Association of IL-1ra and Adiponectin With C-Peptide and Remission in Patients With Type 1 Diabetes

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OBJECTIVE—We investigated the association of anti-inflammatory cytokine interleukin (IL)-1 receptor antagonist (IL-1ra), adiponectin, proinflammatory cytokines IL-1β, IL-6, and CCL2, and tumor necrosis factor-α with β-cell function, metabolic status, and clinical remission in patients with recent-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS—Serum was obtained from 256 newly diagnosed patients (122 males and 134 females, median age 9.6 years). Stimulated C-peptide, blood glucose, and A1C were determined in addition to circulating concentration of cytokines at 1, 6, and 12 months after diagnosis. Analyses were adjusted for sex, age, and BMI percentile.

RESULTS—Anti-inflammatory IL-1ra was positively associated with C-peptide after 6 ($P = 0.0009$) and 12 ($P = 0.009$) months. The beneficial association of IL-1ra on β-cell function was complemented by the negative association of IL-1β with C-peptide after 1 month ($P = 0.009$). In contrast, anti-inflammatory adiponectin was elevated in patients with poor metabolic control after 6 and 12 months ($P < 0.05$) and positively correlated with A1C after 1 month ($P = 0.0004$). Proinflammatory IL-6 was elevated in patients with good metabolic control after 1 month ($P = 0.009$) and showed a positive association with blood glucose disposal after 12 months ($P = 0.047$).

CONCLUSIONS—IL-1ra is associated with preserved β-cell capacity in type 1 diabetes. This novel finding indicates that administration of IL-1ra, successfully improving β-cell function in type 2 diabetes, may also be a new therapeutic approach in type 1 diabetes. The relation of adiponectin and IL-6 with remission and metabolic status transfers observations from in vitro and animal models into the human situation in vivo. ***Diabetes*** **57:929–937, 2008**

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IL, interleukin; TNF, tumor necrosis factor.

Type 1 diabetes is an immune-mediated disease leading to selective destruction of insulin-producing β-cells in which cytokines play an important role (1). Cytokines related to the innate immune response, such as interleukin (IL)-1β (2–6), IL-1 receptor antagonist (IL-1ra) (7), monocyte chemoattractant protein (MCP)-1/CCL2 (8–10), tumor necrosis factor (TNF)-α (2,11), IL-6 (3,12), and adiponectin (13,14), are thought to be associated with β-cell destruction and disease status in humans and in animal models. So far, no association of these markers with endogenous C-peptide secretion and metabolic status has been demonstrated in patients with type 1 diabetes. A recent small study has described slightly decreased circulating concentrations of IL-1ra 3 months after type 1 diabetes onset in patients not undergoing remission; however, no data on C-peptide were available in these subjects (7).

Type 1 and type 2 diabetes are characterized by progressive β-cell failure, although the time courses and mechanisms by which cytokines and nutrients trigger β-cell death seem to differ (2). Nevertheless, the immune response in type 2 diabetes is thought to play a pathogenic role for disease development (15), perhaps similarly but not identically as for type 1 diabetes. Larsen et al. (16) showed that administration of exogenous anti-inflammatory IL-1ra in type 2 diabetic patients could preserve endogenous insulin production and attenuated inflammation. IL-1ra is the natural antagonist of IL-1β that induces programmed cell death (apoptosis) in β-cells (17).

Proinflammatory cytokines like CCL2 and TNF-α are known to impair insulin signaling (18–20), and therefore it is not surprising that there is an association between cytokines and insulin resistance (21–23). Interestingly, adipose tissue plays an important role for cytokine secretion and may actually be a major source of proinflammatory cytokines (12,23–25), but it is also a source of IL-1ra and adiponectin, which display anti-inflammatory and insulin-sensitizing effects (17,26,27). Some cytokines, like the proinflammatory IL-6 and insulin-sensitizing plus anti-inflammatory adiponectin, not affect only insulin signaling but also reveal an insulin-independent role in glucose disposal (27,28).

In the current study we investigated in the longitudinally prospectively performed Hvidøre Study patients with recent-onset type 1 diabetes. We determined how pro- and anti-inflammatory cytokines IL-1β, IL-1ra, adiponectin, IL-6, CCL2, and TNF-α that are associated with β-cell survival or insulin action are related to endogenous β-cell function, metabolic control, glucose disposal, and clinical status.

RESEARCH DESIGN AND METHODS

Patients were recruited consecutively in 18 centers throughout Europe (n = 252) and Japan (n = 4) from the Hvidøre Study. The design and characteristics of the Hvidøre Study have been explained elsewhere (29–31). In brief, prospective clinical and biochemical data were available from diagnosis up to 1 year for 256 children and adolescents (134 girls and 122 boys, median age 9.6 years, range 3 months to 16.8 years) of 275 initially investigated patients at baseline (response rate 93.1%). Only these 256 patients entered subsequent analyses. Exclusion criteria were non–type 1 diabetes (maturity-onset diabetes of the young, secondary diabetes, and other) or initial treatment outside the centers for more than 5 days. Patients were diagnosed with type 1 diabetes according to the World Health Organization criteria (32). The study was performed according to the criteria of the Helsinki II Declaration and was approved by the local ethics committee in each center. All patients (where applicable), their parents, or guardians gave informed consent.

Metabolic parameters. When diabetes was diagnosed, blood pH was determined by routine laboratory methods and was used to assess and adjust for the severity of the metabolic disorder (33). BMI percentiles were used to assess the influence of adipose tissue, since this is more accurate in children and adolescents than the use of BMI alone. Stimulated serum C-peptide as a marker of β-cell function (34) was measured in a central facility at 1, 6, and 12 months after diagnosis. Blood samples were obtained 90 min after the ingestion of a standardized liquid meal (Boost drink, formerly known as Sustacal, 237 ml or 8 fl oz containing 33 g carbohydrate, 15 g protein, and 6 g fat, 240 kcal: 6 ml/kg, maximum 360 ml; Novartis Medical Health, Minneapolis, MN). Serum samples were labeled and frozen at −20°C until shipment on dry ice to Steno Diabetes Center for central determination of C-peptide.

Serum C-peptide was analyzed by a fluoroimmunometric assay (AutoDELFIA C-peptide; PerkinElmer Life and Analytical Sciences, Turku, Finland). The sensitivity was <1 pmol/l, the intra-assay coefficient of variation was <6%, at 20 pmol/l, and recovery of the standard, added to plasma before extraction, was ~100% when corrected for losses inherent in the plasma extraction procedure.

Glycemic control as assessed by A1C was measured at diagnosis and 1, 3, 6, 9, and 12 months after diagnosis. A1C was determined centrally by ion-exchange high-performance liquid chromatography (normal reference range 4.1–6.4%) at Steno Diabetes Center, Gentofte, Denmark (31,35–37).

We used different definitions to classify patients by their clinical outcome, i.e., partial remission and improved C-peptide secretion. To define remission, values of A1C and insulin requirement 6 months after diagnosis were used. First, a more classical definition of partial remission was applied, A1C <7.5% and daily insulin <0.4 units/kg (remission 7.5) (7). However, partial remission discriminated by A1C <7.5% is not always indicative for a euglycemic status. Therefore, we used in addition a stricter definition of partial remission that was A1C <6.5% and daily insulin <0.4 units/kg (remission 6.5). For determination of complete remission, patients would ideally not require any insulin; however, it is recommended to support patients with low doses of insulin even in case of "complete" transient remission, and therefore such patients were not available. Second, patients were classified according to whether C-peptide improved from 1 to 6 months after diagnosis with a lower limit of 100 pmol/l. To account for interassay variation of C-peptide determination, an increase of at least 20% was defined as improved C-peptide secretion. The difference of blood glucose was determined before and 90 min after ingestion of the standardized liquid meal and was taken as a measure of blood glucose disposal.

Cytokines and chemokines. Blood was drawn 90 min after ingestion of the standardized liquid meal. Serum samples were labeled and frozen at −20°C until shipment on dry ice to the German Diabetes Centre for determination of cytokines. Serum samples were measured at time points 1, 6, and 12 months after diabetes diagnosis. Concentrations of IL-6 and total adiponectin were measured by enzyme-linked immunosorbent assay. IL-6 was determined using matched antibody pairs (PeliKine ELISA kit; Sanquin, Amsterdam, The Netherlands) as described (22) and total adiponectin by commercially available kits (Quantikine; R&D Systems, Wiesbaden, Germany) (38). IL-1β, IL-1ra, CCL2, and TNF-α were determined by multiplex-bead technology using commercially available kits (Fluorokine MAP; R&D Systems). All cytokines were measured in a blinded fashion, e.g., clinical data were not known when measurements were performed. The detection limits of the assays were 6.7 pg/ml for adiponectin, 13.6 pg/ml for IL-1ra, 0.4 pg/ml for IL-1β, 0.15 pg/ml for IL-6, 1.8 pg/ml for CCL2, and 1.35 pg/ml for TNF-α. Determinations of cytokine concentrations lower than the detection limit were assigned a value half of the detection limit (IL-1ra, n = 0; adiponectin, n = 0; IL-6, n = 0; CCL2, n = 0; and TNF-α, n = 31). Because IL-1β was only detectable in 12% (n = 86) of all samples, this cytokine was treated detectable or not detectable in all analyses. The immunoassays showed inter-assay variations <20% and intra-assay variations <10%.

Statistical methods. Cytokine concentrations showed no Gaussian distribution, and data are described by medians. Differences between cytokine concentrations during follow-up were analyzed first by the Friedmann test followed by Wilcoxon's test in case of significance to investigate differences between two time points. Distribution and differences between follow-up of IL-1β were not investigated because too many values were below the detection limit. Log-transformed cytokines were approximately normally distributed and entered into Spearman correlation analysis to investigate correlations between cytokines and into linear regression analysis to investigate associations between cytokines and metabolic parameters. Regression analysis included cytokines as the dependent variable and sex, age, BMI percentile, blood pH, C-peptide, and A1C as independent variables. Analyses investigating the influence of cytokines on Δblood glucose included cytokines as the dependent variable and sex, age, BMI percentile, and Δblood glucose as independent variables. For the analysis of IL-1β and metabolic parameters, logistic regression was performed using the same independent variables as in the linear regression analysis. Associations are descriptive and were not corrected for multiple testing. Adjustment for BMI percentiles are based on the 2000 Centers for Disease Control growth charts (www.cdc.gov/growthcharts). Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) and GraphPad Prism version 4 for Windows (GraphPad Software, San Diego, California).

RESULTS

Longitudinal analysis of circulating cytokine concentrations. First we investigated circulating cytokines of patients with newly diagnosed type 1 diabetes during the 1st year after diagnosis. Circulating concentrations of adiponectin ($P < 0.0001$), IL-6 ($P = 0.0008$), and CCL2 ($P < 0.0001$) were significantly higher 1 month compared with 6 and 12 months after diagnosis despite a large overlap between time points (Fig. 1). TNF-α and IL-1ra did not statistically differ during follow-up ($P = 0.16$ and $P = 0.77$, respectively), demonstrating that there is no general upregulation of all cytokines measured 1 month after diagnosis. Because too many values were below the detection limit, differences of IL-1β were not investigated.

Associations between serum cytokines. It is well known that cytokines and chemokines are part of a complex network. Spearman's correlation analysis including all cytokines was performed to investigate associations between cytokines (Table 1). Interestingly, similar association patterns 1 and 6 months after diagnosis were seen, whereas 12 months after diagnosis cytokines seemed related less often. To our surprise, all statistically significant correlations between cytokines found were positive. Adiponectin was the only cytokine that exhibited no association at any time with the cytokines investigated. One month after diagnosis anti-inflammatory IL-1ra revealed associations to proinflammatory TNF-α, IL-6, CCL2, and IL-1β (Table 1). Within the proinflammatory cytokines CCL2 was positively associated with TNF-α, IL-6 with IL-1β, and IL-6 with IL-1β.

Six months after diagnosis, analyses revealed an association pattern similar to that 1 month after diagnosis (online appendix 1 [available at http://dx.doi.org/10.2337/db07-1697]). Twelve months after diagnosis fewer associations were found (online appendix 2). Interestingly, the associations of anti-inflammatory IL-1ra with IL-6, CCL2, and IL-1β were maintained.

Basic characteristics of patients classified by their clinical outcome. We next classified patients by their clinical outcome, such as remission and improved C-peptide secretion (Table 2). Patients with incomplete data record with respect to classification were excluded from the respective analyses: 22 patients missed data for the classification of remissions, and 45 patients had incomplete data on C-peptide values. At baseline, both defini-

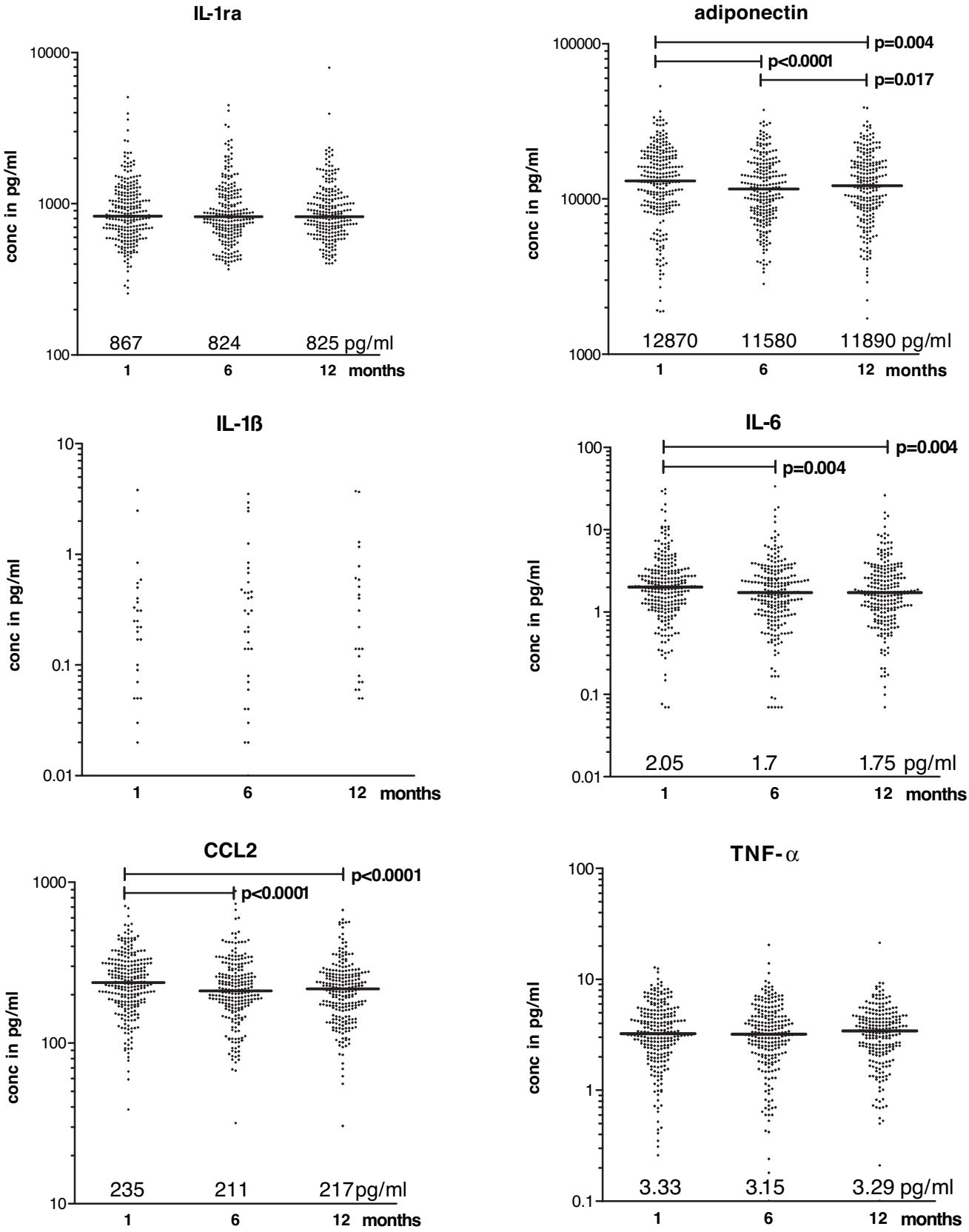

FIG. 1. Circulating cytokine concentrations of patients with type 1 diabetes 1, 6, and 12 months after diagnosis. *P* values for nonparametric testing for paired data (Friedman test) were as follows: adiponectin and MCP-1, $P < 0.0001$; IL-6, $P = 0.0008$; TNF-α, $P = 0.16$; and IL-1ra, $P = 0.77$. In case of significance, *P* values were calculated from comparison of two time points that are indicated in the graph. Bars represent medians. Exact values for medians are depicted above the *x*-axis for each month and time point (pg/ml). For IL-1β neither medians nor differences were investigated because of too many values below the detection limit.

TABLE 1
Correlation between cytokines during the 1st month after diagnosis

	IL-1ß		IL-6		CCL-2		TNF-α		Adiponectin	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-1ra	**0.176**	**0.005**	**0.282**	**<0.0001**	**0.220**	**0.0004**	**0.196**	**0.002**	0.060	0.342
IL-1ß			**0.157**	**0.012**	**0.222**	**0.0003**	0.096	0.127	−0.01	0.874
IL-6					**0.148**	**0.019**	0.087	0.166	0.024	0.704
CCL-2							**0.167**	**0.007**	0.017	0.785
TNF-α									0.098	0.118

Statistically significant correlations are indicated in bold.

tions of remission revealed higher C-peptide and BMI percentiles and lower A1C for remitters in contrast to nonremitters. Nonremitters showed lower blood pH in the classification of remission 7.5; for the stricter definition of remission 6.5, age was significantly higher in remitters. The classification of improved C-peptide secretion revealed no significant differences.

Association of circulating cytokines with clinical status. To assess the association of cytokines with clinical status, we searched for a relation of circulating cytokines and the classification regarding clinical outcome. Regression analysis was applied to take differences of cytokine concentrations due to age and sex differences into consideration. In the first approach, regression analysis was performed adjusting for sex and age; in the second approach, regression analysis was performed adjusting for sex, age, and BMI percentiles, since adipose tissue is known to be an origin of cytokine secretion. As before, patients with an incomplete data record with respect to classification were excluded from analysis: 22 patients for the classification of remissions and 45 patients for improved C-peptide secretion.

The first regression model revealed elevated anti-inflammatory IL-1ra in remitters of both definitions of remission compared with nonremitters. Furthermore, IL-1ra was also elevated in patients with increased C-peptide secretion (Fig. 2). In contrast, the anti-inflammatory adiponectin was lower in remitters of both definitions of remission but unrelated to C-peptide classification (Fig. 2). Interestingly, the pro-inflammatory IL-6 was elevated in patients in remission 7.5 and in patients with increased C-peptide secretion (Fig. 2).

In the second regression model, which included BMI percentile as a covariable in order to account for the effect of cytokine secretion by adipose tissue, we observed the same association for IL-1ra with increased C-peptide 1 month after diagnosis ($P = 0.016$), suggesting a BMI percentile–independent association. In contrast, associations of IL-1ra with both definitions of remission were lost while adjusting for BMI percentiles.

Adiponectin showed associations similar to those in the analysis without adjustment for BMI percentile in remission 6.5 (6 [$P = 0.037$] and 12 [$P = 0.019$] months after diagnosis). Classification of remission 7.5 no longer showed association with adiponectin.

When adjusting for BMI percentile, IL-6 revealed a similar association with remission 7.5 1 month after diagnosis ($P = 0.0097$) and improved C-peptide secretion 6 ($P = 0.005$) and 12 ($P = 0.03$) months after diagnosis as in analyses without additional adjustment.

Association of circulating cytokines with A1C and C-peptide. To address the relationship of circulating cytokines and β-cell function in more detail, we investigated the association of cytokines with β-cell function measured by stimulated C-peptide and glycemic control determined by A1C. First, regression analysis included cytokines, sex, age, blood pH, C-peptide, and A1C. Anti-inflammatory IL-1ra concentrations revealed positive associations with C-peptide 1 (regression coefficient $\beta = 0.00024$, $P = 0.021$), 6 ($\beta = 0.00042$, $P = 0.0001$), and 12 ($\beta = 0.00031$, $P = 0.0013$) months after diagnosis. Anti-inflammatory adiponectin concentrations showed a negative association with C-peptide 12 months ($\beta = -0.00037$, $P = 0.0037$) after diagnosis and related positively with A1C 1 month ($\beta = 0.12$, $P = 0.0002$) after diagnosis. Proinflammatory cytokine IL-1β was negatively associated with C-peptide 1 month after diagnosis ($\beta = -0.0021$, $P = 0.031$).

To account for a possible influence of adipose tissue, BMI percentiles were added to the regression analysis (Table 3). Anti-inflammatory IL-1ra concentrations were associated with BMI percentiles at all time points investigated. IL-1ra showed associations with C-peptide similar to those without adjustment for BMI percentiles, suggesting BMI-independent associations with C-peptide 6 and 12 months after diagnosis (Table 3). Anti-inflammatory adiponectin was independent of BMI percentile, and analysis revealed associations similar to those without adjustment of BMI percentiles; adiponectin was associated with C-peptide 12 months after diagnosis and with A1C 1 month after diagnosis (Table 3). Similar to adiponectin, proinflammatory IL-1β was not associated with BMI percentile and revealed the same association as that without BMI percentile adjustment. IL-1β was negatively associated with C-peptide 1 month after diagnosis (Table 3). Analyses of IL-6, CCL2, and TNF-α revealed no association at any time to any metabolic parameter.

Association of circulating cytokines with Δblood glucose in the liquid meal test. Cytokines not only influence insulin signaling, but also reveal insulin-independent induction of glucose disposal in the case of IL-6 and adiponectin (27,28). To assess the possible influence of cytokines on glycemic control in our study, we investigated whether Δblood glucose in the standardized liquid meal test (taken as a measure of blood glucose disposal) is associated with circulating cytokines (Fig. 3). Smaller Δblood glucose is suggestive of a higher glucose disposal and likely to reflect a more healthy status. Regression analysis included cytokines, Δblood glucose, sex, age, and BMI percentiles.

Anti-inflammatory adiponectin was positively associated with Δblood glucose ($\beta = 0.021$, $P = 0.024$) 6 months after diagnosis. Proinflammatory IL-6 and CCL2 were negatively associated with Δblood glucose 12 ($\beta = -0.035$, $P = 0.047$), 1 ($\beta = -0.021$, $P = 0.036$), and 6 ($\beta = -0.018$,

$P = 0.046$) months after diagnosis. IL-1ra, IL-1β, and TNF-α showed no association with Δblood glucose.

DISCUSSION

Improved β-cell function, reduced insulin resistance, and improved glucose disposal are likely candidates to affect remission in type 1 diabetes. All of these processes have been shown to be influenced by cytokines. Proinflammatory IL-1β induces apoptosis in insulin-producing β-cells, whereas the anti-inflammatory IL-1ra as the specific receptor antagonist of IL-1β preserves β-cells (16,17).

We here show that increased IL-1ra is associated with improved β-cell function (stimulated C-peptide) in type 1 diabetic patients, which is in line with the protective effect of IL-1ra on β-cell in patients with type 2 diabetes (16). We observed a positive association with C-peptide in the regression models that were adjusted for sex, age, and blood pH. In addition, we found that IL-1ra was elevated in patients with improved C-peptide secretion and in patients in remission 7.5. Elevated IL-1ra levels were also maintained at 12 months when more stringent criteria for remission were applied. Additional adjustment for BMI percentiles in the analysis models revealed a positive association of IL-1ra with BMI percentile (Table 3), confirming previous studies and supporting adipose tissue as an important source of IL-1ra (39,40).

Interestingly, IL-1ra showed a BMI percentile–independent protective association with C-peptide secretion when we performed regression analysis investigating metabolic parameters or patients with improved C-peptide secretion. The significant elevation of IL-1ra in patients in remission was attenuated when adjusting for BMI percentile.

Remitters according to both definitions of remission exhibited significantly higher BMI percentiles 1 month after diagnosis in contrast to nonremitters. This elevation of BMI percentiles in remitters may be due to the finding that patients with more severe symptoms at diagnosis including lower BMI and ketoacidosis have more pronounced β-cell destruction and are less likely to undergo remission during follow-up than children with less severe symptoms. Of note, we have confirmed the protective association of IL-1ra to β-cell function in an independent cohort of 99 recent-onset type 1 diabetic patients (C.P. and N.C.S., unpublished data).

Since IL-1ra is antagonistic to IL-1β, it is interesting to note that stimulated C-peptide was negatively associated with circulating IL-1β. However, the interpretation of these data requires caution because we detected IL-1β concentrations in <15% of investigated samples.

For adiponectin, the other anti-inflammatory cytokine expressed by adipose tissue, we expected higher circulating concentrations in remitters than nonremitters, since it has been described that adiponectin leads to improved glucose homeostasis most likely due to improved glucose disposal (27). However, we observed lower adiponectin concentrations in patients in remission of both definitions. We also observed a positive association of adiponectin with A1C and a negative association of adiponectin with C-peptide and with blood glucose disposal. Whether the increased adiponectin concentrations in patients with less endogenous C-peptide secretion and poorer metabolic control resulting in more oxidative stress reflect a compensatory attempt to induce glucose homeostasis cannot be investigated in this type of study. Yet, recent publications report an upregulation of adiponectin during oxida-

TABLE 2
Characteristics of patients classified by remission 7.5, remission 6.5, and improved C-peptide secretion

	n	Sex (male/female)	Age (years)	BMI percentile	pH	A1C (%)	C-peptide (pmol/l)
Patients in remission 7.5	89	41/48	9.9 (6.7–12.5)	**71.2 (45.6–87.8)***	**7,390 (7.350–7.410)***	**8.5 (7.6–9.3)***	**522 (250–818)***
Patients not in remission 7.5	161	87/74	9.4 (5.4–11.3)	**52.7 (26.8–75.3)**	**7,350 (7.250–7.400)**	**9.1 (8.3–9.99)**	**355 (230–516)**
Patients in remission 6.5	46	18/28	**10.8 (7.8–13.5)†**	**74.4 (48.3–87.8)‡**	7,385 (7.291–7.410)	**8.2 (7.2–9.1)***	**529 (226–945)†**
Patients not in remission 6.5	204	110/94	**9.3 (5.8–11.3)**	**55.8 (29.4–76.9)**	7,370 (7.280–7.400)	**9.0 (8.3–9.8)**	**375 (231–559)**
Patients with improved C-peptide secretion	27	15/12	10.2 (5.9–13.1)	62.8 (48.3–87.8)	7,380 (7.250–7.405)	9.1 (7.8–9.6)	392 (238–787)
Patients without improved C-peptide secretion	200	101/99	9.5 (6.4–11.5)	58.2 (31.8–79.3)	7,365 (7.285–7.400)	8.9 (8.2–9.9)	410 (230–586)

Data are median (interquartile range). Due to incomplete data record for classification, 22 patients for both definitions of remission and 45 patients for improved C-peptide secretion were excluded from analysis. For sex, absolute numbers are given. Sex, age, and pH have been determined at diagnosis and BMI percentiles, A1C, and C-peptide 1 month after diagnosis. Dichotomous variables and variables with non-Gaussian distribution were compared within the classification using Fisher's exact test and Mann-Whitney tests, respectively. *$P < 0.001$, †$P < 0.05$, ‡$P < 0.01$. Statistically significant correlations are indicated in bold.

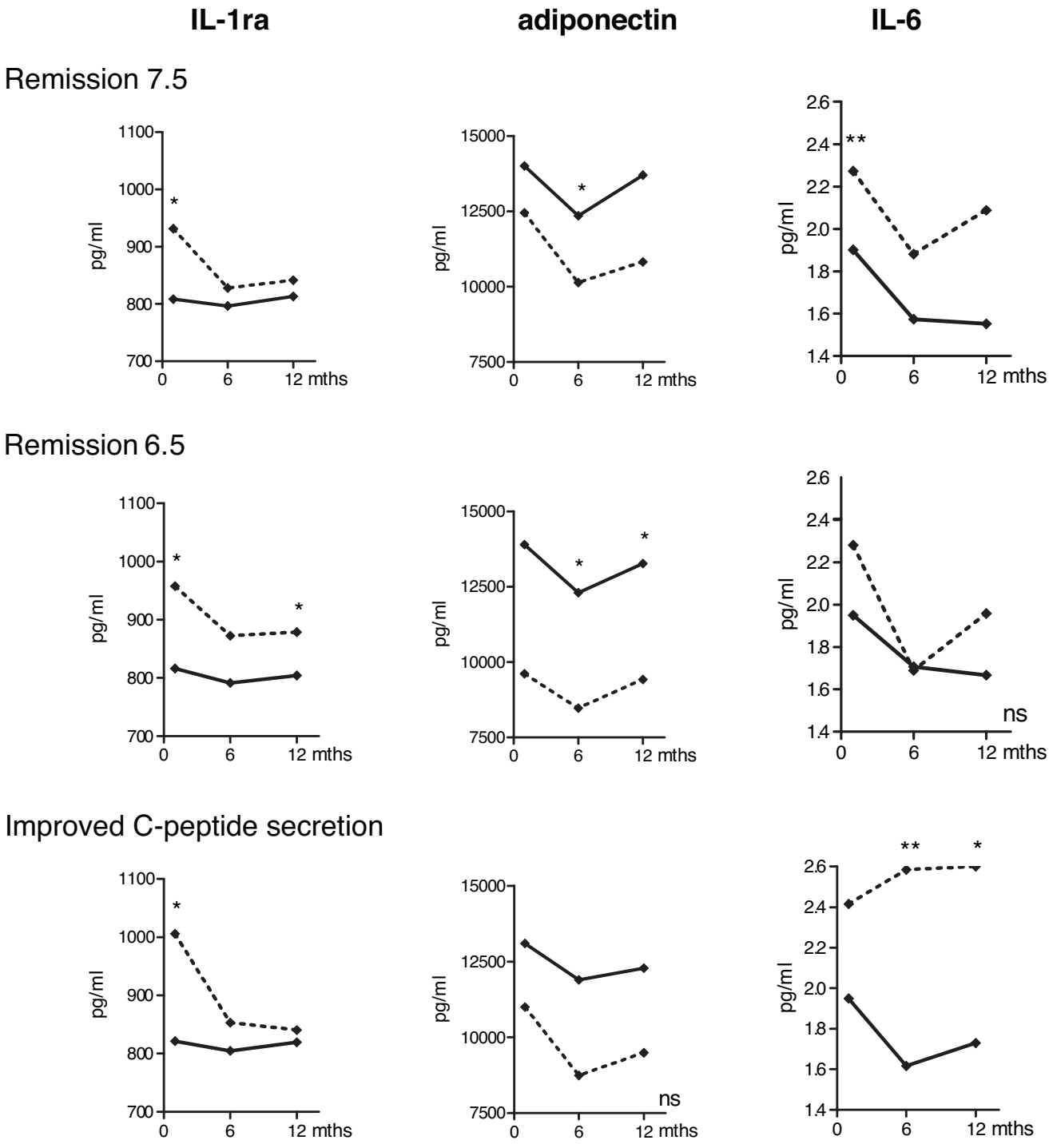

FIG. 2. Follow-up of median circulating cytokine concentrations in patients classified by "remission" or "improved C-peptide secretion." Remission 7.5 (*top panel*) shows data based on the definition of remission A1C <7.5% and <0.4 units/kg daily insulin. Remission 6.5 (*middle panel*) refers to the definition of remission with A1C <6.5% and <0.4 units/kg daily insulin. Patients with improved C-peptide secretion are characterized by an increase of C-peptide from 1 to 6 months after diagnosis of at least 20% with a lower limit of 100 pmol/l. Lines represent medians of the classified groups: dashed lines for patients in "remission" or "increased C-peptide secretion" and solid lines for patients with "no remission" or "no improved C-peptide secretion." *P < 0.05, **P < 0.01 adjusted for sex and age.

tive stress that would support our observation (41,42). Of note, adiponectin revealed no association with BMI percentile in contrast to IL-1ra (Table 3), both secreted by adipose tissue, and was not correlated with the other immune mediators (Table 1). Both findings regarding adiponectin, missing association with BMI percentiles after multiple adjustment and lack of association with circulating concentrations of cytokines, are in line with previous findings from a population-based study (43). We investigated monomeric adiponectin that has been described to be effective (27). Whether high–molecular weight multimers of adiponectin would add or reveal different associations is not clear and is subject to debate (44).

Increased IL-6 has been shown to be linked with inflammation and insulin resistance, especially in patients with

TABLE 3
Association between cytokines and metabolic parameters

	Months after diagnosis	Sex		Age (years)		BMI percentiles		C-peptide (pmol/l)		A1C (%)	
		β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
IL-1ra (pg/ml)	1	−0.11	0.059	−0.010	0.358	**0.0036**	**0.001**	0.00011	0.279	0.006	0.788
	6	−0.062	0.307	**−0.038**	**0.0001**	**0.0027**	**0.048**	**0.00037**	**0.0001**	0.028	0.318
	12	**−0.107**	**0.049**	**−0.022**	**0.009**	**0.0023**	**0.019**	**0.00026**	**0.009**	**0.044**	**0.046**
IL-1β (pg/ml)	1	0.528	0.268	0.145	0.082	−0.0117	0.210	**−0.00263**	**0.009**	−0.095	0.639
	6	−0.189	0.655	−0.054	−0.432	0.0050	0.521	0.00104	0.146	−0.057	0.781
	12	−0.275	0.589	0.073	0.390	0.0051	0.602	−0.00041	0.665	−0.384	0.119
Adiponectin (pg/ml)	1	**−0.172**	**0.027**	**−0.058**	**<0.0001**	0.0011	0.444	0.00004	0.784	**0.121**	**0.0004**
	6	−0.098	0.161	**−0.044**	**<0.0001**	−0.0002	0.891	−0.00018	0.143	0.051	0.111
	12	**−0.197**	**0.005**	**−0.035**	**0.0014**	0.0022	0.090	**−0.00047**	**0.0004**	0.038	0.182

Linear regression analyses were performed for IL-1ra and adiponectin. Cytokines entered the models as log-transformed variables. Logistic regression analyses were applied for IL-1β. Cytokine entered the model as detectable or not detectable. Statistically significant correlations are indicated in bold.

metabolic syndrome (22). Contrary to these findings, we observed in our study elevated IL-6 concentrations in patients in remission and in patients with increased C-peptide secretion who are thought to be characterized by reduced insulin resistance and inflammation (45,46). The negative association of IL-6 with Δblood glucose found in our study that is suggestive of induction of blood glucose disposal by IL-6 might explain why we observed elevation of IL-6 in remission. This suggestion is supported by several studies that found induction of blood glucose disposal by IL-6 (12,28,47,48). To account for the proinflammatory character of IL-6, it is important to note that we observed elevated IL-6 concentrations during the 1st month after diagnosis, suggesting proinflammatory processes around diabetes onset and a decrease during follow-up, which is in line with a previous study (14). Similar to IL-6, CCL2 was elevated 1 month after diagnosis and was associated with higher glucose disposal but not associated with disease stage, as had been assumed previously (9).

In an additional analysis (data not shown), in a small subgroup of patients that were antibody negative, we observed higher IL-1ra concentrations 6 months after diagnosis ($P = 0.017$). This result is in line with our observation of preserved β-cell function during high IL-1ra concentrations, since antibody-negative type 1 diabetic patients are believed to undergo a less aggressive diabetes progression (49). In addition, we observed a negative association of IL-6 with GADAs 1 month after diagnosis ($P = 0.03$). Certainly, it would be interesting to see whether genotypes that have an impact on diabetes are also associated with investigated cytokines and their relation to β-cell function and metabolic status. However, these data were not available in the current study and require future study.

The strength of the current study is that we had access to a well-characterized cohort with relatively big patient numbers of newly diagnosed patients with type 1 diabetes that were followed prospectively and longitudinally for 12 months. To our knowledge, this is the first comprehensive study relating β-cell secretion capacity, metabolic control, and remission status with circulating concentrations of cytokines in pediatric patients. Potential disadvantages come from the multicenter design of the study that combines heterogeneous patient groups throughout Europe. However, at present it will be difficult to obtain equivalent patient numbers from one region only. Also, it should be noted that the results presented here are the descriptive

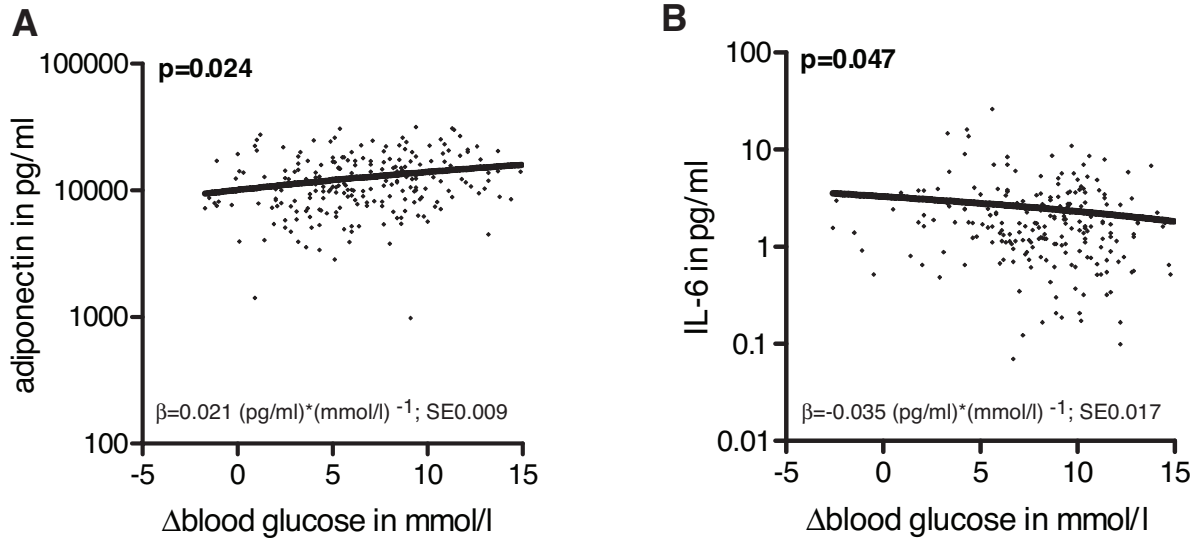

FIG. 3. Correlation of circulating cytokine concentrations with Δblood glucose. Coefficient (β) and *P* values of regression line plotted are adjusted for sex, age, and BMI percentile. *A*: Adiponectin vs. Δblood glucose 6 months after diagnosis. *B*: IL-6 vs. Δblood glucose 12 months after diagnosis.

outcome of associations observed from metabolic data and peripheral blood and thereby a causal relationship cannot be addressed. Furthermore, there were no clamp studies performed to investigate glucose disposal, and therefore the here-reported Δblood glucose gives only an indication of the glucose disposal capacity. Another topic addresses implication of BMI percentiles. We applied BMI percentiles from the U.S., although the patients investigated originate from different centers mainly in Europe. This problem of heterogeneity could be overcome by applying country-specific BMI, but they were not available for all patients.

We conclude that IL-1ra is associated with preserved β-cell capacity in type 1 diabetes. This novel finding indicates that administration of IL-1ra (anakinra), which has been successfully shown to improve β-cell function in patients with type 2 diabetes, may also be a new therapeutical approach for type 1 diabetic patients. The relation of adiponectin and IL-6 with remission and metabolic status in patients with type 1 diabetes transfers observations from in vitro and animal models into the human situation in vivo.

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