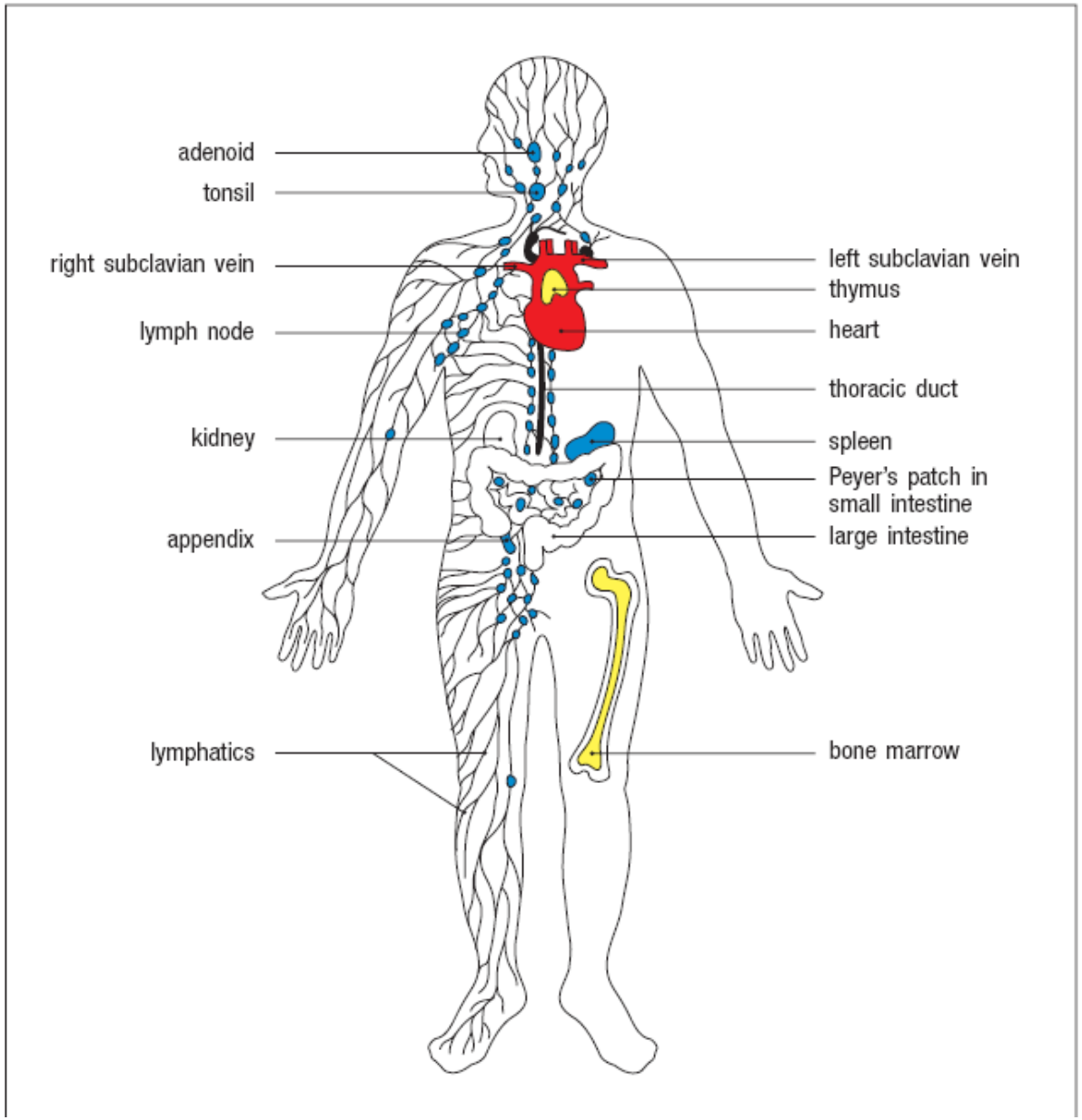
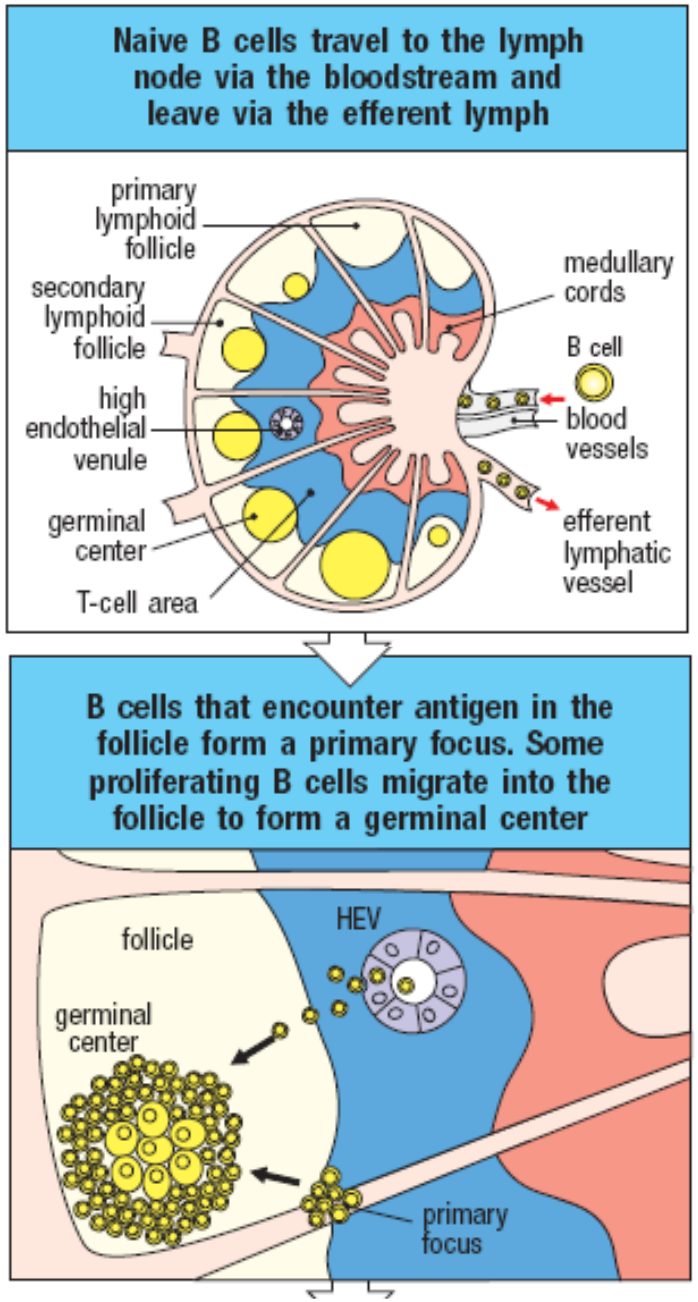
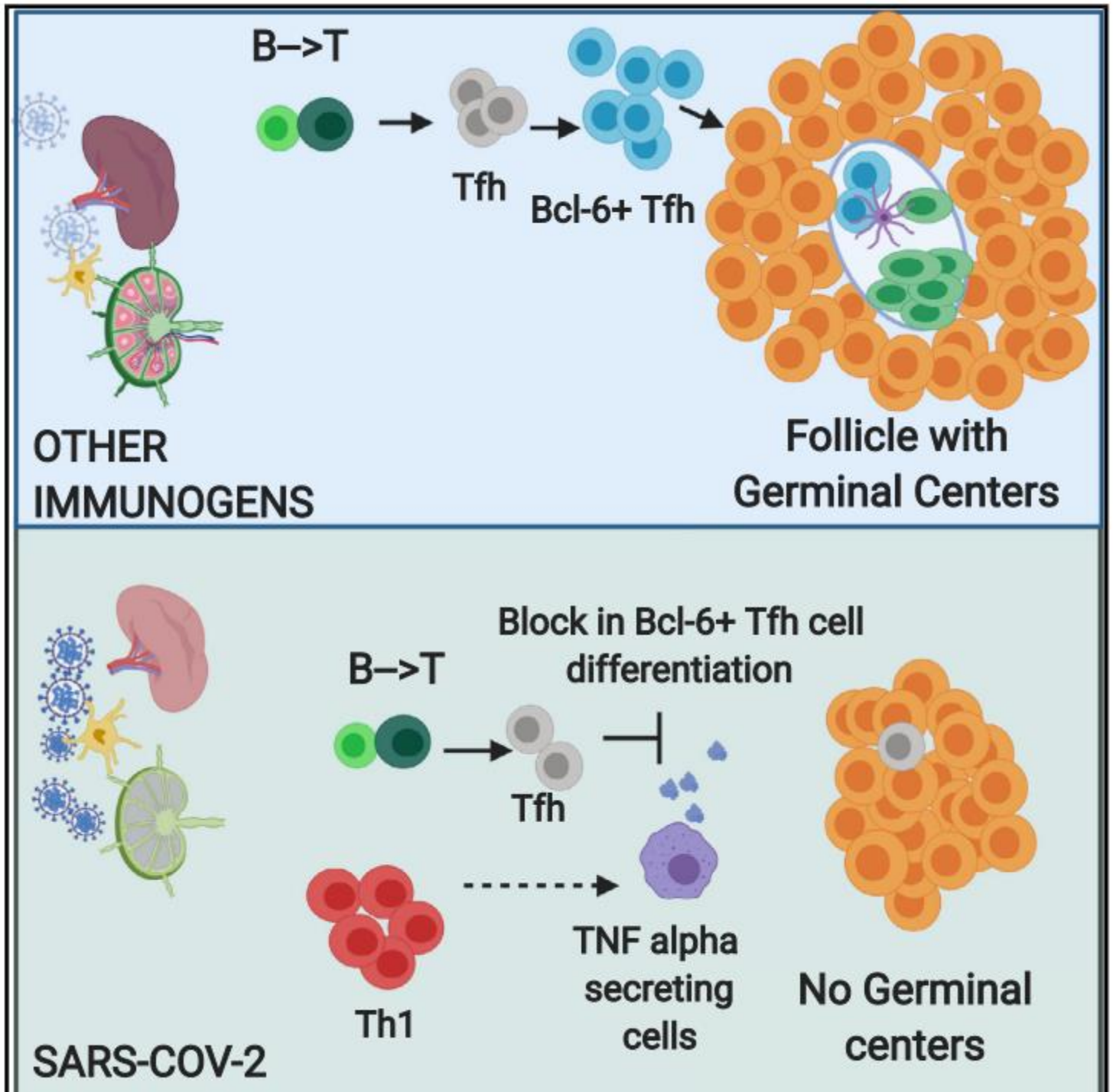
Cell

Article

Loss of Bcl-6-Expressing T Follicular Helper Cells and Germinal Centers in COVID-19


adenoid
tonsil
right subclavian vein
lymph node
kidney
appendix
lymphatics
left subclavian vein
thymus
heart
thoracic duct
spleen
Peyer's patch in small intestine
large intestine
bone marrow


Naive B cells travel to the lymph node via the bloodstream and leave via the efferent lymph
primary lymphoid follicle
secondary lymphoid follicle
high endothelial venule
germinal center
T-cell area
medullary cords
B cell
blood vessels
efferent lymphatic vessel
B cells that encounter antigen in the follicle form a primary focus. Some proliferating B cells migrate into the follicle to form a germinal center
follicle
HEV
germinal center
primary focus



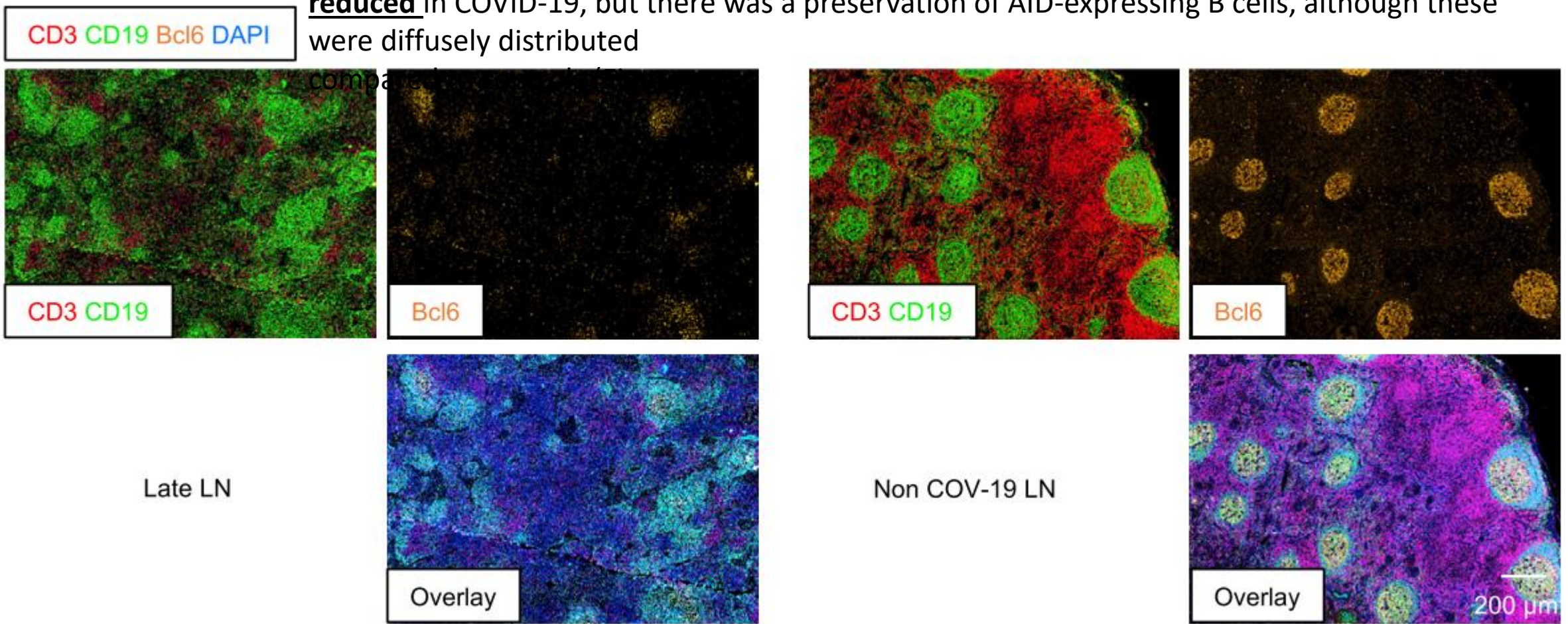
Our results identify a **striking absence of lymph node and splenic germinal centers** and Bcl-6-expressing B cells, **defective Bcl-6+ T follicular helper cell generation and differentiation, and dysregulated**

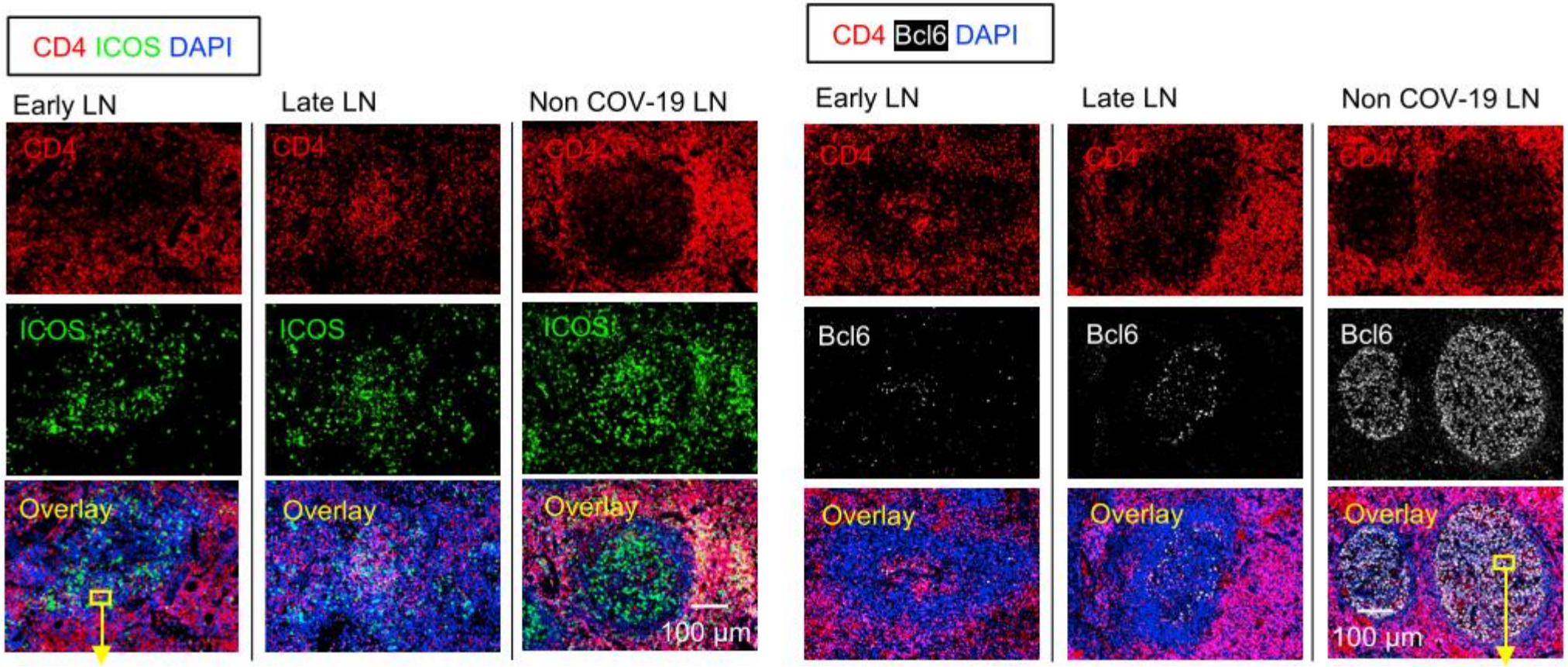
SARS-CoV-2-specific humoral immunity early in COVID-19 disease, providing a mechanistic explanation for the **limited durability of humoral immunity and the less robust somatic hypermutation** seen in this disease following natural infection.

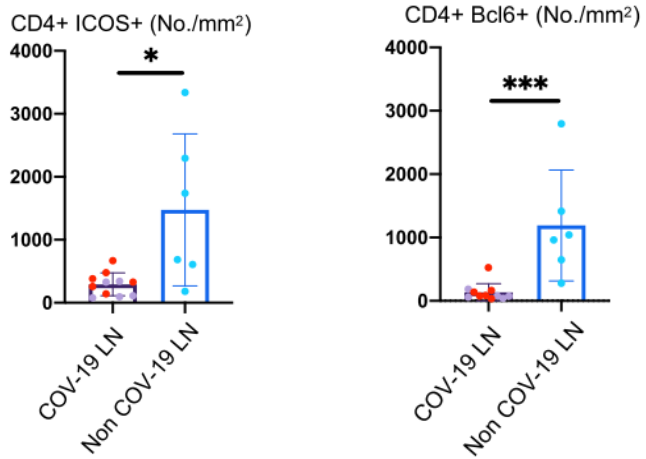
However, **numerous viral and non-viral infections** do give rise to **cytokine storm**, acute respiratory distress, and **lymphopenia** (Tisoncik et al., 2012**). Splenic white pulp atrophy** has also been histopathologically demonstrated in **Ebola and Marburg** disease (Martines et al., 2015; Rippey et. al., 1984) and in **H5N1 influenza**

Thoracic lymph nodes in **severely ill COVID-19 patients who succumbed in less than 8 days after admission** (the

Bcl-6-expressing germinal center B cells were also markedly reduced in COVID-19, but there was a preservation of AID-expressing B cells, although these were diffusely distributed







In both the lymph nodes and spleen, in early as well as late disease, **CD4+ ICOS+ TFH cells were diminished** (Figures 3A, 3C, 3E, and 3G) and CD4+ CXCR5+ TFH cells were present but reduced in numbers (Figure S2), but the **decrease in CD4+ Bcl-6+ germinal center type TFH (GC-TFH) cells was striking** (Figures 3B, 3D, 3F, and 3H). Tissue

These data indicate that the **defect in GC-type TFH cell differentiation is specific and suggest that this defect may be indirectly linked to the strong TH1 response** seen in this disease

The **preservation in COVID-19 of AID+ B cells** and the relatively large proportions of CD4+ CXCR5+ TFH cells (that do not express Bcl-6) and TH1 cells, both known to express CD40L, led us to hypothesize that,even though there **were no germinal centers**, there may be **frequent T-B conjugates in COVID-19 within follicles as well as in extra-follicular locations**.

Based on our findings, we **suspect** that **the very high local levels of TNF-a and possibly other cytokines** at this location in COVID-19 lymph nodes, possibly **induced downstream of TH1 cell activation, block the final step in T follicular helper cell differentiation**

The **absence of Bcl-6+ T follicular helper cells** (and the consequent absence of germinal centers) in COVID-19 secondary lymphoid organs provides an explanation for a phenomenon anecdotally observed in autopsies of **many different severe viral infections**. These findings also provide a mecha-nistic basis for the recent descriptions of non-durable humoral immune responses, impaired humoral immunity, and the **low levels of somatic hypermutation in antibodies from convales-cent COVID-19 patients**

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Humoral Immune Response to SARS-CoV-2 in Iceland

We **measured antibodies in serum samples from 30,576 persons** in Iceland, using six assays

We tested 2102 samples collected from 1237 persons up to 4 months after diagnosis by a quantitative polymerase-chain-reaction (qPCR) assay

Of the 1797 persons who had recovered from SARS-CoV-2 infection**, 1107 of the 1215 who were tested (91.1%) were seropositive**; antiviral antibody **titers** assayed by two pan-Ig assays **increased during 2 months after diagnosis by qPCR and remained on a plateau** for the remainder of the study. Of

Our results indicate that **antiviral antibodies** against **SARS-CoV-2 did not decline within 4 months after diagnosis**.

Seroconversion of most patients with Covid-19 occurs **between 7 and 14 days after diagnosis**.

invited all qPCR- positive persons who had recovered from infection to donate samples, both shortly after recovery and again approximately 3 months after recovery

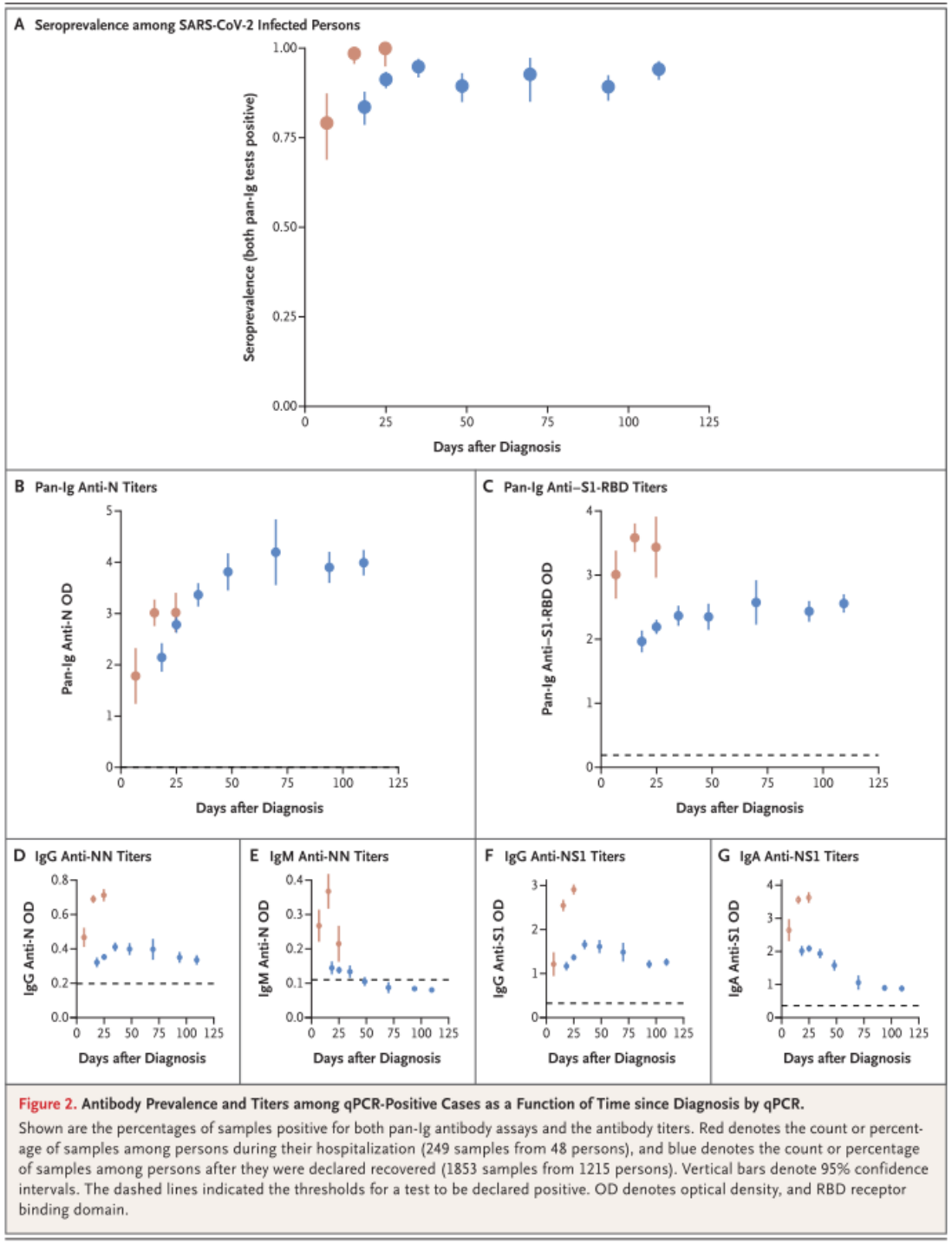


Figure 2. Antibody Prevalence and Titers among qPCR-Positive Cases as a Function of Time since Diagnosis by qPCR.

Shown are the percentages of samples positive for both pan-Ig antibody assays and the antibody titers. Red denotes the count or percentage of samples among persons during their hospitalization (249 samples from 48 persons), and blue denotes the count or percentage of samples among persons after they were declared recovered (1853 samples from 1215 persons). Vertical bars denote 95% confidence intervals. The dashed lines indicated the thresholds for a test to be declared positive. OD denotes optical density, and RBD receptor binding domain.

Since some diagnoses may have been made on the basis of false positive qPCR results, we determined that 91.1% represents the lower bound of sensitivity of the combined pan-Ig tests for the detection of SARS-CoV-2 antibodies among recovered persons.

Antibody levels measured with both **pan-Ig antibody assays increased over the first 2 months** after qPCR diagnosis and **remained at a plateau over the next 2 months** of the study. **IgM anti-N antibody levels increased rapidly soon after diagnosis and then fell rapidly** and were generally **not detected after 2 months.** IgA anti- S1 antibodies decreased 1 month after diagnosis and remained detectable thereafter. **IgG anti-N and anti-S1** antibody levels **increased during the first 6 weeks** after diagnosis and **then decreased slightly**.

SARS-CoV-2

We estimate that during the first wave of the SARS-CoV-2 pandemic, **the incidence of infection in Iceland was 0.9% (95% CI, 0.8 to 0.9) and the infection fatality risk was 0.3%** (95% CI, 0.2)

Previous smaller studies reported reduction of IgG antibodies against the N protein and a peptide representing the S protein within 21 to 28 days and against trimeric S protein within 56 days after a positive test by qPCR. These discrepancies may be explained partly by differences in the **specificity and sensi- tivity of the assays used as well as differences** in the design and performance of the semiquanti- tative assays used, including the antigen target- ed and the analytic sensitivity and range, as well as **differences in the study populations**. For ex- ample, **because of widespread qPCR testing and screening, it is likely that the Icelandic qPCR- positive persons were healthy, as compared with the participants in other studies**.

22 recovered persons who had a negative result (using the combined pan-Ig antibody tests) for an early sample and who had another sample tested at least a month later, 19 (86%) **received a second negative result**. Thus, **either some persons** infected by SARS-CoV-2 produce **no antibodies or undetectable levels of antibodies** reactive to the S1 and N proteins, even 3 months after infection, **or some qPCR delivered false positive results**.

Among recovered persons**, antibody levels are higher in older persons and in those more severely affected by SARS-CoV-2 infection**