The Journal of Infectious Diseases

BRIEF REPORT

Measles Virus Neutralizing Antibodies in Intravenous Immunoglobulins: Is an Increase by Revaccination of Plasma Donors Possible?

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We report a screen of plasma donors confirming that widespread use of childhood measles vaccination since 1963 resulted in a decrease in average measles virus antibody titers among plasma donors, which is reflected in intravenous immunoglobulins (IVIGs). The measles virus antibody titer, however, is a potency requirement for IVIGs, as defined in a Food and Drug Administration regulation. To mitigate the decline in measles virus antibody titers in IVIGs and to ensure consistent product release, revaccination of plasma donors was investigated as a means to boost titers. However, revaccination-induced titer increases were only about 2-fold and short-lived.

Keywords. Primary immunodeficiency; measles virus antibody titer; measles vaccination; intravenous immune globulin; titer decline; antibody boost; FDA regulation 21 CFR 640.104.

Intravenous immunoglobulin (IVIG) is a therapeutic preparation with application as antibody replacement therapy in persons with primary immunodeficiency disorders. These patients critically depend on the presence of a variety of functional antibodies in IVIG, to ensure continued protection against pathogens they might encounter. Each lot of IVIG therefore needs to meet minimum potency requirements, which in the United States include a specification for measles virus antibody titer, defined in the Food and Drug Administration (FDA) regulation 21 CFR 640.104 [1]. This measles potency specification has become increasingly difficult to meet, a topic that has been discussed in detail by the FDA, the Blood Products Advisory Committee, and the Plasma Protein Therapeutics Association [2–4].

Received 23 June 2017; editorial decision 9 August 2017; accepted 16 August 2017; published online August 18, 2017.

Presented in part: 2016 Plasma Protein Therapeutics Association Regulatory Workshop, Washington, DC, 13 June 2016.

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The Journal of Infectious Diseases® 2017;216:977–80

DOI: 10.1093/infdis/jix428

Measles virus antibody titers are lower in children after vaccination than after natural infection [5], and the wide deployment of childhood measles vaccination since 1963 is believed to have resulted in lower average measles virus antibody titers in the US general population, as well as in blood and plasma donors and correspondingly in the IVIG preparations fractionated from these donations [6]. Waning of vaccine-induced immunity might further contribute to the decline in measles virus antibody titers in IVIG. Routine measles vaccination is given twice, typically at age 12–15 months and then around school entry at age 4–6 years [7]. Plasma donors, at a minimum age of 18 years, would be expected to present antibody titers even lower than occurring immediately after vaccination [8].

Because reimmunization with the live-attenuated vaccine resulted in an antibody booster reaction in children [5], this intervention could be considered for applicant plasma donors, such that their subsequent plasma donations would contain higher measles virus antibody titers. Including a certain proportion of such boosted measles virus antibody titer plasma units in every plasma manufacturing pool could ensure that the measles specification for IVIG is consistently met, securing a consistent supply of this important therapeutic.

This study aimed to confirm whether the introduction of measles vaccination in the early 1960s in the United Stated led to an almost complete disappearance of natural infections, that translated into lower measles virus antibody titers in source plasma donors. Subsequently, revaccination of plasma donors was investigated as a means to potentially boost measles virus antibody titers, an intervention that might allow manufacturers to meet the minimum potency requirements as defined by the FDA [1].

METHODS

Evaluation of Measles Virus Antibody Titers in Plasma Donors

Data were obtained from 2 different snapshot studies, done in 2007 by CLS Plasma and in 2015 by BioLife. In 2007, sampling was done for 1 day in June, through random collection of about 20% of the total amount of US plasma donations from that day. The 3312 samples were sorted by birth year and separated into 8 cohorts, spanning 4–5 birth years per cohort except for samples from donors born between 1938 and 1957, which were merged into a single cohort. Sample numbers per cohort ranged from 319 (birth years 1938–1957) to 527 (birth years 1982–1985), and subsequently samples were randomly selected and mixed to create 1 pool per cohort (sample pool size by cohort: 1938–1957, n = 45; 1958–1962, n = 55; 1963–1967, n = 80; 1968–1972, n = 135; 1973–1977, n = 50; 1978–1981, n = 60; 1982–1985, n = 60; and 1986–1989, n = 35), resulting in 8 pools.

Five aliquots from each pool were tested by neutralization assay for measles virus antibodies. In 2015, 103 samples were collected before measles revaccination and single donations were tested by neutralization assay for measles virus antibodies. The data were sorted into 10 cohorts, 8 of which corresponded to the cohorts of the 2007 snapshot study and 2 additional cohorts from the 2015 study. Data from the birth year cohorts that were not available during the initial study in 2007, that is, 1990–1993 (n = 18) and 1994–1997 (n = 41), were included in the overall analysis.

Measles Revaccination Study

A total of 103 male qualified plasma donors [9] born between 1957 and 1997 were enrolled in the study in 2015. They had donated plasma between 3–9 times before study initiation, and written informed consent was obtained. Plasma samples for the determination of measles virus antibody titers were collected at 2 BioLife plasma collection centers (Marquette, Michigan and Green Bay [Isbell], Wisconsin) on the day of vaccination and within 29–45 days after vaccination. For a subset of 20 plasma donors, plasma samples were also collected >150 days after vaccination. The M-M-R II (Measles, Mumps, and Rubella Virus Vaccine Live) vaccine (Merck) was used for revaccination of plasma donors.

Neutralization Assay for Measles Virus Antibodies

Measles virus neutralizing antibody titers were determined 5-fold for pools of plasma samples (2007 study) or once for individual plasma donations (2015 study) in samples that were serially diluted in 2-fold steps and that were mixed 1:2 with infectious measles virus (strain Edmonston; CSL, low-passage strain, FDA [10]; Shire, American Type Culture Collection [catalog No. VR-24]) adjusted to a 10^3 median tissue culture infectious dose per milliliter, incubated for 2–3 hours (CSL, 37°C; Shire, 23 ± 5°C) and titrated on Vero cells (European Collection of Authenticated Cell Cultures; catalog No. 8411301). After incubation for 7–9 (CSL) or 10–12 (Shire) days, cells were evaluated microscopically for the presence of a cytopathic effect, and the measles virus neutralization titer (ie, the reciprocal plasma dilution resulting in 50% virus neutralization) was determined using the Spearman-Kärber formula. The neutralization assays were fully validated according to International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Q2(R1) guidelines [11] and included assay validity criteria (confirmation of virus infectivity, cell viability, neutralization and internal reference standard as neutralization controls). They were calibrated against the third World Health Organization International Standard for Anti-Measles (National Institute for Biological Standards and Control code, 97/648), which allowed for data pooling from different laboratories, and measles virus neutralizing concentrations were expressed in international units per milliliter.

Data Analysis

For the evaluation of measles virus antibody concentrations in plasma donors, the geometric means and standard deviations were calculated for each birth year cohort, using Microsoft Excel 2010 software. Paired *t* tests were performed using GraphPad Prism software (version 5.01 for Windows; GraphPad Software).

RESULTS

Evaluation of measles virus antibody titers in US plasma donations showed a titer decrease over 59 years, the time span covered by the birth year cohorts. A roughly 3-fold decline in measles virus antibody concentrations was seen in plasma donors born after 1968 (geometric mean, 1.2 IU/mL), compared with those born before 1962 (≥3.5 IU/mL) (Figure 1). A mean measles virus antibody concentration of 2.8 IU/mL was determined for plasma donors born between 1963 and 1967, the cohort between generations with high and low measles virus antibody titers. Data from the 2015 snapshot study indicated a further downward drift in measles virus antibody titer among donors born after 1990 (geometric mean, 0.5 IU/mL). The measured decline in measles virus antibody among US plasma donors coincided temporally with the first introduction of a measles vaccine in 1963 and the implementation of a 2-dose measles vaccination scheme in 1989 (Figure 1).

Revaccination of plasma donors (n = 103) resulted in a statistically significant ($P < .001$) increase in measles virus antibody titers, which was about 2-fold, from a mean of 0.8 IU/mL on the day of revaccination (day 0) to a mean of 1.4 IU/mL 29–45 days later (Figure 2A). This increase in measles virus antibody titers after vaccination was short-lived, because the titers at ≥150 days

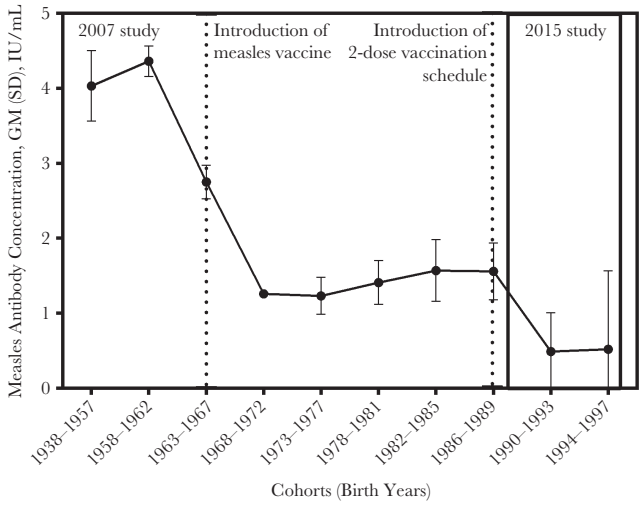

Figure 1. Measles virus neutralizing antibody concentrations in plasma donations, analyzed in donor birth year cohorts. Geometric mean (GM) concentrations (with standard deviation [SD]), are shown for samples collected in the United States in 2007 and 2015, pooled into cohorts according to birth year; data points from the 2007 study represent 5 samples from a single pool per birth cohort, and those from the 2015 study are from individual samples (1990–1993; n = 18; 1994–1997; n = 41). The introduction of measles vaccine programs in the United States is indicated.

(range, 150–230 days after revaccination; mean, 197 days) were almost equal to those on the day of vaccination (n = 20; mean, 0.8, 1.6, and 0.9 IU/mL at day 0, after 29–45 days, and after ≥150 days, respectively) (Figure 2B). At 29–45 days after vaccination, there was no significant correlation between vaccine-induced antibody increase and plasma donor age ($P = .20$).

DISCUSSION

Measles vaccine was first licensed in the United States in 1963 and was implemented in all states by the mid-1980s [3]. By 1989, the 2-dose measles vaccination program was introduced, which resulted in a high level of herd immunity and limited the spread of wild-type measles virus in the population, giving fewer natural opportunities for antigenic boosting, which would also contribute to lower measles virus antibody titers in the general population [3]. The investigation of measles virus antibody titers in the plasma donor population in 2007 indicated a sharp decline in antibody titers for age cohorts born after 1963, which correlated well with the first introduction of measles vaccination in the United States. An additional decline was seen in the snapshot study of 2015, which indicated that the introduction of the 2-dose regime resulted in an even more pronounced reduction of measles virus antibody titers.

In age cohorts born after 1990, antibody titers seem to have leveled off, which might indicate that through vaccination of essentially all younger US plasma donors a "steady state" has been reached. These low levels of measles virus antibody titers in plasma donations translate to lower levels of titers in IVIG [5], which might ultimately endanger the consistent supply of this important therapeutic in the United States, because the minimum potency requirement for measles virus antibodies, as defined in regulation 21 CFR 640.104 [1], has become increasingly difficult to meet. This potency requirement does not apply in Europe or most other parts of the world.

To respond to the observed decline in measles virus antibodies in the plasma donor population, the study in 2015 evaluated the feasibility of revaccination as a means to elicit higher antibody levels in plasma donors. The initial 2-fold increase in antibody titers after revaccination was short-lived, as titers dropped back to similar levels seen just before revaccination approximately 6 months after the booster vaccination. These findings from a plasma donor population 18–58 years of age are consistent with findings of a recent study in which young adults 18–28 years of age showed an overall modest measles virus antibody titer increase 1 month after revaccination. After 1 year, titers had declined to near-baseline levels [12].

In conclusion, the current screen of plasma donations provided further evidence for the continued decline in measles virus antibody titers in the US plasma donor community, which has by now reached a critical level for the supply of IVIG. The current study findings showed that an attempt to boost antibody titers through revaccination cannot be expected to result in a sustainable increase of measles virus antibody titers in plasma donors. It is therefore urgently necessary to consider and investigate alternative measures that will permit a consistent supply of this important therapeutic. A reduction of the measles virus antibody titer required for lot release of IVIG products has already been proposed [3]. With the success of the measles vaccination program and the ongoing World Health Organization efforts to eliminate and ultimately eradicate measles worldwide, a replacement of the measles virus antibody titer as a functional potency requirement for IVIG seems inevitable in the long term.

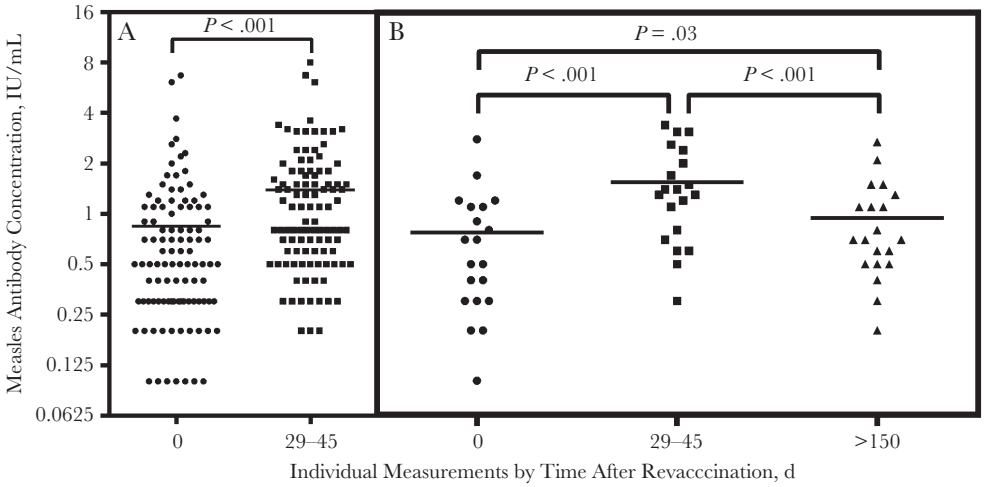

Figure 2. *A*, Measles virus neutralizing concentrations in individuals (n = 103) at revaccination (day 0) and 29–45 days later. Paired *t* tests showed that baseline and post-vaccination data differed significantly ($P < .001$). *B*, Measles virus neutralizing concentrations from the subset of study individuals who, in addition to the sampling at days 0 and 29–45, also provided blood >150 days after revaccination (n = 20). Paired *t* tests showed that the titers measured at 29–45 days differed significantly from both the day 0 titers and those measured at >150 days (both $P < .001$). There was also a significant difference between the baseline titers and those measured after >150 days ($P = .03$). Horizontal bars represent mean values.

Notes

Acknowledgments. The contributions of the entire Global Pathogen Safety team are gratefully acknowledged, most notably those of Philip Rabel (neutralization assays); Veronika Sulzer, Sabrina Brandtner, and Melanie Graf (cell culture); and Eva Ha (virus culture). Malinda Hargitt and Nancy Oakes of the CSL Plasma Laboratory assisted with the organization of the 2007 snapshot, and Mirjam Kuehne of CSL Behring in Bern, Switzerland, supported the assays. Roger Brinser, Julie Quick, Janet Hershman, Peg Pracht, Jim Lenart, and David Zai from BioLife contributed in the revaccination study.

Financial support. CSL and Shire funded the donor snapshot studies, and BioLife funded the revaccination study and analysis of the samples.

Potential conflicts of interest. J. Modrof, B. T., M. R. F., J. A. S., C. M. B., and T. R. K. are employees of Shire, and J. Modrof, M. G., and T. R. K. have stock interest. J. McVey was employed by Baxalta (now part of Shire) at the time of the study, and M. G. supported the work as a contractor. P. F. and T. L. S. are employed by CSL Plasma, a subsidiary of CSL Behring, and have stock interest. 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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