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Received: 31 October 2022 | Accepted: 23 March 2023

DOI: 10.1002/jmv.28707

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

HLA-DQ-conferred risk for type 1 diabetes does not alter neutralizing antibody response to a widely used enterovirus vaccine, the poliovirus vaccine

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Funding information

Academy of Finland; Leona M. and Harry B. Helmsley Charitable Trust; Sigrid Juséliuksen Säätiö; Novo Nordisk Fonden; Reino Lahtikari Foundation; Päivikki and Sakari Sohlberg's Foundation grant; European Commission

Abstract

This study investigated whether children with HLA-DQ-conferred risk for type 1 diabetes (T1D) have an altered immune response to the widely-used enterovirus vaccine, namely poliovirus vaccine, and whether initiation of autoimmunity to pancreatic islets modulates this response. Neutralizing antibodies induced by the inactivated poliovirus vaccine against poliovirus type 1 (Salk) were analysed as a marker of protective immunity at the age of 18 months in a prospective birth cohort. No differences were observed in antibody titers between children with and without genetic risk for T1D (odds ratio [OR] = 0.90 [0.83, 1.06], $p = 0.30$). In the presence of the genetic risk, no difference was observed between children with and without islet autoimmunity (OR = 1.00 [0.78, 1.28], $p = 1.00$). This did not change when only children with the autoimmunity before 18 months of age were included in the analyses (OR = 1.00 [0.85, 1.18], $p = 1.00$). No effect was observed when groups were stratified based on autoantigen specificity of the first-appearing autoantibody (IAA or GADA). The children in each comparison group were matched for sex,

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calendar year and month of birth, and municipality. Accordingly, we found no indication that children who are at risk to develop islet autoimmunity would have a compromised humoral immune response which could have increased their susceptibility for enterovirus infections. In addition, the proper immune response supports the idea of testing novel enterovirus vaccines for the prevention of T1D among these individuals.

KEYWORDS

defective immunity, GADA, IAA, neutralizing antibody, poliovirus type 1, poliovirus vaccine, type 1 diabetes

1 | INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune condition in which complex interactions between risk genes and environmental factors are operative. Among the possible triggering factors, enteroviruses are currently one of the strongest candidates.[1] The genus Enterovirus consists of 15 species of which seven infect humans, namely Enterovirus A, B, C, and D and Rhinovirus A, B, and C.[2] Enterovirus A–D classes comprise 116 virus types,[2] but extensive research in different populations has narrowed down the list of potentially diabetes-associated enterovirus types. Viruses belonging to Enterovirus B, coxsackie B viruses (CVBs) in particular, have most often been linked to the initiation of islet autoimmunity in T1D. They were observed to be associated with the initiation of islet autoimmunity in large prospective birth cohort studies such as "Environmental Determinants of Diabetes in the Young (TEDDY)"[3] and "Type 1 Diabetes Prediction and Prevention (DIPP)" studies,[4–6] supporting previous observations in cross-sectional case control studies[7,8] and demonstrated in a recent meta-analysis.[9] CVBs have clear tropism to the pancreatic islets[10] and the strong expression of the major receptor for these viruses, coxsackie and adenovirus receptor, in pancreatic β cells compared to other islet cells[11] may contribute to this tropism. The accumulated evidence created the base for the development of the PRV 101 vaccine, the first vaccine against CVBs.[12] This formalin inactivated multivalent CVB vaccine containing CVB types 1–5 has recently passed a phase 1 clinical trial showing that it is immunogenic and induces strong antibody responses against CVBs.[13] The goal is to develop a vaccine which can reduce the risk of T1D in genetically susceptible children and in addition prevent several CVB-induced diseases such as myocarditis, cardiomyopathies, meningitis, encephalitis and severe systemic infections in infants.[14] Prototype CVB vaccines have prevented both CVB infection and CVB-induced diabetes and myocarditis in mouse models,[15,16] creating a solid basis for human T1D prevention trials. In addition, it has been shown that the vaccine does not cause diabetes or pancreas pathology in nonobese diabetic mice.[16]

Some studies have shown that the antibody response to certain vaccines, e.g., hepatitis A virus (a virus also in the *Picornaviridae* family) and diphtheria toxoid vaccines is impaired in people with T1D.[17] The antibody response to a vaccine against hepatitis B virus was as well significantly lower in children with T1D compared to healthy controls.[17,18] Other studies also report a defective immune response in adults with T1D, while only limited data is available after the disease manifestation in affected children (Reviewed in detail in Esposito et al.[19] and Kesavadev et al.[20]). Whether this is an inherent defect in these people to respond to pathogens or merely a function of their dysglycaemia needs to be further elucidated. In fact, certain HLA-genotypes are associated with a poor immune response to virus vaccines.[9,21,22]

It has also been implicated that the immune response to natural CVB infections might be incomplete in children at risk for T1D who develop early islet autoimmunity against insulin[23] whereas children with T1D or rheumatic diseases have been shown to have antibody response to poliovirus vaccination similar to the background population.[24] However, these observations are based on small cohorts and no previous studies have specifically investigated the immune responses induced by enterovirus vaccines in nondiabetic children who carry HLA risk genes for T1D (reviewed in detail in Mikk et al.[25]).

Knowing that the children with the genetic risk have altered response to some vaccines discussed earlier, the current study sets out to answer the question whether the response to an inactivated enterovirus vaccine, in this case a poliovirus vaccine, differs in children with genetic risk for T1D compared to children with no such risk, and whether this is modified by autoimmunity status of the children with the HLA risk. Poliovirus vaccine is currently the only licensed enterovirus vaccine and has been widely used since 1950s. A vaccine against enterovirus 71 has recently been licensed in China but is not available in other countries.[26]

Protective immunity against enteroviruses is partially represented by detecting neutralizing antibodies which can block the infectivity of the virus, as shown also in animal models.[27,28] Such circulating antibodies are detected at high titers after natural infections and confer protective immunity against future infections. They can also be used as markers of the efficacy of the enterovirus vaccine applied. Therefore, we set out to analyses the levels of neutralizing antibodies against poliovirus (type) 1 as a representative of the three poliovirus types which are structurally similar.

Additionally, the use of poliovirus 2 in research laboratories is prohibited by World Health Organization (WHO) due to its eradication.[29] All participants had been immunized using a trivalent formalin-inactivated whole poliovirus vaccine (IPV, Salk's vaccine, including polioviruses 1–3). This is a widely administered vaccine on an enterovirus member which is closest to the targeted coxsackievirus B members used in the new PRV 101 vaccine. Polio and PRV-101 vaccines are manufactured using similar technologies.[13] PRV 101 vaccine has been tested in a phase 1 clinical trial in Finland and is designed to be given in the future to infants in multiple doses during the first year of life. Therefore, we analyzed poliovirus antibodies at the age of 18 months when the children had already been immunized at least twice with the poliovirus vaccine.

The neutralizing antibody titers against poliovirus 1 were quantified in young children who carried T1D-associated HLA-DQ genotypes who developed islet autoimmunity at the time of study or later. In addition, the antibody levels were also quantified in children who either shared the same HLA-conferred risk alleles but without any autoimmunity to islet autoantigens or the children who carried protective or neutral HLA alleles for T1D.

The study is based on a longitudinal design making it possible to study the vaccine response also before islet autoimmunity starts. In addition, this design allowed us to study two known endotypes of T1D separately (first appearing autoantibody against either insulin or the glutamic acid decarboxylase isoform 65, GAD65)[30] and compare their neutralizing antibodies to that of children matched with similar T1D-related HLA alleles but without signs of islet autoimmunity.

2 | MATERIALS AND METHODS

2.1 | Study population

The study subjects were recruited from the DIPP study in Finland which follows children with T1D-associated HLA-DQ genotypes until they either develop T1D or reach 15 years of age.[31] The DIPP study started in November 1994. Autoantibodies to GAD65 (GADA), insulin (IAA), tyrosine phosphatase-like protein (islet antigen-2 [IA-2A]), and zinc transporter-8 (ZnT8A) are detected at regular follow-up visits starting at the age of 3 months until the endpoint described earlier.[31] The DIPP study has been approved by the Ethics Committees of the hospital districts involved in the study. Written informed consent has been provided by the parent or guardian. Assent was also given directly by the child where appropriate.

2.2 | Subjects

The participants in the current study ($N = 417$, 41% girls) were selected from the DIPP cohort recruited in the Pirkanmaa Hospital District (Tampere DIPP center) creating the following two sample series:

2.2.1 | Autoantibody-negative series

Autoantibody-negative series (series 1, Table 1) includes citrate plasma samples taken at the age of 18 months (at a mean age of 18.4 months) from 111 "case" children (51.4% girls) who carried HLA-DQ genotypes conferring increased susceptibility to T1D, including high ($N = 36$, 50% girls), moderately increased ($N = 47$, 55.3% girls), and slightly increased ($N = 28$, 46.4% girls) risk genotypes and comparable samples from 84 "control" children (46.4% girls) with HLA-DQ genotypes that are associated with neutral risk ($N = 40$, 47.5% girls), mildly decreased ($N = 19$, 42.1% girls), or strongly decreased ($N = 25$, 48% girls) risk for T1D. The HLA-DQ allele combinations that have been used for this risk classification have been described in detail earlier by Ilonen et al.[32] There was no significant difference in sampling age between girls and boys. The samples have been stored at −80°C until used in the study. The case and control children were individually matched for the calendar year and month of birth, sex and municipality with 1:1 or 1:2 (case: control) ratios. Children with no diabetes-associated HLA-DQ genotypes were recruited in the DIPP only during the period 1999–2001. All children in series 1 remained islet autoantibody negative and nondiabetic by the time when they were recruited to the study. This allowed us to assess the effect of the HLA genotypes before any signs of autoimmunity status had developed in the so called "case" children.

2.2.2 | HLA risk-matched series

HLA risk-matched aeries (series 2, Table 1) similarly includes citrate plasma samples taken at the age of 18 months (at the mean age of 18.5 months) from children who were born in 1995–2009 and carried HLA-DQ genotypes that confer increased risk for T1D among whom 111 (33.3% girls) had developed multiple (≥2) biochemical islet autoantibodies, before ($N = 43$) or later than ($N = 68$) 18 months of age, individually matched for sex, calendar year and month of birth, and the municipality with 111 autoantibody-negative children. There was no significant difference in sampling age between girls and boys. The case children in this set included 54 children who developed IAA as the first appearing islet autoantibody (26% girls) and 57 children who developed GADA as the first-appearing autoantibody (40.3% girls), representing the two main endotypes of T1D (IAA-first or GADA-first endotypes). Individuals testing positive for multiple autoantibodies are highly likely to progress to T1D.[32–35]

The age at seroconversion in the two endotypes was, as expected, significantly different ($p < 0.0001$) with the IAA-first group testing positive for IAA at an earlier age (mean age of 21 months; 95% confidence interval [CI]: 17.2–24.8 months) than the GADA-first group turning positive for GADA (59.8 months; 95% CI: 50.6–69.1 months). Boys and girls did not differ in relation to autoantibody seroconversion age (40.3 months for boys vs. 42.2 months for girls,

TABLE 1 Sub-cohorts and each stratum.

Series	Status	Risk (*N#*)	Sex	*N#*	Sampling age, months mean (range)	MAAb age, months mean (*SE*)	Neutralizing titres mean $\log_{(2)}$ (95% CI)	T1D cases (*N*)	T1D age, months mean (*SE*)
Series 1[a]	HLA-risk	High risk	F	18	18.5 (17.7–19.4)	–	9.1 (8.1–10.1)	–	–
		(36)	M	18	18.5 (17.5–20.0)	–	7.8 (6.2–9.4)	–	–
		Moderate risk	F	26	18.5 (18.0–19.7)	–	9.5 (8.7–10.4)	–	–
		(47)	M	21	18.4 (12.5–24.2)	–	8.1 (6.7–9.5)	–	–
		Slightly increased risk	F	13	18.4 (16.3–20.3)	–	9.0 (7.4–10.5)	–	–
		(28)	M	15	18.5 (17.6–19.1)	–	6.5 (4.6–8.4)	–	–
		Total	F	57	18.5 (16.3–20.3)	–	9.3 (8.7–9.8)	–	–
		(111)	M	54	18.4 (12.5–24.2)	–	7.6 (6.7–8.4)	–	–
	No HLA-risk	Neutral risk	F	19	18.6 (18.2–19.8)	–	8.7 (7.9–9.6)	–	–
		(40)	M	21	18.8 (17.3–24.4)	–	7.5 (5.7–9.4)	–	–
		Mildly protective	F	8	18.5 (18.3–18.8)	–	9.8 (8.7–10.8)	–	–
		(19)	M	11	18.5 (18.2–18.9)	–	9.6 (8.6–10.7)	–	–
		Strongly protective	F	12	18.4 (18.2–18.7)	–	9.8 (8.6–11.1)	–	–
		(25)	M	13	18.4 (18.1–18.7)	–	8.0 (6.6–9.4)	–	–
		Total	F	39	18.5 (18.2–19.8)	–	9.3 (8.7–9.9)	–	–
		(84)	M	45	18.6 (17.3–24.4)	–	8.2 (7.2–9.1)	–	–
Series 2[b]	IAA-first	MAAb[c] positive (case)	F	14	18.4 (12.5–24.2)	18 (2.3)	10 (9.0–11)	8	57 (17)
		(54)	M	40	18.5 (18.2–19.8)	22 (2.4)	9.3 (8.7–9.9)	26	80 (10)
		MAAb negative (control)	F	14	18.6 (17.3–24.4)	–	10.1 (9.3–11)	–	–
		(54)	M	40	18.5 (18.3–18.6)	–	9.6 (8.8–10.3)	–	–
	GADA-first	MAAb positive (case)	F	23	18.5 (18.2–18.7)	57 (5.9)	9.7 (8.9–10.4)	10	116 (10)
		(57)	M	34	18.5 (18.3–18.7)	62 (6.7)	10.3 (9.5–11.1)	7	119 (24)
		MAAb negative (control)	F	23	18.5 (18.3–18.6)	–	10 (9.1–10.9)	–	–
		(57)	M	34	18.6 (18.4–18.8)	–	9.8 (8.9–10.7)	–	–

Note: The table shows demographic data on study subjects and a summary of the neutralizing titres of the subgroups against polio 1.
Abbreviations: CI, confidence interval; F, female; M, male; N#, number, *SE*, standard error.
[a]Autoantibody-negative series.
[b]Risk HLA-matched series.
[c]MAAb = multiple (≥2) autoantibodies to islet autoantigens.

$p = 0.77$). This was true for both IAA-first (22.1 months for boys vs. 17.6 months for girls, $p = 0.30$) and GADA-first subtypes (61.6 months for boys vs. 57.2 months for girls, $p = 0.64$).

Children in both the IAA-first and in the GADA-first groups[30] have eventually developed multiple islet autoantibody status and 51 of them (46%) developed T1D. Altogether 34 of 54 case children (63%) in the IAA first and 17 out of 57 children with GADA first subtypes (30%) had developed T1D by the time they were selected for the present study. The median age of the diagnosis of T1D was 74.2 (95% CI: 56–92) months in the IAA-first subtype, significantly younger compared to the GADA-first subtype with 117.2 (95% CI: 94–140) months at the diagnosis ($p = 0.005$).

2.2.3 | Vaccination history

Before year 2005 the national immunization program schedule in Finland included Inactivated polio vaccine (IPV, brand name Imovax Polio) injections given at the age of 6 and 12 months followed by booster injections later than 2 years of age. In 2005, Finland replaced the IPV with the DTaP-IPV-Hib vaccine (5-in-1 combination, diphtheria, tetanus, acellular pertussis, inactivated polio, and haemophilus influenzae; Infanrix Penta and other brand names) in the national immunization program[36] to be used at 3, 5, and 12 months of age, also repeated later after 2 years of age. Only 28 children participating in our study (all in series 2) were born in 2005 or later

and had received three doses of IPV before the age of 18 months, while 389 children were born earlier and had thus received two doses of IPV before the age of 18 months. Since children in both series were matched in comparison sets for the calendar year and month of birth, they have followed the same vaccination schedule. The sample taken at the age of 18 months is accordingly taken 6 months after the last IPV in all children representing an optimal time-point for the analysis of vaccine-induced humoral immunity. None of the children in the study reported with symptoms of poliomyelitis.

2.3 | Virus and cell lines

Low passaged poliovirus type 1 vaccine strain Sabin (henceforward called polio 1) was used to identify titres of neutralizing antibodies in the citrated plasma samples in a mycoplasma-free African Green Monkey Kidney cell line. Both the virus and cell line were received from the Finnish Institute for Health and Welfare, Helsinki, Finland. Neutralizing hyperimmune monkey serum against polio 1 was originally provided by the National Bacteriological Laboratory, Stockholm, Sweden. It contained a high titre of neutralizing antibodies (1:12 000) against polio 1.

2.4 | Antibody response

Neutralizing antibodies against polio 1 were quantified using a standard plaque reduction assay.[6] The poliovirus vaccine included all three poliovirus serotypes, but neutralizing antibodies are type specific. Hence it was possible to analyse the response only to polio 1. Samples with at least an 80% reduction in the number of virus plaques compared to the control tests infected with untreated virus were considered positive. The end-point titre of the neutralizing antibodies in plasma was identified by applying fourfold dilution series of individual plasma samples, starting from 1:4, to approximately 100 plaque forming units of polio 1. The virus induced CPE in form of plaques was visualized using formaldehyde-crystal violet solution and the number of plaques in each test was quantified visually. The highest plasma dilution blocking ≥80% of viral infectivity was taken as the end-point titre for neutralizing antibodies. Antibody titres ranging from 4 to 16 are generally considered as protective against poliovirus infection[37] depending on the technique used. We set titre 4 as the cut off for antibody positivity since we had used more virus particles and a higher limit for blocking infectivity (80% or more reduction in plaques) compared to the protocol recommended by the WHO which uses microneutralization and 50% blockage of the virus infectivity.[38] Each sample was tested once, and positive and negative control sera were included in each test run.

2.5 | Statistical analysis

The sets of matched children in both series were analysed using conditional logistic regression[39] test to compare the titres of neutralizing antibodies. In this analysis, antibody titre was used as a covariate to compare the immune status of the children matched in the sets. Neutralizing antibody titers are expressed as $\log_2$ of dilution identified as the endpoint titer. The odds ratio (OR), 95% confidence interval (95% CI), and the p value are presented for this type of comparison. The mean of the titres was compared across the groups using the analysis of variance (ANOVA) test. The significance level was set to 95%. In the autoantibody-negative series (series 1) some children with no HLA-conferred risk for T1D were reused as a matched comparison for another child carrying an HLA genotype conferring increased risk for T1D.[39]

3 | RESULTS

Participants in the autoantibody-negative series (series 1) consisted of 111 children with HLA risk alleles for T1D who had no autoimmunity to islet autoantigens (assigned to cases) and 84 children with no such HLA conferred risk and no autoimmunity (assigned to controls). Participants in HLA risk-matched series (series 2) consisted of 222 children who all had HLA-conferred risk to T1D, half of whom had autoimmunity to islet autoantigens (assigned to cases) while the other half did not have autoimmunity (assigned to controls).

The antibody titers in whole study population varied considerably ranging from $\log_{(2)}$ titer = 1–16 (with a mean titer 9.2, *SD* 2.53). This translates to a titer ranging from 2 to 65536 as the reciprocal of the dilution factor used to analyse the titers (Figure 1). Only one child from series 1, despite a history of receiving two doses of IPV according to the standard schedule, had no neutralizing antibodies against polio 1 (titer 2, Figure 1). Sixteen children (all in series 1) had low neutralizing antibody levels (titer 4) out of whom only one subject was female and 10 had increased risk of diabetes (based on the HLA genotyping). Only 28 children (all in series 2) were born in 2005 onwards and received three doses of the polio vaccine since the vaccination schedule in Finland was revised in 2005. The mean titers of neutralizing antibodies did not significantly differ between children with three versus two vaccine doses (mean $\log_{(2)}$ titer 9.4 vs. 9.2, respectively, $p = 0.64$).

Polio 1 antibody levels did not differ between case and control children in either of the series 1 and 2 (Figure 2). In series 1, neutralizing antibody titers against polio 1 were similar in children with HLA-conferred risk and in matched children with no HLA risk (OR = 0.90 [95% CI: 0.83, 1.06], $p = 0.30$). The mean $\log_{(2)}$ titers were 8.4 and 8.7 (ANOVA, $p = 0.52$), respectively. Titers of the neutralizing antibodies in six different HLA sub-groups in series 1 are presented in Figure 3C.

In series 2, polio 1 neutralizing titers were similar in children with islet autoantibodies and matched children without signs of islet autoimmunity (OR = 1.00 [95% CI: 0.85, 1.18], $p = 1.00$; Figure 2) having the same mean in both groups ($\log_{(2)}$ titer 9.8; Figure 3A). Series 2 was also stratified into IAA first and GADA-first endotypes, and the neutralizing titers against polio 1 showed no difference between the matched sets (with and without autoantibodies) in either

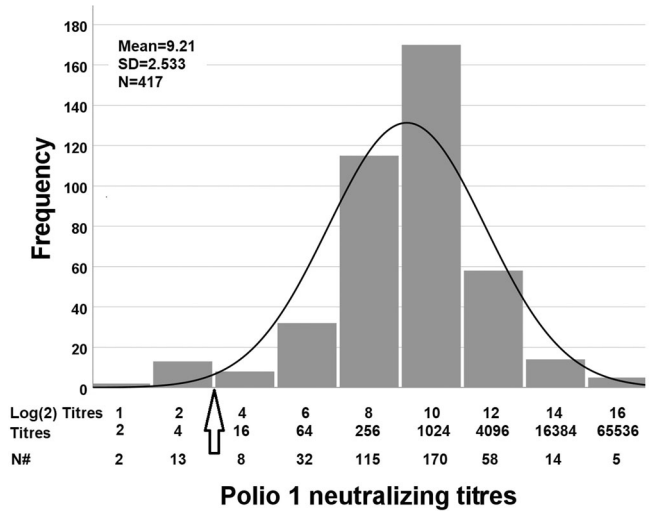

FIGURE 1 Distribution of the neutralizing antibody titres against polio 1 in all participants in the study. The titres are the reciprocal of the dilution factor used to dilute the plasma samples which prevents ≥80% of the plaques generated by the virus. The $\log_{(2)}$ transformed titres are also presented to compare the titre values. The standard curve is shown to indicate how much the observed distribution deviates from a normal distribution. The arrow depicts the cut-off limit for positivity. The number of subjects is shown for each titre category.

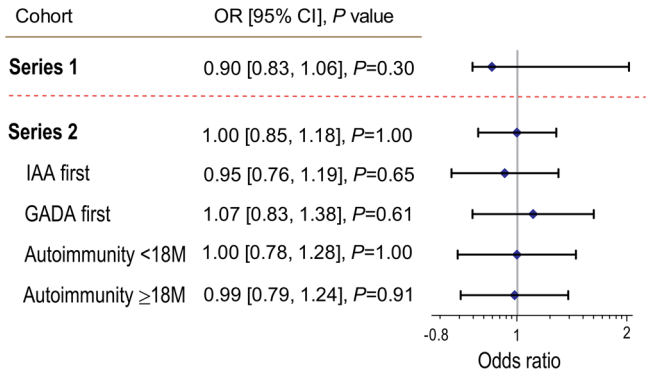

FIGURE 2 Forest plot representation of the odds ratios of differences between cases and controls in different series and sub series in neutralization titers against polio 1. Conditional logistic regression analysis was carried out to compare matched case-control series. Cases and controls in series 1 and 2 have been compared for the titers of neutralizing antibodies against polio 1. In Series 1 children with no islet autoimmunity were compared based on HLA risk-no risk criteria. In series 2 the children with the same HLA risk were compared by the presence and absence of the autoimmunity to beta cells. Children in series 2 were grouped based on IAA or GADA as the first appearing autoimmunity or when grouped based on autoimmunity before 18 months or after that.

of the groups (OR = 0.95 [95% CI: 0.76, 1.19], $p = 0.65$ for the IAA-first group and OR = 1.07 [95% CI: 0.83, 1.38], $p = 0.61$ for the GADA-first group; Figure 2). The mean $\log_{(2)}$ titers were 9.6 versus 9.7 in the IAA-first group (ANOVA, $p = 0.7$) and 10.0 versus 9.9 in the GADA first group (ANOVA, $p = 0.7$) in cases against controls (Figure 3A). Altering the positivity cut off for neutralizing titers from 4 to 6, 8, 10, and 12 did not result in any significant differences between the matched children in any of the series using conditional logistic regression analysis (data not shown).

In series 2, 45 children developed islet autoantibodies before the time of sampling (6 of 57 children with GADA-first status and 39 of 54 children with IAA-first status). To investigate the possible effects of the autoimmunity process on the immune response to the vaccine we compared neutralizing antibody titers of case children ($N = 45$) with developed autoimmunity before the age of 18 months (578 days) to their matched controls. Neutralizing antibodies against polio 1 ($\log_{(2)}$) did not differ between these case children and the matched control children (OR = 1 [95% CI: 0.78, 1.28], $p = 1$; Figure 2). The mean $\log_{(2)}$ titers were 9.69 versus 9.64, in cases compared to controls, respectively (ANOVA, $p = 0.92$). Similar results were shown for the children ($N = 62$) who turned autoantibody positive after the age of 18 months (OR = 0.99 [95% CI: 0.79, 1.24], $p = 0.91$; Figure 2). The mean $\log_{(2)}$ titers were 9.88 versus 9.91, in cases compared to controls, respectively (ANOVA, $p = 0.93$). Stratifying by IAA first and GADA first did not change this result (data not shown).

Girls had slightly higher neutralizing antibody titers compared to boys in the whole cohort. The mean polio 1 neutralizing antibody titer in girls was 9.6 compared to 9.0 in boys, ANOVA, $p = 0.023$. This difference was most conspicuous in children with HLA-conferred risk in series 1 (girls 9.3 vs. boys 7.6, ANOVA, $p = 0.001$, Table 2, see also Figure 3B).

4 | DISCUSSION

The current study shows that children who are genetically at risk for progression to T1D mount a proper antibody response to an inactivated poliovirus vaccine. Their vaccine response was comparable to children who lacked T1D-associated HLA-DQ risk alleles. In addition, children who developed islet autoimmunity or T1D responded to the vaccine similarly to autoantibody-negative peers.

Dysglycaemia, caused by diabetes, is known to dim the immune system at some level for example against hepatitis B virus vaccine where only 45% of the children with T1D responded.[40] However, we did not identify any poor neutralizing antibody response to polio 1 vaccine in children at risk of T1D when they were analysed before developing T1D. This suggests that the previously described impaired vaccine responses in patients with T1D to some other vaccines e.g., hepatitis A vaccine[41] can be linked to metabolic disturbances that might have affected the functions of their immune system.

Since poliovirus is structurally similar to other enteroviruses, it is highly likely that also other enterovirus vaccines would induce a protective antibody response in children at increased HLA-conferred risk for T1D. This is an important message for the ongoing development of the new multivalent CVB vaccine, PRV-101,[13] since one of its intended indications is to prevent potentially diabetes associated CVB infections in genetically susceptible children. PRV-

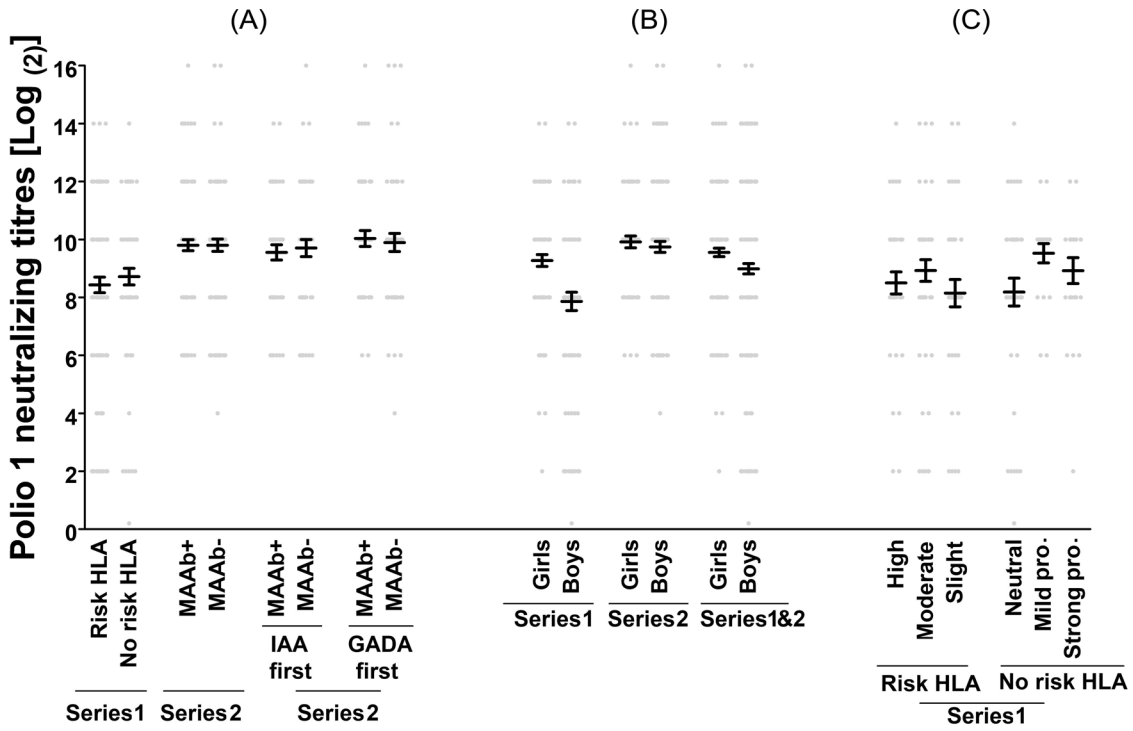

FIGURE 3 Distribution of polio 1 neutralizing antibody titres in two series and in the subgroups. Polio 1 neutralizing antibody titres are presented as $\log_{(2)}$ neutralizing antibody titres in all panels. The thick lines and the error bars represent the mean and standard error of the mean, the dots represent the actual neutralizing titres of the study subjects. (A) Represents the distribution of anti-polio 1 titres in series 1 (autoantibody-negative series, 111 children with HLA conferred risk genotypes and 84 children with no HLA risk), series 2 (HLA risk-matched series, 111 pairs of children with or without multiple [≥2] autoantibodies [MAAb] to islet autoantigens), and sub phenotypes of series 2 (IAA-first, 54 pairs of children; and GADA-first, 57 pairs of children) compared by the presence of islet multiple autoantibodies in each group. The titres are presented as $\log_{(2)}$ neutralizing antibody titres measured using a standard virus plaque neutralization assay. (B) Represents polio 1 neutralizing antibody titres in girls and boys in series 1 (96 girls and 99 boys) and series 2 (74 girls and 148 boys) separately and combined (170 girls and 247 boys). (C) Represents the distribution of $\text{Log}_{(2)}$ neutralizing antibody titres against polio 1 in series 1. HLA risk subgroups are shown as a gradient risk from High ($N = 44$), Moderately increased ($N = 57$), to Slightly increased ($N = 41$) risk groups; and the comparison group with a gradient of protective effect from Neutral ($N = 44$), Mild protective ($N = 21$), to Strong protective ($N = 26$). pro., protective.

101 is produced based on the same technology as the poliovirus vaccine that was used in the participants in the current study—both including highly purified enteroviruses inactivated by formalin. Nevertheless, theoretically it is still possible that subtle structural differences between different enterovirus types could have some influence and the results of the present study are not necessarily applicable for all inactivated vaccines within the genus *Enterovirus*.

The antibody response to polio 1 vaccine was equally good in children whose autoimmune process started by the appearance of either IAA or GADA as the first appearing autoantibody. These two autoantibody patterns are associated with different risk genes for T1D,[30] and it has been implicated that they represent two different endotypes of T1D potentially with different etiopathogenesis[42] In fact, a recent study suggested that the IAA-associated subtype was linked to CVB1 infections.[6] Thus, it is important that enterovirus vaccine can induce robust antibody response also in this subgroup of children.

Another key point relates to the previous findings showing that a prolonged course of enterovirus infections is associated with an increased risk of islet autoimmunity.[3] The reason to this is not known but possible weaknesses in the enterovirus-specific immune response is one possibility. Such a weakness might explain prolonged shedding of the virus in these children, since immune deficiencies are known to predispose to prolonged and even chronic enterovirus infections.[43] In fact, previous observations suggested that the antibody response to enterovirus infections may be weak in children who develop early islet autoimmunity, particularly in young children with insulin autoantibodies.[23,41] That study[23] analysed antibodies against structural proteins of CVBs in a sample taken from islet autoantibody positive and negative children using an immunoprecipitation assay. Antibodies against VP1 protein were at low levels in insulin autoantibody positive children while no such difference was seen in antibodies against other virus proteins. This contrasts with the present study where no such weakness was seen in polio 1 antibodies in the IAA-first subgroup. Thus, even though the vaccine-induced response was normal in the present study it is possible that insulin autoantibody positive children could mount abnormally low antibody response to natural enterovirus infections. On the other hand, the study by Ashton et al.[23] did not use neutralizing antibodies in this comparison and therefore

TABLE 2 Effect of sex.

Subset	Subgroups	MAAb status	Girls	Boys	*p* value
Series 1[a]	All	Negative	9.3	7.8	<0.001
	HLA-risk (case)[b]	Negative	9.2	7.6	0.001
	No HLA-risk (control)[c]	Negative	9.2	8.2	0.061
Series 2[d]	All	Positive/ negative	9.9	9.7	0.566
		Positive (case)	9.8	9.8	0.947
		Negative (control)	10.1	9.7	0.411
	IAA-first	Positive/ negative	10.1	9.5	0.186
		Positive (case)	10	9.4	0.321
		Negative (control)	10.1	9.6	0.386
	GADA-first	Total	9.8	10.1	0.586
		Positive (case)	9.7	10.3	0.258
		Negative (control)	10	9.8	0.786
All	All	Positive/ negative	9.6	9.0	0.023

Note: Neutralizing antibody titres against polio 1 in different study groups stratified according to sex presented as $\log_{(2)}$ of the titres.

[a]Autoantibody-negative series.

[b]Slightly/moderately/highly increased HLA risk of T1D.

[c]Neutral or mildly/strongly protective HLA alleles for T1D.

[d]Risk HLA-matched series.

cross-reactions between different enterovirus serotypes could have caused uncontrolled variation in their immunoprecipitation assay.

Even though the present study was primarily designed to study antibody response to enterovirus vaccinations but not the infections, the results may also help to understand the nature of enterovirus antibody immunity in diabetes-prone children more broadly. From this point of view the main advantage of the current study is that poliovirus vaccinations represent a standardized exposure to enterovirus antigens. This makes it possible to compare immune responses between children with HLA-conferred risk of T1D to children without the HLA risk as well as comparison between children with and without islet autoimmunity. However, we must acknowledge that the immune response to natural enterovirus infections can be regulated differently compared to that to an inactivated vaccine. In addition, we did not study cell-mediated or innate immune responses. In any case, bearing these limitations in mind, the current findings do not support the hypothesis that prolonged enterovirus infections could be due to an impaired antibody response in children with increased susceptibility to T1D.

It is important to note that due to the age of sampling at 18 months of age, maternal antibodies were not present to intervene with the quantification of the neutralizing antibodies against polio 1.

In conclusion, our study suggests that the children at risk for developing T1D do not have any inherited weakness in their ability to mount protective immunity in the form of neutralizing antibodies in response to a formalin-inactivated enterovirus vaccine. Therefore, the results support the idea of testing the efficacy of vaccines that target T1D-associated enteroviruses for primary prevention of T1D.

AUTHOR CONTRIBUTIONS

Amir-Babak Sioofy-Khojine: designed the study, performed the analysis, researched the data, and wrote the manuscript. **Amir-Babak Sioofy-Khojine**: have access to the data and taking responsibility for the contents of the article. **Jussi P. Lehtonen, Noora Nurminen, and Jutta E. Laiho**: researched the data and reviewed/edited manuscript. **Johanna Lempainen** researched the HLA typing. **Johanna Lempainen**, **Jorma Ilonen, Riitta Veijola, and Jorma Toppari**: reviewed/edited the manuscript. **Heikki Hyöty and Mikael Knip**: designed the study and reviewed/edited the manuscript. All authors agreed to the final manuscript version.

ACKNOWLEDGMENTS

The authors wish to thank Mervi Kekäläinen, Eveliina Paloniemi, Maria Ovaskainen, and Minta Lumme for their excellent technical assistance. The study was approved by the ethics committees of the participating university hospital, and the parents gave their written informed consent to the participation in the study.

This study was funded by—The Leona M. and Harry B. Helmsley Charitable Trust (Heikki Hyöty; Grant No. 2010-04139), the Academy of Finland (Heikki Hyöty; Grant No. 288671), Sigrid Juselius Foundation (Heikki Hyöty), Novo Nordisk Foundation (Heikki Hyöty; Grant No. NNF18OC0034080), Reino Lahtikari Foundation (Heikki Hyöty), Päivikki and Sakari Sohlberg's Foundation grant (Jutta E. Laiho), and the European Commission (Heikki Hyöty; Persistent Virus Infection in Diabetes Network [PEVNET] Frame Program 7, Contract No. 261441). DIPP study has additionally been funded by the Competitive Research Funding of the University Hospitals in Tampere, Oulu and Turku.

CONFLICT OF INTEREST STATEMENT

Heikki Hyöty and Mikael Knip are minor shareholders and members of the board of Vactech Ltd., which develops vaccines against picornaviruses. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author upon reasonable request. The data are not publicly available due to privacy or ethical restrictions.

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How to cite this article: Sioofy-Khojine A-B, Lehtonen JP, Nurminen N, et al. HLA-DQ-conferred risk for type 1 diabetes does not alter neutralizing antibody response to a widely used enterovirus vaccine, the poliovirus vaccine. *J Med Virol*. 2023;95:e28707. doi:10.1002/jmv.28707