Subjects who developed SARS-CoV-2 specific IgM after vaccination show a longer humoral immunity and a lower frequency of infection

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Summary

Background We have previously shown that eliciting SARS-CoV-2-specific IgM after vaccination is associated with higher levels of SARS-CoV-2 neutralizing IgG. This study aims to assess whether IgM development is also associated with longer-lasting immunity.

Methods We analysed anti-SARS-CoV-2 spike protein IgG and IgM (IgG-S, IgM-S), and anti-nucleocapsid IgG (IgG-N) in 1872 vaccinees at different time points: before the first dose (D1; w0), before the second dose (D2; w3) at three (w6) and 23 weeks (w29) after D2; moreover, 109 subjects were further tested at the booster dose (D3, w44), at 3 weeks (w47) and 6 months (w70) after D3. Two-level linear regression models were used to evaluate the differences in IgG-S levels.

Findings In subjects who had no evidence of a previous infection at D1 (non-infected, NI), IgM-S development after D1 and D2 was associated with higher IgG-S levels at short (w6, $p < 0.0001$) and long (w29, $p < 0.001$) follow-up. Similar IgG-S levels were observed after D3. The majority (28/33, 85%) of the NI subjects who had developed IgM-S in response to vaccination did not experience infection.

Interpretation The development of anti-SARS-CoV-2 IgM-S following D1 and D2 is associated with higher IgG-S levels. Most individuals who developed IgM-S never became infected, suggesting that IgM elicitation may be associated with a lower risk of infection.

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Keywords: SARS-CoV-2; COVID-19; BTN162b2 vaccine; IgG; IgM; Spike; Breakthrough infection

Introduction

The duration and nature of immune responses to SARS-CoV-2 infection are still not entirely clear. It is assumed that humans are immunologically naïve to SARS-CoV-2 and show a primary immune response to infection. However, pre-existing immunological memory toward other human coronaviruses (hCoVs) must be considered. In addition to SARS-CoV-2, four seasonal hCoV (229E, NL63, HKU1, OC43) are known to cause mild upper respiratory diseases. A large proportion of non-neutralizing cross-reactive antibodies (Abs) shows cross-reactivity with circulating hCoVs, suggesting the recall of pre-existing memory B cells elicited by previous hCoVs encounters.[1] Similarly, immune response elicited by the vaccine may be affected by these cross-reactive responses toward the SARS-CoV-2 Spike protein used in the vaccine formulation. To what extent endemic hCoVs may influence the dynamics of SARS-CoV-2 infection and vaccine response is a hot research topic.[2,3]

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Research in context

Evidence before this study

The duration and nature of immune responses to SARS-CoV-2 infection and vaccination are still not entirely clear. The anti-SARS-CoV-2 humoral response may be influenced by pre-existing immunity to other previously encountered coronavirus. In particular, it has been observed that individuals with an acute infection may lack an early response represented by the development of anti-Spike IgM (IgM-S), and instead immediately develop the class-switched, high affinity IgG. Interestingly, patients who fail to develop IgM-S have a worse clinical outcome compared to individuals who did not develop IgM-S. Similarly, the immune response elicited by the vaccine may be affected by these cross-reactive responses. In a previous study, we showed that the development of IgM-S in response to vaccination was associated with higher anti-Spike IgG (IgG-S) levels and neutralizing activity after the first two vaccine doses.

Added value of this study

We longitudinally analysed the SARS-CoV-2 specific humoral response in 1872 health care workers (HCW) who had received the BNT162b2 vaccine, up to 6 months after the second dose and, in a smaller subgroup, up to 6 months after the third booster dose. The cohort included 1599 subjects who did not experience SARS-CoV-2 infection before vaccination (non-infected, NI) and 273 individuals who were infected before vaccination (IBV). In NI subjects, the development of IgM-S in response to vaccination was associated with higher IgG-S levels at short ($p < 0.0001$) and long ($p < 0.001$) follow-up and persisted even after the booster dose. In the IBV subjects, the antibody response was partially confounded by the pre-existing immunity to SARS-CoV-2 developed during the natural infection, but it was still possible to appreciate higher IgG-S levels over time in subjects with evidence of IgM-S presence following vaccination. Finally, we observed that most of the NI subjects who did not experience infection had evidence of IgM-S as compared to those who were infected after the booster dose.

Implications of all the available evidence

This study highlights that vaccine-induced IgM-S might represent a correlate of immune protection against infections following SARS-CoV-2 vaccination. Our results suggest that individuals who have developed IgM-S after vaccination maintain a longer anti-SARS-CoV-2 humoral response measured as IgG-S levels and may have a lower risk of getting a breakthrough infection. These findings suggest that the assessment of IgM-S development in future vaccination campaigns can help clinicians in the identification of individuals who may benefit of additional vaccine doses to improve the duration of humoral response and protection.

In a previous work, we studied the anti-SARS-CoV-2 Spike protein IgM and IgG (IgM-S and IgG-S) humoral response after two doses of vaccine up to 3 weeks after the second dose in Health Care Workers (HCWs).[4] HCWs are a population at high risk of SARS-CoV-2 infection, so they represent an optimal population to study the response to the vaccine. We observed that a group of patients had developed the early-stage response represented by the presence of SARS-CoV-2 IgM-S, while others had immediately developed the class-switched IgG-S, possibly due to the recall of cross-reactive immunity against the Spike of other hCoVs. Interestingly, the subjects eliciting IgM-S and IgG-S after vaccination showed a higher IgG-S response than those eliciting IgG-S only or eliciting IgM-S after IgG-S. However, because of the short follow-up, it was not possible to evaluate whether the higher IgG-S response also translates into a longer duration and/or a slower anti-SARS-CoV-2 IgG-S decay. The present work attempts to fill this knowledge gap by analysing the IgG-S dynamics at six months after the second vaccine dose, as well as after the booster dose, in HCWs who developed or not IgM-S after the initial SARS-CoV-2 vaccine administration.

Methods

Study design and population

The study cohort included 1872 HCWs who had received an anti-SARS-CoV-2 vaccination (BNT162b2 mRNA, Pfizer-BioNTech; herein also referred to as COVID-19 vaccination) by March 2021, with a second dose (D2) that was administered 3 weeks after the first dose (D1) and a third booster dose (D3) administered at 44 weeks after 1st dose. Serological samples were collected at 6 different time point as depicted in Fig. 1, to analyse the anti-vaccine humoral response after D1, D2 and D3. The population included HCWs, thus lacking paediatric subjects, as well as the elderly, but it is representative of a category of workers at high risk of contracting the virus. SARS-CoV-2 infections were documented by a positive nasal swab and/or IgG positivity against the Nucleocapsid protein (IgG-N). Subjects were routinely screened by nasopharyngeal swab analyses, as per hospital policy.

The population included 273 HCWs with a documented history of SARS-CoV-2 infection before the 1st vaccine dose (infected before vaccination, IBV) and 1599 individuals with no evidence of a previous infection at D1 (non-infected, NI). As depicted in Fig. 1, some of the subjects experienced breakthrough

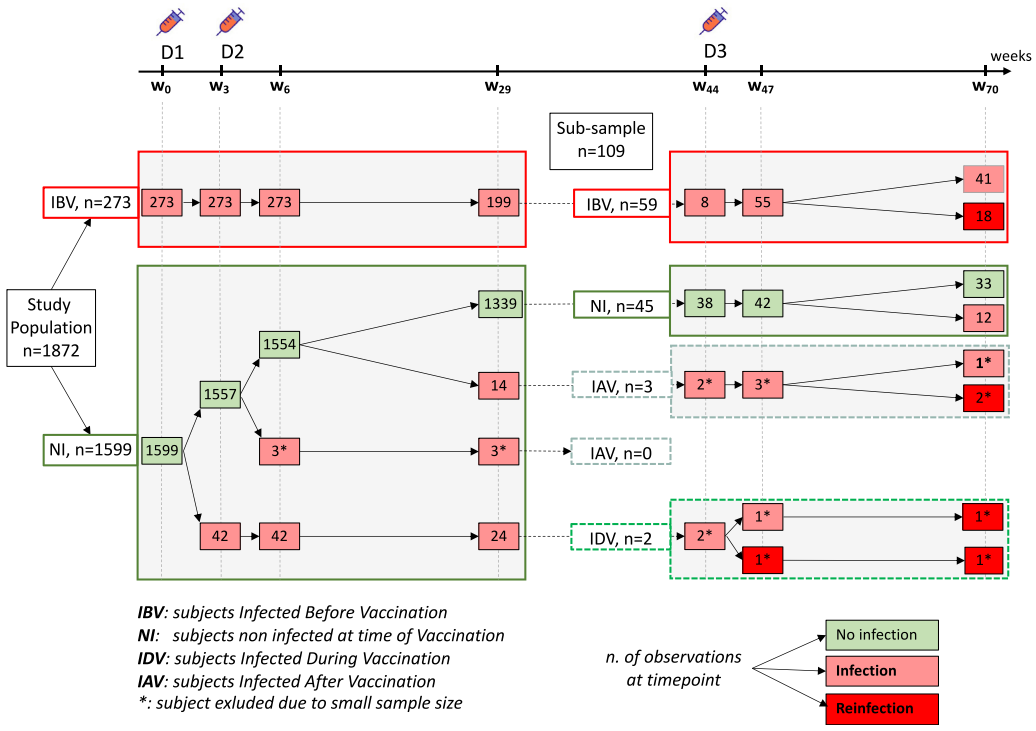

***Fig. 1:* Study cohort.** Vaccine was administered at week 0 (w0, 1st dose/D1), week 3 (w3, 2nd dose/D2) and week 44 (w44, booster dose/D3). Blood samples were collected from 1872 subjects up to w29 and from a sub-sample of 109 subjects at w44, w47 and w70. The number of observations for each time point is reported in squares according to the following colour coding: green, no infection; light red, infection; dark red, re-infections. IAV: Infected After Vaccination; IBV: Infected Before Vaccination; IDV: Infected During Vaccination; n: number of subjects; NI: Non-Infected at vaccination. The asterisks indicate observations excluded from the analyses due to the small sample size.

infection between D1 and D2 (infected during vaccination, IDV), or within 3 weeks after D2 (infected after vaccination, IAV). A limited number of individuals (109/1872, 6%) agreed to participate in the study up to 6 months after the administration of the booster dose (D3). These subjects had similar demographic characteristics at baseline (w0) as compared to the 1763 HCWs who did not participate in the study after w29 (Table 1). The sub-group of 109 HCWs participating in the study after w29 included 59 IBV, 45 NI, 2 IDV, and 3 IAV subjects; these latter 2 groups were excluded from the analysis giving the reduced number of participants. Some of these individuals were re-infected (18 IBV subjects) or experienced their first infection (12 NI subjects) after D3. Subjects also had similar baseline characteristics according to infection status (Supplementary Table S1) and IgM-S groups (Supplementary Table S2).

Ethics

The study protocol received ethical clearance by the local Ethics Committee (Comitato Etico per la Sperimentazione Clinica delle Province di Verona e Rovigo, Prot n. 2916 of 19/01/2021). Participants received written and oral information and signed an informed consent form. Samples were collected and stored in the Tropica Biobank of the IRCCS Sacro Cuore Don Calabria Hospital.

	Sample A	Sample B	p-value
n	1763	109	
Age, median age (IQR)	44 (21)	46 (18)	0.077 (Kruskal–Wallis rank test)
Female, % (vs male)	63.8	58.7	0.306 (Fisher's Exact test)

***Table 1:* Demographic characteristics at baseline (w0) of the 1763 subjects who did not participate in the study after the booster dose (sample A) and of the 109 subjects who agreed to participate in the study up to 6 months after the administration of the booster dose (sample B).**

Serology

All individuals were tested for IgG against the RBD domain of the Spike glycoprotein (IgG-S), IgG against the Nucleocapsid protein (IgG-N) and IgM against the Spike glycoprotein (IgM-S).

IgM-S and IgG-N were measured using the SARS-CoV-2 IgG-N and the SARS-CoV-2 IgM-S assays (Abbott, Ireland), respectively, and IgG-S (RBD) were tested using the SARS-CoV-2 IgG II Quant assay (Abbott, Ireland) as previously described.[4–6] Samples were run in single replicate. Briefly, the chemiluminescent microparticle immunoassays (CMIA) tests were performed according to the manufacturer's instructions using the ARCHITECT i2000 System (Abbott). For IgG-N and IgM-S, the results were reported as assay index (S/C) with a positive cut-off ≥1.4 for IgG-N and ≥1 for IgM-S. For IgG-S (RBD) results were reported as binding antibody Unit/mL (BAU/mL), providing a good correlate of virus neutralization.[4,7–9] Samples with values >5680 BAU/mL (upper limit of quantification) were diluted 1:2 and measured again. Concentrations were reported considering the dilution factor. For the IgM-S assay, the reported positive predicted value (PPV) is 92.07% (IC 95%: 87.07, 95.24) and the reported negative predicted value (NPV) is 99.82% (IC 95%: 99.47, 99.94). For the IgG II Quant assay, the manufacturer reports a PPV of 92.11% (IC 95%: 85.87, 95.73) and a NPV of 99.97% (IC 95%: 99.76, 100.00).

The serological tests we used are highly automatized and standardized diagnostic commercial tests by Abbott. Data about inter- and intra-assay validation studies were reported by the manufacturer indicating that they were conducted according to the clinical and Laboratory standards Institute (CLSI) approved guidelines document EP05-A3.

Statistical analysis

Kruskal–Wallis rank test and Fisher's exact test were used when needed in the descriptive analysis. IgG-S levels were ln-transformed [ln (IgG-S)] to resemble the normal distribution. Two-level linear regression models (measurement: level 1 unit; subject: level 2 unit) were used to estimate the mean of ln (IgG-S) levels according to time of examination (w0, w3, w6, w29, w44, w47, w70) and infection status group (Fig. 2), or according to IgM-S group (separately for NI and IBV subjects) (Figs. 3 and 4). The models had a random intercept term at level 2 and time of examination, infection status/IgM-S group, their interaction term, age at w0, and sex as fixed effect covariates. A first-order autoregressive error was included at level 1 in order to take the correlation of the within-subject observations over time into account. After estimation, the Wald test was used to verify composite linear hypotheses about the parameters of the regression models. All statistical analyses were performed by using STATA software (release 17; StataCorp, College Station, TX).

Role of funding source

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Results

IgG-S levels according to the infectious status

The IBV individuals showed detectable IgG-S prior to the vaccination, as reflection of the previous infection (Fig. 2, left panel). As expected, D1 increased IgG-S in IBV and NI groups, with higher immunoglobulin levels at w3 among the IBV subjects as compared to the values among the NI individuals ($p < 0.001$). D2 induced a greater increase of IgG-S between w3 and w6 in the NI group than in the IBV group ($p < 0.001$, Wald test). Between w6 and w29, IgG-S levels significantly decreased in both the IBV and NI groups ($p < 0.001$), apart from the IAV subjects who showed the highest values at w29 (similar to that measured at w6), due to the response to the breakthrough infection. The IDV subjects had similar IgG-S levels at w3 and w29 to that observed among the NI individuals, suggesting that infection acquired during vaccination did not result in higher IgG-S levels over time, as previously documented.[10]

IgG-S levels increased among IBV and NI subjects in response to D3 (between w44 and w47), with no differences between the two groups at both short (w47) and long follow-up (w70) (Fig. 2, right panel). The NI subjects who had experienced a breakthrough infection after w47 received a further boost reflected by the increase in IgG-S level measured at w70, whereas the reinfected IBV subjects did not experience a further significant increase driven by reinfection after D3.

IgM-S development at time of vaccination is associated with persistently higher IgG-S levels

All the subjects experiencing vaccine breakthrough infections (IDV and IAV) were excluded from this analysis. The development of IgM-S after vaccination in the NI subjects resulted in higher IgG-S levels at w3 ($IgM\text{-}S^{POS}_{w3}$ vs $IgM\text{-}S^{NEG}$, $p < 0.001$) and w6 ($IgM\text{-}S^{POS}_{w3}$ vs $IgM\text{-}S^{NEG}$, $IgM\text{-}S^{POS}_{w6}$ vs $IgM\text{-}S^{NEG}$, $IgM\text{-}S^{POS}_{w6}$ vs $IgM\text{-}S^{POS}_{w3}$, $p < 0.001$), as compared to the values that were found among the individuals who had not elicited IgM-S (Fig. 3, left panel). The significant differences observed at w6 were also present at longer follow up (w29, $p < 0.001$), after the Abs decay. These differences were not statistically significant at the same time point in the sub-sample of NI subjects (n = 109) who were followed up after the booster dose, perhaps as reflection of the reduced sample size (Fig. 3, right panel).

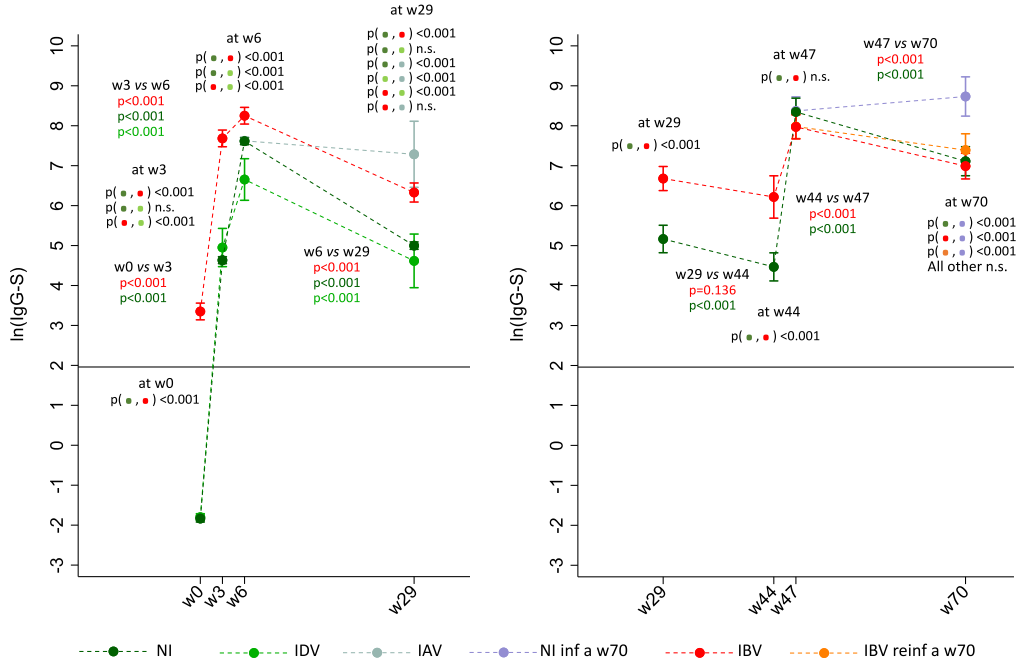

***Fig. 2:* IgG-S levels by infection status.** IgG-S levels over time in patients with different infection status. NI: Non-Infected (dark green lines); IDV: Infected During Vaccination (bright green lines); IAV: Infected After Vaccination (grey lines); NI inf at w70: previous NI with infection detected at w70 (light purple lines); IBV: Infected Before Vaccination (red lines); IBV re-inf at w70 = IBV with re-infection detected at w70 (orange lines). The number of observations per each time point is reported as follows. Left panel. w0: 1559 NI and 273 IBV; w3: 1557 NI, 42 IDV, and 273 IBV; w6: 1554 NI, 42 IDV, and 273 IBV; w29: 1339 NI, 24 IDV, 14 IAV, and 199 IBV. Right panel. w29: 45 NI and 59 IBV; w44: 38 NI and 8 IBV; w47: 42 NI and 55 IBV; w70: 33 NI, 12 NI inf w70, 41 IBV, and 18 IBV reinf w70 (p-values were obtained by two-level linear regression models).

In the IBV subjects, no statistically significant differences in IgG-S levels were observed between IgM-S groups after the first dose (Fig. 4).

The majority of individuals who developed IgM-S at time of vaccination did not experience infection during the study

Over the 70-week follow-up period, some HCWs became infected at different times. In particular, out of the 45 NI subjects who had completed the 3-doses vaccination cycle (Fig. 1), 12 (27%) individuals experienced infection following the booster and 33 (73%) never got infected (Table 2a). We further explored whether there was any association between the development of IgM-S and the presence of breakthrough infection. When looking at infection rates comparing the IgM-S^{POS} vs IgM-S^{NEG} groups, we observed that only 18% (6/34) IgM-S^{POS} individuals became infected compared to the 55% of IgM-S^{NEG}. When looking at the infected vs non-infected groups, among the 12 infected subjects, 6/12 (50%) never showed detectable IgM-S, 5/12 (42%) developed IgM-S after the first two doses and only 1/12 (8%) developed IgM-S even after D3 (Table 3b). On the other hand, the majority of non-infected subjects (28/33, 85%) developed IgM-S in response to vaccination, showing that individuals that did not get infected were more likely to have developed IgM-S in response to vaccination. Despite the small sample size, the difference was significant (Table 2a, p = 0.044). Of note, we observed that only 1/33 NI subjects had evidence of IgM-S presence at all time points following the first vaccine dose (Table 3a). Furthermore, in 21/33 IgM-S were detected up to w6, 5/33 developed an IgM-S boost after the third dose and 3 out the 5 maintained a detectable quantity of IgM-S till w70 (Table 3a). For IBV individuals, no association between the presence of IgM-S at time of vaccination and re-infection could be appreciated (Table 2b).

Discussion

Short-lasting humoral protection against SARS-CoV-2 supports the need to maintain infection prevention, particularly in HCWs, through periodic vaccination boosts.[11] The duration and effectiveness of immunity are relevant for planning interventions, including the timing of vaccine boosters.[12] The role of IgM in monitoring response to infection and/or vaccination has been generally neglected, despite recent studies showing their importance.[13,14] In our previous study,[4] we reported that

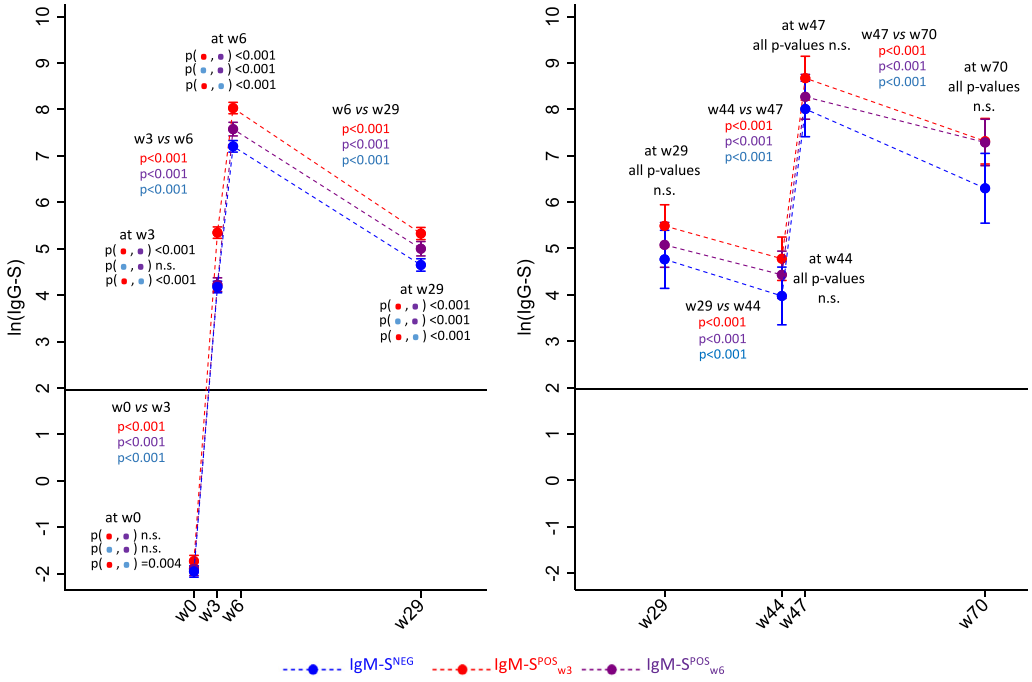

***Fig. 3:* IgG-S levels by IgM-S status in NI subjects.** IgG-S levels over time in patients who developed or not IgM-S in response to the first two doses of vaccination. IgM-S^{NEG}: individuals who did not develop IgM-S (blue lines); IgM-S^{POS}_{w3}: individuals who developed IgM-S at w3 (red lines); IgM-S^{POS}_{w6}: individuals who developed IgM-S at w6 (purple lines). The number of observations per each time point is reported as follows. Left panel. w0: 577 IgM-S^{NEG}, 604 IgM-SPOS$_{w3}$, and 418 IgM-S^{POS}_{w6}; w3: 565 IgM-S^{NEG}, 584 IgM-S^{POS}_{w3}, and 408 IgM-S^{POS}_{w6}; w6: 564 IgM-S^{NEG}, 584 IgM-S^{POS}_{w3}, and 406 IgM-S^{POS}_{w6}; w29: 483 IgM-S^{NEG}, 500 IgM-S^{POS}_{w3}, and 356 IgM-S^{POS}_{w6}. Right panel. w29: 9 IgM-S^{NEG}, 18 IgM-SPOS$_{w3}$, and 16 IgM-S^{POS}_{w6}; w44: 9 IgM-S^{NEG}, 16 IgM-S^{POS}_{w3}, and 13 IgM-S^{POS}_{w6}; w47: 10 IgM-S^{NEG}, 16 IgM-S^{POS}_{w3}, and 16 IgM-S^{POS}_{w6}; w70: 5 IgM-S^{NEG}, 14 IgM-S^{POS}_{w3}, and 14 IgM-S^{POS}_{w6} (p-values were obtained by two-level linear regression models).

the development of IgM-S is associated with higher IgG-S levels and virus neutralizing activity. In the present study, we found that the development of vaccine-induced IgM-S is associated with the maintenance of higher IgG-S levels over time. Furthermore, individuals with evidence of IgM-S following vaccination were also less likely to experience infection.

In this work, we followed up 1872 subjects up to 6 months after the second vaccine dose, showing that the development of IgM-S was associated with higher levels of IgG-S at different time points. Indeed, follow-up at w29 in NI subjects showed that, even several months after the second dose and despite the significant decay in IgG-S levels, those who elicited IgM-S after the first (IgM-S^{POS}_{w3}) or the second dose (IgM-S^{POS}_{w6}) maintained significantly higher IgG-S levels compared to subjects who did not develop IgM-S (IgM-S^{NEG}) (Fig. 3, left panel). Higher IgG-S levels averages continued to be present in the subjects who had developed IgM-S after the two vaccine doses (IgM-$S^{POS}_{w3/w6}$) even at longer follow-up times, at the booster dose (w44) and up to 6 months after the booster (w70), although in the latter the observed differences were not statistically significant, possibly due to the small sample size (Fig. 3, right panel). Overall, the increase in IgG-S levels was higher following the booster dose rather than following the second dose (Fig. 2, w47 vs w44 and w6 vs w3), in line with a recent study.[15]

The observed differences between IgM-S^{POS} and IgM-S^{NEG} groups were not influenced by the demographic characteristics of our cohort. Indeed, as reported in Table 1, the participants included in the long-term analysis, up to w70, were representative of the larger initial recruited cohort, where we observed, across all the analysed groups, a homogeneous lower IgG-S antibody response with increasing age (difference in ln IgG-S for one-year increase of age = –0.015, $p < 0.001$) and a higher IgG-S response in females (difference in ln IgG-S between females and males = 0.1, $p = 0.007$).[4] Moreover, the majority of HCWs in our cohort was young (median age about 45) and with no significant comorbidities or medical treatments. Whilst this increase was statistically significant in individuals who were naïve to the infection before vaccination (NI), it could be less appreciated in IBV subjects. The reason could derive from the presence of a hybrid immunity derived from both infection and vaccination.

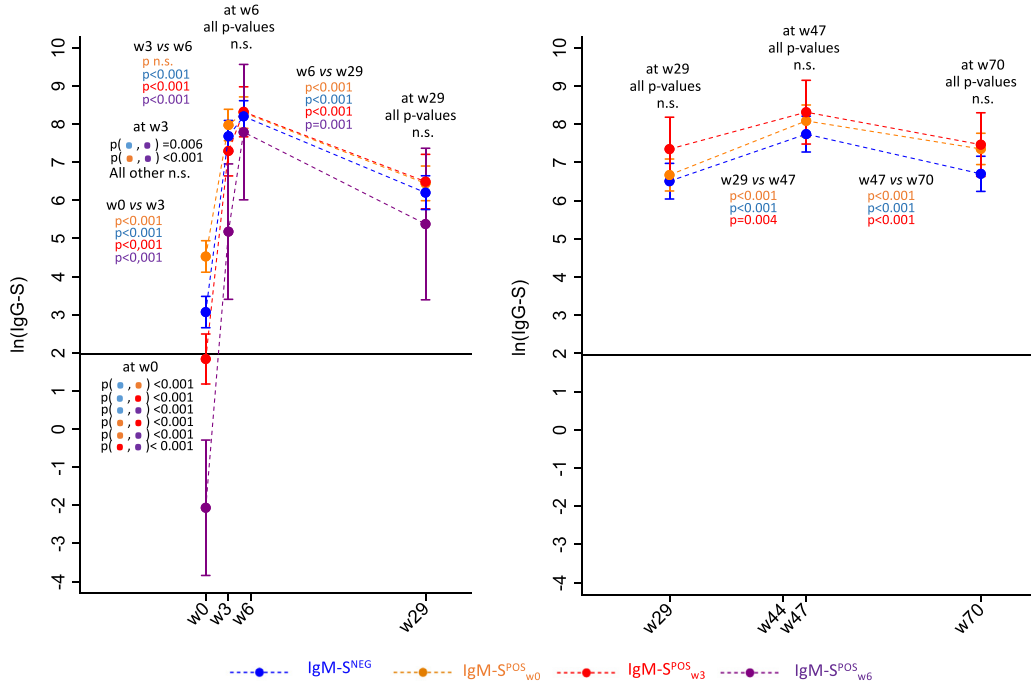

Fig. 4: **IgG-S levels by IgM-S status in IBV subjects.** IgG-S levels over time in patients who presented or not IgM-S as reflection of a recent past infection or in response to vaccination. IgM-S^{NEG}: individuals with no evidence of IgM-S presence (blue lines); IgM-S^{POS}_{w0}: individuals that already presented IgM-S at w0 (orange lines); IgM-S^{POS}_{w3}: individuals who developed IgM-S at w3 (red lines); IgM-S^{POS}_{w6}: individuals who developed IgM-S at w6 (purple lines). The number of observations per each time point is reported as follows. Left panel. w0, w3 and w6: 122 IgM-S^{NEG}, 111 IgM-SPOS$_{w0}$, 44 IgM-S^{POS}_{w3}, and 6 IgM-S^{POS}_{w3}; w29: 85 IgM-S^{NEG}, 78 IgM-S^{POS}_{w0}, 32 IgM-S^{POS}_{w3}, and 4 IgM-S^{POS}_{w3}. Right panel. w29: 22 IgM-S^{NEG}, 27 IgM-SPOS$_{w0}$, and 7 IgM-S^{POS}w3; w47: 21 IgM-S^{NEG}, 27 IgM-S^{POS}_{w0}, and 7 IgM-S^{POS}_{w3}; w70: 23 IgM-S^{NEG}, 29 IgM-SPOS$_{w0}$, and 7 IgM-S^{POS}_{w3}. Only for 8 subjects were samples available at w44 and therefore these were excluded from the analysis (p-values were obtained by two-level linear regression models).

The contribution of pre-existing immunity to the development of durable vaccine-induced anti-SARS-CoV-2 immunity is currently under investigation. It has been proposed that IgG induced by the vaccine may not be specific for SARS-CoV-2, but rather may represent the expansion of the circulating population that was elicited by previous human coronaviruses (hCoVs) infections.[16] Since this type of response does not include the conventional development of IgM before IgG, it may even weaken the overall humoral response, as reported in natural infection.[17,18] In this work, we similarly observed that the lack of IgM-S development was associated with lower levels of IgG-S over time. It remained to be determined whether this association resulted in higher frequency of infection following vaccination, thus suggesting a putative protective role of IgM-S elicitation, as discussed in a recent editorial.[19] In our study, the majority (85%) of the global NI population who had never experienced infection developed IgM-S (Table 2). Despite the small number of subjects, the risk of infection in the absence of vaccine-induced IgM-S was significantly higher than in individuals who showed evidence of IgM-S. This observation suggests that subjects who were not infected prior to vaccination and who developed IgM-S after the first two doses of vaccine may have greater protection and a lower risk of becoming infected, reinforcing the assumptions discussed above. We then checked whether this apparent protection could be mediated by IgM-S persistence, indicating an active role of IgM-S in mounting a more

	IgM-S^{NEG}	IgM-S^{POS}	Total
a) NI subjects			
Infection negative	5	28	33
Infection positive	6	6	12
Total	11	34	45
b) IBV subjects			
Infection negative	17	24	41
Infection positive	6	12	18
Total	23	36	59

a) p-value Fisher's exact test = 0.044. b) p-value Fisher's exact test = 0.773.

Table 2: **Association between IgM-S development and SARS-CoV-2 infection in a) non-infected (NI) and b) infected before vaccination (IBV) subjects.**

a) NI subjects

w0 1st dose	w3 2nd dose	w6	w29	w44 booster	w47	w70	Number of subjects (total n = 33)
–	–	–	–	–	–	–	3
–	–	–	–	na	–	–	1
–	–	–	na	na	na	–	1
–	–	+	–	–	–	–	10
–	–	+	–	–	+	+	2
–	–	+	–	na	–	–	2
–	+	–	–	–	–	–	1
–	+	+	–	–	–	–	9
–	+	+	–	–	+	–	2
–	+	+	–	na	na	–	1
–	+	+	+	+	+	+	1

b) NI subjects found infected at w70

w0 1st dose	w3 2nd dose	w6	w29	w44 booster	w47	w70	Number of subjects (total n = 12)
–	–	–	–	–	–	–	5
–	–	–	na	–	–	–	1
–	–	+	–	–	–	–	1
–	–	+	–	na	–	–	1
–	+	–	–	–	–	–	1
–	+	+	–	–	–	–	1
–	+	+	–	–	na	–	1
–	+	+	+	na	+	–	1

+: IgM-S presence; –: IgM-S absence; na: data not available; w: week.

Table 3: **IgM-S presence following vaccinations in subjects who were never infected (NI, a) and NI subjects who were infected after the boost (NI infected at w70, b).**

efficient anti-SARS-CoV-2 humoral response. We found no such indication through the study, as only 1/33 NI subjects who had never become infected showed evidence of IgM-S presence at all the analysed time points after w0. In our study, therefore, protection does not appear to be mediated by the persistence of IgM-S but rather by the increased IgG-S response in subjects who elicited IgM-S. A recent study, in which higher levels of IgG-S were associated with a lower risk of infection,[20] supports this hypothesis.

Taken together these results suggest an important role of IgM development in the maintenance of the anti-SARS-CoV-2 humoral response and possibly in the protection against new infections mediated by a higher, sustained IgG-S response, proving a possible model of anti-SARS-CoV-2 humoral dynamic following vaccination (Fig. 5).

Several groups have reported a strong back-boost effect to conserved Spike regions of seasonal hCoVs following SARS-CoV-2 infection,[21] which does not necessarily lead to cross-protection[22–24] but rather could negatively modulate the antibody profile,[25] hindering effective immunity against SARS-CoV-2,[16] and being detrimental to the host.[26] Taken together these data, including ours, draw attention on the so-called "original immunological sin",[27] whereby an immune response conditioned by prior immunity against other hCoVs could result in a non-specific SARS-CoV-2 humoral immunity after vaccination, impairing the immune protection.[16]

Furthermore, it has been reported that the expansion of hCoV-specific IgG correlates negatively with the induction of SARS-CoV-2 specific IgG/IgM[25] and consequentially with protection against infection and hospitalizations.[20] Moreover, it was shown that if a second boost, in this case vaccination, recalls the primary antigen, this trigger will define future immune responses leading to the expansion of recalled IgG rather than antigen-specific IgG.[26] In accordance with these observations, in our study none of the subjects who had not developed IgM-S in response to vaccination showed evidence of IgM-S following the breakthrough infection, suggesting that the initial immunological imprinting after vaccination shaped the consequential response.

Our study also further supports the fact that the vaccine is very effective and remains effective even in those subjects who appear to have a response conditioned by the aforementioned original antigenic sin. Original immunological sin is still a strongly debated issue.[28] Understanding the immune response to

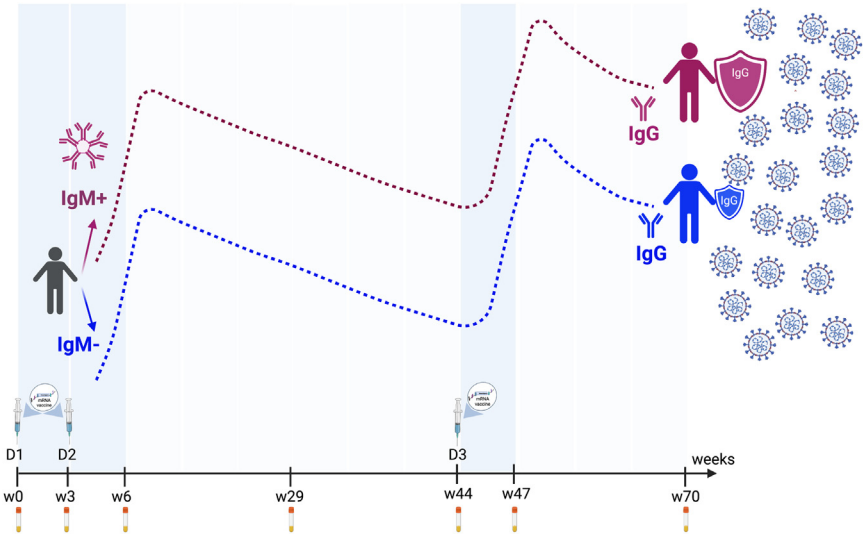

Fig. 5: **Higher IgG-S levels and persistence are associated with the development of IgM-S after vaccination.** Individuals who developed IgM-S following vaccination showed higher levels of IgG-S over time and potentially a stronger protection. IgM+: presence of IgM-S; IgM−: absence of IgM-S; bigger purple shield: greater protection; smaller blue shield: reduced protection; D1, D2 and D3: first, second and booster dose, respectively. w: week.

vaccination, infection and/or hybrid immunity will be helpful to address the role of the original antigenic sin.[27] Additional studies are needed to determine the impact of this mechanism for the development and implementation of SARS-CoV-2 vaccines.[29]

This study has some limitations that deserve discussion. The limited sub-sample studied in the follow up did not allow us to observe significant associations at longer follow-up. In addition, we did not have access to swab or PBMC samples to explore both the virological characteristic of the infections and the anti-SARS-CoV-2 specific cellular responses, and no data about BMI or other factors were collected for this population. Furthermore, it could be argued that IgM production may have peaked before the 3 weeks post-vaccinations, resulting in some IgM positive subjects missing out. However, it has been reported[17,30] that IgM peak around 20 days, thus the 3 week time point should coincide with the peak.

We found that the development of IgM-S after vaccination correlated with longer-term immunity. Further studies are needed to understand the mechanisms of this association, and to determine whether IgM-S development plays a role in improved protection against new infections.

We highlight the need to identify immunological biomarkers capable of differentiating between responders and non/low responders, which are essential for defining future, personalized vaccination protocols.

Contributors

D.Z., A.R., Z.B., C.P. conceived the study design, analysed, and discussed data and wrote the manuscript; Z.B., C.P., designed the study, enrolled patients, collected and managed clinical data; L.C. and S.A. performed statistical analysis; S.C., N.T., S.S.L. collected samples and clinical data; C.M., M.V., C.C. participated in data collection and analysis. All authors read, critically revised, and approved the manuscript. C.P., A.R. and L.C. have verified the underlying data.

Data sharing statement

Raw data are available in Zenodo open repository at the following link: https://doi.org/10.5281/zenodo.7158961.

Declaration of interests

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2023.104471.

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