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The Journal of Infectious Diseases

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Measles Vaccination Elicits a Polyfunctional Antibody Response, Which Decays More Rapidly in Early Vaccinated Children

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Background. Measles outbreaks are reported worldwide and pose a serious threat, especially to young unvaccinated infants. Early measles vaccination given to infants under 12 months of age can induce protective antibody levels, but the long-term antibody functionalities are unknown.

Methods. Measles-specific antibody functionality was tested using a systems serology approach for children who received an early measles vaccination at 6–8 or 9–12 months, followed by a regular dose at 14 months of age, and children who only received the vaccination at 14 months. Antibody functionalities comprised complement deposition, cellular cytotoxicity, and neutrophil and cellular phagocytosis. We used Pearson's r correlations between all effector functions to investigate the coordination of the response.

Results. Children receiving early measles vaccination at 6–8 or 9–12 months of age show polyfunctional antibody responses. Despite significant lower levels of antibodies in these early-vaccinated children, Fc effector functions were comparable with regular-timed vaccinees at 14 months. However, 3-year follow-up revealed significant decreased polyfunctionality in children who received a first vaccination at 6–8 months of age, but not in children who received the early vaccination at 9–12 months.

Conclusions. Antibodies elicited in early-vaccinated children are equally polyfunctional to those elicited from children who received vaccination at 14 months. However, these antibody functionalities decay more rapidly than those induced later in life, which may lead to suboptimal, long-term protection.

Keywords. antibody response; early measles vaccination; Fc effector functions; long-term response; polyfunctionality.

Since the introduction of childhood measles vaccination, the number of measles cases has dropped significantly. However, after years of decline in measles incidence, the number of measles cases has been steadily increasing the past couple of years, due to insufficient measles vaccination coverage by routine immunization programs in certain countries [1]. Since 2016, the number of reported measles cases increased with 556% to almost 870 000 in 2019, which are the most reported cases since 1996 [1]. Unvaccinated infants are the main risk group for measles infection and related complications [2], stressing the need for effective vaccination early in life [3].

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Measles-neutralizing antibodies are considered to be the main correlate of protection for clinical measles infection [4, 5]. These antibodies are induced by natural infection or vaccination and block entry of measles virus into target cells. At birth, infants are protected by neutralizing maternal antibodies that are transferred from mother to child through the placenta. In countries with high vaccination coverage over the past decades, maternal immunity against measles is now primarily induced by vaccination. Studies have shown that measles-specific maternal antibodies of children from vaccinated mothers drop below protective levels after 3–4 months of age [6, 7]. This leaves infants unprotected until their first vaccination, which generally occurs between 12 and 15 months of age. Infants aged 6–12 months may receive an early vaccination in case of a measles outbreak.

We previously reported that children who received an early vaccination under 12 months of age showed a more rapid decline of neutralizing antibodies within the 3-year follow-up [8]. Although most children still had a titer that was considered to be protective (>0.12), long-term modeling showed that the group that received an early vaccination at 6–8 months dropped below this protective level of neutralizing antibodies. These differences in decay of neutralizing

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antibodies in the early vaccines indicate unexplained changes in the development of a functional antibody response. Although the neutralizing capacity of antibodies can prevent infection, other effector functions involved in the measles-specific immune response can be crucial in preventing dissemination of the virus. Indeed, measles-specific antibodies were shown to induce complement-mediated lysis after virus infection [9, 10], and measles-specific antibodies that induce antibody-dependent cellular cytotoxicity (ADCC) have been proposed to play a role in clearing measles virus during infection and recovery [10]. Because dissemination of measles virus is predominantly mediated via cell-to-cell transmission [11, 12], a broader range of antibody-dependent effector functions have to develop to combat the virus. However, detailed knowledge on effector functions of measles-specific antibodies is relatively unknown.

In the current study, we performed a broad antibody effector function analysis and investigated whether the (poly) functionality of the antibody response was affected by the timing of the first vaccination. Here, we used a systems serology approach, ie, we assessed various aspects of measles-specific antibody functionalities. This included measuring a broad range of Fc-mediated effector functions, including antibody-dependent complement deposition (ADCD), and ADCC/natural killer (NK) cell degranulation, as well as antibody-dependent neutrophil phagocytosis (ADNP) and antibody-dependent cellular phagocytosis (ADCP) by monocytes. In addition, measles-specific antibody isotypes and subclasses were measured.

A representative selection of a previously described cohort of children who received an early vaccination was used [8]. This cohort consisted of children receiving their first early measles vaccination between 6 to 8 months or 9 to 12 months of age, followed by a regular-timed dose at 14 months. In addition, a control group that was only vaccinated at 14 months of age was included in this study [8]. By in-depth characterization of the functional antibody response to measles vaccination at different ages, we investigated whether reduced levels of measles-specific antibodies resulted in functional changes in the Fc-binding properties of the antibodies as well. This first study into a multitude of measles-specific antibody effector functions will help to provide a better understanding of factors that influence the antibody response to measles vaccination and may improve future vaccination strategies.

METHODS

Study Population

Samples were selected from a previously described cohort of children who received an early additional measles, mumps and rubella (MMR-0) vaccination, evenly distributed between ages 6 and 12 months (6 months, n = 4; 7 months, n = 5; 8 months, n = 4; 9 months, n = 2; 10 months, n = 5; 11 months, n = 4) during a measles outbreak in the Netherlands in 2013–2014 [8]. All children also received regular-timed MMR at 14 months of age (MMR-1). A control group of children (n = 10) only vaccinated at 14 months of age was also included. Children were selected to be representative of the complete cohort, based on age of first MMR vaccination. Furthermore, plasma samples 1 and 3 years after MMR-1 had to be available.

Measles Antigen

All assays were performed using an in-house, concentrated, and purified Edmonston measles strain [13], which was UV-C inactivated for 5 minutes. Measles antigen was biotinylated with Sulfo-LC-LC-Biotin (Thermo Fisher Scientific) before coupling 1:1 for 2 hours at 37°C to fluorescent NeutrAvidin-Labeled 1.0 μm Microspheres (Invitrogen). Afterward, beads were washed twice with 5% bovine serum albumin (BSA)/phosphate-buffered saline (PBS).

Antibody-Dependent Phagocytosis

Plasma samples were diluted 1:100 and added to antigen-coated, green fluorescent microspheres for 2 hours at 37°C to form immune complexes. Nonspecific unbound antibodies were washed away. For ADCP assay, THP-1 cells (monocyte cell line; American Type Culture Collection [ATCC]) were added at a concentration of 1.25×10^5 cells/mL for 16 hours at 37°C. For ADNP assay, primary human leukocytes were isolated from whole blood using ACK red blood cell lysis buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA) and washed with iced-cold PBS. Fresh leukocytes were added in R10 media at a concentration of 2.5×10^5 cells/mL and incubated for 1 hour at 37°C. Afterward, cells were washed, and neutrophils were stained with Pacific Blue CD66b antibody (BioLegend). Cells were fixed in 4% paraformaldehyde for acquisition on the flow cytometer. The phagocytic score was calculated as the percentage of cells that had taken up beads multiplied by the geometric mean fluorescence intensity (gMFI) of bead-positive cells divided by 10 000 as measured by flow cytometry (LSR FortessaX20; BD).

Antibody-Dependent Complement Deposition

For the ADCD assay, plasma samples were diluted 1:10 and added to antigen-coated red fluorescent microspheres for 1 hour at 37°C. Nonspecific, unbound antibodies were washed away. Lyophilized guinea pig complement, reconstituted in ice-cold water, was added in Boston BioProduct Veronal buffer for 20 minutes at 37°C. Complement heat inactivated at 56°C for 30 minutes was used as a control. Afterward, beads were washed in 15 mM EDTA, and complement deposition was detected by fluorescein isothiocyanate (FITC)-conjugated goat-antiguinea pig C3 (MP Biomedicals) antibody staining. Complement deposition was measured by flow cytometry as the FITC gMFI of C3 on complement coated-antigen bound beads.

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Natural Kill Cell Degranulation

Enzyme-linked immunosorbent assay plates were coated with measles antigen and incubated with samples diluted 1:20, or PBS as negative control, for 2 hours at 37°C. Natural killer cells were isolated from buffy coats collected from blood bank donors. CD107a-PE/Cy5 (BD), brefeldin A (Sigma), GolgiStop (BD), and NK cells were added to the antigen-coated plate and incubated for 5 hours at 37°C. Next, cells were transferred to a V-bottom plate and stained with CD56-PE/Cy7, CD16-APC/Cy7, and CD3-AlexaFluor700 (all BD) for 15 minutes at room temperature. Cells were washed and fixed in Fixation Medium A (Life Technologies) before intracellular staining with MIP-1β-PE and IFN-γ-APC (BD) in Permeabilization Medium B (Life Technologies) for 15 minutes at room temperature. The percentages of positive NK cells for CD107a, IFN-γ, and MIP-1β were determined by flow cytometry.

Subclass and Isotype Detection

Measles antigen-coated MagPlex microspheres (Luminex) through a 2-step carbodiimide reaction were added to 1:10 diluted plasma samples, with a final dilution of 1:100 when 5 µL of sample was added to 45 µL antigen-coated beads, and incubated for 2 hours shaking at room temperature. Next, samples were washed and secondary detection antibodies against immunoglobulin (Ig)G, IgG1, IgG2, IgG3, IgG4, IgM, IgA1, and IgA2 were diluted 1:154 in assay buffer (PBS, 0.1% Tween20, 2% BSA) and incubated on a shaker for 1 hour at room temperature. Samples were washed and measured with a Bio-plex Luminex reader. Relative levels of isotype and subclasses were calculated as the median fluorescence intensity of PE. Background was determined by a no-antibody control.

Statistical Analysis

For comparing different groups per functional assay, differences between log-transformed geometric mean concentrations were tested by Brown-Forsythe and Welch ANOVA tests and corrected for multiple comparisons using Tamhane's T2 multiple comparisons test, with individual variances computed for each comparison. To investigate coordination of the responses, Pearson's r correlations were (1) calculated for each group between the different functions measured and (2) further classified as very weak (±0–0.19), weak (±0.2–0.39), moderate (±0.4–0.59), strong (±0.6–0.79), and very strong (±0.8–1). To assess the quality of the response, we determined (per group and time point) the percentage of children who showed a strong, ie, above the median, functional response and combined this into a cumulative functional response.

RESULTS

Baseline Characteristics

In total, 34 children were selected from a previously described cohort [8]. Children had received an early MMR dose (MMR-0) between ages 6 and 8 months (n = 13) or between ages 9 and 12 months (n = 11). A control group did not receive an early MMR dose (n = 10). All children received a regular-timed MMR dose at 14 months of age (MMR-1). No differences between the selection and the full cohort were observed in their baseline characteristics (Table 1). We previously described a reduced measles virus-specific neutralizing antibody response after an early MMR dose, which persisted up to 3 years after MMR-1 vaccination at 14 months of age (Table 1 and [8]). Measles-specific IgG in plasma showed similar response kinetics compared with neutralizing antibody levels (Supplementary Figure 1).

Regular Measles, Mumps and Rubella Vaccination at 14 Months Induces Measles-Specific Antibody Effector Functions

Antibodies induce a variety of effector functions besides blocking viral entry into cells through neutralization. We first examined the different Fc-effector functions mediated by measles-specific antibodies 1 and 3 years after regular MMR-1 vaccination at 14 months of age (Figure 1), ie, at 26 month and 50 months of age. Using our systems serology platform, we measured ADNP, ADCP, ADCD, and antibody-induced NK activation via markers of activation and degranulation: CD107a, IFN-γ, and MIP-1β. First measles vaccination at 14 months induced a functional antibody response that elicited all assessed Fc-effector functions. Kinetics showed a subtle decline in ADNP and ADCD between 1 and 3 years after vaccination (ADNP, $P = .032$; ADCD, $P = .061$) (Figure 1A and C), whereas ADCP appeared to increase slightly ($P = .081$) (Figure 1B). All NK degranulation markers remained stable over time (Figure 1D and F).

Age of First Measles, Mumps and Rubella Dose Does Not Affect Individual Fc-Effector Function of Antibodies

Next, we examined measles-specific Fc-effector functions in all 3 vaccination groups (Figure 2). Again, these effector functions were determined 1 and 3 years after MMR-1, ie, at 26 months and 50 months of age. For this, individual Fc-effector outcomes were normalized for the level of measles-specific IgG (Supplementary Figure 1). No evidence for lower effector functions in the early-vaccinated groups was observed, except for a lower percentage of $CD107a^+$ NK cells in early-vaccinated children 3 years after MMR-1 ($P = .009$) ((Figure 2D). Other NK cell read-outs were not different between the groups (Figure 2E and F). One year after regular MMR-1 at 14 months of age, antibodies from children with a first MMR dose between 6 and 8 months of age induced higher ADCP ($P = .036$) than antibodies from children with a first dose at 14 months of age, although this difference was no longer present 3 years after MMR-1 (Figure 2B). Thus, despite overall lower levels of antibodies in children who received an early measles vaccination (Supplementary

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Table 1. Baseline Characteristics

Parameter	Yes/No	Age First MMR Vaccination								
		6–8 Months			9–12 Months			Control		
		Full Cohort[a] (n = 45)	Selection (n = 13)	*P* Value	Full Cohort[a] (n = 33)	Selection (n = 11)	*P* Value	Full Cohort[a] (N = 41)	Selection (n = 10)	*P* Value
Date of birth		November 2012 - July 2013	November 2012 - July 2013		August 2012 - October 2013	August 2012 - October 2013		August 2012 - August 2013	August 2012 - April 2013	
Male		19 (43.2%)	6 (46.2%)		15 (48.4%)	5 (45.4%)		17 (42.5%)	4 (40.0%)	
Year of birth mother (years)		1982 (1971–1989)	1982 (1971–1989)	.36[b]	1982 (1967–1989)	1982 (1975–1986)	.97[b]	1982 (1971–1988)	1982 (1978–1988)	.51[b]
Duration pregnancy (weeks)		39.5 (31–42)	39 (38–41)	.74[b]	39 (29–42)	39 (38–41)	.69[b]	40 (36–44)	40 (38–42)	.29[b]
Breastfeeding	Yes	24 (54.5%)	7 (53.8%)	.82[c]	13 (41.9%)	4 (36.4%)	.91[c]	18 (45.0%)	4 (40.0%)	.78[c]
	No	20 (45.5%)	5 (38.4%)		18 (58.0%)	6 (54.5%)		22 (55.0%)	6 (60.0%)	
Childhood measles mother	Yes	5 (11.4%)	3 (23.1%)	.55[c]	5 (15.2%)	2 (18.2%)	.95[c]	14 (35.0%)	2 (20.0%)	.40[c]
	No	29 (65.9%)	7 (53.8%)		19 (61.3%)	7 (63.6%)		23 (57.5%)	6 (60.0%)	
	Unknown	10 (22.7%)	3 (23.1%)		7 (22.6%)	2 (18.2%)		3 (7.5%)	2 (20.0%)	
Measles vaccination mother	Yes	38 (86.4%)	10 (76.9%)	.71[c]	25 (80.6%)	6 (54.5%)	0.20[c]	26 (65%)	9 (90.0%)	.30[c]
	No	2 (4.5%)	1 (7.7%)		2 (6.5%)	1 (9.1%)		13 (32.5%)	1 (10.0%)	
	Unknown	4 (9.1%)	2 (15.4%)		4 (12.9%)	4 (36.4%)		1 (2.5%)	0 (0.0%)	
Neutralizing antibodies (IU/mL)[e]	+1 year	1.09 (0.71–1.66)	2.27 (1.19–4.33)	.16[d]	1.91 (1.40–2.61)	1.80 (1.13–2.87)	>.99[d]	3.22 (2.30–4.52)	4.08 (1.74–9.57)	>.99[d]
	+3 years	0.43 (0.27–0.67)	0.62 (0.27–1.43)	.62[d]	0.76 (0.57–1.03)	0.65 (0.41–1.02)	>.99[d]	1.41 (0.93–2.15)	2.01 (0.87–4.67)	>.99[d]

Abbreviations: MMR, ;

NOTE: Data are median (range), no. (%) of infants or geometric mean (95% confidence interval).

[a]Full cohort previously described in Brinkman et al [8].

[b]By unpaired *t* test.

[c]By χ^2 test

[d]By Dunn's test correcting for multiple testing.

[e]A neutralizing antibody concentration of 0.12 IU/mL is internationally considered the minimal cutoff level for clinical protection in measles.

Figure 1), the first MMR dose did not notably influence the Fc-effector capacity on a single antibody level.

Coordination of Antibody Fc-Effector Functions Shifts Over Time

To investigate the relationship between the different Fc-effector functions within and between the 3 different vaccination groups, we calculated a Pearson's r correlation for each group between the different functions measured (Figure 3); a greater number of strong correlations represents a more coordinated response, ie, inducing multiple effector functions simultaneously. In 13 of 15 correlations, early-vaccinated children showed stronger correlations between different Fc-functions than children from the control group 1 year after regular MMR-1 vaccination at 14 months of age (Figure 3A). In contrast, 3 years after MMR-1 vaccination, early vaccination resulted in weaker correlations between Fc-effector functions (6–8 group vs control, 11 of 15 correlations are lower; 9–12 group vs control, 7 of 15 correlations are lower; 6–8 vs 9–12 group, 10 of 15 correlations are lower) (Figure 3B).

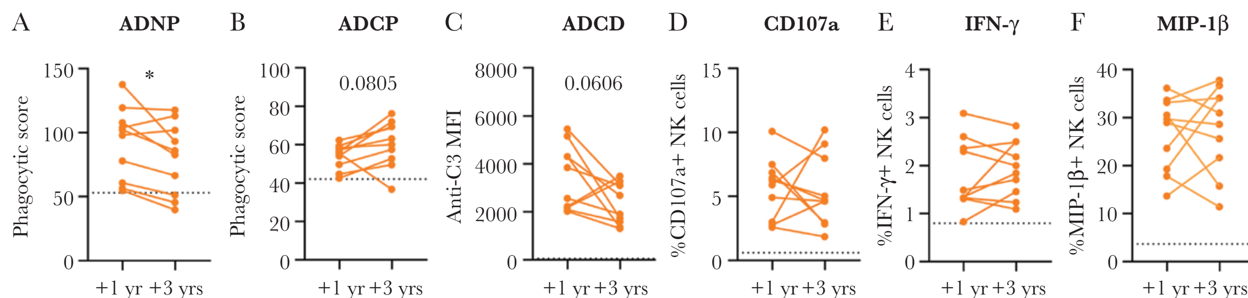

Figure 1. Measles-specific Fc-effector functions induced by antibodies after regular measles, mumps and rubella (MMR)-1 vaccination at 14 months of age (n = 10), measured 1 and 3 years later. Antibody-dependent neutrophil phagocytosis (ADNP) (A) and antibody-dependent cellular phagocytosis (ADCP) (B) are expressed as phagocytic score, calculated as (%FITC + cells) × (geometric mean fluorescence intensity [MFI] of positive cells)/10 000. Antibody-dependent complement deposition (ADCD) (C) is expressed as anti-C3 MFI. Natural killer (NK) cell activation markers are expressed as the percentage of CD107a- (D), IFN-γ- (E), and MIP-1β-positive (F) NK cells. Dotted line indicates the negative control in each experiment. Differences were tested by paired *t* tests. *$P \le .05$.

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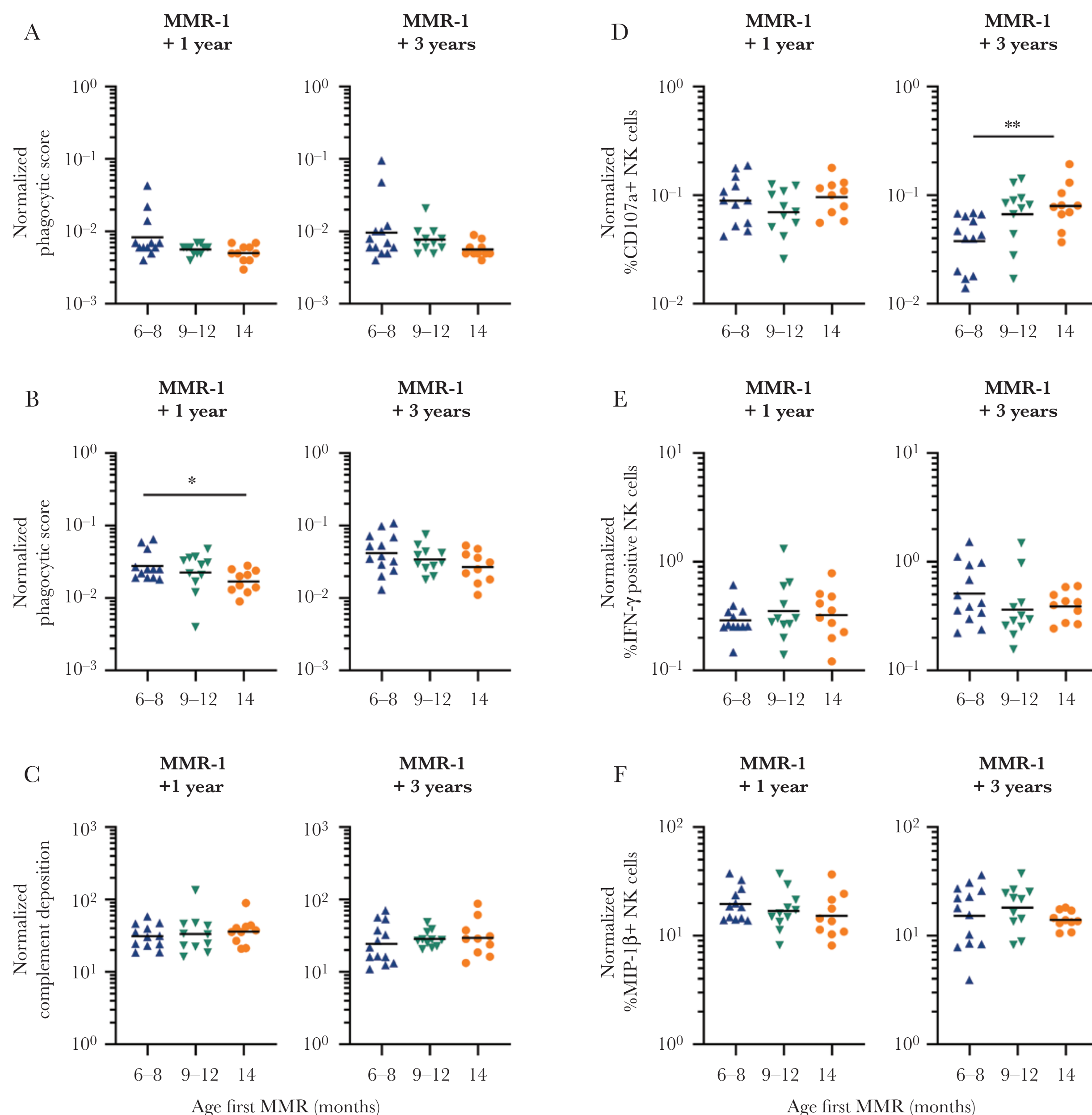

Figure 2. Measles-specific Fc-effector functions normalized for measles-specific IgG concentrations (mean fluorescence intensity) for children who received a first MMR dose between ages 6–8 months (n = 13), 9–12 months (n = 11), or 14 months (n = 10). Early-vaccinated children received additional measles, mumps and rubella (MMR) vaccination at 14 months of age (MMR-1) as well. Fc-effector functions and measles-specific IgG were measured in plasma 1 (left panels) and 3 (right panels) years after MMR-1. Fc-effector functions measured: antibody-dependent neutrophil phagocytosis (ADNP) (A), antibody-dependent cellular phagocytosis (ADCP) (B), antibody-dependent complement deposition (ADCD) (C), and NK cell activation markers CD107a (D), IFN-γ (E), and MIP-1β (F). Differences between log-transformed GMCs were tested by Brown-Forsythe and Welch ANOVA tests and corrected for multiple comparisons using Tamhane's T2 multiple comparisons test $^{*}P \leq .05$ and $^{**}P \leq .01$.

Finally, we classified these correlations according to strength in 5 classes: very weak, weak, moderate, strong, and very strong (Figure 3C and D). One year after MMR-1, children with a first MMR dose between ages 6 and 8 months showed more correlations of higher strength (ie, [very] strong), compared to children with a first dose at a later age (Figure 3C). In contrast, children with a first dose at 14 months of age showed more (very) weak correlations compared to children vaccinated at a younger age (Figure 3C). It is interesting to note that 3 years after MMR-1, this pattern had shifted towards a stronger coordinated response for children from the control group (Figure 3D). Overall, the functional response after 2 doses of MMR, with the first given between 6 and 8 months of age, became less coordinated over time, whereas the functional response after regular MMR vaccination at 14 months became more coordinated after 3 years.

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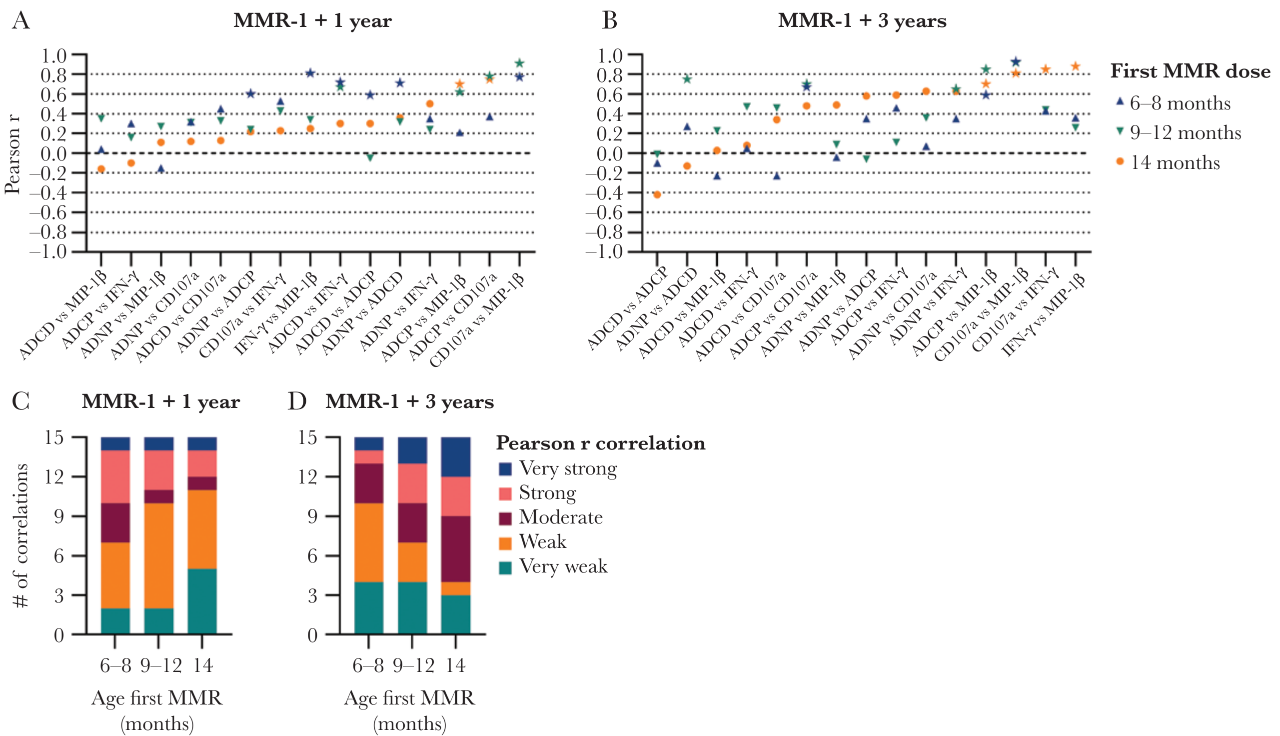

Figure 3. Pearson's r correlations of measles-specific Fc-effector functions for children who received a first measles, mumps and rubella (MMR) dose between 6–8 months (n = 13), 9–12 months (n = 11), or 14 months of age (n = 10). Early-vaccinated children received additional MMR vaccination at 14 months of age (MMR-1) as well. Antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent complement deposition (ADCD), and NK degranulation markers CD107a, IFN-γ, and MIP-1β were measured in plasma 1 (A and C) and 3 years (B and D) after MMR-1. Correlations are indicated on the x-axis and explained in the table (A and B). Each graph shows an increasing correlation for the control group on the x-axis (A and B). Star symbols indicate $P \leq .05$. Correlations are indicated as very weak (±0–0.19), weak (±0.2–0.39), moderate (±0.4–0.59), strong (±0.6–0.79), and very strong (±0.8–1) (C and D).

Different Dynamics in Antibody Polyfunctionality Over Time

Although the correlation of functions demonstrates the coordination between the responses, this analysis alone did not capture the magnitude of the functionality. Therefore, we sought to determine the overall functional patterns across the vaccine groups over time. To this end, we combined the strong responses above the median for each function per vaccination group and compared the number of children with strong versus weak responses between 1 and 3 years based on the age of first MMR dose (Figure 4A). Children who received a first MMR dose between ages 6 and 8 months of age started with a strong cumulative functional response 1 year after MMR-1 (Figure 4A, left panel). However, this cumulative response declined significantly 3 years after MMR-1. In contrast, the cumulative functional response for children with a first MMR dose between ages 9 and 12 months or at 14 months of age increased 3 years after MMR-1 (Figure 4A, middle and right panels). It is notable that the most dramatic changes occurred for markers of NK degranulation.

Next, we compared the functional responses for each child relative to the median of that response, with more functions above the median indicating a higher polyfunctional antibody response, an additional measure to reflect the quality of the response (Figure 4B and C). If mounting 5 to 6 Fc-mediated effector functions to a high level (ie, above the median) defines a strong polyfunctional response, then at 1 year after MMR-1, 40%–60% of children with polyfunctional responses were among the group who received their first dose at 6–8 months (Figure 4B). However, at 3 years, almost 80% of children with polyfunctional responses received a single measles vaccination at 14 months of age (Figure 4C). Children who received a first dose between 9 and 12 months of age also increased in polyfunctionality between 1 and 3 years after the second dose (Figure 4C). These data suggest that the strength of the response for each function as well as total function wanes for children who received a first dose at 6–8 months of age, but this increases among those who received either a first dose at 9–12 months of age or only 1 dose at 14 months.

Age of First Measles, Mumps and Rubella Dose Does Not Influence Antibody Subclasses and Isotypes

To define whether a shift in the quality of the immune response explains differences in the functional evolution of the measles-specific response across age groups, we measured relative levels of measles-specific antibody isotypes and subclasses (IgG1–4, IgA1–2, and IgM) and normalized against the

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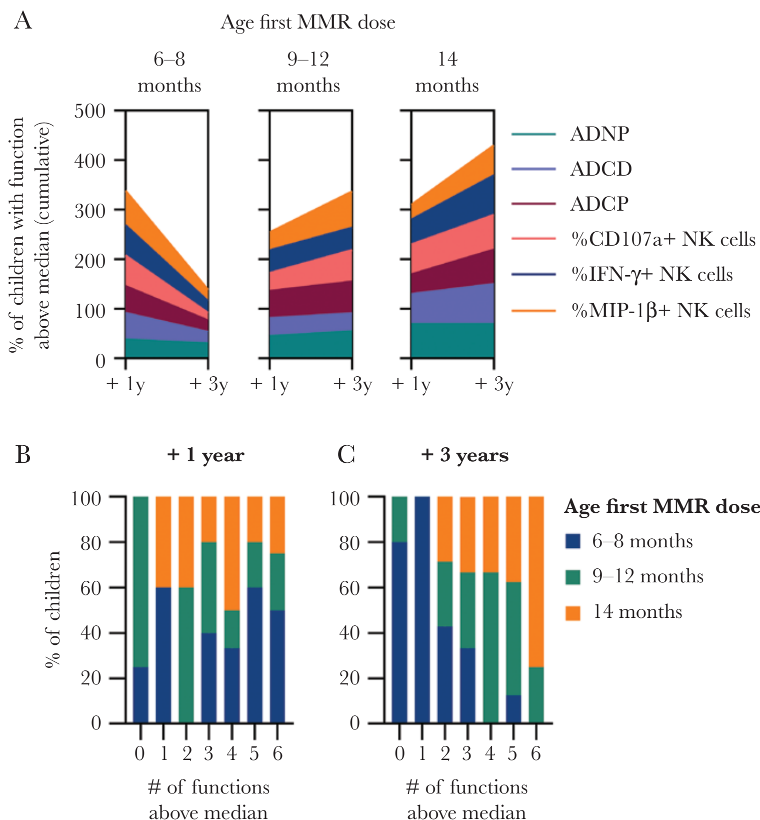

Figure 4. Antibody polyfunctionality for children who received a first measles, mumps and rubella (MMR) dose between 6–8 months (n = 13), 9–12 months (n = 11), or 14 months of age (n = 10). Early-vaccinated children received additional MMR vaccination at 14 months of age (MMR-1) as well. All outcomes were measured in plasma 1 and 3 years after MMR-1. For each time point and Fc-effector function, the median functional response was calculated. The cumulative percentage of children per vaccination group with a function above median is shown (A). We determined for each child the relative number of functional responses above the median, 1 year (B) and 3 years after MMR-1 (C).

dominant subclass IgG1. No differences or shifts in subclass and isotype distribution were observed (Supplementary Figure 2). Next, Pearson's r correlations between antibody subclasses and isotypes and the functional responses were calculated (Supplementary Figure 3), and the geometric mean of the absolute Pearson's r was taken to investigate the differences between the vaccination groups. The overall strength of the correlations between the vaccination groups 1 and 3 years after MMR-1 were not different (Figure 5A and B). Again, to better quantify the correlations, we classified them according to strength. Here, we also observed no major differences between the vaccination groups 1 and 3 years (Figure 5C and D) after MMR-1. The majority of correlations between antibody subclasses/isotypes and antibody functions were (very) weak.

DISCUSSION

This is the first study to show that measles vaccination at childhood leads to the induction of antibodies that can display a broad spectrum of Fc-effector functions, including antibody-dependent complement deposition, antibody-dependent phagocytosis, and ADCC. For other viruses, complement activation and phagocytosis have been described to play a significant role in the immune response and clearance of multiple other viruses [14–22], but, to our knowledge, we are the first to describe measles-specific, antibody-dependent complement activation and phagocytosis upon vaccination.

For ADCC, we focused on 3 NK cell activation markers: CD107a surface expression, IFN-γ, and MIP-1β. All 3 ADCC-related functions were detected after vaccination, even up to 3 years, and followed a similar pattern. The difference we observed in the induction of CD107a expression between children vaccinated before 9 months of age and the control group 3 years after MMR-1 may be due to the lower sensitivity of the assay, because CD107a expression of some children vaccinated between 6 and 8 months was at background levels (data not shown).

Most notably, the antibody response in children who received an early vaccination at 6–12 months of age, showed comparable capacities to mediate Fc-effector functions as antibodies induced by regular-timed measles vaccination at 14 months, despite having lower measles-specific IgG concentrations. More in-depth analysis revealed striking differences in polyfunctionality and coordination of the functional

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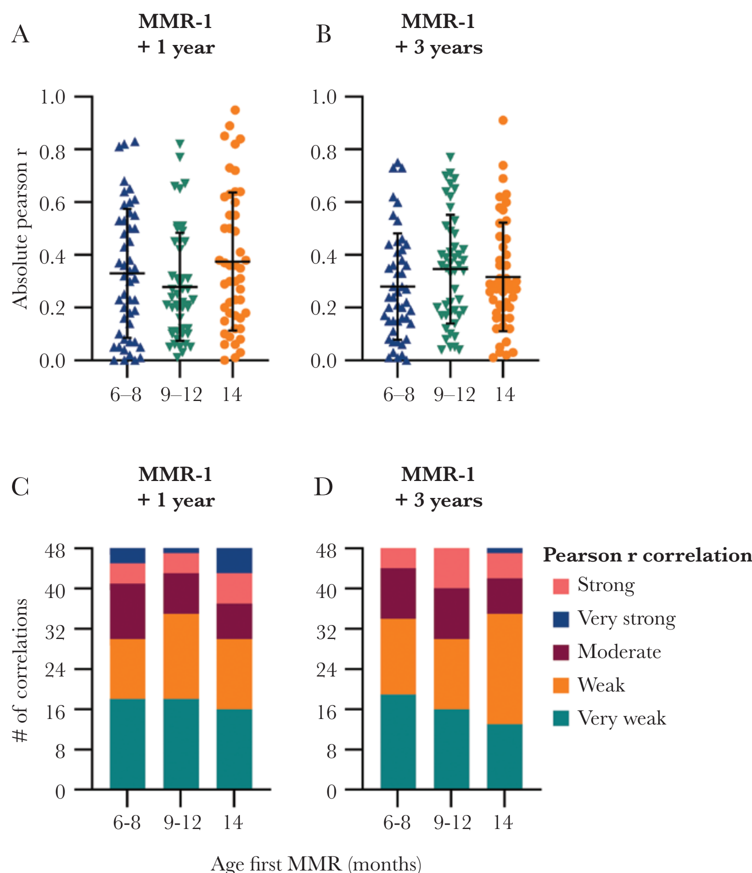

Figure 5. Pearson's r correlations of measles-specific Fc-effector functions for children who received a first measles, mumps and rubella (MMR) dose between 6–8 months (n = 13), 9–12 months (n = 11), or 14 months of age (n = 10). Early-vaccinated children received additional MMR vaccination at 14 months of age (MMR-1) as well. A Pearson's r correlation between IgG, IgG1, IgG2, IgG3, IgG4, IgM, IgA1, and IgA2 and the functional responses antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent complement deposition (ADCD), and NK degranulation markers CD107a, IFN-γ, and MIP-1β was calculated. The absolute Pearson's r for different vaccination groups 1 (A) and 3 (B) years after MMR-1 is shown. Next, Pearson's r correlations were categorized as very weak (±0–0.19), weak (±0.2–0.39), moderate (±0.4–0.59), strong (±0.6–0.79), and very strong (±0.8–1) (C and D).

antibody response between vaccination groups. One year after receiving the MMR-1 vaccine at 14 months, children who had also received an early dose showed a more coordinated and polyfunctional antibody response. However, 3 years after MMR-1 vaccination, the antibody response shifted, with a decrease in the coordination and polyfunctionality of the response for early-vaccinated children and an increase for children only vaccinated at 14 months.

Our data showed a large drop in measles-specific IgG levels for early-vaccinated children over time, whereas this decrease was less significant for children only vaccinated at 14 months [8]. However, we observed variation in the long-term effects of the different antibody-mediated effector functions upon early vaccination. Natural killer-mediated effector functions are significantly reduced 3 years after MMR-1, whereas less decay was observed in phagocytosis and complement deposition. Structural characteristics of the antibody Fc-domain may influence the functional antibody response after vaccination. The different subclasses of IgG have distinct binding affinities to Fc-receptor, to exert their function [23, 24]. IgG3 has the strongest binding affinity, but it also a short halftime. IgG1 also binds stronger to Fc receptors than IgG2 and IgG4. Variant in subclass distribution might thus affect polyfunctionality [23]. However, we did not observe variation in subclass distribution. Another possibility is variation in glycosylation of the antibody Fc over time, which may explain the differences. Altered glycosylation of a single asparagine (Asn297) on the IgG heavy chain can influence the affinity of the antibody for the Fc receptor and subsequently affect the functional response. Investigating the potential change in glycosylation patterns of measles-specific antibodies after vaccination would be of great interest, and it requires further studies. Finally, expression of Fc-receptor on target cells could further steer the ultimate effect of the polyfunctional antibody response. However, the cellular component of the children was not studied here, but it should be taken into consideration to determine the effectiveness of polyfunctional antibody response.

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Thus, early measles vaccination induces a strong and immunologically versatile antibody response in the short term, but this response gradually deteriorates. The immature immune system particularly in young children (6–8 months) may be influencing this long-term response [25]. Our data suggest that measles vaccination of children between 6 and 8 months induces a B cell response, leading to functional antibodies, but that maturation and differentiation to memory B cells and long-lived plasma cells is maybe less sustained at this young age. Nevertheless, our data provide evidence that early vaccination in an outbreak setting may confer effective short-term protection.

CONCLUSIONS

Overall, our results show that antibodies in early-vaccinated children are as capable of inducing Fc-effector functions as antibodies from children who received 1 regular vaccination at 14 months of age. The consequences of the long-term decline in polyfunctionality still must be elucidated, eg, whether these children are at higher risk to get infected by measles virus, or that exposure to the virus sufficiently boost the vaccine-induced memory response. Nonetheless, the spike in functionality in the short term for young vaccinees is promising for children who receive early measles vaccination in endemic locations [26, 27] or in outbreak situations before the first recommended dose. Most importantly, this work has expanded the scope of understanding into the antibody-mediated measles response in both the short and long term after vaccination across multiple age groups.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Author contributions. I. D. B., J. d. W., R. S. v. B., and D. v. B. designed the study. I. D. B. and A. L. B. performed experiments and did the statistical analysis. G. A. and D. v. B. advised on data analysis and interpretation. I. D. B., J. d. W., R. S. v. B., and D. v. B. wrote the first draft of the manuscript, and all authors contributed to subsequent drafts. All authors read and approved the final manuscript.

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Potential conflict of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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