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BRIEF CUTTING EDGE REPORT

Obesity Biology and Integrated Physiology

Individuals with obesity who survive SARS-CoV-2 infection have preserved antigen-specific T cell frequencies

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Abstract

Objective: Obesity is a major risk factor for severe disease in COVID-19, with increased hospitalization, intensive care unit admission, and mortality. This increased impact of COVID-19 in people with obesity (PWO) is likely driven, in part, by the well-described obesity-induced immune dysregulation. Obesity has also been associated with impaired immune memory in many settings, including weakened responses to hepatitis B, tetanus, rabies, and influenza vaccination. Recently, it was reported that PWO who have COVID-19 have reduced IgG antibody titers with defective neutralizing capabilities. However, it remains unknown whether PWO generate durable T cell immunity to SARS-CoV-2.

Methods: This study investigated SARS-CoV-2-specific T cell responses in a cohort of 40 patients ($n = 20$ PWO and $n = 20$ matched control individuals) who had recovered from COVID-19. T cell ($CD4^+$, $CD8^+$) cytokine responses (IFNγ, TNFα) to SARS-CoV-2 peptide pools (spike, membrane) were determined using multicolor flow cytometry.

Results: Circulating T cells specific for SARS-CoV-2 were readily detected in the total cohort. PWO displayed comparable levels of SARS-CoV-2 spike- and membrane-specific T cells, with both T cell subsets responding.

Conclusions: These data indicate that PWO who survive COVID-19 generate robust and durable SARS-CoV-2-specific T cell immunity that is equivalent to that seen in those without obesity.

INTRODUCTION

From an early stage in the COVID-19 pandemic, it has been clear that people with obesity (PWO) have an increased risk of severe disease [1, 2]. This has been observed across multiple studies, with one meta-analysis demonstrating PWO to have a 113% increased risk of hospitalization, a 74% increased risk of intensive care unit admission, and 48% higher mortality [1]. The publication of such studies continues to enhance our understanding of the acute course of SARS-CoV-2 in PWO. In contrast, little is known regarding immune memory following infection in this vulnerable population.

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Our knowledge of immune memory in PWO from other settings provides substantial foundation for concern. Influenza vaccination is an important example of this. A study by Sheridan et al. published in 2012 looked at PWO and control individuals following influenza vaccination. They showed PWO to have significantly lower frequencies of antigen-specific $CD8^+$ T cells and significantly greater declines in antibody titers at 12 months post-vaccination [3]. Deficiencies in immune memory in PWO following vaccination have been observed in multiple other settings, including rabies [4] and hepatitis B vaccination [5].

In the general population, SARS-CoV-2-specific T cells have been shown to have an important protective role against severe disease in acute COVID-19 [6–8]. Furthermore, T cell memory to SARS-CoV-2 has been demonstrated to be durable in large longitudinal cohorts [9, 10] out to at least 15 months post-infection [11]. The aim of this study was to evaluate whether adults (aged ≥ 18 y) with obesity (body mass index [BMI] ≥ 30.0 kg/m^2) who have a history of SARS-CoV-2 infection demonstrate poorer antigen-specific T cell immunity compared with control individuals.

Study Importance

What is already known?

- Obesity is strongly associated with poorer outcomes with severe COVID-19.
- People with obesity (PWO) display defective immune memory following infection or vaccination against H1N1 influenza.

What does this study add?

- Following recovery from COVID-19, PWO generate robust SARS-CoV-2-specific T cell immunity comparable with control individuals without obesity.

How might these results change the direction of research or the focus of clinical practice?

- This study suggests that, following recovery from COVID-19, PWO may no longer represent a major at-risk group because of the generation of SARS-CoV-2-specific T cell immunity.

METHODS

Study cohorts and ethical approval

We performed a cross-sectional analysis of a multicenter cohort study looking at SARS-CoV-2-specific $CD8^+$ and $CD4^+$ cell responses 3 to 9 months following polymerase chain reaction (PCR)-confirmed infection. A total of 40 volunteers (20 adults with obesity [BMI ≥ 30] and 20 matched control individuals [BMI < 30]) were included. Samples were collected from September to December 2020. Patients were included if they were aged ≥18 years, were able to provide informed consent, and had PCR-confirmed SARS-CoV-2 infection 3 to 9 months previously. Exclusion criteria included treatment with certain classes of immunosuppressive medications within the preceding 3 months, obesity induced by other endocrine disorders, lymphoproliferative disorder, and any active illness or medication which, in the opinion of the investigator, precluded participation. Patients were drawn from the All-Ireland Infectious Disease and Obesity Immunology Group cohort studies. All patients were provided written, informed consent. The study was conducted in accordance with the Declaration of Helsinki, and ethical approval was granted by the St. Vincent's Hospital Group Research Ethics Committee.

Preparation of peripheral blood mononuclear cells and flow cytometric analysis

Peripheral blood mononuclear cells were isolated by centrifugation of whole blood through density gradient media (Lymphoprep). Cells were suspended in cell cryopreservation media and stored in a −70 °C freezer. Thawed peripheral blood mononuclear cells were stimulated with SARS-CoV-2 spike peptide pools (Miltenyi PepTivator SARS-CoV-2). After 4 hours, protein transport inhibitor (eBioscience) was added to each well, and samples were incubated at 37 °C for a further 12 hours.

After incubation, cells were centrifuged, resuspended in flow cytometry buffer, then stained for cell surface markers (CD3, CD4, CD8). Following staining, live cells were fixed and permeabilized and intracellular cytokine staining (interferon γ [IFNγ], tumor necrosis

TABLE 1 Participant characteristics

	People with obesity	Control individuals
Total number (%)	20 (50)	20 (50)
Age (y), mean (SD)	49.0 (13.1)	49.2 (17.6)
Male	11 (55)	12 (60)
BMI, median (IQR)	38.5 (34.1-44.7)	23.6 (22.1-26.3)
Days from symptom onset, mean (SD)	179 (40.2)	181 (55.7)
Hypertension	12 (60)	6 (30)
Type 2 diabetes mellitus	3 (15)	3 (15)
Asthma	4 (20)	3 (15)
COPD	1 (5)	1 (5)
Chronic kidney disease	1 (5)	0 (0)
Admitted to hospital	6 (30)	7 (35)

Note: Data are presented as *n* (%) unless otherwise stated.
Abbreviation: COPD, chronic obstructive pulmonary disease.

factor α [TNFα]) was performed. Multicolor flow cytometry was performed using Attune NxT Flow Cytometer. See gating strategy in Supporting Information Figure S1.

Serologic immunoassay

Immunoglobulin G (IgG) against receptor-binding domain (RBD), spike 1 subunit, spike 2 subunit, and nucleocapsid (Sino Biological, Inc.) was measured using a serologic immunoassay developed using an electrochemiluminescence platform, with consumables from Meso Scale Diagnostics, LLC (MSD). Biotinylated SARS-CoV-2 RBD, spike 1 subunit, spike 2 subunit, and nucleocapsid were diluted in ChonBlock enzyme-linked immunosorbent assay (ELISA) buffer (Chondrex, Inc.). Each was incubated with a different "linker" for 30 minutes. Stop and coating solutions were added. Serum samples were diluted in ELISA buffer and incubated for 30 minutes. Detection antibody was added. Plates were washed and MSD GOLD Read Buffer B added. Analysis was performed using an MSD plate reader.

Quantification and statistical analysis

Data and statistical analyses were performed using FlowJo version 10.8.1 and GraphPad Prism version 9. The D'Agostino-Pearson test was used to evaluate whether data were normally distributed.

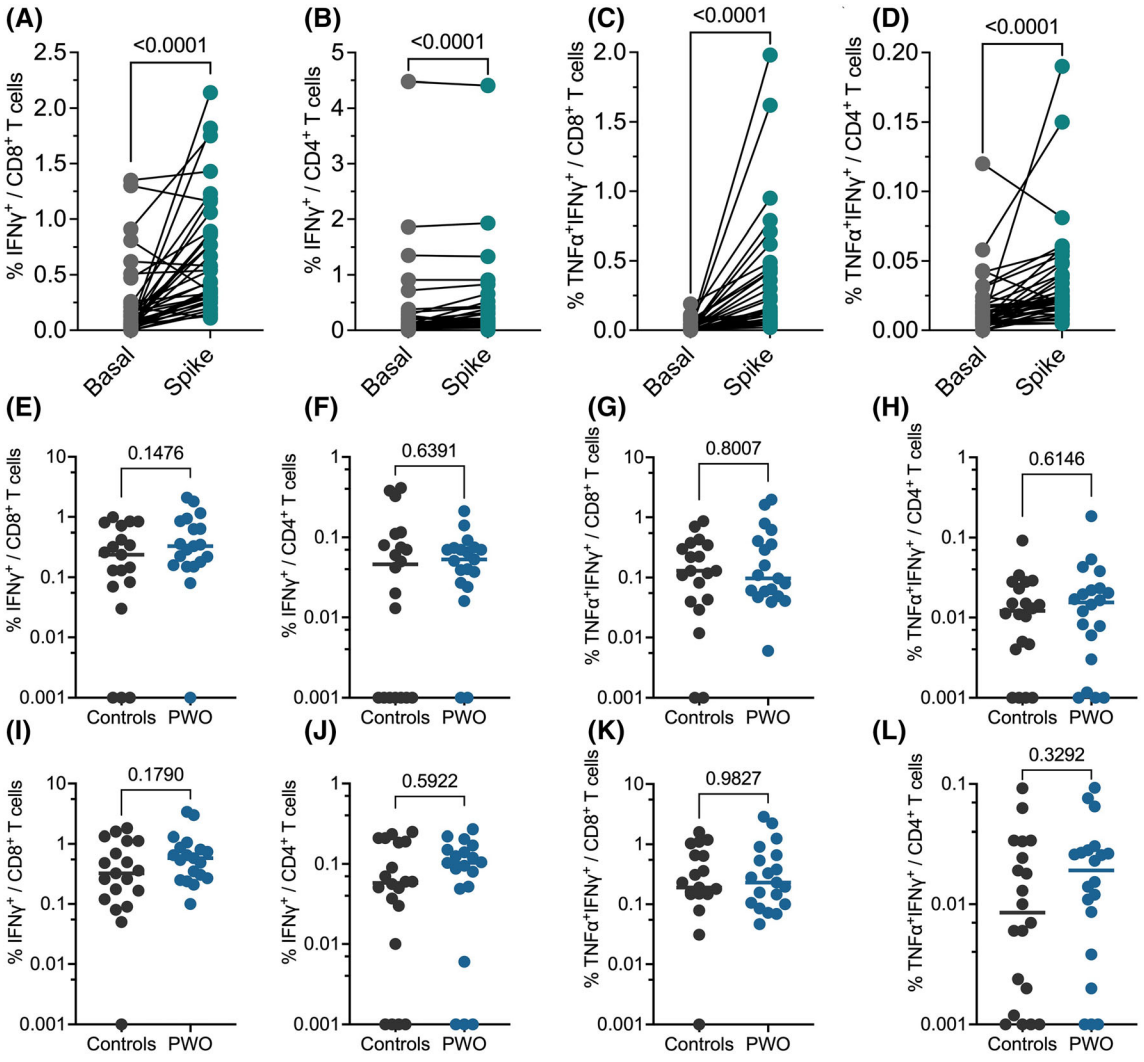

FIGURE 1 SARS-CoV-2 spike-specific T cell responses of patients who have recovered from COVID-19. (**A-D**) Scatterplots showing the frequencies of T cell subsets ($CD8^+$ or $CD4^+$) in individuals who have recovered from COVID-19 producing cytokines (TNFα, IFNγ) in response to overnight stimulation with SARS-CoV-2 spike peptide pools, compared with basal levels. (**E-H**) Frequencies of T cell subsets ($CD8^+$ or $CD4^+$) in individuals who have recovered from COVID-19 with obesity (PWO) or without obesity (control individuals) producing cytokines (TNFα, IFNγ) in response to overnight stimulation with SARS-CoV-2 spike peptide pools. (**I-L**) Frequencies of T cell subsets ($CD8^+$ or $CD4^+$) in individuals who have recovered from COVID-19 with obesity (PWO) or without obesity (control individuals) producing cytokines (TNFα, IFNγ) in response to overnight stimulation with SARS-CoV-2 membrane peptide pools. Where bars are present, they denote median. Statistical comparisons across cohorts were performed with the Wilcoxon test for paired data (panels A-D) and Mann–Whitney *U* test for unpaired data (panels E-L). Data for (panels E-L) were background subtracted against negative control. PWO, people with obesity [Color figure can be viewed at wileyonlinelibrary.com]

Statistical comparisons were performed using the Wilcoxon test for paired data and the Mann–Whitney *U* test for unpaired data. Statistical significance for *p* values was <0.05. Background subtraction was used when calculating antigen-specific T cell frequencies.

RESULTS

The clinical characteristics of the cohort are presented in Table 1. Groups were matched for age, sex, days from symptom onset, and requirement for hospital admission at the time of infection.

Generation of robust SARS-CoV-2-specific T cell responses in PWO

Studies have highlighted the generation of robust T cell responses following SARS-CoV-2 infection. We first confirmed this in our cohort, finding significant frequencies of SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cells producing IFNγ in response to spike and membrane peptides (median $CD8^+$ $IFN\gamma^+$ frequency following spike stimulation: 0.275%; interquartile range [IQR]: 0.130%-0.739%; $CD4^+$: 0.051%; IQR 0.013%-0.075%; Figure 1A-D and Supporting Information Figure S2A-D).

T cells that produce multiple cytokines are known as polyfunctional cells. They are associated with enhanced protection against viral infections [12]. We observed polyfunctional $CD8^+$ and $CD4^+$ cells ($IFN\gamma^+TNF\alpha^+$) in response to both spike and membrane peptides (median $CD8^+$ $IFN\gamma^+TNF\alpha^+$ frequency against spike: 0.114%; IQR: 0.046%-0.365%; $CD4^+$: 0.014%; IQR: 0.004%-0.023%; Figure 1C,D and Supporting Information Figure S2C-D).

Obesity has been associated with significant immune dysregulation, including diminished antiviral immunity following infection or vaccination. We separated our cohort into PWO and control individuals. PWO were seen to have robust SARS-CoV-2-specific T cell responses, with no differences in the frequencies of $CD8^+$ and $CD4^+$ T cells producing IFNγ in response to either spike (Figure 1E,F and Supporting Information Figure S3) or membrane peptide pools (Figure 1I,J and Supporting Information Figure S3). Similarly, frequencies of SARS-CoV-2-specific polyfunctional T cells were the same in people with and without obesity, whether stimulated by spike (Figure 1G,H) or membrane peptide pools (Figure 1K,L). Finally, we evaluated humoral immunity in our cohort and noted comparable responses in PWO when compared with control individuals (Supporting Information Figure S2E-H).

DISCUSSION

Given the substantially increased risk of severe COVID-19 in PWO, there is a critical need to properly characterize immunity in this cohort following both infection and vaccination. We studied persistent antigen-specific T cell responses and humoral immunity in PWO and control individuals at 3 to 9 months following PCR-confirmed SARS-CoV-2 infection. Antigen-specific $CD8^+$ and $CD4^+$ T cells were readily detected across our cohort ($n = 40$). Between-group analysis provides evidence that SARS-CoV-2-specific T cell frequencies are preserved in PWO compared with control individuals.

Obesity is associated with extensive dysregulation of innate and adaptive immunity, leading to chronic systemic inflammation and loss of host protection in many circumstances [13, 14]. Immune memory has been demonstrated to be impaired in PWO in multiple settings. Weakened antibody responses have been seen following rabies [4], tetanus [15], and hepatitis B vaccination [5], and impaired antibody and $CD8^+$ cell responses have been seen following influenza vaccination [3].

Immune memory in PWO following SARS-CoV-2 infection is poorly understood. To date, there has been conflicting evidence regarding antibody responses. One recent publication reported that, in the context of acute infection, PWO ($n = 15$) had dysfunctional antibody responses, with lower peripheral spike-specific IgG titers, impaired neutralization, and potentially pathogenic autoimmune antibodies [16]. In contrast, a recent immunoepidemiology study by Nilles et al. found no difference in peripheral RBD-specific IgG titers or neutralizing capability compared with control individuals [17].

Our study matched PWO and control individuals with samples taken early in the pandemic. No patients had undergone vaccination. Multicolor flow cytometry was used. These data show that PWO who survive their infection have robust antigen-specific T cell responses along both $CD8^+$ and $CD4^+$ cell lines. These data agree with IFNγ ELISpot data from Nillles et al., which did not detect any significant difference between PWO ($n = 12$) and control individuals. The abundant presence of IFNγ-expressing T cells implies not just antigen recognition but functional capacity to inhibit viral replication and mobilize the immune response. Polyfunctional T cells (readily detectable in PWO in our study) have been seen to correlate with favorable disease evolution and protection in multiple settings [12, 18, 19]. Additionally, PWO were seen to have similar titers of SARS-CoV-2-specific IgG relative to control individuals. Interestingly, this finding correlates with observational and case control data, which have demonstrated that obesity is not associated with an increased risk of recurrent disease [20].

The findings of this study are positive for this vulnerable population but do have some limitations. Although it is a strength of this study that the groups are matched across multiple characteristics, including age, sex, and days from symptom onset, our sample size is limited. However, to our knowledge, it is the largest cohort to date examining antigen-specific T cells in PWO following SARS-CoV-2 infection. We welcome further confirmatory studies with clinical correlation.

In summary, we measured SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cell responses in PWO following PCR-confirmed infection, showing features associated with protective immunity and providing evidence that antigen-specific T cell frequencies are not impacted by obesity.O

AUTHOR CONTRIBUTIONS

Neil E. Wrigley Kelly and Féaron C. Cassidy designed and optimized the experimental protocol. Neil E. Wrigley Kelly performed the T cell experiments. Grace Kenny performed the antibody experiments. Conor De Barra provided technical assistance. Neil E. Wrigley Kelly, Donal O'Shea, Grace Kenny, Alejandro A. Garcia-Leon, and Patrick W.G. Mallon recruited, processed, and analyzed the clinical cohorts.

Neil E. Wrigley Kelly and Andrew E. Hogan analyzed the data and drafted the manuscript. Andrew E. Hogan, Donal O'Shea, Patrick W.G. Mallon, and Neil E. Wrigley Kelly conceptualized/designed the study and approved the final manuscript as submitted.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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REFERENCES

1. Popkin BM, Du S, Green WD, et al. Individuals with obesity and COVID-19: a global perspective on the epidemiology and biological relationships. *Obes Rev*. 2020;21:e13128. doi:10.1111/obr.13128
2. Gao M, Piernas C, Astbury NM, et al. Associations between body-mass index and COVID-19 severity in 6.9 million people in England: a prospective, community-based, cohort study. *Lancet Diabetes Endocrinol*. 2021;9:350-359.
3. Sheridan PA, Paich HA, Handy J, et al. Obesity is associated with impaired immune response to influenza vaccination in humans. *Int J Obes (Lond)*. 2012;36:1072-1077.
4. Banga N, Guss P, Banga A, Rosenman KD. Incidence and variables associated with inadequate antibody titers after pre-exposure rabies vaccination among veterinary medical students. *Vaccine*. 2014;32:979-983.
5. Weber DJ, Rutala WA, Samsa GP, Bradshaw SE, Lemon SM. Impaired immunogenicity of hepatitis B vaccine in obese persons. *N Engl J Med*. 1986;314:1393.
6. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell*. 2020;183:158-168.e14.
7. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. *Immunity*. 2020;52:971-977.e3.
8. Moderbacher CR, Ramirez SI, Dan JM, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell*. 2020;183:996-1012.e19.
9. Jung JH, Rha MS, Sa M, et al. SARS-CoV-2-specific T cell memory is sustained in COVID-19 convalescent patients for 10 months with successful development of stem cell-like memory T cells. *Nat Commun*. 2021;12:4043. doi:10.1038/s41467-021-24377-1
10. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. 2021;371:eabf4063. doi:10.1126/science.abf4063
11. Marcotte HA, Piralla A, Zuo F, et al. Immunity to SARS-CoV-2 up to 15 months after infection. *iScience*. 2022;25:103743. doi:10.1016/j.isci.2022.103743
12. Goncalves Pereira MH, Figueiredo MM, Queiroz CP, et al. T-cells producing multiple combinations of IFNgamma, TNF and IL10 are associated with mild forms of dengue infection. *Immunology*. 2020;160:90-102.
13. Green WD, Beck MA. Obesity impairs the adaptive immune response to influenza virus. *Ann Am Thorac Soc*. 2017;14(suppl 5):S406-S409.
14. Karppelin M, Siljander T, Vuopio-Varkila J, et al. Factors predisposing to acute and recurrent bacterial non-necrotizing cellulitis in hospitalized patients: a prospective case-control study. *Clin Microbiol Infect*. 2010;16:729-734.
15. Eliakim A, Schwindt C, Zaldivar F, Casali P, Cooper DM. Reduced tetanus antibody titers in overweight children. *Autoimmunity*. 2006;39:137-141.
16. Frasca D, Reidy L, Romero M, et al. The majority of SARS-CoV-2-specific antibodies in COVID-19 patients with obesity are autoimmune and not neutralizing. *Int J Obes (Lond)*. 2022;46:427-432.
17. Nilles EJ, Siddiqui SM, Fischinger S, et al. Epidemiological and immunological features of obesity and SARS-CoV-2. *Viruses*. 2021;13:2235. doi:10.3390/v13112235
18. Darrah PA, Patel DT, De Luca PM, et al. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. *Nat Med*. 2007;13:843-850.
19. Guerrera G, Picozza M, D'Orso S, et al. BNT162b2 vaccination induces durable SARS-CoV-2-specific T cells with a stem cell memory phenotype. *Sci Immunol*. 2021;6:eabl5344. doi:10.1126/sciimmunol.abl5344
20. Adrielle Dos Santos L, Filho PGG, Silva AMF, et al. Recurrent COVID-19 including evidence of reinfection and enhanced severity in thirty Brazilian healthcare workers. *J Infect*. 2021;82:399-406.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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