# Inverse Relationship Between Organ-Specific Autoantibodies and Systemic Immune Mediators in Type 1 Diabetes and Type 2 Diabetes: Action LADA 11

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## OBJECTIVE

**We related organ-specific autoantibodies, including diabetes-associated autoantibodies (DAAs) and non-DAAs to systemic cytokines/chemokines in type 1 and type 2 diabetes.**

## RESEARCH DESIGN AND METHODS

**From the European Action LADA (latent autoimmune diabetes in adults) cohort, patients with adult-onset type 1 diabetes ($n$ = 80, of whom 50 had LADA and 30 had classic type 1 diabetes) and type 2 diabetes ($n$ = 626) were analyzed for DAAs (GAD antibody [GADA], IA-2 antigen, islet cell antibody, and zinc transporter T8), non-DAAs (transglutaminase, thyroid peroxide autoantibodies, parietal cell antibodies), and 10 immune mediator concentrations (measured by LUMINEX).**

## RESULTS

**Type 1 diabetes patients (whether having classic type 1 diabetes or LADA), apart from their clinical phenotype, could not be distinguished by either autoantibodies (both DAAs and non-DAAs) or immune mediators. In type 1 diabetes, most immune mediators (9 of 10) were negatively correlated with DAA titers. Type 2 diabetes patients, who by definition were without DAAs, had fewer non-DAAs ($P < 0.0005$), but had higher levels of proinflammatory immune mediators, especially compared with patients with type 1 diabetes who had high GADA titers (interleukin [IL]-6 [$P < 0.001$], soluble E-selectin [$P < 0.01$], and IL-1 receptor antagonist [$P = 0.052$], for trend).**

## CONCLUSIONS

**Patients with type 1 diabetes had more DAAs and non-DAAs than did those with type 2 diabetes, whereas the frequency and nature of these autoantibodies was broadly similar in classic type 1 diabetes and LADA. Systemic immune mediator levels, in the main, were negatively correlated with DAA titers, and, for some, were higher in patients with type 2 diabetes, especially when compared with patients who had high GADA titers. Differences in the clinical classification of diabetes are associated with graded differences in adaptive and innate immune reactivity.**



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The definition of type 1 diabetes is clinically exclusive, encompassing patients with diabetes-associated autoantibodies (DAAs) plus, when diagnosed outside a surveillance program, insulin dependence. Difficulty arises when patients with non–insulin-requiring diabetes, which is clinically like type 2 diabetes, have immunogenetic characteristics of type 1 diabetes. Such patients have been designated as having latent autoimmune diabetes in adults (LADA), which is also called slowly progressive insulin-dependent diabetes or type 1.5 diabetes (1). Adult-onset autoimmune type 1 diabetes (AID) is characterized by DAAs, usually GAD antibodies (GADAs) (1,2), and encompasses patients with both classic type 1 diabetes and LADA, of which the latter is the most prevalent (2). Despite having different clinical presentations and phenotypes, adults with classic type 1 diabetes and LADA resemble each other with regard to their HLA and non-HLA genetic risk, the presence of DAAs (predominantly GADAs), the frequency of thyroid peroxide autoantibodies (TPOAs), the frequency of metabolic syndrome, and levels in peripheral blood of systemic cytokines (interleukin [IL]-6, tumor necrosis factor-α [TNF-α], IL-1 receptor antagonist [Ra], IL-10), chemokines (C-C motif chemokine ligand [CCL] 2, CCL3, CCL4), adhesion molecules (soluble E-selectin [sE-selectin], soluble intercellular adhesion molecule-1 [sICAM-1], and soluble vascular cell adhesion molecule-1 [sVCAM-1]), and peripheral B-cell subsets (2–8). In contrast, patients with type 2 diabetes do not have an HLA genetic association, do not have DAAs (1–4), have low frequency of TPOAs (9), and have a substantially higher frequency of metabolic syndrome (10). Similarly, levels of some systemic serum immune mediators are higher in type 2 diabetes than in type 1 diabetes, including LADA (e.g., systemic cytokines [IL-6, TNF-α, IL-1Ra, IL-10]), chemokines [CCL2, CCL3, CCL4], and adhesion molecules [sE-selectin, sICAM-1, and sVCAM-1]) (5,6).

In previous studies (5,6,11–13), we defined a nonrandom association in individuals with type 1 diabetes between adaptive immune changes (the presence and number of DAAs) and innate immune changes (the serum levels of systemic cytokines and chemokines). Here, we extend and expand these studies to include a range of adult-onset clinical diabetes (both forms of type 1 diabetes as well as type 2 diabetes, each analyzed for both DAAs and non-DAAs, as well as a panel of systemic immune mediators [classic type 1 diabetes cytokines, chemokines, and adhesion molecules]). Our hypothesis is that innate (immune mediators) and adaptive immune reactivity (antibodies) can occur in both of the major types of diabetes; adult-onset type 1 diabetes is predominantly associated with adaptive immune changes, whereas type 2 diabetes is predominantly associated with innate immune changes. Here we show graded differences in adaptive and innate immune changes across the classification of diabetes.

## RESEARCH DESIGN AND METHODS

### Patients

The Action LADA multicenter study was performed to identify immune and clinical risk factors for AID, including its epidemiology, genetic susceptibility, metabolic characteristics, and clinical progression (14). The study population for the current study consisted of 706 individuals ranging in age from 30 to 70 years; all had received a diagnosis of diabetes within 5 years before entering this cross-sectional study from the Action LADA cohort (2). The cohort consisted of 50 subjects with LADA, 30 subjects with type 1 diabetes, and 626 subjects with type 2 diabetes. Patients with other forms of diabetes were excluded.

Serum samples were randomly selected with stratification for age and significant serum samples left for analysis. Patients with classic type 1 diabetes were DAA positive and received insulin treatment shortly after the diagnosis of diabetes. Patients were identified as having LADA when they were GADA positive, had an age range from 30 to 70 years, and did not use insulin treatment for at least 6 months after diagnosis. The focus of antihyperglycemic medication was on insulin, as the time to the start of insulin treatment contributed to the definition of LADA. Medications for the treatment of diabetes, other than insulin, were not evaluated. GADA-negative and other DAA-negative patients who did not use insulin for at least the first year after diagnosis were defined as having type 2 diabetes.

Blood withdrawal from all participants was performed in the fasting state. The local ethics committees of each study center approved the study protocol, in accordance with the Declaration of Helsinki. All patients gave written informed consent for the study.

### Antibody Measurements

The DAAs directed against islets (islet cell antibodies [ICAs]) (11), GADA, IA-2 antigen (IA-2A), and zinc transporter T8 (ZnT8A) were determined (2).

The non-DAAs directed against transglutaminase (transglutaminase antibodies [TGAs]), parietal cell antibodies (PCAs), and TPOAs were measured as described. We chose to measure TPOAs, PCAs, and TGAs because they are frequently known to be positive in patients with autoimmune diabetes and are well-established gold standards to detect autoimmunity against thyroid, parietal cells, and intestinal villous epithelial cells (15–19).

In brief, TPOAs were examined by TPO-Ab RIA (B.R.A.H.M.S. AG) using native thyroid peroxidase as an antigen, with results expressed in arbitrary units (AU) per milliliter per hour: the functional assay sensitivity was 14 AU/mL; the upper limit of detection was 15,000 AU/mL; cutoff for positivity was 60 AU/mL.

Gastric $H^+/K^+$-ATPase IgG autoantibodies (PCAs) were determined by ELISA (Euroimmun AG, Lübeck, Germany) according to the manufacturer instructions (cutoff 20 AU/mL).

Tissue TGAs were measured by IgA-ELISA (Euroimmun AG); the assay has an upper limit of detection of 200 AU/mL and a cutoff of 20 AU/mL. The intra-assay and interassay variations were 3.5% and 6.8%, respectively.

### Systemic Cytokines and Chemokines

Soluble immune mediators were measured as described (5,6). Serum samples were obtained in a standardized form from freshly drawn blood samples from fasting subjects in the morning hours, and were stored at −80°C until the time of the assay without prior thawing. In brief, serum concentrations for sICAM-1, sVCAM-1, sE-selectin, CCL2, CCL3, and CCL4 were determined with commercially available multiplex bead technology kits (Fluorokine MAP; R&D Systems, Wiesbaden, Germany). Intra-assay and interassay coefficients of variations were <5% and <11%, respectively.

The detection limits of the assays were 13.8 ng/mL for sICAM-1, 69.0 ng/mL for sVCAM-1, 17.1 ng/mL for sE-selectin, 40.3 pg/mL for CCL2, 2.4 pg/mL for CCL3, and 0.4 pg/mL for CCL4. At least 95% of serum concentrations were above the detection limit for all markers, except for CCL3 levels, which were detectable in 72% of all

samples. Determinations of immune mediator concentrations lower than the detection limit were assigned a value half of the detection limit, as described previously (5,20).

Serum cytokine concentrations of IL-1Ra, IL-6, TNF-α, and IL-10 were measured by multiplex bead technology using commercially available kits (Fluorokine MAP; R&D Systems). The detection limits of the assays were 9.56 pg/mL for IL-1RA, 0.1 pg/mL for IL-6, 0.08 pg/mL for TNF-α, and 0.25 pg/mL for IL-10. For cytokine concentrations lower than the detection limit, a value half of the detection limit was assigned (IL-6, $n$ = 46; IL-1Ra, $n$ = 0; TNF-α, $n$ = 0). The concentration of cytokine IL-10 was detectable in only 44% of the samples. Immunoassays showed interassay variations of <20% and intraassay variations of <10%.

### Statistical Methods

We performed the analyses in randomly selected serum samples from participants of Action LADA that were stratified by age. Analyses were performed using SAS Enterprise Guide version 4.2 (SAS Institute, Cary, NC) and GraphPad Prism version 4 for Windows (GraphPad Software, La Jolla, CA). Continuous variables are presented as the median and range, if not indicated otherwise. First, the Gaussian distribution of data was assessed using the Kolmogorov-Smirnov test. The Kruskal-Wallis and Mann-Whitney tests were used to compare continuous variables. The Fisher exact test or the $\chi^2$ test was performed to evaluate the differences in categorical data with two or more classes. Tests were not adjusted for multiple comparisons and are therefore descriptive if not indicated otherwise. Univariate correlations among organ-specific antibody titers, sex (men = 1, women = 2), age, BMI, and diabetes duration were described by Spearman correlation ($R$). The Kruskal-Wallis test was performed for the association analysis between systemic cytokines and multiple positivity for DAAs and non-DAAs. Associations of antibodies with cytokines upon adjustments for confounders, including sex, age, BMI, and diabetes duration, were performed with multivariate regression analysis.

## RESULTS

### Patient Characteristics

As expected, patients with AID, including classic adult-onset type 1 diabetes and LADA, and patients with type 2 diabetes differed by age ($P < 0.0001$), BMI ($P < 0.0001$), diabetes duration ($P < 0.0001$), and family history for diabetes ($P < 0.01$). Patients with classic type 1 diabetes were the youngest (median age 44.65 years), patients with type 2 diabetes had the highest BMI (30.13 kg/m$^2$), and patients with LADA had the longest diabetes duration (2.92 years) (Table 1).

### Diabetes-Associated Antibodies

AID patients ($n$ = 80) had by definition, one or more DAAs (GADA, IA-2A, ZnT8A, ICA), and within that group patients with LADA ($n$ = 50) were, by definition, at least positive for GADA and did not start insulin therapy for at least 6 months postdiagnosis. Of the patients with classic type 1 diabetes, who started receiving treatment with insulin close to the time of diagnosis ($n$ = 30), 93.3% were GADA positive and their type of DAA did not differ from that of patients with LADA (Table 1). Patients with type 2 diabetes were, by definition, negative for DAAs. Of all patients with autoimmune diabetes ($n$ = 80), 30 (37.5%) were also positive for ICAs, 16 had IA-2As (20%), and 10 had ZnT8As (12.5%) (Table 1). Of the patients with AID ($n$ = 80), only 5 had all four DAAs (6.25%); 8 (10%) had three DAAs, 23 (28.75%) had two DAAs, and 44 (55%) were positive for a single DAA (Supplementary Table 1). Classic type 1 diabetes did not significantly differ from LADA in this comparison. Similarly, DAA titers did not differ among patients with type 1 diabetes, including those with LADA (data not shown). In patients with AID ($n$ = 80), 54 had high GADA

**Table 1—Characteristics of the patients with LADA, classic type 1 diabetes, and type 2 diabetes**

| | LADA ($n$ = 50) | Classic type 1 diabetes ($n$ = 30) | Type 2 diabetes ($n$ = 626) | $P$ value* |
|---|---|---|---|---|
| Demographics | | | | |
| Sex ($n$ male/$n$ female) | 25/25 | 17/13 | 361/265 | NS |
| Age (years) | 52.28 (31.87–69.10) | 44.65 (33.56–66.13) | 56.08 (30.15–69.83) | <0.0001 |
| BMI (kg/m$^2$) | 25.96 (18.12–48.78) | 23.83 (16.44–53.15) | 30.13 (18.00–71.94) | <0.0001 |
| Diabetes duration (years) | 2.92 (0.05–6.63) | 0.59 (0.02–5.36) | 2.00 (0.0–5.79) | <0.0001 |
| Family history of diabetes, $n$ positive/negative (% positive) | 33/17 (66) | 9/21 (30) | 347/279 (55.4) | 0.0065 |
| Antibody status | | | | |
| DAAs | | | | |
| GADA | 50 (100) | 28 (93.33) | 0 | NS |
| IA-2A | 9 (18.0) | 7 (23.3) | 0 | NS |
| ZnT8A | 6 (12) | 4 (13.33) | 0 | NS |
| ICA | 18 (36.0) | 12 (40) | 0 | NS |
| Non-DAAs | | | | |
| TPOA | 15 (30) | 11 (36.67) | 85 (13.58) | <0.0001 (NS) |
| PCA | 6 (12) | 6 (20) | 75 (11.98) | NS<br>NS |
| TGA | 1 (2) | 1 (3.3) | 5 (0.8) | NS<br>NS |
| TPOA and PCA | 4 (8) | 3 (10) | 18 (2.9) | 0.0245 (NS) |

Data are shown as median (range) and $n$ (%), unless otherwise indicated. NS, not significant. *$P$ values refer to the comparison of the three groups by two-sided nonparametric Kruskal-Wallis test or Fisher exact test. DAA antibody positivity was compared between LADA and classic type 1 diabetes. Non-DAA antibody positivity was compared among LADA, classic type 1 diabetes, and type 2 diabetes (Kruskal-Wallis test, $P$ upper line), and between classic type 1 diabetes and LADA ($P$ lower line).

titers (i.e., ≥200 WHO [World Health Organization] units, as defined by an inflection point in signal from the cohort previously described) (2), and the remainder ($n$ = 26) were designated as having low GADA titers (i.e., <200 WHO units). Patients with high GADA titers compared with those with low GADA titers did not differ in terms of the number of DAAs or non-DAAs (data not shown), nor were the groups with high versus low GADA titers clinically different, including age, BMI, and diabetes duration (data not shown).

### Non-DAAs

Non-DAAs were detected more often in patients with AID, whether LADA (19 of 50, 38%) or classic type 1 diabetes (15 of 30, 50%), when compared with patients with type 2 diabetes (145 of 626, 23.2%; $P$ = 0.0004). TPOAs were more frequent in patients with AID (26 of 80, 32.5%) than in those with type 2 diabetes (85 of 626, 13.58%) ($P < 0.0001$); whereas their frequency in LADA (15 of 50, 30%) did not differ from that in classic type 1 diabetes (11 of 30, 36.67%; $P$ = 0.538) (Table 1). Positivity for both TPOAs and PCAs was marginally greater in patients with AID, both classic type 1 diabetes (3 of 30, 10%) and LADA (4 of 50; 8%), compared with patients with type 2 diabetes (18 of 626, 2.9%; $P$ = 0.024). The clinical groups did not differ in terms of TGA positivity or PCA positivity (Table 1). PCA outliers (defined as PCA concentration of >80 AU/mL) are associated with positive results for TPOAs in four of five patients with LADA, but not in patients with type 2 diabetes or type 1 diabetes. TGA outliers (defined as TGA concentration of >30 AU/mL) did not correspond with other non-DAAs (PCAs and TPOAs). TPOA outliers (defined as TPOA concentration of >300 AU/mL) associated with positive results for PCAs in 1 of 5 cases of classic type 1 diabetes and in 4 of 18 cases of type 2 diabetes. Overall, outliers of one non-DAA were not significantly associated with other non-DAAs except for PCAs and TPOAs in LADA cases.

#### *Non-DAA Titers*

Among patients with AID, high and low titers for GADAs were not associated with their classification as either classic type 1 diabetes or LADA, or with the number or titer of non-DAAs (data not shown); and titers of TPOA, TGA, or PCA did not differ between patients with LADA and those with classic type 1 diabetes (Fig. 1). Compared with patients who had type 2 diabetes, patients who had AID had lower mean TGA titers (Kruskal-Wallis test $P$ = 0.003; post hoc Dunn comparison $P < 0.05$), whereas the mean TPOA titers in patients with classic type 1 diabetes were higher (Kruskal-Wallis test $P$ = 0.008; post hoc Dunn comparison $P < 0.05$) (Fig. 1).

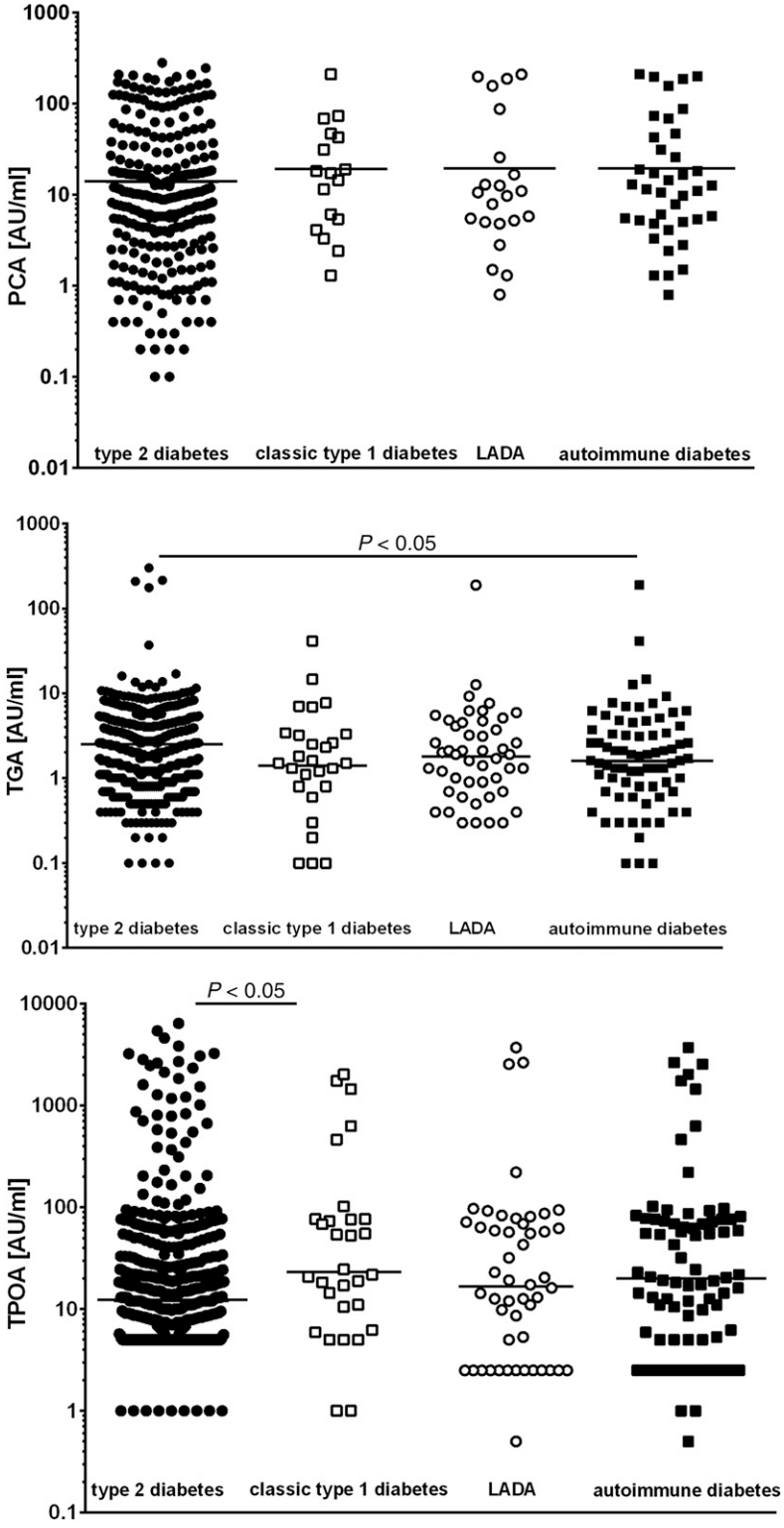


**Figure 1**—Levels of non-DAAs PCA, TGA, and TPOA, in type 2 diabetes, classic type 1 diabetes, LADA, and AID (classic type 1 diabetes and LADA combined). The cutoff for positivity was 20 AU/mL for PCA, 20 AU/mL for TGA, and 60 AU/mL for TPOA. Shown are all individual data and means. $P$ values were determined using the Dunn multiple comparison test.

### Association of DAAs and Non-DAAs With Clinical Demographics

There was no significant relationship between DAA concentrations and sex, age, BMI, or diabetes duration, with the exception that ZnT8A titers correlated positively with BMI ($P = 0.001$, $r = 0.548$) in patients with classic type 1 diabetes. Statistically significant associations were detected for non-DAAs but did not impact our conclusions (Supplementary Table 2). More specifically, sex was significantly related to PCA in patients with type 1 diabetes in that PCA titers were increased in males, and age was positively associated with PCA in patients with LADA ($r = 0.29$, $P = 0.038$) and type 2 diabetes ($r = 0.15$, $P = 0.003$). Age was negatively related with TPOAs ($r = -0.17$, $P = 0.001$). BMI was positively associated with PCA ($r = 0.374$, $P = 0.03$).

### Associations of DAAs and Non-DAAs With Systemic Immune Mediators

First, we investigated associations of antibodies with immune mediators without adjustment for demographic effects. In patients with AID ($n = 80$), we detected significant associations of systemic immune mediators and antibody titers in the following 14 combinations: 10 with DAAs (9 negative, 1 positive) and 4 with non-DAAs (TGAs, TPOAs) (2 negative, 2 positive) (Table 2). Interestingly, no significant association of GADAs with immune mediators was observed in this analysis.

As we detected some association of sex, age, and BMI with non-DAA concentrations (Supplementary Table 2), we adjusted for these parameters in further analysis. After adjustment for sex, age, BMI, and diabetes duration, non-DAAs associated significantly only for PCA and IL-10 ($P = 0.001$, negative correlation). Two DAAs were negatively correlated with immune mediators upon adjustments: IA-2A and TNF-α ($P = 0.0278$); ICA and sVCAM-1 ($P = 0.0065$); and sICAM-1 ($P = 0.011$) and TNF-α ($P = 0.011$). The ZnT8A titer was associated with IL-6 ($P = 0.004$, positive correlation). That means that, of the 14 combinations found in the unadjusted analysis, 5 remained significant after adjustment, 1 association was newly significant (Table 2), and most of them were negatively correlated.

In patients with type 2 diabetes ($n = 626$), without adjustment for demographic effects, we detected six associations of systemic immune mediators and non-DAA titers, all of them negative: PCA with IL-1Ra ($r = -0.148$, $P = 0.025$); IL-6 ($r = -0.146$, $P = 0.027$), IL-10 ($r = -0.133$, $P = 0.044$), and CCL2 ($r = -0.133$, $P = 0.043$); and TPOA with TNF-α ($r = -0.157$, $P = 0.018$) and sVCAM-1 ($r = -0.198$, 0.0026). TGAs were excluded from these analyses because so few patients were positive. After adjustment for sex, age, BMI, and diabetes duration, non-DAAs were not associated with any of the immune mediators in patients with type 2 diabetes ($n = 626$).

**Table 2—Correlation analysis of immune mediators and antibody titers in patients with AID (classic type 1 diabetes and LADA combined)**

| | AID ($n = 80$) | | | | | |
|---|---|---|---|---|---|---|
| | Non-DAAs* | | DAAs | | | |
| | PCA | TPOA | ICA | IA2 | ZnT8A | GADA |
| IL-1Ra | | | | | | |
| *r* | −0.01 | 0.09 | −0.092 | −0.20 | 0.12 | 0.13 |
| *P* | 0.93 | 0.36 | 0.34 | 0.03 | 0.34 | 0.30 |
| IL-6 | | | | | | |
| *r* | 0.06 | −0.01 | 0.03 | **−0.26** | **0.27*** | −0.05 |
| *P* | 0.52 | 0.90 | 0.77 | **0.006** | **0.0314** | 0.67 |
| IL-10 | | | | | | |
| *r* | −0.05* | −0.02 | 0.14 | 0.08 | −0.19 | −0.20 |
| *P* | 0.64 | 0.85 | 0.14 | 0.40 | 0.14 | 0.10 |
| CCL2 | | | | | | |
| *r* | −0.04 | 0.06 | 0.14 | −0.08 | −0.14 | 0.07 |
| *P* | 0.69 | 0.53 | 0.14 | 0.43 | 0.27 | 0.56 |
| CCL3 | | | | | | |
| *r* | 0.01 | **0.43** | 0.13 | −0.09 | 0.08 | 0.02 |
| *P* | 0.90 | **<0.0001** | 0.16 | 0.38 | 0.54 | 0.90 |
| CCL4 | | | | | | |
| *r* | 0.037 | 0.12 | −0.03 | 0.04 | 0.17 | −0.02 |
| *P* | 0.71 | 0.20 | 0.72 | 0.65 | 0.17 | 0.89 |
| TNF-α | | | | | | |
| *r* | −0.13 | **−0.20** | **−0.38*** | **−0.31*** | 0.03 | −0.02 |
| *P* | 0.17 | **0.04** | **<0.0001** | **0.0009** | 0.81 | 0.9 |
| sICAM-1 | | | | | | |
| *r* | 0.10 | −0.09 | **−0.34*** | **−0.26** | 0.08 | 0.13 |
| *P* | 0.28 | 0.33 | **0.0004** | **0.007** | 0.54 | 0.32 |
| sVCAM-1 | | | | | | |
| *r* | 0.05 | **−0.24** | **−0.34*** | −0.09 | 0.22 | 0.20 |
| *P* | 0.61 | **0.01** | **0.0004** | 0.35 | 0.08 | 0.10 |
| sE-selectin | | | | | | |
| *r* | **0.24** | 0.002 | **−0.20** | **−0.28** | −0.11 | 0.07 |
| *P* | **0.01** | 0.98 | **0.04** | **0.003** | 0.38 | 0.60 |

Shown are *r* and *P* values from Spearman analysis. Significant correlations are in bold. TGAs were not analyzed because only 2 of 80 patients with AID were positive for TGA. *Significant association after adjustment for sex, age, BMI, and diabetes duration.

### Association of High GADA Titers With Cytokines

We compared patients with AID who had high GADA titers (>200 units/mL) and low GADA titers (<200 units/mL), and patients with type 2 diabetes (all GADA negative). Patients with high GADA titers had lower levels of IL-6 ($P < 0.001$) and sE-selectin ($P < 0.01$), and a trend toward decreased levels of IL-1Ra ($P = 0.052$), with a significant trend across the three diabetes cohorts for the former two ($P < 0.01$), indicating an inverse association between an adaptive autoimmune response (GADA) and these systemic immune mediators (Fig. 2). There were no significant differences for the other cytokines tested, including IL-10, TNF-α, CCL2, CCL3, CCL4, sICAM-1, and sVCAM-1.

## CONCLUSIONS

We explored the relationship within the two major types of diabetes between adaptive immunity, represented by both DAAs and non-DAAs, and innate immunity, represented by 10 systemic immune mediators, including cytokines, chemokines, and adhesion molecules in the Action LADA cohort. It is well established that type 1 diabetes is associated with DAAs

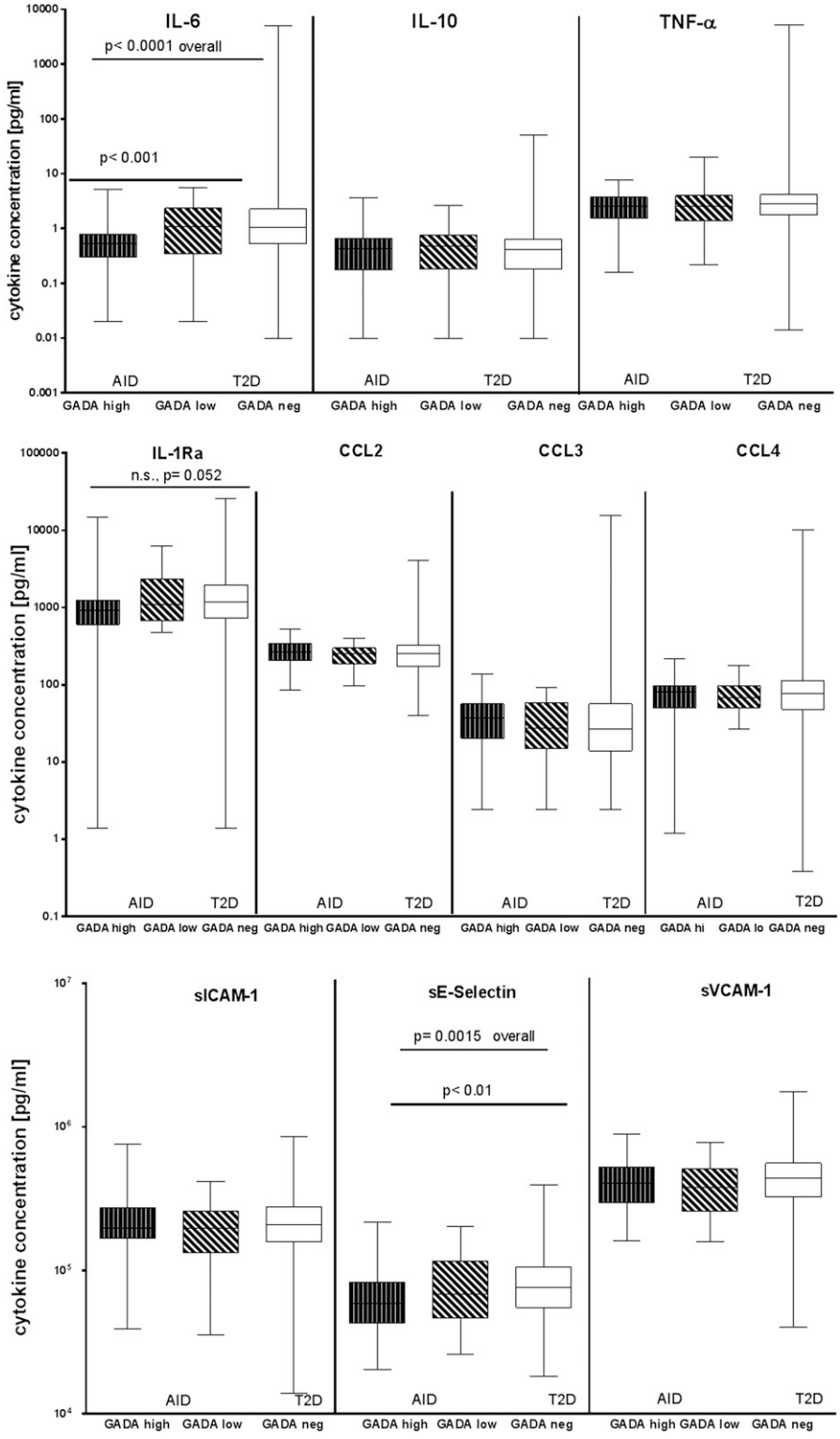


**Figure 2**—Concentrations (box and whiskers) of cytokines, chemokines, and soluble adhesion molecules in patients with AID who had high GADA concentrations (>200 units/mL) or low GADA concentrations (<200 units/mL), and patients with type 2 diabetes who were GADA negative (neg). *P* values were determined for the upper line using the nonparametric Kruskal-Wallis test, and for the lower line using the Dunn multiple-comparison test. T2D, type 2 diabetes.

irrespective of the clinical phenotype or age of the patient at diagnosis. In our study, levels of DAAs, non-DAAs, and immune mediators could not distinguish classic type 1 diabetes from LADA.

As expected, we detected DAAs in a large series of >6,000 adult patients in whom diabetes had been diagnosed (2). We selected patients from that series with DAAs based on their immediate need for insulin treatment (classic type 1 diabetes) and the lack of that need for at least 6 months (LADA), plus the availability of sufficient sera from the same sample. The majority of these

adult-onset cases had a single DAA, and the dominant autoantibody was GADA. There was no difference in the frequency or titer of these DAAs and the need for insulin therapy or initial clinical phenotype. Type 1 diabetes is associated with other autoimmune diseases, including pernicious anemia and Hashimoto thyroiditis, respectively characterized by the presence of PCAs and TPOAs (21). The increased frequency of TPOAs and PCAs has been well described in patients with type 1 diabetes but also in those with LADA (e.g., the Italian NIRAD study and the Chinese LADA study) (9,22–24). As expected, the frequency of non-DAAs here was increased in patients with type 1 diabetes compared with patients with type 2 diabetes, irrespective of the initial need for insulin treatment that occurs both in patients with classic type 1 diabetes and in those with LADA; however, that increase was due mainly to TPOAs, and to a lesser extent PCAs, but not to TGAs. Although some non-DAAs are a feature of type 1 diabetes, these autoantibodies, notably TPOAs, were also found in a proportion of patients with type 2 diabetes. The percentage of non–DAA-positive patients with LADA in our Action LADA cohort was similar to that in other cohorts (9,24).

Importantly, and unique to this study, we found in patients with AID that significantly altered immune mediator levels were nearly all (9 of 10) negatively correlated with DAA titers and 2 of 4 were negatively associated with non-DAA titers. Serum concentrations of some immune mediators (i.e., a cytokine and an adhesion molecule) were overall slightly increased in type 2 diabetes compared with patients with AID who had high GADA titers. There was a graded increase in IL-6 and sE-selectin across the main types of diabetes, with levels being highest in patients with type 2 diabetes and lowest in those patients with classic type 1 diabetes, whereas LADA patients had intermediate levels without significant differences from patients with classic type 1 diabetes. As in most studies, there was a considerable overlap of cytokine concentrations in patients with classic type 1 diabetes, LADA, and type 2 diabetes, and innate immune markers measured in serum are not sufficiently diagnostic to dissect out the different forms of diabetes. This is not surprising because innate as well as adaptive immune alterations may have a role in all diabetes types including type 1 diabetes, LADA, and type 2 diabetes (5–7,13,25). The level of the GADA titer was not associated with classic type 1 diabetes or LADA, and similarly, levels of the immune effector molecules were not different between these two clinically distinct forms of autoimmune diabetes and were lower than in type 2 diabetes. It follows that there is an inverse relationship between adaptive immunity (antibodies, DAAs, non-DAAs) associated with type 1 diabetes and innate immune effector molecules (cytokines, chemokines, adhesion molecules) associated with type 2 diabetes, with a graded change across the clinical categories. Whether the observed inverse innate and adaptive immune responses are causal or merely associated cannot be addressed because our study was exploratory. However, one potential explanation for the inverse relationship between adaptive and innate immune responses is that immune regulation of one component limits the potentially exaggerated, and therefore harmful, response of the other component. Alternatively, since altered innate immune responses are common to both major types of diabetes but adaptive immune responses are a characteristic only of AID, it is the possible that their effect is additive and, therefore, the greater the adaptive immune effect, the less the innate immune effect is required for the development of clinical disease.

Numerous studies have demonstrated clinical heterogeneity in autoimmune diabetes, especially adult-onset diabetes such that some patients present with severe insulin-dependent diabetes but others present with non–insulin-requiring diabetes (1). Yet both of these forms of diabetes are characterized by the presence of DAAs, usually GADAs, associated with genetic susceptibility through common HLA haplotypes. This clinical spectrum extends into childhood-onset autoimmune type 1 diabetes in which insulin-dependent patients have the same DAAs, though not predominantly GADAs, with a stronger HLA genetic susceptibility. That clinical heterogeneity is also reflected in the striking variation in insulin secretion, as illustrated by C-peptide levels, across the types of diabetes. Recent large studies (26,27) have confirmed that there can be substantial serum C-peptide levels in patients with adult-onset type 1 diabetes, suggesting that it is difficult to distinguish some forms of adult-onset type 1 diabetes from type 2 diabetes based on C-peptide levels alone. This present study confirms the results of previous studies indicating that LADA and classic type 1 diabetes have, despite clinical differences, a similar cytokine profile, with similar levels of serum IL-6 and sE-selectin, with each being lower than their corresponding levels in type 2 diabetes. We have now, for the first time, analyzed that relationship in more detail and found that there was an inverse relationship between DAA titers and the levels of four immune mediator molecules, such that high titers of one (e.g., ICA) were associated with lower levels of the other (e.g., sVCAM-1, sICAM-1, and TNF-$\alpha$). It is unclear what pathogenetic mechanism accounts for the relationship between these effector molecules and the two major types of diabetes, but that inverse relationship is detected even within autoimmune diabetes; the group with the highest levels of GADA showed decreased levels of IL-6 and sE-selectin. It will require further study to define the reason for this inverse relationship between adaptive and innate immune changes across a range of forms of adult-onset diabetes.

In summary, we present the first evidence for an effect in both adaptive and innate immunity across type 1 diabetes and type 2 diabetes. Since this graded effect was seen in patients with type 1 diabetes and type 2 diabetes, and even those with autoimmune diabetes, the change in immune effectors likely reflects the presence of some pathogenetic mechanism that is common between the major types of the disease.

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**Duality of Interest.** N.C.S. is employed at Lilly Germany, Bad Homburg. M.N.P. is employed at the Novo Nordisk Research Center, Seattle, WA. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** N.C.S., M.N.P., M.I.H., and R.D.L. measured, researched, analyzed the data, and wrote the manuscript. P.P., W.A.S., H.K., S.H., G.S., and C.T. collected and/or researched patient data and revised the manuscript. M.S. and J.S. measured non-DAA. N.C.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility

for the integrity of the data and the accuracy of the data analysis.

## Appendix

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## References


1. Leslie RD, Palmer J, Schloot NC, Lernmark A. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. Diabetologia 2016;59:13–20
2. Hawa MI, Kolb H, Schloot N, et al.; Action LADA consortium. Adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: Action LADA 7. Diabetes Care 2013;36:908–913
3. Buzzetti R, Di Pietro S, Giaccari A, et al.; Non Insulin Requiring Autoimmune Diabetes Study Group. High titer of autoantibodies to GAD identifies a specific phenotype of adult-onset autoimmune diabetes. Diabetes Care 2007;30:932–938
4. Zhou Z, Xiang Y, Ji L, et al.; LADA China Study Group. Frequency, immunogenetics, and clinical characteristics of latent autoimmune diabetes in China (LADA China study): a nationwide, multicenter, clinic-based cross-sectional study. Diabetes 2013;62:543–550
5. Pham MN, Hawa MI, Pfleger C, et al.; Action LADA Study Group. Pro- and anti-inflammatory cytokines in latent autoimmune diabetes in adults, type 1 and type 2 diabetes patients: Action LADA 4. Diabetologia 2011;54:1630–1638
6. Pham MN, Hawa MI, Roden M, et al.; Action LADA Study Group. Increased serum concentrations of adhesion molecules but not of chemokines in patients with type 2 diabetes compared with patients with type 1 diabetes and latent autoimmune diabetes in adult age: Action LADA 5. Diabet Med 2012;29:470–478
7. Pham MN, Kolb H, Mandrup-Poulsen T, et al.; European C-Peptide Trial. Serum adipokines as biomarkers of beta-cell function in patients with type 1 diabetes: positive association with leptin and resistin and negative association with leptin and resistin and negative association with adiponectin. Diabetes Metab Res Rev 2013;29:166–170
8. Deng C, Xiang Y, Tan T, et al. Altered peripheral B-lymphoctye subsets in type 1 diabetes and latent autoimmune diabetes in adults. Diabetes Care 2016;39:434–440
9. Zampetti S, Capizzi M, Spoletini M, et al.; NIRAD Study Group. GADA titer-related risk for organ-specific autoimmunity in LADA subjects subdivided according to gender (NIRAD study 6). J Clin Endocrinol Metab 2012;97:3759–3765
10. Hawa MI, Thivolet C, Mauricio D, et al.; Action LADA Group. Metabolic syndrome and autoimmune diabetes: action LADA 3. Diabetes Care 2009;32:160–164
11. Hanifi-Moghaddam P, Schloot NC, Kappler S, Seissler J, Kolb H. An association of autoantibody status and serum cytokine levels in type 1 diabetes. Diabetes 2003;52:1137–1142
12. Kaas A, Pfleger C, Kharagjitsingh AV, et al.; Hvidoere Study Group on Childhood Diabetes. Association between age, IL-10, IFN-γ, stimulated C-peptide and disease progression in children with newly diagnosed Type 1 diabetes. Diabet Med 2012;29:734–741
13. Strom A, Menart B, Simon MC, et al. Cellular interferon-γ and interleukin-13 immune reactivity in type 1, type 2 and latent autoimmune diabetes: action LADA 6. Cytokine 2012;58:148–151
14. Leslie RD, Kolb H, Schloot NC, et al. Diabetes classification: grey zones, sound and smoke: Action LADA 1. Diabetes Metab Res Rev 2008;24:511–519
15. Amin M, Eckhardt T, Kapitza S, et al. Correlation between tissue transglutaminase antibodies and endomysium antibodies as diagnostic markers of coeliac disease. Clin Chim Acta 1999;282:219–225
16. Schott M, Eckstein A, Willenberg HS, Nguyen TB, Morgenthaler NG, Scherbaum WA. Improved prediction of relapse of Graves' thyrotoxicosis by combined determination of TSH receptor and thyroperoxidase antibodies. Horm Metab Res 2007;39:56–61
17. Domberg J, Liu C, Papewalis C, et al. Circulating chemokines in patients with autoimmune thyroid diseases. Horm Metab Res 2008;40:416–421
18. Toh BH, Kyaw T, Taylor R, Pollock W, Schlumberger W. Parietal cell antibody identified by ELISA is superior to immunofluorescence, rises with age and is associated with intrinsic factor antibody. Autoimmunity 2012;45:527–532
19. Wolf J, Hasenclever D, Petroff D, et al. Antibodies in the diagnosis of coeliac disease: a biopsy-controlled, international, multicentre study of 376 children with coeliac disease and 695 controls. PLoS One 2014;9:e97853
20. Pfleger C, Mortensen HB, Hansen L, et al.; Hvidøre Study Group on Childhood Diabetes. Association of IL-1ra and adiponectin with C-peptide and remission in patients with type 1 diabetes. Diabetes 2008;57:929–937
21. Wang B, Hawa MI, Rijsdijk FV, et al. Heritability of thyroid peroxidase autoantibody levels in type 1 diabetes: evidence from discordant twin pairs. Diabetologia 2015;58:2079–2086
22. Delitala AP, Pes GM, Fanciulli G, et al. Organ-specific antibodies in LADA patients for the prediction of insulin dependence. Endocr Res 2016;41:207–212
23. Jin P, Zhou ZG, Yang L, et al. Adult-onset latent autoimmune diabetes and autoimmune thyroid disease. Zhonghua Nei Ke Za Zhi 2004;43:363–367
24. Jin P, Huang G, Lin J, et al. High titre of antiglutamic acid decarboxylase autoantibody is a strong predictor of the development of thyroid autoimmunity in patients with type 1 diabetes and latent autoimmune diabetes in adults. Clin Endocrinol (Oxf) 2011;74:587–592
25. Brooks-Worrell BM, Boyko EJ, Palmer JP. Impact of islet autoimmunity on the progressive β-cell functional decline in type 2 diabetes. Diabetes Care 2014;37:3286–3293
26. Barker A, Lauria A, Schloot N, et al. Age-dependent decline of β-cell function in type 1 diabetes after diagnosis: a multi-centre longitudinal study. Diabetes Obes Metab 2014;16:262–267
27. Davis AK, DuBose SN, Haller MJ, et al.; T1D Exchange Clinic Network. Prevalence of detectable C-Peptide according to age at diagnosis and duration of type 1 diabetes. Diabetes Care 2015;38:476–481