Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Background: Young adults are now considered major spreaders of coronavirus disease 2019 (COVID-19) disease. Although most young individuals experience mild to moderate disease, there are concerns of long-term adverse health effects. The impact of COVID-19 disease and to which extent population-level immunity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exists in young adults remain unclear.

Objective: We conducted a population-based study on humoral and cellular immunity to SARS-CoV-2 and explored COVID-19 disease characteristics in young adults.

Methods: We invited participants from the Swedish BAMSE disease group* (Barn [Children], Allergy Millieu, Stockholm, Epidemiology) birth cohort (age 24-27 years) to take part in a COVID-19 follow-up. From 980 participants (October 2020 to June 2021), we here present data on SARS-CoV-2 receptor-binding domain–specific IgM, IgA, and IgG titers measured by ELISA and on symptoms and epidemiologic factors associated with seropositivity. Further, SARS-CoV-2–specific memory B- and T-cell responses were detected for a subpopulation (n = 108) by ELISpot and FluoroSpot.

Results: A total of 28.4% of subjects were seropositive, of whom 18.4% were IgM single positive. One in 7 seropositive subjects was asymptomatic. Seropositivity was associated with use of public transport, but not with sex, asthma, rhinitis, IgE sensitization, smoking, or body mass index. In a subset of representative samples, 20.7% and 35.0% had detectable SARS-CoV-2 specific B- and T-cell responses, respectively. B- and T-cell memory responses were clearly associated with seropositivity, but T-cell responses were also detected in 17.2% of seronegative subjects.

Conclusions: Assessment of IgM and T-cell responses may improve population-based estimations of SARS-CoV-2 infection. The pronounced surge of both symptomatic and asymptomatic infections among young adults indicates that the large-scale vaccination campaign should be continued. (J Allergy Clin Immunol 2022;149:65-75.)

Key words: SARS-CoV-2, COVID-19 disease, IgM, IgA, IgG, memory T cells, memory B cells, young adults, population-based cohort, asthma, risk factors

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses a severe threat to public health worldwide. In Sweden, more than 1 million cases and 14,729 deaths have been confirmed up to September 15, 2021. Recently the death rate has decreased, mainly as a result of the vaccination campaign. Still, the number of patients in intensive care units remains at relatively high levels, which may reflect an increased general spread of the infection that is potentially due to more transmissible new variants. Young adults have been considered to be major spreaders of disease since last autumn.1 Even though the risk for severe illness and mortality increases with age, it is feared that the number of younger adults requiring intensive care may increase. Furthermore, there are also concerns for long-term adverse health effects in infected individuals, including young adults. The impact of COVID-19 disease and to what extent population-level immunity against SARS-CoV-2 exists in young adults are currently unclear—and are of particular interest because restrictions and recommendations suggested by the World Health Organization (WHO) to reduce disease spread was implemented rather late in Sweden, so throughout the pandemic, schools have remained open, and no formal lockdown was enforced.
in the viral genome—the spike (S) protein, nucleoprotein (N), membrane (M) protein, and envelope (E) protein—as well as in several other minor proteins.5,6 T-cell responses against SARS-CoV-2 have been described in mild or asymptomatic cases, also without seroconversion.5,6 However, the prevalence of SARS-CoV-2 cellular memory responses at the population level is not well studied, especially among young adults.

We therefore conducted a population-based study on the presence of humoral and cellular immunity to SARS-CoV-2 and explored disease characteristics in young adults (age 24-27 years). We invited participants from the ongoing BAMSE (Swedish acronym for Barn [Children], Allergy Milieu, Stockholm, Epidemiology) birth cohort study5,6 to participate in a COVID-19 follow-up. We here present data on SARS-CoV-2 receptor-binding domain (RBD)-specific IgM, IgA, and IgG titers and on symptoms and epidemiologic factors associated with seropositivity in all unvaccinated participants (October 2020 to June 2021; n = 980). In addition, we present data on memory B- and T-cell responses in 108 unvaccinated participants (October 2020 to January 2021).

METHODS

Study population, study design, and variables

The study population included participants from the prospective birth cohort BAMSE, which originally included 4089 newborns born between 1994 and 1996.5 A 24-year follow-up was conducted from 2016 to 2019, with a total of 2271 participants attending a clinical examination. These participants were invited to an ongoing COVID-19 follow-up for which a phase 1 web-based questionnaire was answered August to November 2020 (n = 1645). A total of 1453 of 1645 subjects were invited to the study’s phase 2 (start October 6, 2020), which included a clinical examination and a new web-based questionnaire.

From the phase 1 and phase 2 questionnaires, we extracted self-reported data on COVID-19–related symptoms, SARS-CoV-2 PCR and/or antibody tests, household members with COVID-19, use of public transport, face mask use, interactions at work, asthma diagnosis, and rhinitis.8 The presence of symptoms was evaluated by the question “Have you had symptoms of suspected COVID-19?” and follow-up questions including type and duration of symptoms and being bedbound or hospitalized. Weight and body fat percentage were measured by an MC 780 body composition monitor (Tanita, Tokyo, Japan), and body mass index was calculated from measured weight and height.

PCR and antibody tests were reported through the questions “When was the last time you performed a nose- or throat test” with the follow-up question “What was the result?” (PCR); and “When was the last time you performed a blood test” with the follow-up question “What was the result?” (antibody test). Positive PCR tests (primarily) and positive antibody tests (secondary) were used to estimate the time in months that elapsed between presumed COVID-19 disease and clinical examination.

Data on IgE sensitization was obtained from the 24-year cohort follow-up. Further details are available in the Methods section in this article’s Online Repository at www.jacionline.org.

The present study included all 1028 participants who completed the clinical examination (October 6, 2020, to June 23, 2021), resulting in a study population of 1011 subjects after exclusion of subjects with insufficient sample material (n = 17). A total of 980 unvaccinated subjects were included in the main study population and 31 vaccinated subjects in a subanalysis (see Fig E1 in this article’s Online Repository at www.jacionline.org). The study was approved by the Swedish ethical review authority (approval 2020-02922). Participants provided written informed consent.

Sample preparation

Venous blood was collected in serum and sodium heparin tubes (BD Vacutainer; Becton Dickinson, San Diego, Calif) at the site of clinical examination (Södersjukhuset, Stockholm, Sweden). Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples by density centrifugation with Lymphoprep (STEMCELL Technologies, Vancouver, British Columbia, Canada). PBMCs were cryopreserved in 90% fetal bovine serum + 10% dimethyl sulphoxide and stored in liquid nitrogen. Serum was stored at −20°C.

Detection of anti–SARS-CoV-2 antibodies

Serum titers of anti–SARS-CoV-2 antibodies were measured for the whole study population (980 unvaccinated subjects and 31 vaccinated subjects). Serum samples were diluted 1:400, and levels of anti-RBD IgM, IgA, and IgG antibodies were determined by an in-house ELISA as previously described.4 In-house standards made by pooled highly positive serum were calibrated by using the WHO international standard for anti–SARS-CoV-2 immunoglobulin (NIBSC, 20/136). One arbitrary unit (AU) per milliliter of in-house serum standard equaled 7.55 AU/mL IgM, 46.4 AU/mL IgA, and 1.03 AU/mL IgG of WHO international standards, respectively. Cutoff values for antibody positivity were determined on the basis of receiver operating characteristic curves with data from convalescent COVID-19 patients and 108 negative historical control samples (outside BAMSE).3 The value with the highest sensitivity and a specificity of at least 99% was selected as the cutoff for each isotype: 14.42 AU/mL for IgM, 2.61 AU/mL for IgA, and 25.09 AU/mL for IgG.

Detection of SARS-CoV-2–specific memory B- and T-cell responses

SARS-CoV-2–specific memory B- and T-cell responses were analyzed for the first 5 samples collected per study week (n = 38-60, October 2020 to January 2021) and for an additional 49 subjects from the same time period to increase group sizes. The number of B cells secreting SARS-CoV-2 RBD-specific IgG was measured using the Human IgG SARS-CoV-2 RBD ELISPOTPLUS kit (Mabtech AB, Cincinnati, Ohio), and the numbers of spike 1 scanning peptide pool (S1) and spike protein/nucleocapsid/membrane protein/open reading frame (ORF-3a and ORF-7a) proteins (S N M O) peptide-specific IFN-γ and IL-2–secreting T cells were detected using the Human IFN-γ/IL-2 SARS-CoV-2 FluoroSpotPLUS kit (Mabtech AB) (see this article’s Online Repository for details).3 The results are expressed as the number of spots per 300,000 cells, after subtracting the background. The cutoff values were set at the highest number of spots detected in 11 prepanademic PBMC control samples.3

Statistical analyses and graphical presentation

The chi-square test, the Fisher exact test, or multiple logistic regression was used for categorical data and the Mann-Whitney U test or the Kruskal-Wallis test was used for continuous data. For multiple comparisons, the Dunn test with Benjamini-Hochberg correction was used. Categorical data are presented
as numbers and percentages, while continuous data are presented as medians and interquartile ranges. Spearman rank correlation was used for associations between 2 continuous variables. Statistical analysis was conducted by Stata 16.0 software (StataCorp, College Station, Tex). \( P < .05 \) was considered statistically significant. Graphical presentations were made by GraphPad Prism 9.1.0 (GraphPad Software, La Jolla, Calif) and RStudio 1.3.1093 (https://rstudio.com/) software.

**RESULTS**

The study flowchart is presented in Fig E1. A total of 277 serum samples (28.4%) were positive for at least 1 SARS-CoV-2 anti-RBD antibody isotype (seropositive), while 700 samples (71.6%) were triple negative (Fig 1, A). IgM, IgA, and IgG antibodies were detected in 11.8%, 6.8%, and 22.4% of samples, respectively (Table I). Individual data from BAMSE participants and prepandemic control samples⁴ are displayed in Fig 1, B.

Among seropositive samples, 8.7% were triple positive and 27.8% were positive for 2 isotypes (14.1% IgM⁺ IgG⁺, 13.0% IgA⁺ IgG⁺, 0.7% IgM⁺ IgA⁺). The remaining samples were single positive (18.4% IgM⁺, 1.8% IgA⁺, 43.3% IgG⁺) (Table 1, Fig 1, C). Analysis of seroprevalence by month revealed a notable increase in the proportion of IgG⁺ and IgA⁺ subjects in January 2021 (33.7% and 15.7%), which is also reflected in a corresponding peak in the overall seropositivity (Fig 1, D). The proportion of IgM⁺ subjects notably increased during April to June 2021, and the overall seropositivity per month increased during the study period (Fig 1, D). Taken together, more than 1 in 4 young adult participants were seropositive, and assessment of IgM in addition to IgG can better estimate the prevalence of infection.

The seropositive and seronegative groups did not differ significantly by age, sex, asthma diagnosis, rhinitis, IgE

---

**FIG 1.** SARS-CoV-2 anti-RBD IgM, IgA, and IgG prevalence and titers. (A) The proportions of SARS-CoV-2 seropositive and seronegative subjects. (B) The titers of SARS-CoV-2 anti-RBD IgM, IgA, and IgG in samples from historical controls collected before the pandemic (\( n = 108 \)) and BAMSE participants (\( n = 980 \)), expressed in arbitrary units, and prevalence of IgM, IgA, and IgG displayed as pie charts for the BAMSE participants. (C) Venn diagram showing the overlap of IgM, IgA, and IgG seropositivity. (D) The percentages of IgM⁺, IgA⁺, and IgG⁺ subjects for each study month. The chi-square test was used for statistical analysis. Red lines indicate median values; green lines, assay cutoff values.
sensitization, smoking, snuff use, body mass index, or body fat percentage (Table II). A higher proportion of seropositive subjects reported suspected/confirmed COVID-19 disease in the household (P < .001), and small but significant differences were noted for regular use of public transport and face mask use (P < .05), while the frequencies of regular interactions with people at work was rather similar (Table II). The seropositive group more often reported having taken a PCR or antibody test (P < .0001, P = .010), and this group more often reported positive results from these tests (P < .0001) (Table II). Among seropositive and seronegative subjects, 85.2% and 59.6% reported at least 1 occasion with possible COVID-19–related symptoms between February 2020 and the clinical visit (P < .0001, Table III, Fig 2, A). A total of 14.8% of seropositive subjects were asymptomatic during the whole study period. When seropositive subjects were divided into IgG⁺ and IgM⁺ IgA⁻ IgG⁻, we noticed a higher prevalence of asymptomatic subjects (P < .001) and a shorter duration of symptoms (P = .047) in the latter group (see Table E1 in this article’s Online Repository at www.jacionline.org).

One-third of seronegative and seropositive symptomatic subjects reported symptoms more than once, resulting in a total of 314 and 554 occasions with symptoms, respectively. The seropositive group more often reported having been bedbound because of their symptoms (51.8% vs 39.3%) (P < .010), and this group more often reported positive results from these tests (P < .0001) (Table II). Among seropositive and seronegative subjects, 85.2% and 59.6% reported at least 1 occasion with possible COVID-19–related symptoms between February 2020 and the clinical visit (P < .0001, Table III, Fig 2, A). A total of 14.8% of seropositive subjects were asymptomatic during the whole study period. When seropositive subjects were divided into IgG⁺ and IgM⁺ IgA⁻ IgG⁻, we noticed a higher prevalence of asymptomatic subjects (P < .001) and a shorter duration of symptoms (P = .047) in the latter group (see Table E1 in this article’s Online Repository at www.jacionline.org).

Ten subjects had positive memory B- and/or T-cell responses within the seronegative group. Among these, 2 had a positive B-cell response, 1 of whom was borderline positive for all 3 antibody isoatypes, was T-cell positive, reported a positive PCR test, and suspected COVID-19 in the household. Among the remaining 8 subjects with T-cell responses, 7 had IgG and/or IgM titers exceeding the median value in the seronegative group, none reported a positive PCR test, 3 reported suspected/confirmed COVID-19 in the household, and 5 reported a history of COVID-19–related symptoms (see Table E4 in this article’s Online Repository at www.jacionline.org). Taken together, SARS-CoV-2 B- and T-cell memory responses were detected in 68.1% and 55.1% of seropositive individuals, respectively. A memory B-cell response was almost exclusively associated with seropositivity, while memory T-cell responses were found in 1 in 5 seronegative subjects.

Data on all 3 arms of adaptive immunity (virus-specific antibodies, and memory B and T cells) were available for 104 subjects. Among these, 60.6% were positive for at least 1 investigated immune parameter with highly variable patterns, suggesting a high heterogeneity of adaptive immune responses in young adults (see Fig E2 in this article’s Online Repository at www.jacionline.org).
| Characteristic                  | Seropositive |          | Seropositive |          |          |          | P value* |
|--------------------------------|--------------|----------|--------------|----------|----------|----------|----------|
|                                | Median       | IQR      | Median       | IQR      | ns       | ns       |          |
| Age in years                   | 25.8         | 23.9-27.3| 25.8         | 24.0-27.2| ns       | ns       |          |
| BMI                            | 22.9         | 15.2-48.3| 22.5         | 14.8-59.9| ns       | ns       |          |
| Body fat percentage            | 23.9         | 18.9-28.4| 22.8         | 17.7-27.2| ns       | ns       |          |
| Sex                            |              |          |              |          |          |          |          |
| Female                         | 177          | 63.9     | 428          | 61.2     | ns       | ns       |          |
| Male                           | 100          | 36.1     | 271          | 38.8     |          |          |          |
| Asthma                         |              |          |              |          |          |          |          |
| Yes                            | 37           | 13.4     | 100          | 14.3     | ns       | ns       |          |
| No                             | 240          | 86.6     | 600          | 85.7     |          |          |          |
| Rhinitis                       |              |          |              |          |          |          |          |
| Yes                            | 45           | 16.2     | 103          | 14.8     | ns       | ns       |          |
| No                             | 232          | 83.8     | 595          | 85.2     |          |          |          |
| IgE-sensitization              |              |          |              |          |          |          |          |
| Yes                            | 111          | 40.2     | 313          | 45.0     | ns       | ns       |          |
| No                             | 165          | 59.8     | 382          | 55.0     |          |          |          |
| Smoking                        |              |          |              |          |          |          |          |
| Yes                            | 39           | 14.2     | 78           | 11.2     | ns       | ns       |          |
| No                             | 236          | 85.8     | 619          | 88.8     |          |          |          |
| Snuff                          |              |          |              |          |          |          |          |
| Yes                            | 67           | 24.4     | 157          | 22.5     | ns       | ns       |          |
| No                             | 208          | 75.6     | 541          | 77.5     |          |          |          |
| BMI categories[^]              |              |          |              |          |          |          |          |
| Overweight/obese               | 75           | 28.2     | 157          | 23.1     | ns       | ns       |          |
| No overweight                  | 191          | 71.8     | 526          | 76.9     |          |          |          |
| COVID-19 in household§         |              |          |              |          |          |          |          |
| Feb 2020-Jul 2020              |              |          |              |          |          |          |          |
| Yes/No/Not sure                | 94/167/14    | 34.2/60.7/5.1 | 125/519/56 | 17.9/74.1/8.0 | <.0001  |          |          |
| Aug 2020-June 2021             | 84/181/8     | 30.8/66.3/2.9 | 103/578/17 | 14.8/82.8/2.4 | <.0001  |          |          |
| Do you use face mask?          |              |          |              |          |          |          |          |
| Yes, often                     | 35           | 12.7     | 102          | 14.6     | .031     |          |          |
| Yes, sometimes                 | 162          | 57.9     | 346          | 49.6     |          |          |          |
| No                             | 78           | 28.4     | 249          | 35.7     |          |          |          |
| Regular interactions with people at work |              |          |              |          |          |          |          |
| Feb 2020-Jul 2020              |              |          |              |          |          |          |          |
| Yes/No                         | 204/73       | 73.6/26.4| 497/203      | 71.0/29.0| ns       |          |          |
| Aug 2020-June 2021             | 211/64       | 76.7/23.3| 491/207      | 70.3/29.7| .045     |          |          |
| Regular use of public transport to work |              |          |              |          |          |          |          |
| Feb 2020-Jul 2020              |              |          |              |          |          |          |          |
| Yes/No                         | 90/187       | 32.5/67.5| 176/524      | 25.1/74.9| .020     |          |          |
| Aug 2020-June 2021             | 91/184       | 33.1/66.9| 174/523      | 25.0/75.0| .010     |          |          |
| PCR-test performed prior to clinical examination (self-reported) |              |          |              |          |          |          |          |
| No                             | 112          | 40.4     | 378          | 54.0     | <.0001   |          |          |
| Yes                            | 165          | 59.6     | 322          | 46.0     |          |          |          |
| Positive                       | 86           | 52.1     | 14           | 4.4      | <.0001   |          |          |
| Negative                       | 79           | 47.9     | 308          | 95.6     |          |          |          |
| Antibody-test performed prior to clinical examination (self-reported) |              |          |              |          |          |          |          |
| No                             | 171          | 61.7     | 492          | 70.3     | .010     |          |          |
| Yes                            | 106          | 38.3     | 208          | 29.7     |          |          |          |
| Positive                       | 59           | 55.7     | 16           | 7.7      | <.0001   |          |          |
| Negative                       | 47           | 44.3     | 192          | 92.3     |          |          |          |

*BMI, Body mass index; IQR, interquartile range.
*Mann-Whitney U-test.
†Chi-square test or Fisher exact test.
[^]Overweight/obese defined as BMI ≥25 kg/m², no overweight defined as BMI <25 kg/m².
§Suspected or confirmed.
[| Corresponding odds ratio 1.5 (95% CI, 1.1-2.0) for regular use of public transport to work during phase 1 after adjustment for age, sex, smoking, having regular interactions at work and occupation.
●Corresponding odds ratio 1.4 (95% CI, 1.0-1.9) for regular use of public transport to work during phase 2 after adjustment for age, sex, smoking, face mask usage, having regular interactions at work and occupation.
We correlated measured antibody titers and cellular responses at the time of clinical examination with the time in months that elapsed between presumed COVID-19 disease primarily on the basis of self-reported positive PCR test (n = 99 for antibody titers and n = 12-13 for cellular responses) and secondarily self-reported positive antibody test (additional n = 56 for antibody titers and n = 6-8 for cellular responses). Of note, the estimation that was based on self-reported positive antibody tests gives a minimum number of elapsed months. IgM, IgA, and IgG titers negatively correlated with time elapsed since presumed disease, regardless of whether the estimation was solely based on positive PCR test (Fig 4, A, IgM: r = -0.31, P = .002; IgA: r = -0.42, P < .0001, IgG: r = -0.36, P < .001) or when combining positive PCR and antibody tests (see Fig E3, A, in this article’s Online Repository at www.jacionline.org). The number of IgG-producing B cells positively correlated with time elapsed since presumed disease (Fig E3, B, P = .007), while T-cell responses did not show clear associations (Fig E3, C and D); however, all investigated cellular responses, except S1-specific IL-2, were measurable 6 months after presumed disease.

Finally, we investigated antibody levels among 31 subjects who had received the first dose of a SARS-CoV-2 vaccine (AstraZeneca, n = 6; Moderna, n = 1; Pfizer, n = 24), 18 of 31 of whom had also received a second vaccine dose. Few vaccinated subjects were IgM⁺, while IgA and IgG responses were robust after the second dose (Fig 4, B).

### DISCUSSION

In this population-based study, we show that more than 1 in 4 young adults were SARS-CoV-2 seropositive, with a significant proportion of subjects being IgM single positive. Seropositivity was associated with COVID-19 disease in the household and weakly with use of public transport. Memory B- and T-cell responses were observed in the majority of IgG⁺ subjects, but T-cell responses were also observed in 1 in 5 seronegative subjects. The proportion of seropositive subjects remained relatively stable October to December 2020, increased to over 30% in January 2021, declined during early spring, and increased again to above 40% close to the summer of 2021. The latter increase was mostly due to an increased number of IgM⁺ subjects, suggesting...
that we detected more cases of recent or ongoing infections during the late spring. These subjects may have been asymptomatic at the time of clinical examination, considering that the participants were asked not to attend if symptomatic. Whether this reflects a higher degree of asymptomatic infections due to virus variants during this time period or less cautiousness is not known. Data from the Swedish Public Health Agency collected late May to early June 2021 report the national IgG seroprevalence to be 52% for subjects aged 20 to 64 years; however, this heterogeneous age group also includes a large proportion of vaccinated subjects. The data suggest that the spread of infection was, and still is, substantial in young adults.

Our results indicate that measuring IgM likely captures a significant proportion of recent or ongoing SARS-CoV-2 infections. However, anti-RBD IgM was less frequently observed in acute and convalescent COVID-19 patients compared to IgG, which is reflected in the lower prevalence of reduced taste or smell, sore throat, fever, pain in bone or muscles, body fatigue, and body fatigue in the IgM seropositive group.
and studies of anti–SARS-CoV-2 IgM indicate the longevity to be 4 months or less among symptomatic individuals.\textsuperscript{4,11} Nevertheless, our result suggests that using only IgG as a readout underestimates the prevalence of SARS-CoV-2 infection in the population, especially when the epidemic is resurgent.

In this cohort, seropositive subjects had more frequently used public transport and more often had household members with suspected or confirmed COVID-19 disease compared to seronegative subjects. These associations have been described by others.\textsuperscript{13,14} Notably, even though public transport areas are likely to contribute to the virus’s spread,\textsuperscript{15} we cannot conclude that participants were infected while using the public transport system because we do not have detailed data on virus spread. Also, we adjusted for potential confounders in the regression model (Table II), but we cannot exclude residual confounding effects (eg, contact with confirmed cases at work or at social events).

In Sweden, public face mask use was not recommended until the beginning of 2021. We found a weak but significant inverse association between face mask use and seropositivity, although the protective effect of such cross-reactive memory T cells to previously encountered infections like SARS-CoV-2 is unknown. Our data show that most seropositive subjects with positive T-cell responses have higher titers (though still below the cutoffs) of anti–SARS-CoV-2 antibodies than seronegative subjects without T-cell responses, suggesting that some subjects mount low anti–SARS-CoV-2 antibodies but these results suggest that undetected infection may be a major public health issue in young adults. Data regarding associations between the magnitude and duration of immune responses and symptom severity during COVID-19 disease are conflicting.\textsuperscript{11,22-24} A connection between mild symptoms and lower T-cell responses has been suggested,\textsuperscript{25} while other studies have failed to find this association,\textsuperscript{26} and we and others have shown that specific T-cell responses are similar in symptomatic and asymptomatic subjects.\textsuperscript{27,28} Furthermore, IgG titers were significantly higher and the median number of memory B cells tended to be higher in symptomatic subjects—results also reported elsewhere.\textsuperscript{29} Others have shown that individuals with mild or severe COVID-19 disease mount comparable and equally durable memory B-cell responses.\textsuperscript{27}

We found that almost 1 in 5 seronegative subjects tested positive in at least 1 T-cell assay. Studies report that 40% to 60% of nonexposed prepanicp brands subjects had SARS-CoV-2–reactive T cells, most likely as a result of cross-reactivity,\textsuperscript{20} and that cross-reactive T cells exist to a higher extent in young adults.\textsuperscript{28} However, the protective effect of such cross-reactive memory T cells to newly encountered infections like SARS-CoV-2 is unknown. Our data show that most seropositive subjects with positive T-cell responses have higher titers (though still below the cutoffs) of anti–SARS-CoV-2 antibodies than seronegative subjects without T-cell responses, suggesting that some subjects mount low antibody responses that are not detected by available assays. Because the full dynamics of the SARS-CoV-2 immune response are not understood, a missing overlap between seropositivity and cellular memory responses may be due to sampling timing. One recent review highlights an urgent need for the development of SARS-CoV-2 T-cell assays,\textsuperscript{28} and Sweden is now one of the first countries to offer T-cell tests to the community. Still, it is unclear how broadscale T-cell testing can increase the understanding of population immunity, guide restrictions, and behavioral recommendations or evaluate the vaccine response. Finally, the question on which T-cell assay would be the most appropriate to choose for broadscale testing of a variety of age groups remains to be addressed.

Several studies have assessed the maintenance of protective levels of antibodies and the durability of cellular responses to SARS-CoV-2 partly as a result of concerns regarding reinfections. Some studies suggest that antibody titers wane within a few months,\textsuperscript{22,30} while others show that titers are maintained

| Characteristic                | IgG\(^*\)         | IgM \(^*\)IgG\(^-\) | IgA \(^*\)IgG\(^-\) | Seronegative |
|------------------------------|-------------------|---------------------|---------------------|--------------|
| B-cell response              | 32/47 (68.1)      | 0/6 (0.0)           | 0/0 (0-3)           | 2/54 (3.7)   |
| T-cell response (any)        | 27/49 (55.1)      | 3/7 (42.9)          | NA                  | 9/52 (17.3)  |
| T-cell response SI           | 21/49 (42.9)      | 3/7 (42.9)          | NA                  | 5/52 (9.6)   |
| IFN-γ                        | 16/49 (32.7)      | 1/7 (14.3)          | 2 (0-5)             | 1/52 (1.9)   |
| IFN-γ/IL-2                   | 7/49 (14.3)       | 1/7 (14.3)          | 2 (0-3)             | 0/52 (0.0)   |
| IFN-γ/IL-2                   | 26/49 (53.1)      | 3/7 (42.9)          | NA                  | 7/52 (13.5)  |
| IL-2                         | 14/49 (28.6)      | 3/7 (42.9)          | 0 (0-30)            | 4/52 (7.7)   |
| IL-2                         | 23/49 (49.4)      | 3/7 (42.9)          | NA                  | 7/52 (13.5)  |
| IL-2                         | 17/49 (34.7)      | 3/7 (42.9)          | 0 (0-3)             | 5/52 (17.3)  |

\(^*\)Median number of positive cells.

\(\text{IQR, Interquartile range; NA, not applicable.}\)

\(\text{Chi-square test/Kruskal-Wallis test.}\)
We used the results of self-reported PCR and antibody tests to make an assumption about when participants had COVID-19 disease. Our results suggest that even though the levels of antibodies declined, they were measurable up until 8 months after infection, and in some cases longer for IgG. For the 14 subjects who were seronegative at clinical examination but who reported infection, and in some cases longer for IgG. For the 14 subjects who were seronegative at clinical examination, and their IgG or IgM levels were measurable but below the assay cutoffs. Four subjects had 3 to 5 months in between presumed disease and clinical examination. Our data indicate that antibody titers decline more rapidly in some individuals, which may be worth commenting on in light of vaccination.

The strengths of this study are the population-based approach targeting young adults and the lengthy consecutive follow-up. We measured IgM and IgA in addition to IgG, as well as specific B- and T-cell responses. We included only unvaccinated subjects in our main analyses to gain knowledge about virus spread and risk factors, as well as knowledge about the level of adaptive immunity among young adults. Vaccination campaigns for this target age group are ongoing in Sweden, and our pilot analyses of
31 vaccinated participants showed adequate serologic responses. Limitations of this study include that we were only able to study mild COVID-19 disease, we lack information regarding exact disease dates for some subjects, and we investigated cellular memory responses in a relatively small group.

In summary, to our knowledge, this is the first population-based cohort study to investigate different arms of adaptive memory responses in a relatively small group. Limitations of this study include that we were only able to study mild COVID-19 disease, we lack information regarding exact disease dates for some subjects, and we investigated cellular memory responses in a relatively small group.

Clinical implications: In young adults, characterizing COVID-19 disease and measuring IgM and memory T-cell responses in addition to IgG improve estimations of SARS-CoV-2–specific immunity and create awareness of disease spread.

REFERENCES
1. Monod M, Blenkinsop A, Xi X, Hebert D, Bershán D, Tietze S, et al. Age groups that sustain resurging COVID-19 epidemics in the United States. Science 2021;371:eabe8372.
2. Naqi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: structural genomics approach. Biochim Biophys Acta Mol Basis Dis 2020;1866:165878.
3. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371:eabf4063.
4. Sherina N, Pirallu A, Wu L, Wan H, Kumagai-Braesch M, Andrè J, et al. Persistence of SARS-CoV-2–specific B and T cell responses in convalescent COVID-19 patients 6-8 months after the infection. Med (N Y) 2021;2:281-95.e4.
5. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Stralin K, Gorin JB, Olsson A, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 2020;183:158-68.e14.
6. Gallais F, Velay A, Nazon C, Wendling MJ, Partisani M, Sibilia I, et al. Intrafamilial exposure to SARS-CoV-2 associated with cellular immune response without seroconversion, France. Emerg Infect Dis 2021;27:113-21.
7. Melén E, Bergström A, Kull I, Almqvist C, Andersson N, Asarnö A, et al. Male sex is strongly associated with IgG-sensitization to airborne but not food allergens: results up to age 24 years from the BAMSE birth cohort. Clin Transl Allergy 2020 May 25;10:15. https://doi.org/10.1186/s13601-020-00319-w. eCollection 2020.
8. Westman M, Åberg K, Apostolovic D, Lupinek C, Gattinger P, Mittermann I, et al. Sensitization to grass pollen allergen molecules in a birth cohort—natural Phl p 4 as an early indicator of grass pollen allergy. J Allergy Clin Immunol 2020;145:1174-81.e6.
9. Ekström S, Andersson N, Lövquist A, Lauber A, Georgelis A, Kull I, et al. COVID-19 among young adults in Sweden: self-reported long-term symptoms and associated factors. Scand J Public Health 2021;49:4821025425. In press.
10. Public Health Agency of Sweden. Antikropp mot COVID-19 ökar i alla grupper. June 20, 2021. Available at: https://www.folkhalsomyndigheten.se/nyheter-och-
11. Rydyzninski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, 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.

12. Piccoli L, Park YJ, Tortorici MA, Cudinochowski N, Walls AC, Beltramello M, et al. Mapping neutralizing and immunodominant sites on the SARS-CoV-2 spike receptor-binding domain by structure-guided high-resolution serology. Cell 2020; 183:1024-42.e21.

13. Costa SF, Giavina-Bianchi P, Buss L, Mesquita Peres CH, Rafael MM, dos Santos LGN, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) seroprevalence and risk factors among oligo/asymptomatic healthcare workers: estimating the impact of community transmission. Clin Infect Dis 2021;73:e1214-8.

14. Martischang R, Iten A, Arm I, Abbas M, Meyer B, Yerly S, et al. Severe acute respiratory coronavirus virus 2 (SARS-CoV-2) seroconversion and occupational exposure of employees at a Swiss university hospital: a large longitudinal cohort study. Infect Control Hosp Epidemiol 2021;1-8.

15. Luo K, Lei Z, Hai Z, Xiao S, Rui J, Yang H, et al. Transmission of SARS-CoV-2 in public transportation vehicles: a case study in Hunan Province, China. Open Forum Infect Dis 2020;7:ofaa430.

16. Ng OT, Marimuthu K, Koh V, Pang J, Linn KZ, Sun J, et al. SARS-CoV-2 seroprevalence and transmission risk factors among high-risk close contacts: a retrospective cohort study. Lancet Infect Dis 2021;21:333-43.

17. Marks M, Millat-Martinez P, Ouchi D, Roberts CH, Alemany A, Corbacho-Monné M, et al. Transmission of COVID-19 in 282 clusters in Catalonia, Spain: a cohort study. Lancet Infect Dis 2021;21:629-36.

18. Skevaki C, Karsonova A, Karaulov A, Xie M, Renz H. Asthma-associated risk for COVID-19 development. J Allergy Clin Immunol 2020;146:1295-301.

19. Rudberg AS, Havervall S, Månberg A, Jern bombed Falk A, Aguilera K, Ng H, et al. SARS-CoV-2 exposure, symptoms and seroprevalence in healthcare workers in Sweden. Nat Commun 2020;11:5064.

20. Zuo J, Dowell AC, Pearce H, Verma K, Long HM, Begum J, et al. Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. Nat Immunol 2021;22:620-6.

21. Abayasingam A, Balachandran H, Agapiou D, Hammoud M, Rodrigo C, Keoshkerian E, et al. Long-term persistence of RBD memory B cells encoding neutralizing antibodies in SARS-CoV-2 infection. Cell Reports Med 2021;2:100228.

22. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26:1200-4.

23. Long Q-X, Jia Y-J, Wang X, Deng H-J, Cao X-X, Yuan J, et al. Immune memory in convalescent patients with asymptomatic or mild COVID-19. Cell Discov 2021;7:18.

24. Cervia C, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A, et al. Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. J Allergy Clin Immunol 2021;147:545-57.e9.

25. Le Bert N, Clapham HE, Tan AT, Chia WN, Tham CYL, Lim JM, et al. Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. J Exp Med 2021;218:e20202617.

26. Wang Z, Yang X, Zhong J, Zhou Y, Tang Z, Zhou H, et al. Exposure to SARS-CoV-2 generates T-cell memory in the absence of a detectable viral infection. Nat Commun 2021;12:1724.

27. Ogega CO, Skinner NE, Blair PW, Park HS, Littlefield K, Ganesan A, et al. Durable SARS-CoV-2 B cell immunity after mild or severe disease. J Clin Invest 2021;131:e145516.

28. Saletti G, Gerlach T, Jansen JM, Molle A, Elbahesh H, Ludlow M, et al. Older adults lack SARS-CoV-2 cross-reactive T lymphocytes directed to human coronaviruses OC43 and NL63. Sci Rep 2020;10:21447.

29. Ameratunga R, Woon S-T, Jordan A, Longhurst H, Leung E, Steele R, et al. Perspective: diagnostic laboratories should urgently develop T cell assays for SARS-CoV-2 infection. Expert Rev Clin Immunol 2021;17:421-30.

30. Barrasso FJ, Fulcher JA, Goodman-Meza D, Elliott J, Hofmann C, Hausner MA, et al. Rapid decay of anti–SARS-CoV-2 antibodies in persons with mild COVID-19. N Engl J Med 2020;383:1085-7.

31. Gaebler C, Wang Z, Lorenzo ICC, Muecksch F, Finkin S, Tokuyama M, et al. Evolution of antibody immunity to SARS-CoV-2. Nature 2021;591:639-44.
METHODS

Extended definition of variables obtained from the questionnaires

From the phase 1 and phase 2 web-based questionnaires, we obtained information regarding type of symptoms: fever, cough, reduced taste or smell, sore throat, running nose, blocked nose, headache, joint or muscle pain, abdominal symptoms, breathing difficulties, tiredness, and body fatigue from the questions: “Has anyone in your household had suspected or confirmed COVID-19 during the pandemic/since August 1, 2020?” “Do you use face mask?” asked only during phase 2, when national face mask recommendations were issued, “Have you regularly met other people at work?,” and “What is your main use of transport to your daily work?” PCR and antibody tests were reported through the questions “When was the last time you performed a nose or throat test” with the follow-up question “What was the result?” (PCR); and “When was the last time you performed a blood test” with the follow-up question “What was the result?” (antibody test). Asthma was defined as doctor’s diagnosis (ever) together with symptoms of breathing difficulties or asthma medication occasionally or regularly in the last 12 months. Rhinitis was defined as doctor’s diagnosis (ever) together with nasal or eye problems without having a cold in the last 12 months. The phase 1 questionnaire covers February 2020 until November 2020, and the phase 2 questionnaire covers August 1, 2020, until the clinical examination.

IgE sensitization was analyzed during the 24-year follow-up and was defined as ≥0.35 kU/L of soluble serum IgE to at least 1 of the tested allergens using ImmunoCAP System (Thermo Fisher Scientific, Waltham, Mass).

Detection of SARS-CoV-2–specific memory B- and T-cell responses

To detect SARS-CoV-2 RBD-specific IgG-secreting memory B cells, PBMCs were cultured for 4 days in RPMI 1640 medium with 10% fetal bovine serum and 1% penicillin–streptomycin, supplemented with 1 μg/mL R848 (resiquimod; a TLR7/8 agonist) and 10 ng/mL recombinant human IL-2. A total of 300,000 cells per well were then loaded onto ELISpot plates precoated with monocolonal anti-human IgG antibodies (Human IgG SARS-CoV-2 RBD ELISpotPLUS kit, Mabtech AB). IFN-γ- and IL-2–secreting T cells were detected using the Human IFN-γ/IL-2 SARS-CoV-2 FluoroSpotPLUS kit (Mabtech AB). A total of 300,000 PBMCs per well were added to FluoroSpot plates precoated with monocolonal anti–IFN-γ and anti–IL-2 antibodies together with the S1 scanning pool containing 16 peptides (#3629-1, Mabtech AB) or the S N M O–defined peptide pool containing 47 synthetic peptides binding to human HLA (#3620-1, Mabtech AB) and 100 ng/mL anti-CD28. The plates were incubated overnight and developed the following day according to the manufacturer’s protocol. ELISpot and FluoroSpot images and spot counts were obtained using an IRIS plate reader. The results are expressed as number of spots per 300,000 cells, after subtracting the background. The cutoff value was set at the highest number of spots detected in 11 prepan-demic PBMC control samples.

REFERENCES

E1. Wang G, Hallberg J, Bergström PU, Janson C, Pershagen G, Gruzieva O, et al. Assessment of chronic bronchitis and risk factors in young adults: results from BAMSE. Eur Respir J 2021;57:2002120.
E2. Ekström S, Andersson N, Lövquist A, Lauber A, Georgelis A, Kull I, et al. COVID-19 among young adults in Sweden: self-reported long-term symptoms and associated factors. Scand J Public Health 2021;14034948211025425.
E3. Melin E, Bergström A, Kull I, Almqvist C, Andersson N, Asarnoj A, et al. Male sex is strongly associated with IgE-sensitization to airborne but not food allergens: results up to age 24 years from the BAMSE birth cohort. Clin Transl Allergy 2020;10:15.
E4. Sherina N, Piralla A, Du L, Wan H, Kumagai-Braesch M, Andrell J, et al. Persistence of SARS-CoV-2–specific B and T cell responses in convalescent COVID-19 patients 6-8 months after the infection. Med (N Y) 2021;2:281-95.e4.
FIG E1. Flowchart of the study protocol. From the BAMSE original cohort (n=4089), we invited all subjects who participated in the clinical phase of the 24-year follow-up to the COVID-19 follow-up (n=2270). Of these, 1644 participants answered the phase 1 web-based questionnaire and were then invited to the clinical examination. Of the 1026 participants who attended the COVID-19 clinical examination, 980 subjects constituted the main study population, whereas 32 vaccinated subjects were grouped for subanalysis. Fourteen subjects were excluded after the clinical examination as a result of lack of or insufficient sample material.
FIG E2. Polar bar plot of SARS-CoV-2–specific adaptive immune responses for subjects with available data on all 3 arms of adaptive immunity (virus-specific antibodies, memory B and T cells). From top in a clockwise direction, the bars indicate the serum anti-RBD IgA, IgG, and IgM titers (red bar), the number of RBD-specific memory B cells (green bar), and the number of T cells (blue bar) specific for the virus protein–derived peptide pools S1 and S N M O that produce IFN-γ, IL-2, or both (Dual). The axis was scaled from 0 to 1 using minimum and maximum log-normalized values. The headers shown in the center of each plot are unrelated to the study participants’ identification numbers used within the BAMSE cohort and cannot be connected to any individual.
FIG E3. Correlation of antibody titer or cellular responses with the time in months that elapsed since presumed COVID-19 disease. (A) Correlation between the number of months that elapsed between self-reported positive PCR test or antibody test to clinical examination and the titers of anti–SARS-CoV-2 IgM, IgA, or IgG. Gray circles indicate PCR test; purple circles, antibody test. (B-D) Correlation between the number of months that elapsed since presumed COVID-19 disease among seropositive subjects based on self-reported positive PCR test (gray circles) or antibody test (pink circles) and the numbers of RBD-specific IgG⁺ B cells (A), the numbers of S1-specific IFN-γ⁺ or IL-2⁺ T cells (B), and the numbers of S N M O-specific IFN-γ⁺ or IL-2⁺ T cells (C). Spearman rank correlation was used for statistical analysis. Green lines represent assay cutoff values.
| Characteristic                                                                 | Variable                                                                 | IgG⁺ | %     | IgM⁺ IgA⁻ IgG⁻ | No. | %     | P value* |
|--------------------------------------------------------------------------------|--------------------------------------------------------------------------|------|--------|-----------------|-----|--------|----------|
| Any COVID-19 related symptoms between Feb 20 and June-21 | Yes                                                                      | 196  | 89.5   | 35              | 68.6|        | <.0001   |
|                                                                                   | No                                                                       | 23   | 10.5   | 16              |     | 31.4   |          |
| Disease symptoms experienced more than once between Feb 20 and June-21          | More than once                                                           | 64   | 32.7   | 14              | 40.0|        | NS       |
|                                                                                   | Once                                                                     | 132  | 67.3   | 21              | 60.0|        |          |
| Total number of occasions with symptoms                                           |                                                                          | 260  | 49     | NA              |     |        |          |
| Bedbound during disease                                                          | Yes, ≥7 days                                                             | 23   | 8.8    | 1               | 2.1 |        | NS       |
|                                                                                   | Yes, 1-6 days                                                            | 114  | 43.8   | 21              | 43.8|        |          |
|                                                                                   | No                                                                       | 123  | 47.3   | 26              | 54.2|        |          |
| Hospitalization                                                                 | Yes                                                                       | 3    | 1.2    | 0               | 0.0 |        | NS       |
|                                                                                   | No                                                                       | 257  | 98.8   | 48              | 100.0|       |          |
| When were symptoms experienced?                                                  | Feb 20                                                                   | 12   | 4.7    | 1               | 2.1 |        | <.0001   |
|                                                                                   | Mar 20                                                                   | 47   | 18.2   | 7               | 14.6|        |          |
|                                                                                   | Apr 20                                                                   | 31   | 12.0   | 6               | 12.5|        |          |
|                                                                                   | May 20                                                                   | 20   | 7.8    | 3               | 6.3 |        |          |
|                                                                                   | Jun 20                                                                   | 13   | 5.0    | 3               | 6.3 |        |          |
|                                                                                   | Jul 20                                                                   | 4    | 1.6    | 3               | 6.3 |        |          |
|                                                                                   | Aug 20                                                                   | 4    | 1.6    | 3               | 6.3 |        |          |
|                                                                                   | Sep 20                                                                   | 15   | 5.8    | 3               | 6.3 |        |          |
|                                                                                   | Oct 20                                                                   | 20   | 7.8    | 10              | 20.8|        |          |
|                                                                                   | Nov 20                                                                   | 31   | 12.0   | 0               | 0.0 |        |          |
|                                                                                   | Dec 20                                                                   | 22   | 8.5    | 1               | 2.1 |        |          |
|                                                                                   | Jan-21                                                                   | 10   | 3.9    | 1               | 2.1 |        |          |
|                                                                                   | Feb-21                                                                   | 7    | 2.7    | 0               | 0.0 |        |          |
|                                                                                   | Mar-21                                                                   | 13   | 5.0    | 4               | 8.3 |        |          |
|                                                                                   | Apr-21                                                                   | 6    | 2.3    | 3               | 6.3 |        |          |
|                                                                                   | May-21                                                                   | 3    | 1.2    | 0               | 0.0 |        |          |
| Duration of symptoms                                                             | <1 week                                                                  | 60   | 23.1   | 21              | 43.8|        | NS       |
|                                                                                   | 1 week                                                                   | 75   | 28.8   | 12              | 25.0|        |          |
|                                                                                   | 2 weeks                                                                  | 73   | 28.1   | 10              | 20.8|        |          |
|                                                                                   | 3 weeks                                                                  | 24   | 9.2    | 2               | 4.2 |        |          |
|                                                                                   | ≥4 weeks                                                                 | 28   | 10.8   | 3               | 6.3 |        |          |

NA, Not applicable.

*Chi-square test or Fisher exact test.
TABLE E2. Prevalence of symptoms for all disease occasions reported by SARS-CoV-2 seropositive and seronegative subjects

| Characteristic | Variable | Seropositive | | Seronegative | | P value* |
|----------------|----------|--------------|-------|--------------|-------|-----------|
|                |          | No. | %    | No. | %    |           |
| Reduced taste or smell | Yes | 169 | 56.0 | 118 | 22.1 | <.0001 |
|                | No       | 133 | 44.0 | 416 | 77.9 |           |
| Sore throat | Yes | 202 | 65.4 | 440 | 80.0 | <.0001 |
|                | No | 107 | 34.6 | 110 | 20.0 |           |
| Fever | Yes | 200 | 65.4 | 279 | 52.0 | <.001 |
|                | No | 106 | 34.6 | 258 | 48.0 |           |
| Pain in bone or muscles | Yes | 164 | 53.4 | 225 | 41.6 | .001 |
|                | No | 143 | 46.6 | 316 | 58.4 |           |
| Body fatigue | Yes | 205 | 66.3 | 330 | 60.1 | .078 |
|                | No | 104 | 33.7 | 219 | 39.9 |           |
| Runny nose | Yes | 234 | 75.5 | 442 | 80.7 | .082 |
|                | No | 76  | 24.5 | 106 | 19.3 |           |
| Tiredness | Yes | 262 | 85.6 | 453 | 82.1 | NS |
|                | No | 44  | 14.4 | 99  | 17.9 |           |
| Cough | Yes | 204 | 65.6 | 338 | 61.6 | NS |
|                | No | 107 | 34.4 | 211 | 38.4 |           |
| Blocked nose | Yes | 216 | 69.7 | 370 | 68.1 | NS |
|                | No | 94  | 30.3 | 173 | 31.9 |           |
| Headache | Yes | 235 | 75.8 | 417 | 76.1 | NS |
|                | No | 75  | 24.2 | 131 | 23.9 |           |
| Abdominal symptoms: | Yes | 87 | 28.3 | 164 | 30.3 | NS |
| stomach pain, nausea, |                    |     |     |     |     |           |
| vomiting, diarrhea | No | 220 | 71.7 | 378 | 69.7 |           |
| Breathing difficulties | Yes | 98 | 31.9 | 158 | 28.7 | NS |
|                | No | 209 | 68.1 | 392 | 71.3 |           |
| Other symptoms | Yes | 22 | 7.9 | 26 | 5.1 | NS |
|                | No | 257 | 92.1 | 484 | 94.9 |           |

*Chi-square test or Fisher exact test.
TABLE E3. Symptom rating for all disease occasions reported by SARS-CoV-2 seropositive and seronegative subjects

| Symptom                  | Rating                | Seropositive |      | Seronegative |      | P value* |
|--------------------------|-----------------------|--------------|------|--------------|------|----------|
|                          | No.       | %            | No.  | %            |      |          |
| Reduced taste or smell   | Yes, very much | 102          | 33.8 | 43           | 8.1  | <.0001   |
|                          | Yes, pretty much    | 24           | 8.0  | 23           | 4.3  |          |
|                          | Yes, a little       | 43           | 14.2 | 52           | 9.7  |          |
|                          | No                    | 133          | 44.0 | 416          | 77.9 |          |
| Sore throat              | Yes, very much       | 23           | 7.4  | 63           | 11.5 | <.0001   |
|                          | Yes, pretty much     | 72           | 23.3 | 157          | 28.5 |          |
|                          | Yes, a little        | 107          | 34.6 | 220          | 40.0 |          |
|                          | No                    | 107          | 34.6 | 110          | 20.0 |          |
| Fever                    | Yes, very much       | 20           | 6.5  | 32           | 6.0  | .002     |
|                          | Yes, pretty much     | 53           | 17.3 | 79           | 14.7 |          |
|                          | Yes, a little        | 127          | 41.5 | 168          | 31.3 |          |
|                          | No                    | 106          | 34.6 | 258          | 48.0 |          |
| Pain in bone or muscles  | Yes, very much       | 37           | 12.1 | 32           | 5.9  | .001     |
|                          | Yes, pretty much     | 63           | 20.5 | 79           | 14.6 |          |
|                          | Yes, a little        | 64           | 20.8 | 114          | 21.1 |          |
|                          | No                    | 143          | 46.6 | 316          | 58.4 |          |
| Body fatigue             | Yes, very much       | 53           | 17.2 | 61           | 11.1 | .005     |
|                          | Yes, pretty much     | 72           | 23.3 | 96           | 17.5 |          |
|                          | Yes, a little        | 80           | 25.9 | 173          | 31.5 |          |
|                          | No                    | 104          | 33.7 | 219          | 39.9 |          |
| Runny nose               | Yes, very much       | 31           | 10.0 | 69           | 12.6 | NS       |
|                          | Yes, pretty much     | 78           | 25.2 | 162          | 29.6 |          |
|                          | Yes, a little        | 125          | 40.3 | 211          | 38.5 |          |
|                          | No                    | 76           | 24.5 | 106          | 19.3 |          |

*Chi-square test or Fisher exact test.
TABLE E4. Characterization of 10 seronegative subjects with SARS-CoV-2 memory B- and T-cell responses

| Characteristic                             | 1A  | 2B  | 3C  | 4D  | 5E  | 6F  | 7G  | 8H  | 9I  | 10J |
|-------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **Cellular memory immunity**              |     |     |     |     |     |     |     |     |     |     |
| Memory B-cell response                    | No  | Yes | No  | No  | No  | No  | No  | No  | Yes | No  |
| Memory T-cell response*                   | Yes | No  | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| **Antibody titers** (cutoff/borderline/median) |     |     |     |     |     |     |     |     |     |     |
| IgG (25.1/22.5/6.5 AU/mL)                | 9.7 | 6.6 | 8.6 | 7.5 | 3.1 | 11.5| 9.2 | 16.2| 23.2| 9.6 |
| IgM (14.4/7.8/3.0 AU/mL)                 | 3.7 | 7.0 | 4.3 | 4.6 | 2.5 | 6.5 | 2.2 | 11.2| 10.5| 3.7 |
| IgA (2.6/1.4/0.1 AU/mL)                  | 0   | 0   | 2.1 | 0   | 0   | 0   | 1.1 | 0.5 | 2.1 | 0.0 |
| **Symptoms**                             |     |     |     |     |     |     |     |     |     |     |
| Any symptoms Feb 20 to Jan 21             | Yes | No  | Yes | No  | No  | Yes | Yes | Yes | Yes | No  |
| Time from symptoms to clinical visit§     | 0   | NA  | 0   | NA  | NA  | 2   | 8   | 10  | 1   | NA  |
| **Other factors**                         |     |     |     |     |     |     |     |     |     |     |
| Self-reported positive PCR test           | No  | No  | No  | No  | No  | No  | No  | No  | Yes | No  |
| COVID-19 in household||||| | No  | No  | Yes | No  | Yes | No  | Yes |

Participant cohort identification numbers are renamed by 1 number and 1 letter that cannot be connected to original identification numbers. NA, Not applicable.

*Positive in at least 1 T-cell assay.
†Bordeline positive antibody titer.
‡Titer exceeding median antibody titer value in seronegative group.
§Time in months between latest episode of reported symptoms and clinical visit.
||Suspected or confirmed COVID-19 disease in household.