SUPPLEMENTARY DATA

Supplementary Table 1. Distribution according to year of birth in the children taking part in the study

Year of birth	No of participants
1995	9
1996	25
1997	39
1998	86
1999	90
2000	71
2001	58
2002	45
2003	73
2004	31
2005	19
2006	3

Supplementary Table 2. Distribution of the sampling age (in months) at the time when the first autoantibody positive sample was drawn in the case children

Age in months	4	6	7	9	11	12	13	14	17	18	19	20	21	23	24	25	26	35	36	37
Number of children	1	2	2	1	2	31	8	3	3	20	8	1	1	1	15	4	2	7	14	9
Age in months	38	39	42	46	47	48	49	50	52	60	61	62	72	73	74	84	95	96	98	121
Number of children	1	2	1	2	3	7	5	4	1	6	2	3	2	2	1	1	1	2	1	1

Supplementary Table 3. Distribution of HLA genotypes among the study subjects. Children carrying the HLA genotype DQB1*02/DQB1*0302 were categorized into the high-risk group and children with the DQB1*0302/x Genotype (x≠DQB1*0301 or *0602) into the moderate risk group.

HLA DQB1 genotype	Number of children	Percentage
*02/*0302	154	28.1
*0302/x	395	71.9
Total	549	100

SUPPLEMENTARY DATA

Supplementary Table 4. Summary of enterovirus strains, their source, seroneutralization method, and cell lines used in the virus isolation and cultivation and the measurement of neutralizing antibodies against different enterovirus serotypes

No	Virus isolate	Source of the virus	Strain†	Neutralization method	Cell line used in neutralization assay
1	CAV4	DIPP	isolate 10433	plaque	RD
2	CAV5	DIPP	isolate P-550/CA5/Kanagawa/2000	MN	RD
3	CAV6	DIPP	isolate CSF-1739/07 VP1	MN	RD
4	CAV10	DIPP	P-2206/CA10/Kanagawa/2003	MN	RD
5	CAV16	DIPP	W42-44/01	MN	RD
6	EV71	DIPP	isolate 03784-MAA-97	MN	Vero
7	CAV9	DIPP	FR-08-2005-149	plaque	GMK
8	CBV1	HUSLAB	isolate CVB1Nm	plaque	GMK
9	CBV2	Laboratory center	FR-CASE4	plaque	GMK
10	CBV3	DIPP	CBV3-18219-02 from Moldova polyprotein	plaque	GMK
11	CBV4-wt#	DIPP	isolate P234pak92	plaque	GMK
12	CBV4-rs*	HUSLAB	Tuscany	plaque	GMK
13	CBV5	DIPP	isolate CVB5-CSF1841/BLR/2003	plaque	GMK
14	CBV6	ATCC	Schmitt [1-15-21]	plaque	GMK
15	Echo-1	Laboratory center	isolate 10429	plaque	GMK
16	Echo-2	Virology, University of Turku	152-77	plaque	GMK
17	Echo-3-wt	Virology, University of Tampere	PicoBank/DM1/E3	plaque	GMK
18	Echo-3-rs	HUSLAB	Morrisey	plaque	GMK
19	Echo-4	ATCC	Pesascek	plaque	GMK
20	Echo-5	ATCC	isolate Noyce	plaque	GMK
21	Echo-6	Virology, University of Tampere	Germany/120/2003	plaque	GMK
22	Echo-7	Laboratory center	FR-07-2000-55	plaque	GMK
23	Echo-9	Laboratory center	clone: No.66	plaque	GMK

24	Echo-11	DIPP	NET/2000-10025	plaque	GMK
25	Echo-12	Laboratory center	isolate: 120-98	plaque	GMK
26	Echo-13	DIPP	isolate FR-06-2000-93	plaque	GMK
27	Echo-14	ATCC	Tow	plaque	GMK
28	Echo-15	HUSLAB	CH6-51	plaque	GMK
29	Echo-17	ATCC	CHHE-29	plaque	GMK
30	Echo-18	HUSLAB	Metcalf	plaque	GMK
31	Echo-19	HUSLAB	isolate 87SD140	plaque	GMK
32	Echo-20	HUSLAB	isolate 10465	plaque	GMK
33	Echo-21	HUSLAB	Farina	plaque	GMK
34	Echo-25	Laboratory center	isolate SE-97-80688	plaque	GMK
35	Echo-26	HUSLAB	Coronel (11-3-6)	plaque	GMK
36	Echo-27	ATCC	Bacon	plaque	GMK
37	Echo-29	ATCC	JV-10	plaque	GMK
38	Echo-30-wt1	Laboratory center	isolate CF2191-01	plaque	GMK
39	Echo-30-wt2	Laboratory center	Bern7/ch1996	plaque	GMK
40	Echo-32	HUSLAB	PR 10	plaque	GMK
41	Echo-33	ATCC	Toluca-3	plaque	GMK
42	EV74	SMI	FRA99-130	plaque	GMK
43	EV78	SMI	Human enterovirus 78 polyprotein gene, partial cds Length=2574	MN	RD
44	EV94	SMI	isolate 19/04 from Democratic Republic of the Congo	MN	A594

#wt= field isolate

*rs = reference strain

†closest strain according to partial VP1-VP3 sequence used as blast search string

MN=microneutralization (Although microneutralization was used to assay these viruses actually all viruses formed plaques or plaque like structures seen under the microscope and therefore they could be isolated as single “plaques” for further cultivation.)

Laboratory center = Laboratory Center, Tampere University Hospital, Tampere, Finland

HUSLAB = Laboratory Center, Helsinki University Central Hospital, Helsinki, Finland

SMI = Smittskyddinstitutet, Stockholm, Sweden

SUPPLEMENTARY DATA

Supplementary Table 5. The median age and quartiles (Q1 and Q3) in months of the children at the various sampling time points used for the analyses of neutralizing antibodies to different enterovirus strains

Sampling time point	Median	Q1	Q3
-12 months	24	9	36
-6 months	12	6	13
At seroconversion to autoantibody positivity	24	13	38

SUPPLEMENTARY DATA

Supplementary Table 6. Effect of different combinations of risk and protective type Coxsackievirus B infections on the risk of β-cell autoimmunity at the time of autoantibody seroconversion (cross-sectional analysis). The reference group in this analysis comprises children with the lowest predicted risk being seropositive for one or more of the protective serotypes but seronegative for CBV1. Altogether 180 case children and 360 control children were used in these analyses. OR = Odds Ratio; 95% CI = 95% Confidence Interval.

Risk serotype CBV1	Protective serotype CBV3 or CBV4 or CBV6*	OR	95% CI	*P* value
neg	pos	1	ref	
neg	neg	1.5	(0.8-2.7)	0.16
pos	pos	1.3	(0.7-2.3)	0.42
pos	neg	2.6	(1.4-4.6)	0.001
CBV1	CBV3 or CBV6			
neg	pos	1	ref	
neg	neg	1.6	(0.9-3.1)	0.12
pos	pos	1.5	(0.8-2.9)	0.20
pos	neg	2.5	(1.4-4.7)	0.003
CBV1	CBV3			
neg	pos	1	ref	
neg	neg	2.3	(0.8-7.0)	0.14
pos	pos	1.5	(0.4-5.8)	0.53
pos	neg	3.6	(1.2-10.7)	0.02
CBV1	CBV6			
neg	pos	1	ref	
neg	neg	1.4	(0.7-2.7)	0.28
pos	pos	1.6	(0.8-3.2)	0.22
pos	neg	2.2	(1.1-4.1)	0.02
CBV1	CBV3 and CBV6			
neg	pos	1	ref	
neg	neg	3.2	(0.4-26.7)	0.28
pos	pos	2.0	(0.18-23.3)	0.56
pos	neg	4.9	(0.6-40.4)	0.14

*This largest group of protective serotypes includes CBV4 since it showed a protective non-significant trend in primary antibody screening and belonged to the same phylogenetic group as CBV1, CBV3 and CBV6 which showed statistically significant effects.

SUPPLEMENTARY DATA

Supplementary Table 7. Effect of CBV1, CBV3 and CBV6 infections on the risk of β-cell autoimmunity when adjusted for the duration of breast feeding (160 case and 317 control children) or the number of older siblings (177 case and 344 control children). Cross-sectional virus antibody analyses were performed at the time of autoantibody seroconversion. Odds ratios (OR) and 95% confidence intervals (95% CI) are adjusted for the duration of exclusive breast-feeding (time when the child has not received any other nutrients than breast-milk), overall duration of breast feeding (the total time when the child has received breast-milk) and number of older siblings at birth.

	Adjustment								
	Duration of exclusive breast-feeding			Overall duration of breast-feeding			Number of older siblings		
	OR	95% Cl	*P* value	OR	95% Cl	*P* value	OR	95% Cl	*P* value
CBV1	1.6	(1.1-2.5)	0.020	1.6	(1.0-2.4)	0.030	1.5	(1.0-2.3)	0.032
CBV3	0.3	(0.2-0.8)	0.008	0.3	(0.1-0.6)	0.002	0.3	(0.2-0.7)	0.005
CBV6	0.7	(0.4-1.1)	0.088	0.7	(0.4-1.1)	0.092	0.8	(0.5-1.2)	0.288

SUPPLEMENTARY DATA

Supplementary Table 8. The effect of the order of infection on the risk of β-cell autoimmunity. The reference group comprises children who had none of the CBV infections studied or who had been infected by the protective serotypes before being infected by CBV1. Infections were diagnosed by virus antibody seroconversion observed between consecutive follow-up samples taken before or at the sampling date of the first autoantibody positive sample. Altogether 183 case children and 366 control children were entered into these analyses. OR = Odds Ratio; 95% CI = 95% Confidence Interval

Protective serotype: CBV3, CBV4 or CBV6 *			
Risk serotype: CBV1	OR	95% CI	P value
Protective serotype first or negative for both protective serotype and CBV1	1	ref	
CBV1 first	1.5	(1.0-2.2)	0.06
CBV1 and protective serotype simultaneously**	1.2	(0.7-1.0)	0.54
Protective serotype: CBV3 or CBV6			
Risk serotype: CBV1	OR	95% CI	P value
Protective serotype first or negative for both protective serotype and CBV1	1	ref	
CBV1 first	1.4	(0.9-2.1)	0.10
CBV1 and protective serotype simultaneously**	1.1	(0.7-1.8)	0.64
Protective serotype: CBV3			
Risk serotype: CBV1	OR	95% CI	P value
Protective serotype first or negative for both protective serotype and CBV1	1	ref	
CBV1 first	1.5	(1.0-2.2)	0.03
CBV1 and protective serotype simultaneously**	1.4	(0.5-4.4)	0.53

SUPPLEMENTARY DATA

Supplementary Figure 1. Neutralizing antibody response induced by coxsackievirus B3 (CBV3) infection in one DIPP child. CBV3 was detected in a stool sample taken at the age of 1.2 years. Neutralizing antibody titers against CBV3 are shown for all follow-up serum samples.