed for use, but there is concern that they may be less effective against emerging variants of the virus. Stamatatos et al. now report that the mRNA vaccines designed against earlier variants of SARS-CoV-2 also elicit and boost levels of cross-neutralizing antibodies (nAbs) to newer B.1.351 variants, but are less potent against these variants.

The two mRNA vaccines that have received emergency use authorizations — BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna) — encode a stabilized ectodomain version of the spike (S) protein from the Wuhan-Hu-1 variant of SARS-CoV-2 (isolated in 2019). These vaccines elicit nAbs to the virus and show more than 94% efficacy in preventing disease. However, emerging variants of SARS-CoV-2 contain S protein mutations that could potentially evade the nAb responses induced by the vaccines. Of particular concern are variants that have emerged in the United Kingdom (B.1.1.7), South Africa (B.1.351) and Brazil (P.1) as they have mutations that may enhance transmissibility and disease severity, as well as vaccine evasion.

The authors collected sera from 15 individuals who had previously been




Credit: S. Bradbrook/Springer Nature Limited

In line with the known cytoskeletal regulation by MRTF proteins, transcriptome analysis showed that MRTF-overexpressing cancer cells had strongly increased expression of actin and related genes. Thus, the authors speculated that MRTF overexpression by cancer cells might increase mechanotransduction through the cytotoxic synapse. Indeed, MRTF overexpression increased the stiffness of all cell lines examined.

To separate the effects of cytoskeletal changes from potential unknown changes in cell-surface ligands, they showed that giant plasma membrane vesicles (GPMVs) derived from MRTF-overexpressing cancer cells were not more stimulatory for cytotoxic lymphocytes than GPMVs from control cells. Furthermore, transient transfection of MRTF-overexpressing cells with an actin-severing agent markedly reduced their stiffness and reversed their increased killing.

The results suggest that cancer cells increase their rigidity through MRTF activation to metastasize but that this results in a biophysical vulnerability to immune killing. Importantly, MRTF overexpression was shown to sensitize metastasizing cancer cells to checkpoint blockade through CTLA4. Furthermore, humans with the RAC1/2$^{P29S/L}$ form of melanoma, in which MRTF signalling is strongly induced by constitutive RAC1 activity, had a significantly improved response to anti-CTLA4 therapy according to survival data from immune checkpoint blockade datasets. Thus, mechanosurveillance of metastasis might identify those patients most likely to benefit from immunotherapy.

Kirsty Minton

ORIGINAL ARTICLE Tello-Lafoz, M. et al. Cytotoxic lymphocytes target characteristic biophysical vulnerabilities in cancer. *Immunity* https://doi.org/10.1016/j.immuni.2021.02.020 (2021)
RELATED ARTICLE Huse, M. Mechanical forces in the immune system. *Nat. Rev. Immunol.* **17**, 679–690 (2017)

infected with SARS-CoV-2 and 13 individuals who had not been infected, both before and after immunization with one of the mRNA vaccines. They used pseudovirus systems to assess the ability of the sera to neutralize viruses expressing the Wuhan-Hu-1 S protein or two different versions of the B.1.351 S protein. Prior to vaccination, sera from 12 of the 15 previously infected donors (but not from any of the naive donors) neutralized the Wuhan-Hu-1 variant. By contrast, the sera from the unvaccinated previously infected donors showed weak and only sporadic neutralizing activity against the B.1.351 variants.

A single vaccine dose in previously infected individuals with pre-existing virus-specific antibodies induced higher levels of virus-specific IgG and IgA than two vaccine doses in naive individuals. A single vaccination in previously infected individuals also boosted nAb titres against all three of the SARS-CoV-2 variants tested. Again, this was to a greater extent than two vaccine doses in naive individuals, with the vaccinated previously infected individuals showing 10-fold and 20-fold higher levels of nAbs to the Wuhan-Hu-1 and B.1.351 variants, respectively. Antibody-depletion studies indicated that the majority of cross-neutralizing antibodies generated by the mRNA vaccines target the receptor-binding domain of the S protein. However, the serum of vaccinated previously infected individuals was 3- to 10-fold less efficient in neutralizing the B.1.351 variants than the Wuhan-Hu-1 variant.

Overall, these results suggest that the available mRNA vaccines for SARS-CoV-2 do induce cross-variant neutralizing antibodies and highlight the importance of vaccinating previously infected as well as naive individuals to promote immunity to emerging variants. Another key finding was that a second dose of the vaccine within 3–4 weeks did not further boost nAb levels in the previously infected individuals, so second vaccine doses could be delayed in such cases.

Yvonne Bordon

ORIGINAL ARTICLE Stamatatos, L. et al. mRNA vaccination boosts cross-variant neutralizing antibodies elicited by SARS-CoV-2 infection. *Science* https://doi.org/10.1126/science.abg9175 (2021)

Journal Club

PREPRINT WATCH

INNATE IL-13 LICENSES DERMAL TYPE 2 DENDRITIC CELLS FOR EFFICIENT T HELPER 2 CELL RESPONSES

Dendritic cells (DCs) are specialized in antigen presentation for T cell priming. Type 2 DCs (DC2s) excel at the induction of T helper 2 (T_H2) cell and T_H17 cell responses. However, the signals that control the ability of DC2s to prime T_H2 cells versus T_H17 cells remain unknown.

Previous work has shown that loss of *Klf4* expression in DCs leads to the loss of migratory CD11b^{low} DC2s in the skin-draining lymph nodes and loss of T_H2 cell responses to house dust mite and *Schistosoma mansoni* infection. Now, Mayer, Lamiable and colleagues (preprint; non-peer reviewed) build on these results by showing that the differentiation of *Klf4*-dependent dermal CD11b^{low} DC2s is dependent on IL-13 produced by group 2 innate lymphoid cells (ILC2s) and, to a lesser extent, by dendritic epidermal T cells in the normal skin. They also show that the absence of *Klf4*-dependent dermal CD11b^{low} DC2s is associated with a strong reduction of T_H2 cell responses against *Nippostrongylus brasiliensis* together with reduced transport of *N. brasiliensis* to the draining lymph nodes. By contrast, T_H17 cell responses against *Candida albicans* were increased in mice lacking CD11b^{low} DC2s.

> This preprint reveals the role played by environmental IL-13 in shaping the balance of DC2 molecular composition in the dermis

This study suggests that DC2-intrinsic IL-13-induced signalling through STAT6 promotes the differentiation of dermal CD11b^{low} DC2s and subsequent polarization of T_H2 cells over T_H17 cells. These results are in line with a previous study showing that ILC2-derived IL-13 in the lung promotes DC migration to the draining lymph nodes and T_H2 cell polarization. This preprint reveals the role played by environmental IL-13 in shaping the balance of DC2 molecular composition in the dermis, which thereby affects the nature of T cell effector function in the skin. Further studies are warranted to define the DC2-associated innate trigger that promotes T_H17 cell polarization over T_H2 cell polarization.

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ORIGINAL ARTICLE Mayer, J. U. et al. The skin environment controls local dendritic cell differentiation and function through innate IL-13. Preprint at *bioRxiv* https://doi.org/10.1101/2021.01.05.425466 (2021)
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