Antibody Responses to Immunizations in Children with Type I Diabetes Mellitus: a Case-Control Study

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Type I diabetes mellitus (DM) has been associated with abnormalities of T cells. Our objective was to assess whether antibody responses to T-cell-dependent and -independent antigens in children with DM are lower than those of children without DM. We performed a case-control study matching children with DM to children without DM by age and by assessing antibody levels to pneumococcal serotypes, Haemophilus influenzae, and tetanus and diphtheria toxoids and reassessing antibody levels in patients with antibody levels below protective thresholds after booster immunization. We recruited 36 children with DM and 36 age-matched controls. The mean age was 10 years. There was no difference between groups in antibody levels against the antigens tested. Pneumococcal antibody levels below the protective threshold were found in 35.9% of DM patients after conjugate pneumococcal vaccination with no difference between groups. Booster immunization with unconjugated pneumococcal vaccine resulted in a median level against pneumococcal serotypes of 2.3 µg/ml (range, 0.05 to 664.7 µg/ml) in children with DM and 6.1 µg/ml (0.12 to 203.36 µg/ml) in children without DM (P = 0.013). Over 85% of children had levels above the protective threshold after booster immunization with no difference between groups. There was no evidence for a reduced antibody response to T-cell-dependent antigens given during childhood immunizations in children with DM. There was a reduced antibody response to antigens of pneumococcal strains in children with DM given unconjugated pneumococcal polysaccharide vaccine compared to that of children without DM without being associated with a difference in percentage of antibody levels below the protective threshold between groups.

Type I diabetes mellitus (DM) has been associated with multiple abnormalities of T-cell function and quantities. In the first ground-breaking studies, decreased CD4/CD8 lymphocyte ratios, reduced lymphocyte blastogenesis, and acquired defects in interleukin-2 production were observed in people affected by DM (1, 2, 3). Subsequent research revealed reduced T-cell primary responses to protein antigens (4). Other investigations demonstrated features of a suppression of a T-helper cell 1 phenotype, with reduced expression of Th1-associated chemokine receptors and decreased secretion of Th1 cytokines (5).

A previous study showed that compared to healthy controls, adult DM patients mounted a significantly impaired primary antibody response to T-cell-dependent primary protein antigens used for immunization, like hepatitis A vaccine and diphtheria toxoid, while the response to the T-cell-independent pneumococcal polysaccharide vaccine was not different. Patients with type II diabetes showed a normal response to immunization, illustrating that hyperglycemia is not involved in a change of the immune response (6).

People with DM are susceptible to bacterial and particularly pneumococcal infection and are at increased risk of morbidity and mortality from bacteremia due to Streptococcus pneumoniae (7, 8).

There are no studies assessing the antibody response to primary immunizations in children with DM. There are no studies in adults or children with DM assessing antibody response to conjugated pneumococcal vaccines, the response to which is dependent on intact T-cell responses, which are impaired in DM. If a reduced response to conjugated pneumococcal vaccines and other T-cell-dependent conjugated vaccines is identified, vaccination schedules may have to be modified to incorporate additional booster immunizations.

We posited that the T-cell-dependent antibody response to bacterial antigens used in childhood immunizations is reduced in children with DM. We examined levels of antibody to diphtheria and tetanus toxoids, haemophilus antigens, and pneumococcal antigens in children with DM versus age-matched controls without DM. We also examined levels of antibody to pneumococcal polysaccharide vaccines in patients with DM versus controls.

MATERIALS AND METHODS
To assess antibody responses in children with DM, blood was taken as part of routine blood sampling, e.g., during an annual review of children with DM. At the annual review, blood samples are taken routinely from all children with DM; the measurement of antibody levels for this study was added to the routine. As a control group, children without diabetes mellitus were recruited from a routine blood sampling clinic. To avoid the need for additional venepuncture, samples from routine blood sampling were used to determine antibody levels against the four antigens included in this study. Controls were selected only if they matched with previously recruited cases of DM by age. In other words, children who had DM as well as children born within a year of the DM patients but without DM were recruited. The mean age in children of 10.4 years for those with DM and 10.3 years for those without DM. Blood was analyzed for antibodies to diphtheria and tetanus toxoids, Haemophilus influenzae antigen, and invasive pneumococcal serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F, and 19A. Blood samples for antibodies were analyzed at national reference laboratories at the Public Health England Laboratories in Colindale, London (diphtheria and tetanus antigens), Manchester (Streptococcus pneumoniae, tetanus toxoid, and Haemophilus influenzae antigen).
pneumoniae), and Birmingham (Haemophilus influenzae), United Kingdom, by standard methods, including IgG enzyme-linked immunosorbent assay (ELISA) (Haemophilus influenzae and tetanus toxoids), inhibition of toxoid effects in Vero cell cultures (diphtheria toxoid), and Bioplex platform testing for IgG against Streptococcus pneumoniae. If an antibody level below the protective threshold (defined for pneumococcal serotypes as IgG levels of >0.35 μg/ml according to WHO guidelines [9], for Haemophilus influenzae as >0.12 μg/ml, for tetanus toxoid as >0.1 IU/ml, and for diphtheria toxoid as 0.01 IU/ml) was identified, the principal investigator wrote to the general practitioner (GP) of the patient and asked for a booster immunization with the recommended childhood vaccine. The booster recommended depended on the antigen against which antibody levels were below the protective threshold for diphtheria, tetanus, haemophilus, or pneumococcal vaccine (the GP’s choice was pneumococcal polysaccharide vaccine) or a combination. Regarding the antigens used for antibody measurement in this study, the immunization regimen in the United Kingdom includes diphtheria, tetanus, and Haemophilus influenzae vaccines at 2, 3, and 4 months and diphtheria and tetanus boosters at 3 years 4 months and around 14 years of age. The conjugated pneumococcal vaccine used was Prevnar 13, containing the strains 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, at 2 months, 4 months, and between 12 and 13 months of age (the latter was introduced in 2010). Between 2006 and 2010, Prevnar 7 was used, which contained antigens of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Patients with an insufficient antibody response to any of the vaccine antigens investigated had a repeat antibody level determined at least 6 weeks after the booster with the corresponding vaccine.

Participants. Carers of children aged 15 months to 16 years attending the diabetes clinic at Luton & Dunstable University Hospital NHS Foundation Trust were approached. Controls matched 1:1 by age to the children with DM were recruited from the phlebotomy clinic of children’s outpatient departments after informed written consent was obtained by the principal investigator. The study protocol was approved by the National Research Ethics Service of the United Kingdom and the Research & Development Department of Luton & Dunstable University Hospital National Health Service Foundation Trust.

Exclusion criteria. The following exclusion criteria were used: known T-cell or antibody deficiency; known autoimmune disease other than DM for the control group (a case with DM can have other associated autoimmune diseases); and lack of any of the childhood immunizations investigated in this project.

Statistical analysis. Antibody levels in cases versus controls were compared by Mann-Whitney test for independent samples because of non-parametric data distribution. For parametric data, the t test for independent samples was used. Chi-square test or Fisher’s exact tests were used to compare categorical data as appropriate. For comparison of antibody levels within the same person before and after booster immunization, the Wilcoxon signed-rank test for dependent samples was used. A P value of <0.05 was used to indicate statistical significance.

Sample size (power calculation). Based on a previous study (6), we estimated that the response to diphtheria toxoid antibodies for controls was a mean of 6.38 IU/ml, and for DM patients the response was a mean of 0.94 IU/ml with an estimated standard deviation (estimated as interquartile range divided by 1.3) of 5.38. To demonstrate a statistically significant difference with a power of 95% at a significance level of 1%, there should be at least 35 patients in each group.

RESULTS
We recruited 36 children with DM and 36 age-matched controls without DM. Mean age was 10 years in both groups (Table 1). Comorbidities in patients (numbers) with DM were asthma (4), eczema (4), hay fever (2), and migraine, hearing loss, autoimmune hypothyroidism, and food allergies or costochondritis (one person each). In the children without DM, the comorbidities were anorexia nervosa (6), asthma (4), epilepsy (2), absent thyroid gland (2), and tuberculosis, hemiplegia, nephrolithiasis, migraine, hay fever, or learning difficulties (one person each). There were 10 patients with atopy among children with DM and 5 in the group of children without DM, with no difference between groups (P = 0.24). There was no difference in antibody levels against the antigens tested or number of levels below the protective threshold between groups of children with and without DM (Table 2 and Table 3). Children with previous conjugated pneumococcal immunization had significantly higher pneumococcal antibody lev-

| Parameter | Value(s) for: | DM (n = 36) | Age-matched controls (n = 36) | P value |
|-----------|---------------|-------------|-----------------------------|---------|
| Age (yr; mean [SD]) | 10.4 (3.2) | 10.3 (3.1) | 0.92 |
| Comorbidities* (n) | 14 | 16 | 0.81 |
| Previous pneumococcal vaccination (n) | 19 | 16 | 0.63 |
| Time interval between previous pneumococcal vaccination and measurement of antibody levels (yr; mean [SD]) | 5.4 (2.4) | 6.0 (1.7) | 0.42 |
| Previous booster with diphtheria, pertussis, tetanus vaccine following course of primary immunization (n) | 32 | 35 | 0.35 |
| Time interval between diphtheria and tetanus immunizations and measurement of antibody levels (yr; mean [SD]) | 6.7 (2.9) | 5.4 (3.1) | 0.07 |
| Time interval between haemophilus influenzae immunization and measurement of antibody levels (yr; mean [SD]) | 10.0 (3.2) | 9.5 (3.8) | 0.55 |

*For details, see the text.

Table 1 Baseline characteristics of patients with DM and controls

| Antibody level measured | Value(s) for: | Children with DM (n = 36) | Children without DM (n = 36) | P value |
|-------------------------|---------------|---------------------------|-----------------------------|---------|
| Pneumococcal (µg/ml; median [range]) | 0.33 (0.05–166.28) | 0.33 (0.05–34.49) | 0.67 |
| Haemophilus influenzae (µg/ml; median [range]) | 0.98 (0.11–9.00) | 1.03 (0.11–9.00) | 0.92 |
| Tetanus toxoid (IU/ml; median [range]) | 0.42 (0.03–8.83) | 0.36 (0.11–7.0) | 0.93 |
| Diphtheria toxoid (IU/ml; median [range]) | 0.25 (0.01–4.10) | 0.25 (0.01–4.10) | 0.58 |
els. There was no difference in levels between groups with or without previous pneumococcal immunization (Table 4). In the group with antibody levels below the protective threshold who were given pneumococcal polysaccharide immunization (22/34 patients in the group with diabetes and 13/35 in the control group) containing serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F, children with DM had significantly lower antibody levels postvaccination than children without DM. Comorbidities in this subgroup (numbers) for patients with DM were asthma (2), hay fever (2), and migraine, eczema, hearing loss, or costochondritis (one each), and for participants without DM they were anorexia nervosa (2) and asthma, migraine, hay fever, or congenital hypothyroidism (one each). The immunization resulted in a reduction of the percentage of levels below the protective threshold to a percentage not significantly different between the two groups (Table 5).

**DISCUSSION**

The results of our study did not confirm the hypothesis of an impaired antibody response to T-cell-dependent antigens, including pneumococcal conjugated vaccine, *Haemophilus influenzae* conjugated vaccine, and tetanus and diphtheria toxoids, in children with DM. Our study actually investigated the persistence of such an antibody response beyond 5 years and could not exclude a difference in the antibody response in the short term. That a persistent response was measured for pneumococcal immunization is evident from the fact that children with conjugated pneumococcal immunization more than 5 years ago had significantly higher pneumococcal antibody levels than children who did not have pneumococcal vaccination. We found a reduced response to unconjugated pneumococcal polysaccharide vaccine in patients with DM compared to controls about 8 weeks after immunization. However, it is important to note that the percentages of pneumococcal antibody levels below the putative protective threshold following this unconjugated pneumococcal immunization were not different between the groups. This result is in keeping with a previous study, which, after unconjugated pneumococcal polysaccharide immunization, found higher pneumococcal antibody levels in children with chronic respiratory diseases without DM than in children with DM (10). This result is in contradiction to previous studies showing no difference between pneumococcal antibody levels in patients with and without DM after pneumococcal polysaccharide vaccination (6, 11, 12). A reason for this discrepancy may be that none of these previous studies were based on a power calculation aimed at demonstration of equivalence of groups, and their calculations were probably underpowered to exclude a statistically significant difference. Clinically relevant differences in protective antibody responses may have been missed. The control group in our study contained individuals with anorexia nervosa with a low body mass index. We did not exclude individuals with low body mass index from the control group, because previous studies did not find a reduction in antibody responses to vaccines in children with severe acute or chronic malnutrition (13, 14, 15, 16, 17, 18).

The nonparametric distribution of antibody levels in our study did not allow calculation of parameters essential for a sample size calculation (means and standard deviations) to demonstrate inferiority of antibody response to unconjugated pneumococcal polysaccharide vaccine in children with DM. None of the previously reported studies were designed as double-blind randomized controlled trials allocating patients in age-matched groups with and without DM to pneumococcal immunizations or placebo. In the future, such trials need to contain trial arms with conjugated and unconjugated pneumococcal polysaccharide vaccines and controls given placebo. Given the results of previous studies, a sample size calculation should assume equivalence of unconjugated and

**TABLE 4 Response to pneumococcal immunization in children with and without DM**

| Group | History of pneumococcal conjugate immunization | No history of previous pneumococcal conjugate immunization | P value |
|-------|-----------------------------------------------|----------------------------------------------------------|---------|
| With DM | 0.55a (0.05–78.45) | 0.23a (0.05–166.28) | <0.01 |
| Without DM | 0.67 (0.05–34.49) | 0.22 (0.05–20.09) | <0.01 |

a P = 0.25 compared to children without DM.
b P = 0.75 compared to children without DM.

**TABLE 3 Antibody levels below protective threshold**

| Parameter | Value for: | Children with DM | Children without DM | P value |
|-----------|------------|------------------|---------------------|---------|
| Levels against pneumococcal serotypes below protective threshold (n/N [%]) | 216/432 (50.0) | 223/432 (51.6) | 0.63 |
| No. of participants with any level below protective threshold (n/N [%]) | 34/36 (94.4) | 35/36 (97.2) | 1.00 |
| Levels against pneumococcal serotypes below protective threshold (n/N [%]) | 82/228 (35.9) | 61/192 (31.7) | 0.09 |
| With previous conjugated pneumococcal immunization | 123/204 (60.2) | 162/240 (67.5) | 0.11 |
| Without previous pneumococcal immunization | 2/36 (5.5) | 1/36 (2.7) | 0.55 |
| Diphtheria | 3/36 (8.3) | 0/36 (0.0) | 0.07 |
| levels below the protective threshold who were given pneumococcal polysaccharide vaccine | 1/36 (2.7) | 1/36 (2.7) | 1.00 |

n refers to the number of antibody levels against pneumococcal serotypes below the protective threshold, and N refers to the total number of antibody levels against pneumococcal serotypes. Antibody levels to 12 serotypes were measured per patient.
TABLE 5 Response to unconjugated pneumococcal polysaccharide booster immunization in children with and without DM

| Parameter | Value(s) for: | Children with DM (n = 22) | Children without DM (n = 13) | P value |
|-----------|---------------|---------------------------|-----------------------------|---------|
| Age at booster immunization (yr; mean [SD]) | 11.5 (2.9) | 10.6 (3.6) | 0.38 |
| No. with comorbidities (no. with atopy) | 8 (5) | 6 (2) | 0.83 (0.68) |
| No. with previous conjugated pneumococcal immunization | 9/22 | 5/13 | 0.83 |
| Time interval from booster immunization with pneumococcal polysaccharide vaccine and repeat pneumococcal serology (median wk [range]) | 8.0 (4–40) | 7.0 (6–17) | 0.82 |

Pneumococcal antibody level before or after booster immunization with pneumococcal polysaccharide vaccine (µg/ml; median [range])

| Before | After |
|--------|-------|
| 0.22 (0.05–166.28) | 2.31 (0.05–664.71) |
| 0.25 (0.05–34.49) | 6.10 (0.12–203.36) |
| 0.21 | 0.01 |

Percentage of pneumococcal serotype antibody levels below protective threshold of 0.35 µg/ml before or after booster immunization

| Parameter | Value(s) for: | Before | After |
|-----------|---------------|--------|-------|
| Percentage of pneumococcal serotype antibody levels below protective threshold of 0.35 µg/ml before or after booster immunization | 60.3 | 59.6 | 0.87 |
| | 12.4 | 14.1 | 0.73 |

These children required pneumococcal booster immunization and had it arranged at the investigators’ request.

conjugated pneumococcal polysaccharide vaccine. If there is truly no difference between the antibody responses against the two vaccines, then 554 patients (277 in each immunized group) are required for a power of 90% at a significance level of 5% so that the limits of a two-sided 90% confidence interval will exclude a difference between the groups of more than 10% of antibody levels below the protective threshold (http://www.sealedenvelope.com/power/binary-equivalence/).

It is concerning that over 30% of antibody levels against invasive pneumococcal serotypes in children both with and without DM were below the protective threshold recommended by WHO guidelines (9) after a full course of conjugated pneumococcal immunization in childhood. This highlights the need for further pneumococcal booster immunization, particularly in children from high-risk groups, like those affected by DM.

Conclusions. There was no evidence for a reduced antibody response to T-cell-dependent antigens given during childhood immunizations in children with DM. There was a reduced antibody response to antigens of pneumococcal strains in children with DM given unconjugated pneumococcal polysaccharide vaccine compared to children without DM without being associated with a difference in percentage of antibody levels below the protective threshold between groups.

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