J Clin Immunol (2015) 35:213–217
DOI 10.1007/s10875-015-0131-y

ORIGINAL RESEARCH

Antibody Levels to *Bordetella pertussis* and *Neisseria meningitidis* in Immunodeficient Patients Receiving Immunoglobulin Replacement Therapy

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Received: 20 June 2014 / Accepted: 15 January 2015 / Published online: 29 January 2015

Abstract
Purpose Patients with antibody deficiency rely on immunoglobulin products for protection against many vaccine-preventable diseases. We measured antibody titers against *Bordetella pertussis* and *Neisseria meningitidis* in patients receiving immunoglobulin (IG) therapy to determine if they have any protection against infections from these organisms.
Methods In an unblinded, prospective assessment we measured antibody titers against *B. pertussis* filamentous hemagglutinin (FHA) and pertussis toxin (PT) antigens and *N. meningitidis* serogroups A, C, W-135, and Y in patients receiving immunoglobulin therapy for primary immune deficiency (PI). We measured steady state levels in patients receiving subcutaneous immunoglobulin therapy while in patients receiving intravenous immunoglobulin therapy we measured titers immediately before and after infusion to more clearly define the contribution of the infused product.
Results Thirty subjects, 17 females and 13 males, participated in the study, 22 were receiving intravenous IG products and 8 were receiving subcutaneous IG products. Diagnoses included common variable immunodeficiency in 12, combined immunodeficiency in 6, specific antibody deficiency in 6, X-linked agammaglobulinemia in 4 and ataxia telangiectasia and hyper-IgE syndrome in one each. All subjects had detectable IgG antibodies against the pertussis antigens measured and most had antibody to the meningococcus serotypes measured. However, only 26.6 % had protective levels (>=2 mcg/mL) against serogroup C at trough or steady state.
Conclusions Patients receiving immunoglobulin therapy have antibodies against *B. pertussis* and most of them have antibodies against the four measured serogroups of *N. meningitidis*. There is significant variability in the levels of antibody between patients and the low titers against group C may suggest a role for active immunization in those who may respond to conjugated polysaccharide vaccine administration.

Keywords Meningococcus · pertussis · immune deficiency · immunoglobulin therapy

Introduction

Patients with deficiencies or dysfunctions in their humoral immune responses rely on regular infusions of human immunoglobulin (IG) for protection against infectious diseases. These immune deficiencies include, among others: common variable immune deficiency (CVID), X-linked agammaglobulinemia (XLA) and selective antibody deficiency (SAD), as well as combined immune deficiency (CID), severe combined immune deficiency (SCID), ataxia telangiectasia, and some patients with HIV-1 [1]. IG is manufactured from plasma obtained from healthy donors and contains antibodies against many organisms for which there are effective vaccines [1–3]. At the present time, however, the donor population from which IG is prepared may be largely underimmunized against *B. pertussis* and *N. meningitidis*, and it is not known if and to what degree IG products contain antibodies against these organisms. Two patients with PI who developed

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meningococcal sepsis while on appropriate IG replacement therapy [4] raise the question of whether patients receiving IG therapy are protected against this and other vaccine preventable organisms.

The objective of this study was to determine the presence and levels of antibodies against *B. pertussis* and *N. meningitidis*, the causative organisms for pertussis and meningococcal meningitis, respectively, in patients receiving IG replacement therapy for PI.

Methods

Patients receiving IG replacement therapy in a hospital-based office setting were invited to participate in this research study. The study was approved by the institutional review board and written informed consent was obtained from all patients or parents/legal guardians. Those that accepted had blood drawn for research purposes at the time of venipuncture for standard laboratory monitoring during a clinic visit. Medical records were reviewed; data extracted included age at time of study, most recent IgG trough level, route of IG therapy, IG product infused, diagnosis as indication for IG therapy, and past receipt of vaccination against *B. pertussis* or *N. meningitidis*, if any. For each subject receiving intravenous immunoglobulin (IVIG), pre- and post-infusion samples were collected. This allowed clear differentiation of the contribution of IVIG to the measured antibody levels and identification of potential loss of protection at the "trough" of IgG. For each subject receiving subcutaneous immunoglobulin (SCIG), the antibody levels were assumed to approximate steady state [5] and to provide an indication of antibody persistence throughout infusion intervals. Antibody levels against *B. pertussis* and *N. meningitidis* were measured at Focus Diagnostics, Inc. (Cypress, CA – owned by Quest Diagnostics, Madison, NJ) using a microsphere-based multianalyte immune detection (MAID) system [6]. This methodology was developed primarily to define antibody responses to specific vaccines (Focus Diagnostics Technical Summary, accessed online October 29, 2011). As no clear serologic threshold for protective immunity to *B. pertussis* and *N. meningitidis* has been defined with the MAID system, results of this study defined qualitatively whether IG infusions provide detectable antibodies to patients with PI. Results below the detectable range for the assay were assigned a number between zero and the minimum detectable level; levels above the quantifiable range were assigned the maximum distinguishable level.

We calculated the median and interquartile range for patients getting IVIG and SCIG for each of the eight antibodies tested, then we calculated the differences between peak and trough for patients receiving IVIG and used the sign test to assess the statistical significance of this difference. Because of the inherent 15 % variability in this test (Quest Diagnostics, personal communication J.C.) we also looked at the proportion of patients with more than a 15 % rise in titers and calculated 95 % confidence intervals for this proportion.

Results

We enrolled 30 patients, 22 of whom were receiving IVIG therapy and 8 of whom were receiving SCIG therapy. There were 17 females and 13 males, ages 2–57 years old. The patients in the study had a variety of underlying primary immune deficiencies, including CVID (n=12), CID (n=6), specific antibody deficiency (n=6), XLA (n=4), ataxia telangiectasia (n=1), and hyper IgE syndrome (n=1). Of the patients receiving IVIG, 15 were using Privigen 10%^{TM} (CSL Behring, King of Prussia, Pennsylvania), 5 patients were receiving Gammagard Liquid 10%^{TM} (Baxter, Deerfield, Illinois), 1 used Carimune NF^{TM} (CSL Behring), and 1 patient was on Gammunex^{TM} (Grifols, Los Angeles, California). Of the patients on SCIG, 7 were on Hizentra^{TM} (CSL Behring) and 1 was on Gammunex^{TM} (Grifols). The patients receiving IVIG had a mean trough IgG level of 1180 mg/dL (range 632–1760) and those receiving SCIG had a mean steady state level of 1262 mg/dL (range 949–1680). All patients in our clinic receive a minimum immunoglobulin dose of 0.5 g/kg per month, which is titrated up as needs be to maintain an adequate trough total IgG level. All 30 patients showed evidence of IgG against the pertussis antigens measured, FHA and PT. The patients receiving IVIG therapy each demonstrated a rise in titers greater than 15 % in their IgG titers for FHA and PT post-infusion and the differences between the peaks and troughs were statistically significant (Table 1). The confidence interval for each of these antibodies rising more than 15 % above trough levels was 84.6–100 %. Titers of IgA against FHA and PT were undetectable in most patients, and when there were detectable levels there was no significant change between peak and trough, suggesting that subjects had pre-existing exposure to *B. pertussis* or *B. pertussis* vaccine.

For meningococcus, 28 of the 30 patients had detectable levels of antibodies against all the serotypes measured. However, the percentages of patients having titers over 2 mcg/mL, the presumed protective level, were low for serotypes C and W-135 (Table 2). Of the 28 patients with titers there were 20 patients receiving IVIG, two of them had titers above the quantifiable range of the assay for both peak and trough for two serotypes each. Therefore we cannot determine the contribution of IVIG to the antibody levels for those serotypes in those two patients, so we analyzed the data without them but included the patients that didn't have detectable antibody (Table 3). Two patients demonstrated no measurable antibody against the four meningococcal serotypes tested at both peak and trough despite having a threefold rise in titers of IgG against FHA and PT post-infusion; both patients had CVID

Table 1 Summary of Antibody Measurements in Patients Receiving IVIG (n=22)

	Trough Measurements			Peak Measurements			Peak-Trough Differences			Sign Test
	Median	Q1	Q3	Median	Q1	Q3	Median	Q1	Q3	P value
BP PT IgG (IU)	5	4	8	18.5	12	23	12.5	8	16	<.0001
BP PT IgA (IU)	0.8*	0.8*	1	0.8*	0.8*	1	0	0	0	1.0000
BP FHA IgG (IU)	39	26	56	109	85	156	74	52	97	<.0001
BP FHA IgA (IU)	0.9	0.8*	4	1	0.8*	5	0	0	0	0.7266
NM Sg A (mcg/mL)	4.5	3.5	7.5	10.7	7.5	17.5	4.95	4	8.7	<.0001
NM Sg C (mcg/mL)	1.2	1	2.1	2.8	2.2	4.3	1.5	1.2	2.9	<.0001
NM Sg Y (mcg/mL)	2.65	1.9	4	7.15	4.6	10.2	4.15	2.2	5.2	<.0001
NM Sg W-135 (mcg/mL)	0.8	0.6	1.2	1.9	1.5	3	0.9	0.6	1.4	<.0001

*Antibody levels below the assay's threshold of detection are assigned non-zero values - this may not represent the presence of antibodies

BP Bordetella pertussis, *NM* Neisseria meningitidis, *FHA* filamentous hemagglutinin, *PT* pertussis toxin, *Sg* serogroup, *Q1* top value of first quartile, *Q3* top value in third quartile

but each received different intravenous formulations of IG. However, other patients receiving these same products had antibodies against these antigens.

No specific trends were noted with relation to titers and underlying diagnosis or IG product infused. We collected pertussis and meningococcal immunization history from these patients, but for many of the patients we were unable to get a complete history that included the vaccine given and the date it was administered. For this reason we have not included these data in the manuscript.

Discussion

The primary mechanism of protection against pertussis and meningococcal infection is likely antigen-specific antibody [7], so it is reasonable to try to protect antibody-deficient patients from these diseases with IG replacement therapy. Unfortunately there is no widely accepted protective antibody level for pertussis. There is, however, evidence to show that risk of infection is inversely proportional to concentration of antibodies against PT and pertactin (a third vaccine protein, not measured in this study) [8]. In meningococcal infection, the commonly referenced protective antibody level is 2 mcg/mL. This value is based on a study done by the Finnish military showing that their adult population had a very low rate of meningococcus A infection and that 60 % of them had titers over 2 mcg/mL to meningococcus A [9]. This has been empirically extrapolated to the other serotypes with the assumption that they would require the same titers for protection. Thus we chose to examine the antibody levels in our subjects to determine if they had levels of antibodies that suggested protection against these diseases.

Table 2 Percentage of patients protected against each tested meningococcus serotype at trough or steady state[a] measurement (n=30)

Serogroup of N. meningitidis	A	C	Y	W-135
Percentage of Patients protected[b]	93.3 %	26.6 %	80 %	10 %

[a] In patients receiving IVIG we got trough levels and in patients receiving subcutaneous immunoglobulins we got steady state levels

[b] This assumes that titers>= 2 mcg/mL are protective – the validity of this assumption is discussed in the accompanying article

For pertussis, we found that subjects universally have IgG against FHA and PT, which implies some level of immunity against pertussis. The presence of these antibodies in patients that cannot make IgG due to XLA or SCID, the consistent increase in titers after IV infusion of IG therapy, and the lack of IgA to these antigens in the presence of IgG against them all support the conclusion that the IG therapy is the source of the antibody. This universal presence of pertussis antibodies is consistent with the observation that to our knowledge severe pertussis has not been reported in patients receiving IG replacement therapy for PI.

Similarly for meningococcal antigens we found that 28 of 30 patients had measurable antibody against the four serotypes we tested. This implies that most patients on IG replacement therapy are afforded some protection against these two diseases. Patients in our study had lower titers against serogroups C and W-135, with only about a quarter of individuals having levels≥2 mcg/mL against serogroup C and 10 % having≥2 mcg/mL against serogroup W-135 at trough or steady state sampling (Table 3). This is potentially concerning because serogroups C and B are the main causes of meningococcal outbreaks in the US. Of note, both of the reported patients with CVID that suffered from meningococcal infections [4] were receiving Sandoglobulin™, now

Table 3 The percent increase in titers after IVIG infusion and proportion of patients with significant increase

	BP PT IgG	BP PT IgA	BP FHA IgG	BP FHA IgA	NM Sg A	NM Sg C	NM Sg Y	NM Sg W-135
Median Percent Increase in titers (Interquartile Range)	231 (200,288)	0 (0,0)	207 (161,271)	0 (0,0)	110 (88,152)	113 (67,150)	149 (79,205)	117 (50,155)
Proportion of Patients with >15 % rise[a] (95 % confidence interval)	22/22=100 % (84.6, 100)	3/22=13.6 % (2.9, 34.9)	22/22=100 % (84.6, 100)	4/22=18.2 % (5.2, 40.3)	19/21=90.5 % (69.6, 99.8)	20/22=90.9 % (70.8, 98.9)	19/21=90.5 % (69.6, 99.8)	18/20=90 % (68.3. 98.8)

[a] The variability in the assay is +/- 15% (Quest Diagnostics, personal communication J.C.), thus this is a measure of the proportion of patients with a measured rise large enough to signify a true rise and not variability in the assay

BP = Bordetella pertussis, NM = Neisseria meningitidis, PT = pertussis toxin, FHA = filamentous hemagglutinin, Sg = serogroup

known as Carimune™ (CSL Behring) in the US. However these two infections occurred over a decade ago in the United Kingdom and were caused by N. meningitidis serogroup B, which is not in any of the meningitis vaccines currently approved in the US. One of our patients was receiving Carimune™ and demonstrated titers suggestive of protection against all four serotypes in current meningococcal vaccines.

The variability in measured titers raises questions about variation within a single formulation of IG, as two patients without antibody against meningococcus were receiving products shared by other participants in this study who had antimeningococcal antibodies from the IG they received. One approach to addressing this would be for manufacturers of IG products to label batches with relevant antibody concentrations that are known to be variable. The low levels of antibody against *Neisseria meningitidis* serogroup C are particularly concerning as this is the most relevant meningococcal serotype for North American patients. This suggests that patients receiving IG therapy but who nevertheless may be able to respond to conjugated polysaccharide vaccines may benefit from meningococcal immunization. Furthermore, clinicians treating patients with IVIG cannot assume that their patients are fully protected against specific infections unless protective levels of antibody are demonstrated in the product.

Acknowledgments This research was supported in part by the Jeffrey Modell foundation and the NIH NCRR CTSI GRANT 1UL1RR031986 and was performed at the CTSI at Children's Hospital Los Angeles.

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