SARS-CoV-2-cross-reactive T cells identified in pre-pandemic lower airway samples

Through access to healthy donors' bronchoalveolar lavage (BAL) samples cryopreserved before the COVID-19 pandemic, we discovered CD4$^+$ and CD8$^+$ T cells able to cross-recognize SARS-CoV-2 among the airway tissue-resident memory pool. Pre-pandemic donors with detectable SARS-CoV-2-cross-reactive T cells in their airways also had stronger immunity to human seasonal coronaviruses.

This is a summary of:
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The question

We recently showed that expansion of pre-existing SARS-CoV-2-cross-reactive T cells is associated with abortive infection characterized by the induction of a sensitive innate biomarker of infection (IFI27) but repeatedly negative PCR and antibody tests[1]. Donors with abortive, compared to overt, infection had more T cells that targeted the SARS-CoV-2 replication transcription complex (RTC), which is highly conserved across human coronaviruses and is expressed first in the viral life cycle. We hypothesized that rapid shutdown of infection, which prevents detection via PCR or antibodies, would require tissue-resident T cells to mediate frontline protection at the site of viral inoculation. A previous elegant study in mice had shown that depletion of the fraction of T cells resident in the lower airways abolished the protection against SARS-CoV achieved by mucosal vaccination[2]. Recent studies had identified SARS-CoV-2-reactive T cells in pre-pandemic tonsils[3] and post-COVID-19 total lung tissue[4,5], but cross-reactive responses specifically located in the lower airways had not yet been identified in humans.

The observation

BAL samples containing T cells localized to human lower respiratory airways had been cryopreserved from healthy donors before the COVID-19 pandemic, which allowed us to probe them for pre-existing sentinel T cells able to cross-recognize SARS-CoV-2. Paired BAL samples and peripheral blood mononuclear cells (PBMCs) from ten donors were stimulated overnight with peptide pools spanning the RTC (nonstructural proteins NSP7, NSP12 and NSP13) and predicted epitopes from SARS-CoV-2 spike protein; CD4$^+$ and CD8$^+$ T cell responses from the two sites were compared by flow cytometry after intracellular cytokine staining. Global and antigen-specific T cells from BAL fluid were phenotyped for markers of tissue residency: CD69 with or without CD103. PBMCs and serum samples were also tested for reactivity to human seasonal coronaviruses, to assess the potential contribution of these closely related viruses to T cell cross-reactivity.

Six of the ten pre-pandemic BAL samples contained T cells that cross-reacted with at least one of the four pools of SARS-CoV-2 peptides tested. CD4$^+$ T cells were more frequent than CD8$^+$ T cells, and some responses were polyfunctional (TNF, IFN-γ and/or CD40L), although the TNF response was most abundant. A large proportion of human airway T cells had the expected tissue-resident phenotype, and SARS-CoV-2-reactive T cells were mainly within this pool. Consistent with tissue retention, BAL samples showed enrichment for SARS-CoV-2-reactive T cells, compared with their frequency in paired blood samples (Fig. 1a). Blood SARS-CoV-2- reactive T cells tended to be more abundant in donors with detectable BAL fluid responses, and the frequency of NSP12-specific T cells was correlated between blood and airways. Donors with SARS-CoV-2-cross-reactive T cells in their airways had stronger T cell and antibody responses to spike protein from seasonal coronavirus (Fig. 1b), supportive of their putative role as one source of cross-reactive T cells.

The implications

The finding of pre-existing cross-reactive T cells in the airways that are able to provide rapid antiviral effector function supports their potential to maintain local sentinel surveillance to efficiently clear infection, or even abort infection before it is fully established. This underscores the potential for mucosal vaccines to induce a large reservoir of pathogen-specific airway-resident T cells in humans. Moreover, although we could not ascertain the trigger and durability of cross-reactive T cells in BAL fluid, our data support the proposal of prior seasonal coronavirus infection as one likely source and highlight the importance of prior immune history in shaping the effectiveness of airway memory T cell cross-protection against new pathogens such as SARS-CoV-2.

A caveat of our study was the fact that donors had participated in a pneumococcal challenge and influenza vaccine trial an average of 4 months before BAL. These common 'real-world' exposures may have increased recruitment of SARS-CoV-2-cross-reactive airway-resident T cells. We focused on responses to spike and RTC proteins, as these have been associated with cross-protection and we did not have enough cells to assess the full extent of airway reactivity to SARS-CoV-2. Caution must be taken in interpreting the expression of CD69 on peptide-stimulated T cells, as it is both a residency marker and an activation marker.

We are now comparing the capacity of SARS-CoV-2 infection and/or vaccination to expand antigen-specific T cells in human airways. In the future, it will be important to test whether nasal or aerosolized vaccines that target conserved SARS-CoV-2 regions can induce broadly cross-reactive tissue-resident T cells better able to provide rapid frontline defense against emerging variants.

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EXPERT OPINION

"This study provides an important insight into resident T cell populations in the airways. What I really like about this paper is that the authors have analyzed antigen-specific T cells against SARS-CoV-2 in the lungs of healthy, uninfected people, which to my knowledge has not been done before. It will be interesting to see to what extent these T cells are protective or what their role is in prevention of infection." **Bali Pulendran, Stanford University, Stanford, CN, USA.**

FIGURE

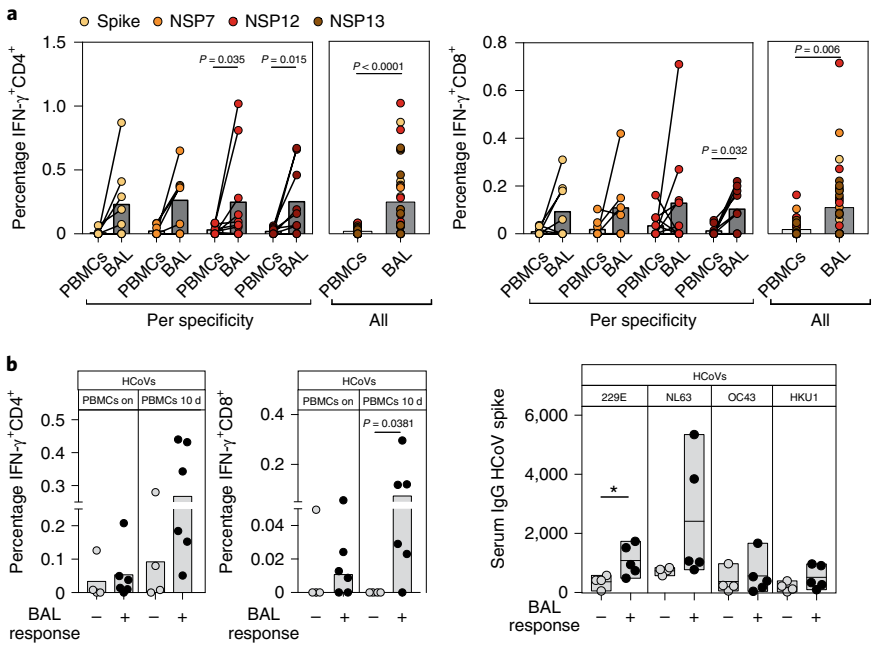

Fig. 1 | The airways show enrichment for pre-existing SARS-CoV-2-reactive T cells. a, Frequency of IFN-γ-producing CD4+ or CD8+ T cells from BAL fluid and PBMCs stimulated with SARS-CoV-2 peptide pools (key). **b**, Frequency of IFN-γ-producing CD4+ or CD8+ T cells after stimulation of PBMCs overnight (on) or for 10 days (10 d) with peptide pools spanning spike protein from seasonal human coronaviruses (HCoVs) (left), or serum IgG directed against spike protein from human coronavirus 229E, NL63, OC43 or HKU1, presented in arbitrary units (right).

BEHIND THE PAPER

Our group at University College London has a longstanding interest in the antiviral capacity and longevity of tissue-resident T cells, so from the outset of the COVID-19 pandemic, we were looking for opportunities to explore their role in SARS-CoV-2 infection. In our search for access to pre-pandemic airway samples, I contacted my long-term friend from Brazil whose research group routinely studies BAL samples obtained after pneumococcal challenge. She generously shared her remaining pre-pandemic samples, and the pressure was on to get maximum data from these few cryopreserved cells. After losing three valuable samples because of high background with the IFN-γ ELISpot assay we had successfully used for PBMCs, we switched to flow cytometry, which generated the robust data in our paper. Additional analysis of seasonal coronavirus immunity was stimulated by insightful reviewers' comments and, to our surprise, showed associations even in this small cohort. **M.O.D.**

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3. Niessl, J. et al. Identification of resident memory CD8+ T cells with functional specificity for SARS-CoV-2 in unexposed oropharyngeal lymphoid tissue. *Sci. Immunol.* **6**, eabk0894 (2021).
Paper that examines a large collection of pre-pandemic tonsils to show that pre-existing SARS-CoV-2-cross-reactive T cells are sequestered in this localized upper airway lymphoid tissue.

4. Poon, M. M. L. et al. SARS-CoV-2 infection generates tissue-localized immunological memory in humans. *Sci. Immunol.* **6**, eabl9105 (2021).
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Authors identify functional SARS-CoV-2-specific T cells in resected lung tissue from seven donors sampled up to 10 months after COVID-19.

FROM THE EDITOR

"This study is unique in that the BAL samples, derived from healthy donors, were cryopreserved before the pandemic. Using these rare samples, the authors are able to show pre-existing human lower airway T cells that cross-react to SARS-CoV-2, which could potentially be harnessed in the future for next-generation mucosal vaccines." **Jamie D. K. Wilson, Chief Editor, *Nature Immunology***