Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Beta variant of concern ( VOC) elicits neutralization by major classes of antibodies from COVID-19 patients and vaccinated individuals. In this study, serum of Beta-infected patients revealed reduced cross-neutralization of wild-type virus. From these patients, we isolated Beta-specific and cross-reactive receptor-binding domain (RBD) antibodies. The Beta-specificity results from recruitment of VOC-specific clonotypes and accommodation of mutations present in Beta and Omicron into a major antibody class that is normally sensitive to these mutations. The Beta-elicited cross-reactive antibodies share genetic and structural features with wild type–elicited antibodies, including a public VH1-58 clonotype that targets the RBD ridge. These findings advance our understanding of the antibody response to SARS-CoV-2 shaped by antigenic drift, with implications for design of next-generation vaccines and therapeutics.
immune responses against wild-type RBD in convalescent and vaccinated individuals (2), in which neutralization of SARS-CoV-2 Beta was ~8- to ~14-fold reduced compared with wild-type virus (1–6). No positive correlation was found between neutralizing antibodies against Beta and the time point of sample collection relative to first positive PCR test (fig. S1G). Neutralizing antibodies against Beta modestly correlated with age (fig. S1H), but no statistically significant gender difference was observed (fig. S1). Collectively, these data show that sera from Beta-infected patients exhibit reduced cross-reactivity to wild-type SARS-CoV-2, therefore affecting diagnostic antibody testing when using wild-type antigens and adding complexity to the concept of defining a threshold for protective antibody titers.

To investigate the effect of this difference in reactivity between RBD Beta and wild-type RBD at the level of mAbs elicited by SARS-CoV-2 Beta infection, we isolated CD19+CD27+ memory B cells from the peripheral blood of 12 donors in our cohort by means of fluorescence-activated cell sorting using a recombinant RBD Beta probe (fig. S2, A and B). Using single-cell Ig gene sequencing (15, 16), we derived 289 pairs of functional heavy (IGH) and light (IGL) chain sequences from IgG mAbs (table S2). Sequence analysis showed enrichment of certain genes compared with mAbs derived from healthy, noninfected individuals—including VH-β-58, VH-β-30, VH-β-48, and VH-β-33—illustrating a preferential recruitment of certain VH genes (Fig. 2A), IGH-JH gene combinations (fig. S3A), and variable light chain genes (fig. S3B). For some genes such as VH-β-58 and VH-β-33, enrichment has previously been identified in CoV-AbDab, a database of published SARS-CoV-2 mAbs (9, 17). We confirmed this finding for all human wild-type RBD mAbs in CoV-AbDab (Fig. 2A). Consistent with reports from wild-type SARS-CoV-2 infections (18–20), the somatic hypermutation (SHM) count was generally low in mAbs of our cohort (fig. S3C). Together, these findings argue for conservation of certain antibody sequence features between antibody responses in different donors and between antibody responses elicited against Beta and wild-type virus. Hence, we compared antibody sequences after Beta infection with all previously published wild-type RBD mAbs and identified several clonotypes shared between both datasets (Fig. 2B), some of which were present in multiple patients of our study (Fig. 2C). Thus, a subset of the antibodies to RBD Beta and wild-type RBD converge upon recruitment of specific germline genes.

However, other gene enrichments found in our study, such as VH-β-39, have not been identified within the CoV-AbDab mAbs (Fig. 2A) (9), exemplifying concurrent divergence in the antibody response to the different RBDS. VH-1-2, one of the most common genes contributing to the RBD antibody response to wild-type SARS-CoV-2, was strongly reduced in our dataset (Fig. 2A and table S2), which is consistent with the dependence of VH-1-2 mAbs on E484 (9). VH-β-53/VH-β-66 antibodies bind to wild-type RBD in two canonical binding modes, which involve residues K417 and E484, respectively; binding and neutralization of these antibodies are strongly affected by the K417N and E484K mutations in RBD Beta (9, 21). We therefore hypothesized a similarly reduced recruitment of VH-β-53/VH-β-66 mAbs after Beta infection. Unexpectedly, we identified 15 VH-β-53/VH-β-66 mAbs, albeit at a reduced frequency compared with that of the CoV-AbDab dataset (4.7 versus 19.4%), but still at an increased frequency compared with that of healthy donors (Fig. 2A), thus indicating either a noncanonical binding mode or accommodation of these mutations into the known binding modes.

To determine the binding properties of antibodies elicited by SARS-CoV-2 Beta, we selected representative mAbs for expression (table S2). We identified 81 mAbs with strong binding to RBD Beta (table S3). Of those, 44 revealed comparable binding to wild-type RBD and were considered cross-reactive mAbs, whereas 37 mAbs did not bind wild-type RBD and were considered Beta-specific. There were no differences in V gene SHMs, CDR H3/L3 hydrophobicity, and ACE2-binding inhibition between Beta-specific and cross-reactive antibodies (fig. S4, A to C), but the cross-reactive antibodies had a slightly shorter CDR H3/L3 and lower isoelectric point of their CDR H3 (fig. S4, D and E). The neutralization potencies were similar between Beta-specific and cross-reactive mAbs (fig. S4F). All Beta-specific VH-β-30 mAbs paired with JH-6 (fig. S4G), whereas all cross-reactive VH-β-30 mAbs paired with JH-4 (fig. S4H). Competition experiments showed that many of the strongly neutralizing Beta-elicited mAbs compete for RBD binding (fig. S4I), indicating that they target similar epitopes.

Next, we aimed to determine the residues that define the binding selectivity for the 37 RBD Beta-specific mAbs and performed ELISAs with single-mutant constructs of RBD Beta and wild-type RBD. For all three Beta-defining RBD mutations (K417N, E484K, and N501Y), we identified mAbs with RBD binding that depended on a single residue. The Beta-specificity of the other mAbs was dependent on multiple residues (Fig. 3A). RBD Beta–specific mAbs were encoded by diverse VH genes (Fig. 3A and table S2), and 26 of the RBD Beta-specific mAbs (70.3%) neutralized the authentic SARS-CoV-2 Beta isolate (Fig. 3A). All nine Beta-specific VH-4-39 mAbs from three different patients were Y501-dependent, comprising 81.8% of all Y501-dependent mAbs. This finding suggests a common mode of binding these clonally unrelated mAbs that depends on Y501—which is a residue present in RBD Beta, Alpha, Gamma, and Omicron but not Delta—and may explain the frequent use of VH-4-39 in mAbs to RBD Beta (Fig. 2A). VH-4-39 Y501–dependent mAbs revealed few SHMs in VH genes but no uniform pattern in other sequence features (fig. S5A). Although all VH-4-39 RBD Beta-specific mAbs bind to a Y501-dependent epitope, their neutralization activity showed noticeable differences (IC_{50} ranging from 5.2 to 947 ng/ml) (fig. S5B). Surface plasmon resonance measurements of these mAbs to RBD Beta revealed equilibrium dissociation constants (K_{D}) between 3.39 and 80.4 nM (fig. SSC) with correlation to their PRNT-derived activity.

**Fig. 1. Authentic virus neutralization of sera from individuals after infection with SARS-CoV-2 Beta.** (A and B) Neutralizing activity of sera of patients infected with SARS-CoV-2 Beta variant was measured by using a plaque-reduction neutralization assay with the indicated authentic virus. Results are given as reduction of plaque number at indicated serum dilutions. Patients SA1 and SA2 mounted the strongest antibody response, which are highlighted in red and blue, respectively. Means of duplicate measurements are shown. Values below zero indicate no plaque reduction. (C) Change in neutralization activity against SARS-CoV-2 Beta and wild-type SARS-CoV-2 based on area-under-the-curve (AUC) calculations from authentic virus PRNT curves [shown in (A) and (B)]. Mean fold change is indicated above the P value. Statistical analysis was performed by using a Wilcoxon matched-pairs signed-rank test with two-tailed P value.
**Fig. 2. Germline gene usage and clonotype analysis of Beta-elicited antibodies.** (A) VH gene usage of 289 RBD Beta IgG mAbs from this study (red) is compared with 1037 wild-type RBD mAbs from 96 previously published studies (blue, CoV-AbDab) (17). Frequencies of mAbs encoded by each VH gene are shown as bars. Enrichment of indicated VH genes is compared with that of healthy individuals (3I), with fold-enrichment shown as number next to bars. VH gene frequencies that were not reported in healthy individuals (3I) are indicated with an asterisk. Only VH genes with a frequency of at least 2% in CoV-AbDab are shown, and VH genes are ordered by frequency in CoV-AbDab. (B) Circos plot shows the relationship between 289 IgG mAbs from this study (Beta) and 1037 previously published human mAbs reactive to wild-type RBD (CoV-AbDab) from 96 studies (17). Interconnecting lines display clonotypes shared between both datasets, as defined by the usage of the same V and J gene on both heavy and light chain. Thin black lines at the outer circle border indicate expanded clonotypes within the respective data set. (C) Circos plot displaying the 289 IgG mAbs from this study grouped per patient. Interconnecting colored lines indicate clonotypes found in more than one patient. Small black at the outer circle border indicate clonally expanded clones within one patient. In (B) and (C), colored interconnecting lines depict clonotypes found in more than one patient of our cohort.
constructs of VOCs Alpha through Delta and SARS-CoV. Whereas only two mAbs (10%) strongly detected SARS-CoV RBD, the majority of cross-reactive antibodies bound the RBD of Alpha, Gamma, and Delta (Fig. 4A). In PRNT assays with further authentic virus isolates, 15 (75%) Beta-neutralizing cross-reactive mAbs also neutralized wild-type virus, and 14 (70%) neutralized a Delta virus isolate, which of the VOCs was the most antigenically distinct from the others at the time of testing (Fig. 4A). Six cross-neutralizing antibodies were encoded by VH1-58 (Fig. 4A). VH1-58 is the most enriched germline VH gene in RBD antibodies in both Beta and wild-type infection (Fig. 2A) (26). VH1-58 RBD antibodies almost exclusively pair with JH3 (Fig. S3A). This VH1-58/JH3/VK3-20/JK1 clonotype has been described in individuals infected with wild-type virus (20, 26) and found in several patients within our cohort (Fig. 2C and Table S6), representing 2.4% of all Beta-elicited IgG mAbs analyzed in this study (Table S2).

To elucidate the structural basis of this public broadly reactive clonotype, we determined crystal structures of CS44 and CV07-287, a mAb of the same clonotype that was isolated from a wild-type–infected individual (19), in complex with RBD Beta and wild-type RBD, respectively (Fig. 4B). We compared the structures of CS44 and CV07-287 with other published VH1-58 antibodies including COVOX-253 (27), SZE12 (28), A23-58.1, and B1-182.1 (26). These antibodies all target the RBD in the same binding mode (Fig. 4B), which suggests that this public clonotype is structurally conserved. The dominant interaction of VH1-58 antibodies is with the RBD ridge region (residues 471 to 491), which accounts for ~75% of the entire epitope surface. Most of the VOC mutations occur outside of the ridge region (for example, residues 417, 452, and 501) and are distant from the binding sites of VH1-58 antibodies CV07-287 and CS44 (Fig. 4, C and D). T478 interacts with VH1-58 antibodies, but mutation to a lysine can be accommodated (Fig. 4A) (26). V11 W50 and Y52 in CDR H2 provide hydrophobic interactions with the RBD (Fig. 4, E and F). CDR H3 also forms extensive interactions with the RBD (Fig. 4, G and H). The CDR H3 sequences of 38 antibodies that belong to this clonotype (Fig. 4I) (77) are highly conserved, and all contain a disulfide bond between V11 C97 and C100b, with four relatively small residues (G, S, and T) in between (Fig. 4, I, G, and H). V11 D100d is also conserved (Fig. 4I), forming H-bonds with S477 and T478 (Fig. 4, G and H). In addition, the conserved V11 H95 and F100f (Fig. 4I) stack with RBD-F486 together with V11 W50, V11 Y91, and V11 W96 (Fig. 4, G and H). Although E484 is often an important residue for antibody binding on the ridge region, here it is 5 Å distant from the antibodies, and mutations at this site have not

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**Fig. 3. Binding, neutralization, and structures of Beta-specific antibodies.** (A) Neutralization of indicated Beta-specific mAbs against authentic Beta virus is shown in purple. Binding to single-point mutant RBD constructs with the indicated amino-acid residues at positions 417, 484, and 501 is shown in green, normalized to RBD Beta. (B to G) Structural comparison of VH3-53 mAbs between Beta-specific CS23 and wild-type–specific CC12.1 and CC12.3. (B) CC12.3 and CS23 adopt the same binding mode. The crystal structure of CC12.3 (pink) in complex with wild-type RBD was superimposed onto CS23 (yellow) in complex with RBD Beta. Only the variable domains of the antibodies are shown for clarity. A small local conformational difference was observed between CS23-bound RBD Beta and CC12.3-bound wild-type RBD (191 Å, root mean square deviation = 0.8 Å). [(C) and (D)] Comparison of the (C) CDR H1 (“NY” motif) and (D) CDR H2 (“SGGS” motif) between CS23 and CC12.3. [(E) to (G)] Structures of CDR H3 of (E) CC12.1, (F) CC12.3, and (G) CS23. A modeled side chain of K417 is shown as transparent pink sticks, which would be unfavorable for binding to CS23, where V11 M98 occupies this pocket. Structures of CC12.1 (PDB 6X3C, cyan), CC12.3 (PDB 6XC4, pink), and CS23 (this study, yellow) are used throughout this figure, and the RBD is shown in white. Hydrogen bonds, salt bridges, or cation–π bonds are represented with black dashed lines.
been reported as being sensitive for VH1-58 antibodies.

Thousands of anti-SARS-CoV-2 mAbs were isolated before the VOCs started to emerge (17), many of which are highly potent but with varying sensitivity to VOCs. We characterized the antibody response to the RBD after SARSCoV-2 Beta infection to provide insights into diverging and converging features of antibodies elicited by this lineage compared with wild-type–elicited antibodies. Recently, the highly mutated Omicron variant has further increased the complexity of SARS-CoV-2 cross-variant immunity and resembles Beta as an antigenically distant VOC. On the basis of their shared RBD mutations, we hypothesized that some Beta-elicited mAbs also bind Omicron. Accordingly, VH3-53 mAb CS23, which binds the shared mutated residues N447 and Y501, showed comparable binding to Omicron and Beta (Fig. 4J). By contrast, Y501-dependent VH4-39 antibodies CS43 and CS170 did not bind to Omicron, suggesting that other mutations in Omicron impede binding of this clonotype. Similarly, VH1-58 mAbs CS44 and CS102 also showed a drastic reduction in affinity to Omicron (Fig. 4J), suggesting that Omicron may not be efficiently neutralized by this public clonotype that exhibits ultra-high potency and high resistance to VOCs Alpha, Beta, Gamma, and Delta (26). These findings emphasize the antigenic complexity and high temporal dynamics that define antibody immunity against SARS-CoV-2 in the context of ongoing antigenic drift and provide insights for next-generation vaccine design and antibody therapeutics. For example, simultaneous or sequential immunization with vaccines based on diverse RBD sequences could be evaluated for superiority in induction of cross-variant immunity. Although large-scale production of novel vaccine candidates based on the Omicron sequence have been initiated, those based on the Beta sequence already show promising novel vaccine candidates based on the Omicron sequence have been initiated, those based on the Beta sequence already show promising
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SUPPLEMENTARY MATERIALS

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Materials and Methods

Figs. S1 to S6

Tables S1 to S6

References (32–45)

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SARS-CoV-2 Beta variant infection elicits potent lineage-specific and cross-reactive antibodies

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Beta variant antibody responses
Several severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern that either enhance infectivity or resist neutralization by sera from vaccinated or convalescent individuals have emerged. The variants Beta and Omicron in particular no longer bind many neutralizing antibodies that target the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein. Reincke et al. isolated antibodies from Beta-infected patients and showed that although some bound both the Beta and the wild-type RBD, others were specific for Beta. Some of the Beta antibodies had genetic characteristics similar to wild-type antibodies that were sensitive to the Beta mutations. Whereas some antibodies appeared to use a noncanonical binding mode, others accommodated Beta mutations into known binding modes. This work provides insights for the design of next-generation vaccines and antibody therapeutics. —VV

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