

Protective effect of IgM against colonization of the respiratory tract by nontypeable *Haemophilus influenzae* in patients with hypogammaglobulinemia

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Background: Primary immunoglobulin deficiencies lead to recurrent bacterial infections of the respiratory tract and bronchiectasis, even with adequate immunoglobulin replacement therapy. It is not known whether patients able to secrete IgM (eg, those with hyper-IgM [HIGM] syndrome) are as susceptible to these infections as patients who lack IgM production (eg, those with panhypogammaglobulinemia [PHG]).

Objective: This study is aimed at identifying specific microbiological and clinical (infections) characteristics that distinguish immunoglobulin-substituted patients with PHG from patients with HIGM syndrome.

Methods: A cohort of patients with HIGM syndrome (n = 25) and a cohort of patients with PHG (n = 86) were monitored

prospectively for 2 years while receiving similar polyvalent immunoglobulin replacement therapies. Regular bacterial analyses of nasal swabs and sputum were performed, and clinical events were recorded. In parallel, serum and saliva IgM antibody concentrations were measured.

Results: When compared with patients with PHG, patients with HIGM syndrome were found to have a significantly lower risk of nontypeable *Haemophilus influenzae* carriage in particular (relative risk, 0.39; 95% CI, 0.21-0.63). Moreover, patients with HIGM syndrome (including those unable to generate somatic hypermutations of immunoglobulin genes) displayed anti-nontypeable *H influenzae* IgM antibodies in their serum and saliva. Also, patients with HIGM syndrome had a lower incidence of acute respiratory tract infections.

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Conclusions: IgM antibodies appear to be microbiologically and clinically protective and might thus attenuate the infectious consequences of a lack of production of other immunoglobulin isotypes in patients with HIgM syndrome. Polyvalent IgG replacement therapy might not fully compensate for IgM deficiency. It might thus be worth adapting long-term antimicrobial prophylactic regimens according to the underlying B-cell immunodeficiency phenotype. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: *Haemophilus influenzae*, infections, immunoglobulins, IgM, immunodeficiency

Primary B-lymphocyte immunodeficiencies are characterized by defective immunoglobulin production¹ and thus result in high susceptibility to recurrent bacterial infections of the upper and lower respiratory tracts. Bacteria known to colonize airway epithelia (eg, nonencapsulated nontypeable *Haemophilus influenzae* [NTHi])²⁻⁶ and those that are invasive (eg, *Streptococcus pneumoniae* and *Moraxella catarrhalis*) are the most frequent causes of recurrent pneumonia, bronchitis, sinusitis, and otitis in these immunodeficient patients.⁷⁻¹² A number of studies have highlighted the association between NTHi and the severity of chronic lung disease in children with non-cystic fibrosis-induced bronchiectasis,¹³ patients with chronic obstructive pulmonary disease,¹⁴ and adults with bronchiectasis.¹⁵ Of note, however, NTHi has not been thus far shown to directly cause chronic lung damage in patients with chronic obstructive pulmonary disease-induced bronchiectasis or primary immune deficiency. Untreated hypogammaglobulinemia invariably leads to bronchiectasis, which in turn develops into chronic respiratory insufficiency.^{16,17} Immunoglobulin replacement therapy has profoundly modified the prognosis of these conditions and leads to a very significant reduction in the occurrence of respiratory tract infections and bronchiectasis, notably when treated with appropriate doses.^{16,18} Nevertheless, chronic complications are still observed in a fraction of patients with hypogammaglobulinemia.¹⁷ There are distinct subsets of profound primary B-cell immunodeficiencies; these include panhypogammaglobulinemia (PHG) with X-linked and autosomal recessive agammaglobulinemia, common variable immunodeficiency, and hyper-IgM (HIgM) syndrome, in which IgM production is maintained.¹ It is not clear whether the frequency of bacterial infection and colonization of the respiratory tract varies according to the partial maintenance of IgM antibody production.

We prospectively investigated IgM-mediated protection by assessing nasopharyngeal and oropharyngeal bacterial carriage and clinically recorded infectious events over 2 years in a PHG cohort and an HIgM syndrome cohort. We found that patients with HIgM syndrome carried NTHi less frequently and had anti-NTHi IgM antibodies in their serum and saliva. This finding suggests that IgM antibodies have a distinct anti-infective activity involved in the prevention of respiratory tract bacterial colonization and infection.

METHODS

Patients

Patients given a diagnosis of inherited PHG or HIgM syndrome were eligible for participation in this French, nationwide, multicenter prospective study (n = 10 centers). The main inclusion criteria were profound hypogammaglobulinemia at the time of diagnosis (plasma IgG level <200 mg/dL and plasma IgA level <10 mg/dL for all patients, plasma IgM level <30 mg/dL for

Abbreviations

AID:	Activation-induced deaminase
BTK:	Bruton tyrosine kinase
CD40L:	CD40 ligand
HIgM:	Hyper-IgM
NTHi:	Nontypeable <i>Haemophilus influenzae</i>
PHG:	Panhypogammaglobulinemia
RR:	Relative risk
SHM:	Somatic hypermutation

patients with PHG [regardless of B-cell counts], and normal or increased plasma IgM levels for patients with HIgM syndrome).

Of 122 eligible patients, 11 did not meet the inclusion criteria (10 with PHG and 1 with HIgM syndrome). Immunologic, genetic, and clinical data on the remaining 111 patients (86 with PHG and 25 with HIgM syndrome) are summarized in Table I.

All patients were receiving immunoglobulin replacement therapy with a median IgG trough level of greater than 900 mg/dL, which is considered to be satisfactory for infection prevention.

Immunologic and genetic studies

The immunodeficiency was characterized, as previously described.¹⁹ The investigation included extensive blood T-lymphocyte, natural killer lymphocyte, and B-lymphocyte phenotyping¹⁹; detection of Bruton tyrosine kinase (BTK), CD40 ligand (CD40L), and CD40 with specific antibodies (when appropriate); determination of somatic hypermutation (SHM) frequency in the VH3-23 region of immunoglobulin heavy chain genes¹⁹; and screening for mutations in the *BTK* gene (for patients with PHG) or the *CD40L*, *CD40*, activation-induced deaminase (*AID*), and uracil N-glycosylase genes (for patients with HIgM syndrome).^{1,20} We excluded patients with associated T-cell immunodeficiencies (eg, ataxia-telangiectasia or X-linked immunodeficiency with hypohidrotic ectodermic dysplasia), with the exception of CD40L deficiency.

Study design

Patients were enrolled for 2 years (from August 2006 to September 2008) in a prospective cohort study. On enrollment, we recorded the following data: diagnosis, clinical examination, growth status, chronic sinusitis and bronchiectasis (as assessed by using a computed tomographic scan), past frequency of hospitalization for infections, infection sites and causal microorganisms (when known), and, lastly, therapy (including the type of immunoglobulin replacement therapy and long-term anti-infective prophylaxis). Patients were evaluated every 3 to 6 months, and the following items were recorded: clinical parameters (occurrence of acute infections, chronic infections, or both; drug tolerance; immunoglobulin replacement therapy; and, when administered, prophylactic antibiotics), immunologic and microbial parameters (trough IgG, IgM, and IgA serum levels; direct microbiological examination of sputum swabs, nasopharyngeal swabs, or both; and the presence of 6 determined bacterial species [*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and *H influenzae*]). Infectious events were defined as those requiring treatment with antibiotics. Pneumonia was defined as the occurrence of fever and the presence of a new lung parenchymal anomaly on a chest radiograph, a computed tomographic scan, or both. Episodes of sinusitis and bronchitis were defined as those recorded prospectively by a physician in a clinical examination (with or without a radiologic examination). For these latter sets of infections, this could have influenced the objectivity of event recording, although it should be stressed that patients were all under the care of experienced physicians.

All patients, their legal representatives, or both were given comprehensive information on the study and provided their informed written consent to

TABLE I. Patients' characteristics at the time of study enrollment (n = 111)

	Patients with PHG* (n = 86 [%])	Patients with HlgM syndrome (n = 25)	P value
BTK deficiency	64 (74.4)		
Other types of PHG	22 (25.6)		
CD40L deficiency		12	
AID deficiency		6	
Other HlgM syndrome		7	
Sex (% male)	88.4	96.0	NS
Median age on enrollment (y)	12.6	16.4	NS
Children aged ≤15 y (%)	58.1	44.0	NS
Children aged ≤5 y (%)	12.8	8.0	NS
Immunoglobulin replacement therapy (%)			
Intravenous administration	73.3	48.0	<.02
Subcutaneous administration	26.7	52.0	
Median IgG trough level (mg/dL)	920	950	NS
Antibioprophylaxis (%)	15.1	56.0	<.0001
Cotrimoxazole (%)	5.8	52.0	<.0001
Bronchiectasis (%)	27.9	8.0	<.05
Chronic sinusitis (%)	23.3	8.0	NS

NS, Not significant.

*All patients with PHG were screened for *BTK* gene sequence. BTK protein expression was assessed when a mutation in the *BTK* gene was not found.

participate. The study's objectives and procedures were approved by the Necker Hospital's independent ethics committee.

Bacteriology

We screened for the 6 most frequent bacterial species known to colonize the respiratory tract of patients with B-cell deficiencies (listed in the previous section). The study personnel processing the sputum samples were blind to the subjects' immune deficiency and clinical status. Bacterial cultures were incubated at 37°C on blood, chocolate agar plates, and selective medium plates for *P aeruginosa* (cetrimide agar), *S aureus* (mannitol salt agar), and *N meningitidis* (Thayer-Martin medium containing vancomycin, colistin, and nystatin). Agar plates were analyzed first after 24 to 48 hours and then after 7 days of incubation. Isolates were identified according to standard clinical microbiology procedures. For each *H influenzae* isolate, the capsule phenotype was determined by using the Phadebact *Haemophilus* test (Bactus, Huddinge, Sweden).

Detection of anti-*H influenzae* IgM antibodies in serum and saliva samples

H influenzae antigens were extracted by means of sonication from 10 distinct isolates originating from hypogammaglobulinemic patients and then pooled. Each isolate was characterized by means of sodium sulfate polyacrylamide gel electrophoresis.²¹ Anti-NTHi antibodies were screened for by using a Western blot assay and an ELISA. Serum samples from healthy individual adults (n = 6) and patients with cystic fibrosis (n = 5) were used as controls. Results of anti-NTHi titers were expressed as reciprocal of the highest dilution that resulted in an OD at 490 nm greater than that seen in the negative controls. All samples were analyzed in the same laboratory.

Statistical analyses

Continuous variables are presented with their medians and interquartile ranges. Groups were compared by using the Mann-Whitney *U* test for continuous variables and the χ^2 or Fisher exact tests for categorical variables. Multivariate logistic regression was used to evaluate the association between (1) bronchiectasis and chronic sinusitis at the study's onset and (2) independent variables, such as cotrimoxazole prophylaxis, age, and type of immunodeficiency. We used a log-linear Poisson regression model to calculate the respective overall relative risks (RRs) and 95% CIs for bacterial carriage by patients

with HlgM syndrome and patients with PHG. After an initial univariate analysis, multivariate analysis was used for the final model. NTHi-free survival rates were estimated by using the Kaplan-Meier method. The log-rank test was used to compare patients with HlgM syndrome and patients with PHG in terms of NTHi-free survival rates. SAS software (version 9.1; SAS Institute, Inc, Cary, NC) was used for all statistical analyses.

RESULTS

Bacterial colonization of the respiratory tract

As shown in Table II, 782 and 3152 determinations of bacterial carriage in nasal swabs, sputum, or both were carried out in patients with HlgM syndrome and patients with PHG, respectively. There was a significantly lower global frequency of positive test results in patients with HlgM syndrome (RR, 0.57; 95% CI, 0.44-0.75) than in patients with PHG. Specific analysis of each of the 6 bacterial species showed that (1) the frequency of NTHi detection was almost 3-fold higher in patients with PHG than in patients with HlgM syndrome and (2) there was no statistical difference in carriage of the other bacterial species. We thus focused our analysis on the statistically significant difference between patients with PHG and patients with HlgM syndrome in terms of NTHi carriage (RR, 0.35; 95% CI, 0.21-0.57). As shown in Fig 1, the probability of remaining NTHi free over the 2-year study period was significantly higher in patients with HlgM syndrome ($P = .012$, log rank test).

Carriage of NTHi in the respiratory tract of patients with X-linked agammaglobulinemia (mutated *BTK*) was similar to that seen in other cases of PHG. Because a subgroup of patients with HlgM syndrome was receiving cotrimoxazole because of CD40L deficiency (for *Pneumocystis jiroveci* prophylaxis), we investigated whether this drug influenced NTHi carriage. This was not found to be the case in a multivariate analysis because only a diagnosis of HlgM syndrome and age greater than 15 years were shown to be independently associated with a lower risk of NTHi carriage. Neither cotrimoxazole prophylaxis nor other antibiotic prophylaxis had a significant influence on NTHi carriage (Table III). Also, NTHi carriage was detected as well in patients with PHG without (49/62 [79%]) or with (19/24 [79.1%]) bronchiectasis, indicating that bronchiectasis was not the primary cause of NTHi carriage. For patients with HlgM syndrome, there was no difference in the frequency of NTHi carriage when comparing patients with SHM-positive B cells (33%, n = 6) with those lacking SHM-positive B cells (40%, n = 10).

IgM-mediated antibody responses to NTHi

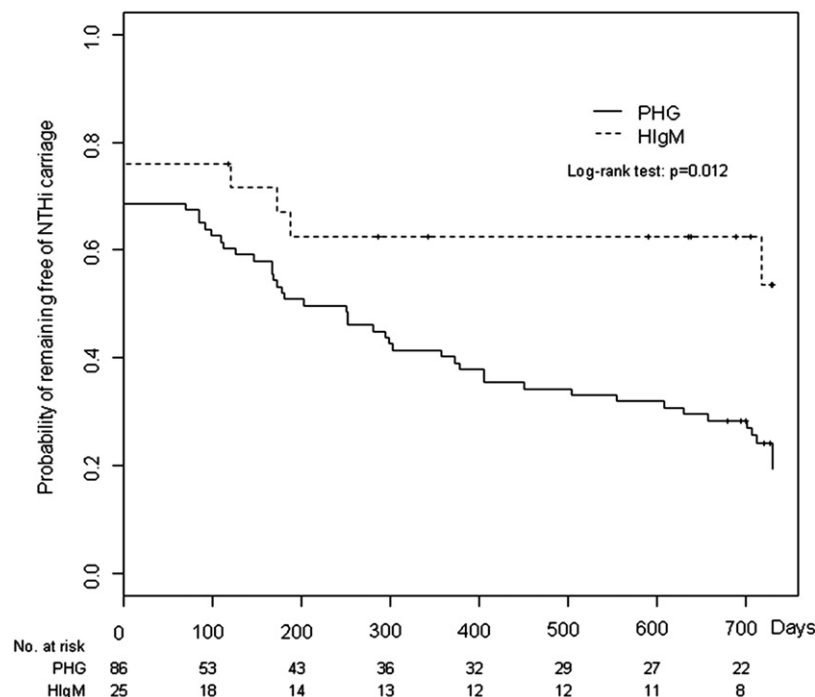
Given that NTHi carriage was significantly lower in patients with HlgM syndrome than in patients with PHG, we next investigated whether these results correlated with the detection of anti-NTHi IgM antibodies in the patients' sera. A Western blot and ELISA analysis showed that patients with HlgM syndrome had detectable serum anti-NTHi IgM levels, whereas patients with PHG did not (Fig 2, A). Serum samples from control subjects and patients with cystic fibrosis had little IgM detectable antibodies (Fig 2, A). Furthermore, serum anti-NTHi IgM titers were higher in patients with HlgM syndrome than they were in healthy blood donors and age-matched patients with cystic fibrosis (Fig 2, B). No correlation was found between anti-NTHi IgM titers and serum IgM levels (data not shown). Because B cells from 10 of 16 tested patients with HlgM syndrome were shown

TABLE II. RR of microbial carriage over the 2-year study period in patients with HIgM syndrome and patients with PHG

	Patients with HIgM syndrome (n = 25)	Patients with PHG (n = 86)	RR* (95% CI)
All bacteria; positive test result (%)	60/782; 7.7	416/3152; 13.2	0.57 (0.44-0.75)
<i>Haemophilus influenzae</i> ; positive test result (%)	17/132; 13.0	195/532; 36.7	0.35 (0.21-0.57)
<i>Moraxella catarrhalis</i> ; positive test result (%)	6/130; 4.6	54/524; 10.3	0.45 (0.19-1.04)
<i>Staphylococcus aureus</i> ; positive test result (%)	16/130; 12.3	100/529; 18.9	0.65 (0.38-1.10)
<i>Streptococcus pneumoniae</i> ; positive test result (%)	18/133; 13.5	52/526; 9.9	1.37 (0.80-2.34)
<i>Pseudomonas aeruginosa</i> ; positive test result (%)	2/130; 1.6	15/521; 2.9	0.53 (0.12-2.34)
<i>Neisseria meningitidis</i> ; positive test result/no. of tests (%)	1/127; 0.1	0/520; –	–

Poisson regression: number of positive tests/number of tests; monovariate analysis for each bacteria. Positive tests/all tests: positive test results as a proportion of all tests for any of the 6 bacteria. The few variations in number of samples reflects culture failure or missing data.

*The reference (RR, 1) is the PHG group.

**FIG 1.** Probability of being free of NTHi over the 2-year study period for patients with PHG (solid line) and patients with HIgM syndrome (dashed line; Kaplan-Meier analysis).**TABLE III.** RR of NTHi carriage according to the underlying disease, age, cotrimoxazole prophylaxis, and other antiobiprophylaxis (n = 111)

	RR (95% CI)*
HIgM syndrome	0.39 (0.21-0.63)
Age >15 y	0.68 (0.51-0.93)
Cotrimoxazole prophylaxis	1.16 (0.72-1.85)
Other antiobiprophylaxis	1.26 (0.81-1.95)

*The references (RR, 1) of each variable included in the multivariate model are as follows: PHG, age of 15 years or less, absence of cotrimoxazole prophylaxis, and absence of other antiobiprophylaxis.

to be unable to undergo SHM, we looked at whether patients with HIgM syndrome with germline IgM had lower anti-NTHi titers. There was no detectable correlation between SHM frequency and antibody titers (as assessed by means of ELISA in 14 patients, Fig 3). It is noteworthy that the anti-NTHi IgG antibodies in both

patients with HIgM syndrome and patients with PHG originated from their polyvalent IgG replacement therapy (Fig 2) because these antibodies were detected in the administered IgG preparations. As a surrogate marker for mucosal antibody detection, we measured total IgM levels and anti-NTHi IgM levels in the saliva of patients with HIgM syndrome and control subjects. These antibodies could be detected in the saliva of 8 of 8 tested patients with HIgM syndrome, 6 of whom had negative SHM results (Fig 4, A). The saliva titers correlated well with the serum anti-NTHi IgM antibody titers (Fig 4, B). Of note, we did not detect a correlation between saliva anti-NTHi IgM titers and the total saliva IgM concentration. As shown in Table IV, there was a lower acute infection rate in patients with HIgM syndrome (RR, 0.38; 95% CI, 0.22-0.68) than in patients with PHG during the study period. The multivariate analysis clearly showed the protective role of a diagnosis of HIgM syndrome (Table V). Older age and cotrimoxazole prophylaxis were associated with a significant, although moderate, risk of acute infection (Table V).

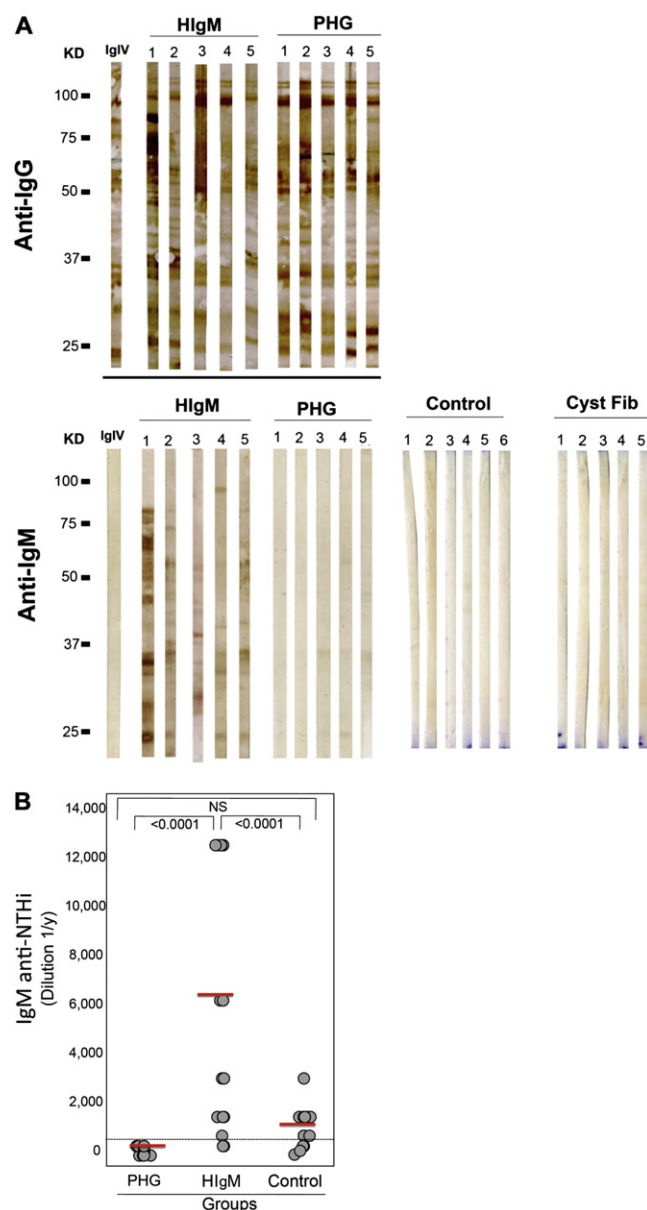


FIG 2. IgM antibodies in the sera of patients with HlgM syndrome specifically bind to NTHi antigens. **A**, Serum IgG and IgM antibodies were tested against a pool of *H influenzae* antigens (previously separated by means of SDS-PAGE). Anti-NTHi IgG antibodies in the sera of patients with HlgM syndrome and patients with PHG (*upper panel*) had the same profile as that obtained for an intravenous immunoglobulin preparation. The presence of anti-NTHi IgM antibodies in patients with HlgM syndrome was compared with that seen in patients with PHG and 2 control groups (healthy control subjects and patients with cystic fibrosis). *Control*, Control noninfected subjects ($n = 6$); *Cyst Fib*, patients with cystic fibrosis ($n = 5$) as a control group of patients with chronic *H influenzae* infections; *IgIV*, intravenous immunoglobulins. HlgM: *lane 1*, AID deficiency; *lanes 2 and 3*, CD40L deficiency; *lanes 4 and 5*, unknown. PHG: *lanes 1-3*, BTK (-); *lanes 4 and 5*, BTK (+). All the serum immunoblots were performed with the same dilution (1:50). **B**, Serum anti-NTHi IgM assay results. Individual and median values (*bars*) for the patients with PHG ($n = 19$), patients with HlgM syndrome ($n = 19$), and control subjects ($n = 18$) are shown. Values are expressed as the highest dilution enabling the detection of anti-NTHi IgM. ANOVA indicated significant differences ($P < .001$) among the 3 groups. P values for the nonparametric comparisons for each group are shown in the Figure. NS, Nonsignificant.

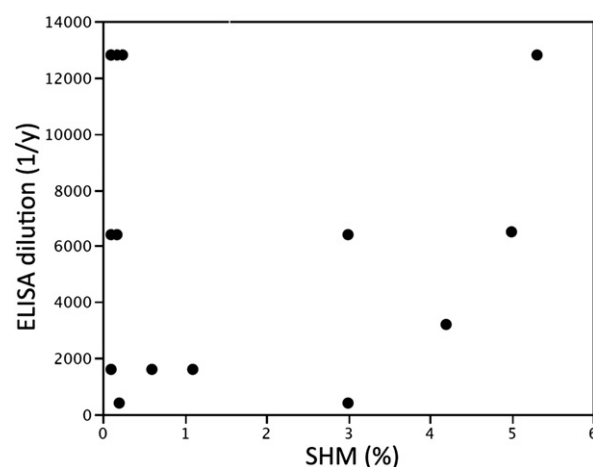


FIG 3. Lack of correlation between SHMs and the level of serum anti-NTHi IgM antibodies. Fourteen patients were tested (AID, $n = 6$; CD40L, $n = 4$; and unknown, $n = 4$). $R^2 = 0.03$.

DISCUSSION

The results of this study show that carriage of bacteria in general and NTHi in particular in nasal swabs and sputum is more frequent in patients with PHG than in patients with HlgM syndrome. This difference is not related to adjunct, potentially confounding variables, such as antibiotherapy administration, age, and IgG trough level. The frequency of NTHi carriage is essentially similar in BTK⁻ and BTK⁺ patients with PHG, excluding an additional potential role of BTK defects in other cells than B lymphocytes. NTHi is known to commonly be associated with upper respiratory tract infections (notably in immunocompetent children)³ and sinusitis, bronchitis, and pneumonia in patients with hypogammaglobulinemia.⁷⁻¹² Our present results strongly indicate that IgM protects against pharyngeal NTHi carriage and possibly against related respiratory tract infections in patients with HlgM syndrome. Indeed, anti-NTHi IgM antibodies were detected by means of Western blotting and ELISA in the saliva (used here as a surrogate for mucosal surfaces) and sera of patients with HlgM syndrome. Remarkably, even the IgM of patients with SHM-negative B cells (as observed in patients with AID deficiency²²) contained antibodies to NTHi; hence antibody titers did not correlate with the frequency of SHM detection. However, this finding does not rule out the possibility that unmutated anti-NTHi IgM antibodies were less affine than those produced by patients able to undergo immunoglobulin SHM, even though patients with HlgM syndrome with SHM-positive B cells and those with SHM-negative B cells were less susceptible to NTHi carriage to a similar extent.

Anti-NTHi IgM antibodies have previously been detected in the sera of patients with bronchiectasis²³ but not, to our knowledge, in saliva. A protective role for anti-NTHi antibodies has been reported in animal models.²⁴⁻²⁷ *In vitro* human NTHi antibodies can facilitate complement-mediated killing of bacteria and neutrophil intracellular killing of NTHi.²⁷ It is also conceivable that antibodies binding to NTHi surface proteins (eg, HMW, Hap, and Hif, which are known to mediate attachment to the upper respiratory tract epithelium) can prevent bacterial adhesion and thus colonization.²⁸⁻³² Like IgA (but not IgG), IgM antibodies are part of mucosal immunity. The J-chains containing

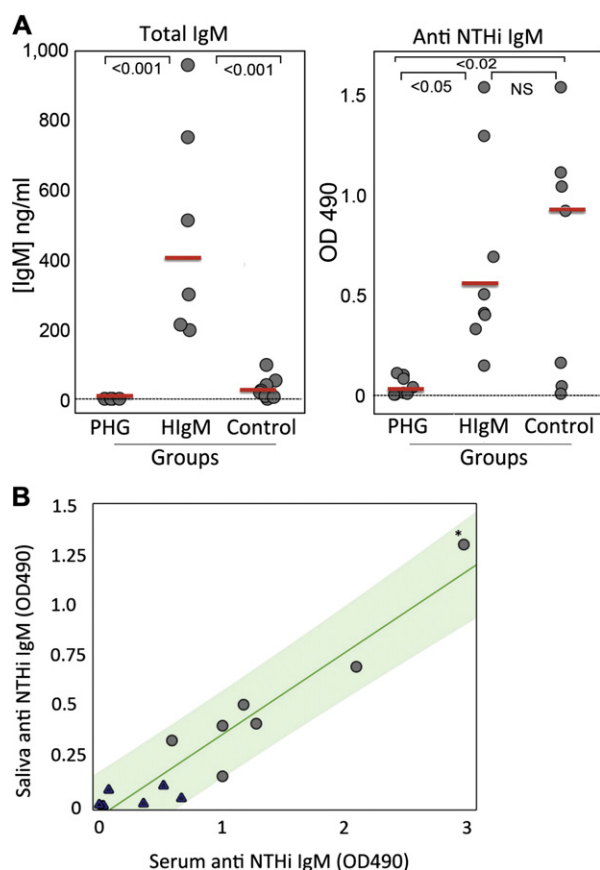


FIG 4. Detection of anti-NTHi IgM antibodies in saliva. **A**, Individual and median values (bars) for total IgM antibodies (expressed in nanograms per milliliter of IgM) and anti-NTHi IgM detected in the saliva (expressed as the OD at 490 nm) are shown for the 3 groups: patients with PHG ($n = 8$), control subjects (healthy adults, $n = 9$), and patients with HIgM syndrome ($n = 8$). Nonparametric ANOVA revealed a significant difference for total IgM ($P < .001$) and anti-NTHi IgM antibodies ($P < .005$) in the saliva. The P values for comparisons of patients with PHG and patients with HIgM syndrome with the control group are indicated in the figure. **B**, Correlation between serum and saliva anti-NTHi IgM levels. Anti-NTHi IgM levels in serum and saliva (determined in an ELISA) are expressed as the OD at 490 nm (after background subtraction) for the 1:50 dilution. The linear regression was statistically significant ($R^2 = 0.9$, $P < .001$). *Denotes an out-of-range value in the ELISA at the tested dilution. Triangles, Patients with PHG; circles, patients with HIgM syndrome.

pentameric IgMs are exported by the polyimmunoglobulin receptor to mucosal surfaces.³³ IgM antibodies could therefore (like IgA but not IgG) act at epithelial surfaces to clear bacteria and prevent colonization and invasion. IgM-producing cells account for 6% (nasal glands) to 18% (duodenum/jejunum) of the antibody-producing cells in mucosae.³³ It is therefore plausible that IgM isotype antibodies might, at least in part, protect patients with HIgM syndrome against bacteria known to colonize the upper and lower respiratory tract epithelium.^{2,6} Interestingly, it has been suggested that the increased frequency of IgM plasma cells found in the mucosae of patients with selective IgA deficiency accounts for their lower frequency of infectious episodes.³³ It has also been reported that AID-deficient mice show a marked increase in IgM⁺ plasma cells, at least in the gut.³⁴ IgD antibodies, which are produced in the upper respiratory mucosa, might also be involved in protection. However, patients with HIgM

TABLE IV. Occurrence of acute infections over the 2-year study period in patients with HIgM syndrome and patients with PHG

Occurrence	Mean no. of infections (range)		
	Patients with HIgM syndrome ($n = 25$)	Patients with PHG ($n = 86$)	RR (95% CI)*
Bronchitis	0.4 (0-2)	1.0 (0-7)	0.40 (0.21-0.78)
Sinusitis	0	0.23 (0-5)	—
Pneumonia	0	0.06 (0-1)	—
Otitis	0.08 (0-1)	0.02 (0-1)	3.44 (0.48-24.4)
All infections	0.52 (0-2)	1.35 (0-10)	0.38 (0.22-0.68)

Actual numbers of infected patients were as follows: bronchitis — patients with HIgM syndrome, $n = 9$; patients with PHG, $n = 38$; sinusitis — patients with HIgM syndrome, $n = 0$; patients with PHG, $n = 14$; pneumonitis — patients with HIgM syndrome, $n = 0$; patients with PHG, $n = 5$; and otitis — patients with HIgM syndrome, $n = 2$; patients with PHG, $n = 2$.

*The reference (RR, 1) is the PHG group.

TABLE V. RR of acute infections over the 2-year study period in patients with HIgM syndrome and patients with PHG: multivariate analysis ($n = 111$)

	RR (95% CI)*
Patients with HIgM syndrome	0.24 (0.13-0.47)
Age >15 y	1.73 (1.22-2.50)
Cotrimoxazole prophylaxis	2.02 (1.21-3.39)
Other antibioprophyllaxis	0.56 (0.24-1.27)

*The references (RR, 1) of each variable included in the multivariate model are as follows: PHG, age of 15 years or less, absence of cotrimoxazole prophylaxis, and absence of other antibioprophyllaxis.

syndrome (or at least those with AID deficiency) are also IgD deficient.³⁵

The present study therefore provides the first indication of a protective role of IgM antibodies in a context of deficient IgG and IgA production. It has been shown that anti-influenza virus IgM antibodies were able to protect AID-deficient mice against experimental influenza infection³⁶ and death, whereas a role for IgM in immunity to various microorganisms has been shown in murine experimental models, as reviewed by Ehrenstein and Notley.³⁷ In addition to the statistically significant difference between patients with HIgM syndrome and patients with PHG in terms of NTHi carriage, one cannot rule out more subtle and less visible roles of IgM antibodies in protection against other mucosal pathogens. That no statistical difference could be observed for other bacteria might reflect rarity of carriage, lack of power, or a specific function of secretory IgM in the prevention of NTHi adhesion to epithelial cells. It is tempting to propose that the presence of IgM antibodies at the mucosal surfaces in patients with HIgM syndrome accounts for the observed lower frequency of upper and lower respiratory tract infections in general and pneumonia in particular. The fact that patients with HIgM syndrome exhibit less frequent chronic sinusitis and bronchiectasis compared with that seen in patients with PHG could also result from a protective role of IgM antibodies, notably those directed against the common pathogen NTHi.

However, we should acknowledge that these data are only correlative; in the absence of appropriate, well-characterized animal models, it will be very difficult to provide definitive evidence of a causal link. This observation nevertheless points to an incomplete efficacy of IgG replacement therapy in patients with PHG, even

when high trough IgG levels are obtained, as was the case for the participants in the present study. IgG cannot be transported to the surfaces of epithelial cells and is potentially less effective in preventing colonization/infections by bacteria, such as NTHi. Hence to further reduce the occurrence of infectious episodes and irreversible bronchial lesions in patients with PHG, additional therapeutic measures should be considered. Long-term antimicrobial prophylaxis for reducing this type of bacterial colonization might be warranted in patients with IgM-deficient hypogammaglobulinemia and should be evaluated in prospective clinical trials.

In conclusion, this prospective study provides evidence for a protective role of IgM antibodies against colonization of the respiratory tract by NTHi. This protection might have attenuated the infectious consequences of the immunoglobulin immunodeficiency. Our data might provide a rationale for fine tuning the prevention of infections and bronchiectasis in patients with PHG through the introduction of additional anti-infective treatments.

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Key Messages

- This report shows that patients with HIGM syndrome are less prone to carry NTHi than patients with PHG.
- Because patients with HIGM syndrome were found to have IgM anti-*H influenzae* antibodies in both sera and saliva, these results point to a protective role of IgM that might reduce the risk of acute infections and chronic respiratory tract disease in these patients.
- IgG substitution might be completed by additional therapeutic measures to prevent infections in patients with PHG.

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