Development of an Effective Immune Response in Adults With Down Syndrome After Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Vaccination

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Background. Immune dysregulation in individuals with Down syndrome (DS) leads to an increased risk for hospitalization and death due to coronavirus disease 2019 (COVID-19) and may impair the generation of protective immunity after vaccine administration.

Methods. The cellular and humoral responses of 55 individuals with DS who received a complete SARS-CoV-2 vaccination regime at 1 to 3 (visit V1) and 6 (V2) months were characterized.

Results. SARS-CoV-2–reactive CD4+ and CD8+ T lymphocytes with a predominant Th1 phenotype were observed at V1 and increased at V2. Likewise, an increase in SARS-CoV-2–specific circulating Tfh (cTfh) cells and CD8+ CXCR5+ PD-1hi lymphocytes was already observed at V1 after vaccine administration. Specific immunoglobulin G (IgG) antibodies against SARS-CoV-2 S protein were detected in 96% and 98% of subjects at V1 and V2, respectively, although IgG titers decreased significantly between both time points.

Conclusions. Our findings show that DS individuals develop an effective immune response to usual regimes of SARS-CoV-2 vaccination.

Keywords. Down syndrome; COVID-19; SARS-CoV-2; vaccination; immune system.

The new coronavirus disease 2019 (COVID-19) has been a global health emergency since March 2020. Although COVID-19 entails no or mild symptoms in most individuals, several patient subgroups develop a severe form of the disease, leading to acute respiratory distress syndrome (ARDS) [1, 2]. Individuals with Down syndrome (DS), are particularly vulnerable to the disease, with a 4-fold increased risk for COVID-19–related hospitalization and a 3- to 10-fold increased risk for COVID-19–related mortality [3–5].

Although this increase can be partially attributed to a higher prevalence of comorbidities associated with a poorer COVID-19–related prognosis, such as obesity, congenital heart disease, or respiratory diseases [6, 7], the susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be further explained by immune dysregulation. Patients with DS show higher levels of interferon (IFN)-stimulated genes, which explains their higher basal levels of IFN-α signaling and hypersensitivity to IFN stimulation [8]. Indeed, 4 subunits of IFN receptors are encoded on HSA21 (IFNAR1, IFNAR2, IFNγR2, and IL10RB), and an increase in IFNAR2 expression was observed in DS donors, whereas nod-like receptor family pyrin domain containing 3 (NLRP3) downregulation and increased interleukin (IL)-10 production could be involved in the higher risk of bacterial infectious complications in these patients [10, 11]. Individuals with DS have high basal levels of proinflammatory cytokines such as IL-2, IL-6, or tumor necrosis factor α (TNF-α), which may enhance the “cytokine storm” that underlies ARDS [11]. Although the overall effect of these alterations provides some proof for the worse outcomes following...
SARS-CoV-2 infection, the net balance between all of these interplaying factors is far from clear.

This immune dysregulation may also impair the generation of protective immunity after vaccine administration in individuals. In fact, although people with DS usually develop protective antibody responses after vaccination, overall antibody levels tend to be lower and decline faster than in individuals without DS [12]. Due to their excess mortality risk and these concerns over vaccine protection, most countries have included adults with DS, especially those over 40 years, in prioritized vaccination groups [13–15]. However, data on the immune response elicited by SARS-CoV-2 vaccines in the DS population are lacking, and the degree of protection achieved is unknown. A thorough characterization of the humoral and cellular immune mechanisms underlying the response of individuals with DS to COVID-19 vaccines is needed to appropriately tailor future vaccination campaigns for this population. Moreover, this knowledge may also shed light onto general immune mechanisms involved in the response to SARS-CoV-2, and in the development of postvaccination responses.

**METHODS**

Details on the study population, determination of specific immunoglobulin G (IgG) antibodies, analysis of T-cell response, and statistical analysis are provided in the Supplementary Methods.

**Ethical Considerations**

This project was approved by the Institutional Review Board (IRB) at La Princesa University Hospital (register number: 4386), meets international standards of data protection, and is in line with practice guidelines established in the Declaration of Helsinki.

**RESULTS**

**T-Cell Response to SARS-CoV-2 Vaccination in Down Syndrome Donors**

To assess immune response to SARS-CoV-2 vaccine in DS, we recruited 55 DS, 50 non-DS A, and 39 non-DS B donors, whose demographic and clinical characteristics are shown in Table 1. They were inoculated with 2 doses of a SARS-CoV-2 vaccine following the recommended schedule (Table 1), and both T-cell and antibody responses were analyzed before vaccine administration (V0) and 1–3 months (V1) and 6 months (V2) after the second vaccination. Fifty-one subjects with DS (93%), 50 non-DS A (100%), and 39 non-DS B (100%) donors were enrolled after having received at least 1 vaccination dose. To avoid any delay in official vaccination strategies, we admitted the use of biobank-stored samples (from earlier than February 2020) as baseline (V0) in 44 subjects with DS, 48 controls from non-DS cohort A, and none from cohort B. COVID-19 infection prior to vaccination occurred in 15 individuals with DS (27%), 2

| Age, y | 44 ± 10 | 44 ± 13 |
| Sex (male) | 25 (44%) | 25 (50%) |
| Living situation | N/A | N/A |
| Degree of intellectual disability | N/A | N/A |
| Comorbidities | N/A | N/A |
| Gastrointestinal disorders | N/A | N/A |
| Obstructive sleep apnea | 19 (34%) | ... |
| Congenital heart disease | 10 (18%) | ... |
| Alzheimer disease | 11 (20%) | ... |
| Epilepsy | 2 (4%) | ... |
| Allergy | ... | ... |
| Multiple sclerosis | ... | 1 (2%) |
| Glaucoma | ... | 1 (2%) |
| Vaccine type | BNT162b2 (Comirnaty, Pfizer/BioNTech) | mRNA-1273 (Spikevax, Moderna) |
| To first vaccine dose | 6 (11%) | 7 (12.5%) |
| To second vaccine dose | 6 (11%) | 50 (100%) |
| Vaccinated in the past 12 months | ... | ... |

**Table 1. Demographic and Clinical Characteristics of the Study Sample**

Data are presented as n (%) unless otherwise indicated. Abbreviations: HT, hypertension; N/A, non-applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2;

*Only comorbidities that have been linked to a worse prognosis of SARS-CoV-2 infection or to immune dysregulation are presented.

*Noted adverse reactions were all mild (involving either local pain, fever, or malaise, lasting <24 hours).

*Other than vaccination against SARS-CoV-2 (ie, influenza or pneumococcal vaccination).
Median (interquartile range) time from the last dose of the recommended vaccination schedule to the first blood extraction (V1) was 62 (42–70) days for DS donors, 75 (43–84) days for non-DS A donors, and 98 (84–100) days for non-DS B donors. In the case of V2, median times were 159 days (150–172) for DS donors, 182 (173–190) for non-DS A donors, and 174 (161–183) for non-DS B donors.

To assess the percentage of responsive T cells, we used an activation-induced marker assay based on the detection of CD4+ OX40/CD137 double-positive and CD8+ CD69/CD137 double-positive cells after 24 hours in the presence of peptide pools from SARS-CoV-2 (NCAP, S1, S2, RBD domain, VME1, and Mpro proteins for V0 and S1, S2, and RBD domain for V1 and V2). We included a human actin-derived peptide pool as a negative control, and Staphylococcal enterotoxin B (SEB) as a positive control.

As shown in Figure 1 and Supplementary Table 1A, we found a predominant CD4+ response in DS donors (73.21% patients with specific CD4+ at V2), while the percentage of specific CD8+ lymphocytes increased from 10% at V1 to 33.33% at V2 (Supplementary Table 1B and Supplementary Figure 2A). Of note, all non-DS donors showed a specific CD4+ response at the same time point (Supplementary Table 1B and Supplementary Figure 2A). Neither the existence of a previous SARS-CoV-2 infection in DS nor the recruitment date modified cellular immune responses to vaccination (Supplementary Figure 3A and 3B). Of note, all non-DS donors showed a specific CD4+ response at V1, while the percentage of specific CD8+ lymphocytes increased from 10% at V1 to 33.33% at V2 (Supplementary Table 1B and Supplementary Figure 2A).

SARS-CoV-2 mRNA vaccines promote a T-helper (Th) 1 (Th1) reaction in the general population [16]. Individuals extracted prior to February 2020 as the baseline determination. Median (interquartile range) time from the last dose of the recommended vaccination schedule to the first blood extraction (V1) was 62 (42–70) days for DS donors, 75 (43–84) days for non-DS A donors, and 98 (84–100) days for non-DS B donors. In the case of V2, median times were 159 days (150–172) for DS donors, 182 (173–190) for non-DS A donors, and 174 (161–183) for non-DS B donors.

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After vaccination, the percentage of individuals with DS with SARS-CoV-2–responsive T lymphocytes increased from 23.8% at V1 to 73.21% at V2 for CD4+, and from 8.93% at V1 to 16.36% at V2 for CD8+. We detected vaccine-elicted specific CD4+ and CD8+ directed against S1, S2, and RBD, with a similar pattern at V1 and V2 (Figure 1A and 1B and Supplementary Table 1A). Neither the existence of a previous SARS-CoV-2 infection in DS nor the recruitment date modified cellular immune responses to vaccination (Supplementary Figure 3A and 3B). Of note, all non-DS donors showed a specific CD4+ response at V1, while the percentage of specific CD8+ lymphocytes increased from 10% at V1 to 33.33% at V2 (Supplementary Table 1B and Supplementary Figure 2A).

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SARS-CoV-2 mRNA vaccines promote a T-helper (Th) 1 (Th1) reaction in the general population [16]. Individuals
with DS present a higher Th1:Th2 ratio than euploid donors, a finding related to an increase in baseline IFN-γ levels [17]. We evaluated T-helper populations in SARS-CoV-2–specific CD4+ lymphocytes in DS donors, and found comparable percentages of Th1 and Th2 in V1. However, a significant increase in Th1 but not Th2 could be observed at V2 (P < .01). As a specificity control, we determined Th1 and Th2 distribution in SEB-stimulated CD4+ cells and found no significant differences between both subsets at either V1 or V2 (Supplementary Figure 4A). A similar pattern was detected regardless of the peptide pool (S1 or S2) analyzed (Supplementary Figure 4A). The percentage of Th1-reactive CD4+ cells was significantly higher in non-DS individuals than in DS donors both at V1 and V2, whereas we found no significant differences in Th2 response at either visit (Figure 2B). In addition, there were no gender differences in Th1 and Th2 behavior, except for a significantly higher Th2 response at V1 in male DS donors (Supplementary Figure 4B). Notably, although a Th1 increase at V2 could be observed in all individuals with DS, it was higher in individuals aged 40 years and younger (Supplementary Figure 4C).

T-follicular helper cells (Tfh) constitute a specialized subset of CD4+ T cells that collaborates in the generation of high-affinity antibodies. In vaccinated DS donors, we found a SARS-CoV-2–specific circulating Tfh (cTfh) population with sustained levels between V1 and V2 (Figure 3A). We detected similar percentages of cTfh in non-DS donors at both V1 and V2 (Figure 3B). PD-1hi cTfh cells represent a subset of recently activated cTfh cells [18]. Accordingly, we detected SARS-CoV-2–specific CD4+ CXCR5+ PD-1hi cells at V1 in DS donors, whose levels were slightly reduced at V2 (Figure 3A). Likewise, in non-DS individuals we found a CD4+ CXCR5+ PD-1hi population whose levels at V2 were significantly higher than those of individuals with DS (Figure 3B).

A population of CD8+ CXCR5+ PD-1hi cells with Tfh-like properties has been recently identified [19]. We therefore investigated the presence of SARS-CoV-2–specific CD8+ CXCR5+ PD-1hi cells in vaccinated patients with DS and found that these cells were detectable at V1, and their levels significantly increased at V2 (P < .01) (Figure 3C). Because of the reduced number of non-DS donors with SARS-CoV-2–specific CD8+ lymphocytes at V1 (Supplementary Figure 2B), we could not analyze the behavior of the CD8+ CXCR5+ PD-1hi population in this cohort. Hence, our results show that SARS-CoV-2 vaccination efficiently induces the differentiation of specific
populations in DS that may collaborate in an effective antibody response.

Humoral Response to SARS-CoV-2 Vaccination in Individuals With Down Syndrome

Individuals with DS exhibit alterations in B-lymphocyte maturation and function that may account for an impaired humoral response to vaccination. Therefore, we analyzed humoral response to SARS-CoV-2 vaccination in DS donors. No patients were positive for anti-SARS-CoV-2 IgG before vaccination; however, we found detectable titers of specific IgG in 96.4% of patients at V1 (median, 62 days). Two patients did not develop anti-S IgG antibodies at V1, one of whom showed detectable titers of IgG at V2 (median, 159 days). The percentage of positive donors was similar at V2, when detectable titers of specific IgG were observed in 98.1% of patients (2 patients with no data on humoral response at this time point) (Supplementary Figure 2B and Supplementary Table 2A). Likewise, in non-DS donors, we did not find humoral response in prepandemic samples. However, 97.4% of individuals showed detectable

Figure 3. Circulating Tfh in patients with DS after SARS-CoV-2 vaccination. A, Graphics show the percentage of circulating CD4+ CXCR5+ (left) and CD4+ CXCR5+ PD-1hi (right) cells at visits 1 (V1) and 2 (V2). Mean ± SD values are shown. B, Graphics show the percentage of CD4+ CXCR5+ (left) and CD4+ CXCR5+ PD-1high (PD-1hi) (right) cells at V1 and V2 in non-DS and DS donors. C, Graphics show the percentage of SARS-CoV-2–specific CD8+ CXCR5+ (left) and PD-1hi expression within the CD8+ CXCR5+ population (right) at V1 and V2 (n = 111). **P < .01. Abbreviations: DS, Down syndrome; ns, nonsignificant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; Tfh, T-follicular helper cells.
antibody titers at V1 (median, 98 days), which reached 100% at V2 (median, 174 days) (Supplementary Table 2B and Supplementary Figure 2B).

A significant decrease in IgG titers was found in 92.6% of individuals with DS between both time points (Figure 4A and Supplementary Table 1A). Average specific IgG titers declined from 1520.84 binding antibody units (BAU)/mL at V1 to 780.88 BAU/mL at V2. This decrease was statistically significant in individuals older than 40 years but not in those younger than 40 years of age (Figure 4B). In addition, we did not find significant differences in specific IgG titers between genders in these patients (Supplementary Figure 4D). Similar to T-cell responses, IgG titers were analogous in COVID-19–convalescent and non-convalescent DS donors, and did not vary with the date of admission in the study (Supplementary Figure 3C). In non-DS individuals, we found an average IgG titer of 483.2 BAU/mL at V1, which decreased to 282.7 BAU/mL at V2. Our data indicate that individuals with DS develop a detectable humoral response after SARS-CoV-2 vaccination, comparable to that of healthy donors.

**DISCUSSION**

In the present study we investigated the immune response to SARS-CoV-2 vaccination in adults with DS. Our results provide evidence of the presence of SARS-CoV-2–reactive CD4+ and CD8+ T lymphocytes with a predominant Th1 phenotype at V1, which increases at V2. A sustained increase in SARS-CoV-2–specific cTfh cells was observed in these individuals 1–3 months after vaccine administration. Specific IgG antibodies against the SARS-CoV-2 S protein were detected at V1, but significantly decreased at V2.

We observed S1- and S2-responsive CD4+ cells in a single individual with DS at V0 (Figure 1A). Conversely, SARS-CoV-2 S-specific CD4+ lymphocytes have been frequently detected in healthy non-DS donors, and their presence is explained by a possible cross-reaction with pathogens that contain SARS-CoV-2 homologous sequences [20]. In addition, unlike in our DS donors, NCAP-specific CD4+ T cells have also been identified in healthy non-DS individuals [21], a finding that has been attributed to former exposure to SARS-CoV-2 [22]. Because most V0 samples in our study were collected...
before the beginning of the pandemic, previous asymptomatic contact with SARS-CoV-2 can be reliably excluded. After vaccination, we detected CD4+-specific activation in a reduced percentage of individuals with DS at V1 (21.86%), which reached frequencies similar to non-DS donors at V2 (74.55%) (Figure 1 and Supplementary Figure 2A). Thus, CD4+ cell responses to SARS-CoV-2 vaccination seem to be delayed in comparison to non-DS donors, which usually reach a higher percentage of individuals with specific T-cell immunity within 4–7 weeks post–SARS-CoV-2 vaccination (Supplementary Figure 2A and reference [23]).

The Tfhl-like CD8+ CXCR5+ PD-1hi subset has been recently reported to increase in acute immunization settings and collaborate in specific antibody response [24]. An increase in this population was found after SARS-CoV-2 vaccination in individuals with DS (Figure 3B), suggesting that this cell subset could also cooperate in antibody generation after vaccine administration, although the actual functional significance of this population remains to be determined.

A reduced frequency of switched memory B cells specific to vaccine antigens has been described in DS [25]. However, the degree of humoral response in individuals with DS is highly variable among different vaccines. Thus, influenza A/H1N1, pneumococcal capsular polysaccharide, or hepatitis B virus vaccination induce a partial antibody response [26, 27], while conjugated pneumococcal or hepatitis A virus vaccines elicit adequate antibody titers. We detected specific IgG antibodies against SARS-CoV-2 S in 96% and 98% of subjects at V1 and V2, respectively (Figure 4A), indicating that an effective humoral response was indeed elicited by SARS-CoV-2 vaccination in DS. These findings are in accordance with a recent report, which shows a proper antibody response in individuals with DS that declines over time with a rate similar to that of non-DS donors (reference [28] and Figure 4). Our results show an uncoupled humoral and cellular response in these patients, with antibody titers peaking already at V1 (Figure 4A), whereas CD4+ T-cell activation peaks at V2 (Figure 1). A dissociation between both arms of adaptive immunity has been described in euploid individuals infected with SARS-CoV-2, and the development of an overt antibody response is not always accompanied by detectable T-cell–specific activation [29, 30]. This phenomenon has been attributed, among other causes, to higher levels of IP-10, PD-1 expression, or an increased presence of apoptotic T lymphocytes [31]. It is possible that an increase in the production of IP-10 due to the intrinsic activation of the IFN pathway, or in the expression of lymphocyte PD-1 [32], could participate in the uncoupling of humoral and cellular responses to SARS-CoV-2 vaccine in DS.

These findings show that an immune adaptive response is achieved in individuals with DS 3 months after SARS-CoV-2 vaccination lasting, at least, up to 6 months after vaccination. The degree of protection conveyed by these vaccination-elicited immunological changes should nonetheless be carefully interpreted. Recent reports still show an increased hazard ratio for COVID-19 hospital admission (2.55-fold increase) and mortality rate (12.7-fold increase) in patients with DS, despite vaccination with 1 or 2 doses of either ChAdOx1 nCoV-19 (AstraZeneca) or BNT162b2 (Pfizer/BioNTech) [33]. It is likely that other mechanisms beyond viral infection, and probably related to the intrinsic proinflammatory status present in patients with DS, operate to lead to a worse outcome even in vaccinated individuals. Our findings support the generalization of current recommendations to promote booster doses in the DS population. Moreover, because we observed a more robust response to vaccination in individuals with DS younger than 40 years, we believe our results lend further support to the recommendation of prioritizing the administration of an additional vaccine booster dose preferably in adults with DS over 40 years.

Our study presents several additional limitations. It could be argued that the higher rates of institutionalization might have increased the COVID-19 exposure risk prior to vaccination. However, only a minority of prior COVID-19 infections were documented among institutionalized individuals in our sample. To further control for COVID-19 exposure prior to vaccination, an ideal control group would have included individuals with non–trisomy 21 intellectual disabilities. However, this would have been an extremely difficult population to recruit. Likewise, we acknowledge that the potential (deleterious) role played by certain comorbidities, such as obesity or cardiac disease, has not been explored. However, this does not weaken the robustness of our conclusion, that individuals with DS elicit good immune responses to COVID-19 vaccines despite their increased-risk phenotype.

Yet unexplored but interesting issues are whether and how long vaccine-elicited responses persist over time in these individuals. We are currently collecting samples to assess the evolution of humoral end cellular immunity up to 12 months after vaccination. Meanwhile, individuals with DS older than 40 years are already being administered a third dose of mRNA SARS-CoV-2 vaccine in Spain at the time of this publication. Analyzing the impact of this additional booster dose in their immune response will also be of great interest.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Author contributions. Project coordination, original idea: A. A., A. G.-C., and D. R. d. A. Subject recruitment and follow-up: F. M. and D. R. d. A. Database compilation: G. M.-J., A. Y.-C., and L. E.-P. Sample processing, collection preservation/storage: G. M.-J., A. Y.-C., L. E.-P., N. R., and A. L.-S. Serological assays: A. G.-C. and A. Y.-C. Cellular
immunity assays: A. L.-S., L. E.-P., and N. R. Statistical analysis: A. G.-C., A. A., and D. R. d. A. Results evaluation, manuscript drafting and publication: all authors.

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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