Reduction in White Blood Cell, Neutrophil, and Red Blood Cell Counts Related to Sex, HLA, and Islet Autoantibodies in Swedish TEDDY Children at Increased Risk for Type 1 Diabetes

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Islet autoantibodies (IAs) precede the clinical onset of type 1 diabetes (T1D); however, the knowledge is limited about whether the prodrome affects complete blood counts (CBCs) in 4- to 12-year-old children with increased genetic risk for T1D. This study tested whether CBCs were altered in 4- to 12-year-old children without (*n* = 376) or with one or several IAs against insulin, GAD65, or IA-2 (*n* = 72). CBC was analyzed during longitudinal follow-up in 448 Swedish children enrolled in The Environmental Determinants of Diabetes in the Young (TEDDY) study. A linear mixed-effects model was used to assess potential association between IA and CBC measurements over time. The white blood cell and neutrophil counts were reduced in children with IAs, primarily in boys. In contrast, girls had lower levels of hemoglobin and hematocrit. Positivity for multiple IAs showed the lowest counts in white blood cells and neutrophils in boys and red blood cells, hemoglobin, and hematocrit in girls. These associations were primarily observed in children with the HLA-DR3-DQ2/DR4-DQ8 genotype. We conclude that the reduction in neutrophils and red blood cells in children with multiple IAs and HLA-DR3-DQ2/DR4-DQ8 genotype may signal a sex-dependent islet autoimmunity detected in longitudinal CBCs.

Autoantibodies against the β-cell autoantigens insulin (IAA), GAD antibody (GADA), or protein tyrosine phosphatase-like (IA-2A) precede the clinical onset of autoimmune type 1 diabetes (T1D). Children at genetic risk for T1D were monitored from birth in The Environmental Determinants of Diabetes in the Young (TEDDY) study for a first appearing islet autoantibody (IA), be it IAA or GADA (1–3). The IAA incidence rate was highest in the 1st year of life (18 months in Sweden) and declined over the following 5 years, whereas the incidence rate of GADA increased during the first 2 years (2.5 years in Sweden) of life and remained seemingly constant until the age of 6 years (1–3). The appearance of IAA or GADA as the first IA was related to the HLA-DR/DQ genotype (1,2). After an initial event that triggers autoimmunity reflected by a first appearing IAA or GADA, the pathogenesis progresses toward the clinical onset of disease more rapidly with an increasing number of IAs (4,5), which may be critical because islet β-cells are thought to be destroyed by autoreactive T cells, not by autoantibodies (6,7). So far, β-cell–specific autoantibodies are the best predictors of an ongoing autoimmune process resulting in clinical onset (8). Notwithstanding the critical role of the adaptive immune response in the etiology and pathogenesis of T1D (reviewed in [9–12]), there is growing evidence that the innate immune response also contributes to the pathogenesis (12–14). Neutrophils are thought to contribute to the etiological triggering and the pathogenesis of T1D in mouse models (reviewed in [15]). A reduction in peripheral blood neutrophils has recently been reported in healthy IA-positive children all having a first-degree relative with the disease (16). Similarly, compared with healthy children,

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white blood cells, neutrophils, and lymphocytes were reduced in IA-positive children with a family history of T1D (17), and there is an unknown effect of the peripheral immune cell counts on the pathogenesis of T1D (18).

We have studied children in Sweden who are enrolled in the TEDDY study (19,20) and monitored from birth to determine the first appearing IA (1) as well as progression to multiple autoantibodies and clinical onset of diabetes (4,5). We specifically questioned whether complete blood count (CBC) was associated with the IA status in 4- to 12-year-old Swedish TEDDY children and whether the association differed by sex and HLA DQ-DR genotype.

RESEARCH DESIGN AND METHODS

TEDDY Design

The TEDDY study is a prospective cohort study funded by the U.S. National Institutes of Health with the primary goal to identify environmental causes of T1D. TEDDY includes six clinical research centers—three in the U.S.: Colorado, Georgia/Florida, and Washington, and three in Europe: Finland, Germany, and Sweden. Detailed study design and methods have been previously published (19,21). Written informed consents were obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and participation in prospective follow-up. The current study was approved by the Regional Ethics Review Board in Lund and is monitored by an External Advisory Board formed by the National Institutes of Health.

CBC Measurements in TEDDY Children

The study cohort consists of 448 children, 4–12 years old, from the TEDDY clinic in Malmö, Sweden (Table 1). The CBC measurements were initiated in June 2014 and completed in February 2017. The samples were analyzed at their scheduled visits and within 8 h after sample draw. The TEDDY protocol requires visits every 3 months for children who were IA positive and every 6 months for children who remained autoantibody negative.

CBC

CBC was determined in a multiparameter automated hematology analyzer (CELL-Dyn Ruby; Abbott Laboratories, Diagnostic Division, Abbott Park, IL) (22). The instrument was operated according to the instructions by the manufacturer, including a daily calibration. Counts (cells $\times 10^9$/L) of white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets, red blood cells (cells $\times 10^{12}$/L) and red blood cell parameters; hemoglobin (g/L), hematocrit (L/L), mean corpuscular volume (fL), mean corpuscular hemoglobin (MCH) (pg), MCH concentration (MCHC) (g/L), and red cell distribution width (% coefficient of variation) were obtained.

HLA-DR-DQ Typing

Genotype screening (20) was conducted using a dried blood spot punch or a small volume whole-blood lysate specimen format, as previously published (23). Infants from the general population were eligible for the study if they had any one of the following HLA genotypes (excluding those with DR4 subtype DRB1*04:03):

1. DR4-DQA1*03:0X-DQB1*03:02/DR3-DQA1*05:01-DQB1*02:01
2. DR4-DQA1*03:0X-DQB1*03:02/DR4-DQA1*03:0X-DQB1*03:02[1]
3. DR4-DQA1*03:0X-DQB1*03:02/DR8-DQA1*04:01-DQB1*04:02
4. DR3-DQA1*05:01-DQB1*02:01/DR3-DQA1*05:01-DQB1*02:01

Note: [1]Acceptable alleles in this haplotype include both DQB1*03:02 and *03:04.

Infants with a first-degree relative with T1D were eligible for enrollment if they had any of the following HLA genotypes:

1. DR4-DQA1*03:0X-DQB1*03:02[1]/DR3-DQA1*05:01-DQB1*02:01
2. DR4-DQA1*03:0X-DQB1*03:02[1]/DR4-DQA1*03:0X-DQB1*03:02[1]
3. DR4-DQA1*030X-DQB1*0302[1]/DR8-DQA1*04:01-DQB1*04:02
4. DR3-DQA1*05:01-DQB1*02:0[1]/DR3-DQA1*05:01-DQB1*02:01 (24)

Table 1—Characteristics of TEDDY children (*n* = 448) investigated for CBC when negative or positive for one or several IAs

	IA negative (*n* = 376)	IA positive (*n* = 72)
Children, *n* (%)	376 (84)	72 (16)
Sex, *n*		
Girls	182	30
Boys	194	42
Age at first CBC (months), median (min–max)	91 (52–145)	101.5 (59–139) n.s.
Girls	91 (53–144)	103 (59–139)
Boys	94 (52–145)	99 (59–137)
CBC measures per child, min–max	1–6	1–9
Months of follow-up, min–max	1–30	1–30
IAs, *n*		
1	0	25
2	0	16
3	0	31
Change in IA status	None	None
HLA DR-DQ, *n* (%)		
DR3/4-DQ2/8	151 (40.2)	39 (54.1)
DR4/4-DQ 8/8	87 (23.1)	12 (16.7)
DR4/8-DQ 8/4	40 (10.6)	12 (16.7)
DR3/3-DQ 2/2	91 (24.2)	9 (12.5)
DR4/1-DQ 8/5	4 (1.1)	0
DR4/13-DQ 8/6	2 (0.5)	0
HLA ineligible	1 (0.3)	0
Total	376 (100)	72 (100)

5. DR4-DQA1*03:0X-DQB1*03:02[1]/DR4-DQA1*03:0X-DQB1*02:0X
6. DR4-DQA1*03:0X-DQB1*03:02[1]/DR12-DQA1*0101-DQB1*05:01[2]
7. DR4-DQA1*030X-DQB1*03:02[1]/DR13-DQA1*01:02-DQB1*06:04
8. DR4-DQA1*03:0X-DQB1*03:02/DR4-DQA1*03:0X-DQB1*03:04
9. DR4-DQA1*03:0X-DQB1*03:02[1]/DR9-DQA1*03:0X-DQB1*03:03 (23)
10. DR3-DQA1*05:01-DQB1*02:01/DR9-DQA1*03:0X-DQB1*03:03

Notes: [1]Acceptable alleles in this haplotype included both DQB1*03:02 and *03:04. [2]In this DQB1*05:01 haplotype, DR10 was excluded. Only DR1 was eligible.

Autoantibody Measurements

IAA, GADA, or IA-2A were measured in two laboratories by radiobinding assays (25,26). In Europe, all sera were assayed at the University of Bristol, Bristol, U.K. All samples positive for IA and 5% of negative samples were retested at the Barbara Davis Center for Childhood Diabetes at the University of Colorado, Denver, and deemed confirmed if concordant. Both laboratories have previously shown high sensitivity and specificity as well as concordance (27).

IA Analyses

Persistent IA positivity was defined as confirmed positive IAA, GADA, or IA-2A on at least two consecutive study visits. The first appearance of persistent confirmed IA in the follow-up was considered and counted.

Statistical Analysis

Considering correlations between measures from the same subject (within-subject correlation), a linear mixed-effects model was used to assess the association of islet autoimmunity on each CBC measurement. We first examined whether the association between the status of IA and each CBC measurement was different by sampling age, but no significant difference was noted in all CBC analyses. Hence, the model included random intercept and random slope as well as sampling age and the status of IAs as fixed effects. Unstructured within-subject correlation was assumed for the random error. The regression coefficient for the status of IAs was assessed to determine whether the associations of islet autoimmunity were ignorable or not. The association between age and each CBC measurement was assessed among IA-negative children. Age of initial measurement was compared using the Wilcoxon rank sum test, and the proportions of girls and HLA-DR3-DQ2/DR4-DQ8 subjects were compared using the Fisher exact test. Age-dependent effects were observed in most CBC parameters and later corrected for in the linear mixed-effects model. Due to this procedure, the observed effects of an increasing number of IAs on neutrophils and red blood cell parameters were all corrected for age.

Table 2—Effects of age (years) on each CBC measurement in IA-negative subjects (n = 376)

CBC	Estimate	SE	P value
White blood cells (10^9 cells/L)	**−0.123**	**0.044**	**0.005**
Neutrophils (10^9 cells/L)	−0.060	0.031	0.053
Lymphocytes (10^9 cells/L)	**−0.036**	**0.013**	**0.006**
Monocytes (10^9 cells/L)	**−0.010**	**0.004**	**0.015**
Eosinophils (10^9 cells/L)	−0.002	0.008	0.766
Basophils (10^9 cells/L)	−0.001	0.001	0.306
Red blood cells (10^{12} cells/L)	**0.040**	**0.015**	**0.010**
Hemoglobin (g/L)	**0.167**	**0.040**	**<0.0001**

Values in boldface type are statistically significant ($P < 0.05$).

Two-sided P values of less than 0.05 were considered for statistical significance. All analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC).

RESULTS

CBC in Relation to Age in IA Negative Children

CBC was examined in 448 children participating in the longitudinal TEDDY study. Children were examined at one to six visits if negative (84%) or one to nine visits (16%) if positive for any IA. The number of CBC measurements was therefore higher in the IA-positive subjects (n = 72; median, 3) than in the IA-negative subjects (n = 376; median, 2), but the significant difference in the age when the CBC measurements started was not significant (Table 1). We examined whether CBC varied with age in the IA-negative children (Table 2). In agreement with earlier studies, older age was significantly associated with decreasing cell counts in the different white blood cell types, except for a non-significant association in eosinophils and basophils (Table 2). In contrast to the nucleated cells, red blood cell counts and hemoglobin levels increase with age, which was also confirmed in our study (28,29).

CBC in Children With and Without IAs

In the next step, differences in the peripheral blood cell counts were examined between children with or without IAs (Table 3). Children with IAs had reduced white blood cell counts (P = 0.046) as a result from a reduction in neutrophil cell counts (P = 0.017). The two red blood cell parameters, hemoglobin (P = 0.026) and hematocrit (P = 0.031), were also reduced in children with IA. The neutrophil-to-lymphocyte ratio did not differ between the two groups.

CBC in Relation to Sex in Children With and Without IAs

Stratified analyses by sex were performed to evaluate sex differences (Table 3). Reduction of white blood cell counts (P = 0.02) caused by a reduction of neutrophil (P = 0.012) and basophil (P = 0.029) counts showed a significant difference between children with and without IAs in boys, whereas a reduction of hemoglobin (P = 0.012) and hematocrit (P = 0.047) was found in girls with IA.

Table 3—The association between IA and each CBC measurement and association to sex

CBC	Estimate	SE	*P* value
White blood cells (10^9 cells/L)			
All subjects	**−0.315**	**0.157**	**0.046**
Girls	−0.025	0.212	0.906
Boys	**−0.540**	**0.229**	**0.019**
Neutrophils (10^9 cells/L)			
All subjects	**−0.239**	**0.099**	**0.017**
Girls	−0.031	0.128	0.807
Boys	**−0.386**	**0.151**	**0.012**
Lymphocytes (10^9 cells/L)			
All subjects	−0.034	0.057	0.553
NLR (all)	−0.115	0.067	0.090
Girls	0.048	0.082	0.555
Boys	−0.084	0.080	0.295
Monocytes (10^9 cells/L)			
All subjects	−0.024	0.015	0.108
Girls	−0.024	0.022	0.281
Boys	−0.024	0.021	0.248
Eosinophils (10^9 cells/L)			
All subjects	−0.023	0.030	0.437
Girls	−0.031	0.045	0.490
Boys	−0.028	0.040	0.486
Basophils (10^9 cells/L)			
All subjects	−0.004	0.002	0.064
Girls	−0.001	0.003	0.702
Boys	**−0.007**	**0.003**	**0.029**
Platelets (10^9 cells/L)			
All subjects	−8.190	6.519	0.210
Girls	0.995	8.731	0.909
Boys	−16.260	9.542	0.090
Red blood cells (10^{12} cells/L)			
All subjects	−0.089	0.050	0.077
Girls	−0.134	0.077	0.084
Boys	−0.065	0.065	0.322
Hemoglobin (g/L)			
All subjects	**−0.297**	**0.133**	**0.026**
Girls	**−0.491**	**0.192**	**0.012**
Boys	−0.180	0.185	0.337
Hematocrit (L/L)			
All subjects	**−0.800**	**0.366**	**0.031**
Girls	**−1.170**	**0.581**	**0.047**
Boys	−0.518	0.521	0.328
Mean corpuscular volume (fL)			
All subjects	0.042	0.374	0.911
Girls	−0.089	0.544	0.870
Boys	0.330	0.500	0.510
Red cell distribution width (%CV)			
All subjects	0.109	0.075	0.146
Girls	0.094	0.121	0.440
Boys	0.130	0.095	0.171

Values in boldface type are statistically significant ($P < 0.05$). CV, coefficient of variation; NLR, neutrophil-to-lymphocyte ratio.

CBC in Relation to the Number of IAs and HLA Genotype

We investigated whether the number of IAs was associated with CBC by comparing the different cell counts (Table 4). The number of IAs was counted as the appearance of any of the following three IAs—IAA, GADA, or IA-2A—in the follow-up. Of the 72 children with IA, all 3 IAs appeared in 31 children, 2 IAs appeared in 16 children, and 1 IA appeared in 25 children. The number of IAs was inversely correlated with the number of white blood cells, neutrophils, and lymphocytes. Sex differences were also identified in this analysis. Children with three IAs and therefore at the greatest risk for T1D showed a reduction in white blood cell counts ($P = 0.007$), primarily in boys ($P = 0.019$) and in children with HLA-DR3-DQ2/DR4-DQ8 ($P = 0.045$). The reduction of white blood cells was mainly caused by the reduction in neutrophil counts ($P = 0.003$), primarily in boys ($P = 0.004$) and in children with HLA-DR3-DQ2/DR4-DQ8 ($P = 0.010$), but also the reduction of lymphocyte counts ($P = 0.038$) in all children.

Red cell parameters were also associated with the number of IA, especially when all three IAs appeared. The red blood cell count was reduced in all children with two IAs ($P = 0.026$), particularly in girls ($P = 0.002$) and in children with the HLA-DR3/4-DQ2/8 genotype and three IAs ($P = 0.006$) (Table 4). These reductions in red blood cell counts were reflected in reduced concentrations of both hemoglobin and hematocrit in the presence of two or more IAs in girls and in children with the HLA-DR3-DQ2/DR4-DQ8 genotype (Table 4). Furthermore, in children with the HLA-DR3-DQ2/DR4-DQ8 genotype and one IA, the MCH was also decreased ($P = 0.042$). In contrast, boys with one positive IA had increased mean corpuscular volume levels ($P = 0.044$), particularly in children not carrying the HLA-DR3-DQ2/DR4-DQ8 genotype ($P = 0.008$).

DISCUSSION

The major findings of CBC in TEDDY children were lower numbers of white blood cells, primarily of neutrophils in boys and in children with HLA-DR3-DQ2/DR4-DQ8 with an increasing number of IAs. Also, an increasing number of IAs was related to lower numbers of hemoglobin and hematocrit in girls and in children with HLA-DR3-DQ2/DR4-DQ8. These findings may be related to an interaction between HLA risk and development of chronic islet autoimmunity. In TEDDY, chronic islet autoimmunity is defined as the length of being persistent confirmed IA positive after seroconversion and also in relation to the number of different IAs (30,31). The major finding that levels of neutrophils, primarily in boys, as well as in children with HLA-DR3-DQ2/DR4-DQ8, decreased with an increasing number of IAs is a novel finding, which to our knowledge has not been reported before. Our data are otherwise consistent with a reduction in peripheral blood neutrophils as recently reported in healthy IA-positive children all with a first degree relative with the disease (16,17). However, in contrast to one of these reports, a reduction in platelet counts in children positive for one or several IAs was not detected. The difference may be explained by the fact that our study was longitudinal, including a relatively larger number of children. Our results take these previous reports to a next step, indicating that the larger the number of IA, the lower the number of neutrophils. Reduction of red blood cells, hemoglobin, and hematocrit in girls positive for two or more IAs has not been reported previously.

Table 4—Association between the number of IA and each CBC measurement in 72 subjects (boys *n* = 42)

CBC	*n* IAs	Estimate	SE	*P* value
White blood cells (10^9 cells/L)				
All subjects	1	0.142	0.257	0.582
	2	−0.379	0.293	0.197
	3	**−0.613**	**0.225**	**0.007**
Boys	1	−0.001	0.392	0.998
	2	−0.701	0.404	0.086
	3	**−0.789**	**0.332**	**0.019**
HLA-DR3-DQ2/DR4-DQ8	1	0.512	0.401	0.203
	2	−0.392	0.415	0.347
	3	**−0.729**	**0.360**	**0.045**
Neutrophils (10^9 cells/L)				
All subjects	1	0.081	0.161	0.616
	2	−0.328	0.178	0.068
	3	**−0.427**	**0.141**	**0.003**
Boys	1	0.033	0.251	0.897
	2	−0.479	0.259	0.068
	3	**−0.634**	**0.216**	**0.004**
HLA-DR3-DQ2/DR4-DQ8	1	0.270	0.245	0.275
	2	−0.420	0.246	0.091
	3	**−0.572**	**0.216**	**0.010**
Lymphocytes (10^9 cells/L)				
All subjects	1	0.092	0.091	0.315
	2	0.025	0.109	0.822
	3	**−0.175**	**0.0840**	**0.038**
Girls	1	0.080	0.124	0.519
	2	**0.368**	**0.175**	**0.038**
	3	−0.115	0.118	0.332
Monocytes (10^9 cells/L)				
All subjects	1	0.003	0.0244	0.885
	2	**−0.057**	**0.0281**	**0.044**
	3	−0.026	0.0214	0.231
Red blood cells (10^{12} cells/L)				
All subjects	1	0.019	0.080	0.813
	2	**−0.200**	**0.089**	**0.026**
	3	−0.112	0.073	0.125
Girls	1	0.060	0.119	0.616
	2	**−0.471**	**0.142**	**0.002**
	3	−0.109	0.104	0.295
HLA-DR3-DQ2/DR4-DQ8	1	0.029	0.129	0.826
	2	−0.211	0.136	0.125
	3	**−0.320**	**0.113**	**0.006**
Hemoglobin (g/L)				
All subjects	1	−0.031	0.210	0.882
	2	**−0.635**	**0.235**	**0.008**
	3	−0.282	0.193	0.147
Girls	1	−0.038	0.300	0.900
	2	**−1.248**	**0.335**	**0.0007**
	3	−0.407	0.255	0.116
HLA DR3/4-DQ2/8	1	−0.255	0.372	0.495
	2	−0.614	0.388	0.116
	3	**−0.710**	**0.326**	**0.031**
Hematocrit (L/L)				
All subjects	1	0.147	0.577	0.799
	2	**−1.801**	**0.640**	**0.006**
	3	**−1.034**	**0.525**	0.050
Girls	1	0.395	0.907	0.664
	2	**−3.635**	**0.990**	**0.0007**
	3	−1.011	0.776	0.200
HLA-DR3-DQ2/DR4-DQ8	1	−0.375	0.996	0.707
	2	−1.922	1.029	0.065
	3	**−2.514**	**0.853**	**0.004**
Mean corpuscular volume (fL)				
All subjects	1	0.854	0.597	0.153
	2	−0.481	0.734	0.513
	3	−0.351	0.544	0.519
Boys	**1**	**1.728**	**0.850**	**0.044**
	2	−0.505	0.900	0.576
	3	−0.118	0.716	0.869
Not HLA-DR3-DQ2/DR4-DQ8	**1**	**2.210**	**0.826**	**0.008**
	2	−0.605	1.246	0.628
	3	−0.192	0.722	0.791
MCH (pg)				
HLA-DR3-DQ2/DR4-DQ8	**1**	**−0.861**	**0.418**	**0.042**
	2	0.034	0.433	0.938
	3	0.562	0.354	0.116

Values in boldface type are statistically significant ($P < 0.05$).

Continued on p. 5

The strength of the current study is that we have been able to determine CBC during almost 3 years in this TEDDY subset of 448 children who visit the Malmö clinic. They represent ~15% of all children in TEDDY. During this period of investigation, children with IAs underwent CBC measurements more than three times and children with no IA one to two times because they only visit every 6 months. Another strength is that the CBC was done at random because our TEDDY laboratory could only perform the CBC in a maximum of eight children per day with blood samples collected in the morning. During the current period of investigating these 4- to 12-year-olds, we did not expect that we would come across a child converting to IA because of small numbers of IA-positive children monitored in this study. Although TEDDY aims to identify the environmental factors behind seroconversion, the strength of the current study was to contribute to the second end point in TEDDY, which is to identify factors that predispose to T1D, to determine the pathogenic mechanisms that eventually result in clinical onset of T1D.

The weakness was that none of the children changed IA status during the follow–up, and therefore, we cannot test whether the observed changes in CBC may precede seroconversion. Therefore, our data underscore the importance to investigate CBC when children at increased genetic risk for T1D are monitored from birth in the future. Another limitation is the number of IA-positive children monitored for CBC. Therefore, we continue the CBC follow-up in children with and without IAs to understand underlying mechanisms of altered CBC. Validation of these results outside of the TEDDY cohort may be accomplished in studies monitoring newborns, such as in the recently initiated Primary Oral Insulin Trial (POInT) study (32).

Pediatric blood reference intervals are mainly based on retrospective data from hospitalized individuals (28,33). However, the data in this study are prospective from healthy children with a genetic risk for T1D. This premise makes our data more reliable and useful when IA-positive

children are compared with those negative for any IA. HLA risk eligibility for TEDDY in Sweden represented 7.5% of all newborns (20). Further CBC analyses are needed because there is an apparent lack of information of CBC neutrophil and red blood cell parameters in relation to HLA-DR-DQ risk not only for T1D but also celiac disease, thyroiditis, multiple sclerosis, and other HLA-associated organ-specific autoimmune disorders.

In agreement with numerous studies, white blood cell counts varied during the 1st years of life and decreased slightly thereafter. Moreover, the red blood cell count is known to increase by age in children, which was found also in this study (28,33). Furthermore, we examined the association between age and CBC in the IA-negative children considered as the general population with the above limitations. As revealed in Table 2, age-dependent effects were observed in most CBC parameters and later corrected for in the linear mixed-effects model. Due to this procedure, the observed associations between the increasing number of IAs and the reduction in neutrophils and red blood cell parameters were all corrected for age.

A diminished number of white blood cells was reported in several autoimmune diseases (17,18,34). Even though neutrophils are innate immune cells, they are involved in the activation and recruitment of both innate and adaptive immune cells (35,36). An impaired neutrophil response is thought to cause or initiate several autoimmune diseases, including systemic lupus erythematosus, vasculitis, and multiple sclerosis (35,37). Our results demonstrate a reduction in neutrophils in boys and in HLA-DR3-DQ2/DR4-DQ8 children positive for three IAs. Recent studies have also reported a reduction in circulating neutrophils in patients newly diagnosed with T1D (who did not have diabetic ketoacidosis at onset) and in IA-positive healthy individuals, suggesting β-cell specific autoimmunity (16,17,38). Neutrophils are thought to be recruited to infiltrate pancreatic islets by the physiological death of β-cells and by a signaling cross talk with other innate immune cells activating the autoreactive T cells (36). Recent studies have also suggested that the reduction of neutrophil numbers may be related to an accumulation of neutrophils in the pancreas (16,17), perhaps associated with insulitis (39). These data would be consistent with the observations that the larger the number of IAs, the higher the risk for insulitis (40,41). However, further studies need to dissect the neutrophil count reduction in relation to pathophysiological changes that occur in the pancreas before the clinical onset of T1D.

Mild neutropenia is a common finding in children with viral infections (42). The reduction in the neutrophil counts in healthy Swedish TEDDY boys and children with the HLA-DR3-DQ2/DR4-DQ8 genotype could be due to an impaired hematopoietic cell production in the bone marrow, impaired maturation, apoptosis, peripheral consumption or damage, pooling to other organs such as the pancreas, and perhaps tissue detention (43,44). The reduction in neutrophil counts associated with a presence of three IAs may contribute to an accelerated pathogenesis by potentiating the autoimmune attack on β-cells, by increasing the risk for infection, or by some other processes. However, it can also be a secondary phenomenon due to detention of neutrophils in the pancreas when the β-cell destruction is more pronounced. Alterations in the CBC among autoantibody-positive children may be caused by an impaired hematopoiesis caused by infections or toxins (42,45).

Under normal circumstances, no sex differences in neutrophil, lymphocyte, or basophil counts at the age of 4–12 years have been reported (28). Boys in this study had reduced basophil and neutrophil counts and increased MCHC counts associated with the status of being positive for three IAs. The MCHC count is normally decreasing by age (28). Viral and bacterial infections may alter neutrophil, basophil, and lymphocyte counts; for instance, whooping cough is known to decrease basophil numbers. Basophils have been shown to migrate to secondary lymphoid organs where they cross talk with T- and B-lymphocytes thereby linking the T-helper cell environment as a contributor to the development of autoimmune systemic lupus erythematosus, and as a consequence, the basophils in the circulation decrease (24). Girls with two IAs had increased levels of lymphocytes which could be associated with viral infections because many viruses, such as rotavirus, mumps virus, and others, have been associated with the pathogenesis of T1D (46,47).

The reduction of red blood cells, hemoglobin and hematocrit associated with two IAs in girls may be explained by a peripheral destruction of red blood cells, impaired hematopoiesis, or infections. Previous studies have associated rotavirus and enterovirus with islet β-cell autoimmunity and T1D (48,49). Hence, whether a reduction in CBC is caused by or contributes to the development of the second or third IA is unclear. Our results in TEDDY girls suggest that an alteration of CBC was observed with a second IA.

We concluded from the current study that deviations in CBC after seroconversion were common primarily in boys and in children with the HLA-DR3-DQ2/DR4-DQ8 genotype. Our statistical models indicate that the reduction of neutrophil counts in boys and HLA-DR3-DQ2/DR4-DQ8 children positive for three autoantibodies may be consequences of islet β-cell autoimmunity, because the reduction in neutrophil levels correlated with the number of IAs. Additional factors influencing the CBC in children at genetic risk for T1D and positive for one to three IAs were also affected by sex, HLA genotype, and number of IAs. The cellular and molecular mechanisms behind the aggravating CBC alterations different in boys or girls with an increasing number of IAs need to be further explored.

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SUPPLEMENTARY DATA

Supplementary Table 1. Complete blood count (CBC) (median (range)) at first visit in 448 TEDDY children without or with one or several islet autoantibodies (IA).

	IA negative	**IA positive**
n	**376**	**72**
White blood cells (WBC) (10E9 Cells/L)	4.87 (2.09-12.3)	4.54 (2.08-8.02)
Neutrophil (10E9 cells/L)	2.13 (0.59-9.42)	1.95 (0.85-5.05)
Lymphocyte (LYM) (10E9 cells/L)	1.79 (0.50-3.93)	1.81 (0.80-3.22)
Neutrophil Lymphocyte Ratio (NLR)	1.20 (0.28-7.73)	1.18 (0.42-4.45)
Monocyte (MONO) (10E9 cells/L)	0.37 (0.11-1.07)	0.37 (0.18-0.78)
Eosinophils (EOS) (10E9 cells/L)	0.23 (0.01-1.88)	0.21 (0-1.05)
Basophil (BASO) (10E9 cells/L)	0.05 (0.01-0.15)	0.05 (0.01-0.10)
Platelets (PLT) (10E9 cells/L)	255 (99.4-496)	235 (113-350)
Red blood cells (RBC) (10E12 cells/L)	3.99 (2.13-6.66)	3.92 (1.99-5.35)
Red blood cell parameters		
Hemoglobin (HGB) (g/L)	11.4 (6.66-19.7)	11.0 (5.79-14.9)
Hematocrit (HCT) (L/L)	31.1 (17.1-51.7)	30.1 (15-41)
Mean corpuscular volume (MCV) (fL)	77.5 (68.7-88.7)	77.6 (70.6-87.1)
Mean corpuscular hemoglobin (MCH) (pg)	28.7 (23.4-51.3)	28.4 (24.3-31.4)
Mean corpuscular hemoglobin concentration (MCHC) (g/L)	36.9 (33-65.7)	36.7 (34.3-41)
Red cell distribution width (RDW) (%CV)	11.8 (10.5-15.4)	11.8 (10.7-13.5)

SUPPLEMENTARY DATA

Supplementary Table 2. The Association between islet autoantibodies (IA) and each complete blood count (CBC) measurement by HLA-DR3-DQ2/DR4-DQ8.

HLA DR3/4-DQ2/8	CBC	Estimate	Standard error	P-value
Yes	White blood cells (10E9cells/L)	-0.240	0.247	0.333
No		-0.329	0.207	0.116
Yes	Neutrophils (10E9cells/L)	-0.275	0.155	0.078
No		-0.192	0.139	0.171
Yes	Lymphocytes (10E9cells/L)	0.040	0.086	0.640
No		-0.055	0.075	0.461
Yes	Monocytes (10E9cells/L)	-0.006	0.019	0.766
No		**-0.047**	**0.023**	**0.042**
Yes	Eosinophils (10E9cells/L)	-0.020	0.044	0.651
No	Model does not fit			
yes	Basophils (10E9cells/L)	-0.002	0.003	0.557
No		-0.006	0.003	0.071
Yes	Platelets	-4.171	10.201	0.683
No		-13.258	8.721	0.130
Yes	Red blood cells	**-0.177**	**0.080**	**0.030**
No		0.003	0.062	0.966
Yes	Hemoglobin	**-0.548**	**0.224**	**0.016**
No	Model does not fit			
Yes	Hematocrit	**-1.712**	**0.599**	**0.005**
No	Model does not fit			
Yes	Mean corpuscular volume	-0.463	0.539	0.392
No		0.614	0.524	0.242
Yes	Mean corpuscular hemoglobin	-0.010	0.255	0.969
No	Model does not fit			
Yes	Red cell distribution width	0.096	0.107	0.372
No		0.144	0.101	0.156

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