Profile of the Immune and Inflammatory Response in Individuals With Prediabetes and Type 2 Diabetes

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Diabetes Care 2015;38:1356–1364 | DOI: 10.2337/dc14-3008

OBJECTIVE

The inflammatory and immune systems are altered in type 2 diabetes. Here, the aim was to profile the immune and inflammatory response in subjects with prediabetes and diabetes in a large population-representative sample.

RESEARCH DESIGN AND METHODS

In total, 15,010 individuals were analyzed from the population-based Gutenberg Health Study. Glucose status was classified according to HbA_{1c} concentration and history of diagnosis. All samples were analyzed for white blood cells (WBCs), granulocytes, lymphocytes, monocytes, platelets, C-reactive protein (CRP), albumin, fibrinogen, and hematocrit. Interleukin-18 (IL-18), IL-1 receptor antagonist (IL-1RA), and neopterin concentrations were determined in a subcohort.

RESULTS

In total, 7,584 men and 7,426 women were analyzed (range 35–74 years), with 1,425 and 1,299 having prediabetes and diabetes, respectively. Biomarkers showed varying dynamics from normoglycemic via subjects with prediabetes to subjects with diabetes: *1*) gradual increase (WBCs, granulocytes, monocytes, IL-1RA, IL-18, and fibrinogen), *2*) increase with subclinical disease only (lymphocytes and CRP), *3*) increase from prediabetes to diabetes only (neopterin), and *4*) no variation with glucose status (hematocrit). The strongest relative differences were found for CRP, IL-1RA, and fibrinogen concentrations. Several inflammatory and immune markers were associated with the glucose status independent from cardiovascular risk factors and comorbidities, varied with disease severity and the presence of disease-specific complications in the diabetes subcohort.

CONCLUSIONS

The inflammatory and immune biomarker profile varies with the development and progression of type 2 diabetes. Markers of inflammation and immunity enable differentiation between the early preclinical and clinical phases of the disease, disease complications, and progression.

Type 2 diabetes and its disease-associated complications represent an important and increasing public health burden worldwide (1). Obesity, which leads to metabolic and adipocyte stress, is the most important predisposing factor for type 2 diabetes (2). Obesity-associated insulin resistance, activation of the innate immune system, and chronic increased production of cytokines and adipokines are an

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Received 18 December 2014 and accepted 14 March 2015.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc14-3008/-/DC1.

important link between obesity and type 2 diabetes (3,4). Novel data further suggest that chronic adipose tissue inflammation and β-cell stress cause an activation of the adaptive immune system as well, which may also participate in the progression of the inflammatory response (5). Autoimmune and inflammatory mechanisms during hyperglycemia-induced glucotoxicity could favor an increased expression of several β-cell antigens, thus increasing β-cell apoptosis through autoantibodies (6). Such islet autoantibodies are presented in ~10% of subjects with diabetes, which have distinct clinical and phenotypical characteristics compared with patients with type 2 diabetes without signs of autoimmunity (5,7).

With respect to inflammatory processes, prospective studies have demonstrated that chronic low-grade inflammation precedes the onset of diabetes (8–11). For example, elevated concentrations of biomarkers such as C-reactive protein (CRP), white blood cell (WBC) count, interleukin-1β (IL-1β), IL-1 receptor antagonist (IL-1RA), IL-6, IL-8, IL-18, monocyte chemoattractant protein-1 (MCP-1), interferon-γ–inducible protein-10 (IP-10), haptoglobin, and fibrinogen point to a chronic, often subclinical degree of inflammation and are increased already years before type 2 diabetes onset (11–18). The degree of subclinical inflammation was reported to be similar between subjects with impaired fasting glucose and individuals with diabetes (9,19).

Subjects with diabetes are at increased risk for atherosclerotic cardiovascular disease (CVD), peripheral arterial disease (PAD), and cerebrovascular disease (20) because of the coexistence of multiple cardiovascular risk factors (CVRFs), including hypertension and dyslipidemia, that are more prevalent in individuals having type 2 diabetes compared with subjects with normal homeostasis (20–22). Further, the onset of diabetes leads to several micro- and macrovascular complications. Long-term complications include retinopathy with a potential loss of vision, neuropathy with the danger of foot amputation, and nephropathy leading to renal function decline and dialysis (20). In this context, IL-6 and CRP are the most discussed candidates to predict CVRFs and disease-associated complications in individuals with diabetes (23,24).

Taken together, inflammatory processes are a causal link for the development of vascular complications during (pre)diabetes (8,25). However, there is a lack of data on the overall profile of the immune and inflammatory response in the disease, particularly in subjects with prediabetes.

With the current study, we characterized the immune and inflammatory response of subjects with prediabetes and diabetes in a large population-representative sample. This representative setting offers the possibility to investigate the complete spectrum of the disease from normal metabolic homeostasis over the subclinical to the manifest disease and helping to differentiate between biological effects versus a simple progression of the disease.

RESEARCH DESIGN AND METHODS

The Gutenberg Health Study (GHS) is a population-representative, prospective, observational, single-center cohort study in western mid-Germany and includes a total sample size of 15,010 individuals. The interdisciplinary study, including a detailed biobanking, focuses predominantly on CVD, but also metabolic, ophthalmological, and mental disease. The sample was drawn randomly from the local governmental registry offices. The sampling procedure was stratified 1:1 for sex and residence (urban and rural) and in equal strata for decades of age (range 35–74 years). The study design has been published elsewhere in more detail (26).

The baseline collection started in April 2007 and was completed in April 2012. All individuals were invited for a 5-h baseline examination at the study center, where the clinical data assessment was performed. The study was designed according to the tenets of the revised Helsinki protocol. All participants gave written informed consent to laboratory analyses, clinical examinations, sampling of biomaterial, and the use of data records for research purposes.

Study Population and Diabetes Diagnosis

All individuals with unspecified or types other than type 2 diabetes were excluded from analysis (see Supplementary Fig. 1). HbA_{1c} concentration was determined in all remaining subjects (n = 14,876) using a standardized high-performance liquid chromatography assay. Study participants were categorized according to the International Expert Committee, i.e., <6.0% (<42 mmol/mol hemoglobin) as with normal glucose homeostasis, 6.0–6.4% (42–46 mmol/mol hemoglobin) as having increased risk for diabetes or prediabetes, and ≥6.5% (≥48 mmol/mol hemoglobin) as having diabetes (27). Analyses were performed in a centralized laboratory setup. Information on duration and complications of type 2 diabetes, concomitant diseases, and family history were collected during a comprehensive computer-assisted personal interview. All individuals brought their drugs to the study center for recording current drug intake and, if available, medical records. CVRFs were assessed by clinical examination and laboratory measurements. Individuals with diabetes were classified according to no treatment/dietary treatment, insulin-dependent diabetes, and noninsulin-dependent diabetes.

Laboratory Measurements

Venous blood sampling was performed in lying position, and the prior fasting period was documented. Biosamples were stored at −80°C immediately after blood withdrawal. Plasma CRP concentration (n = 14,983) was measured by a high-sensitivity latex enhanced immunoturbidimetric assay (Abbott Laboratories, Abbott Park, IL). The limit of detection was ≤0.1 mg/L for the ultrasensitive calibrator and ≤0.2 mg/L for the wide-range calibrator. Fibrinogen (n = 14,892) was determined by a derived method with a detection limit of 40 mg/dL (Siemens, Munich, Germany). Albumin (Abbott Laboratories; n = 14,982) and blood cell counts (WBC count, n = 14,973; platelet count, n = 14,959; neutrophil granulocyte count, n = 14,973; lymphocyte count, n = 14,973; monocyte count, n = 14,973) were performed by routine laboratory methods. WBCs were counted, whereas for granulocytes, lymphocytes, and monocytes, relative frequencies were given. Relative frequencies were converted to absolute numbers based on the WBC number. Hematocrit (n = 14,976) was calculated as follows: MCV * erythrocytes. IL-18 (Human IL-18 ELISA Kit; MBL, Woburn, MA; detection limit of 128 pg/mL; n = 4,573), IL-1RA (Quantikine; R&D Systems, Wiesbaden,

Germany; detection limit of 31.2 pg/mL; *n* = 4,967), and neopterin (Thermo Fisher Scientific, Hennigsdorf, Germany; *n* = 3,972) were determined by commercially available assays. HbA_{1c}, WBCs, granulocytes, lymphocytes, monocytes, platelets, CRP, albumin, fibrinogen, and hematocrit were determined in fresh material, whereas IL-1RA, IL-18, and neopterin were assessed in frozen material.

Data Assessment, Data Management, and Statistical Analysis

All clinical and laboratory examinations were performed according to standard operating procedures by specifically trained and certified medical technical assistants. The definitions of CVRFs and comorbidities are presented in the Supplementary Data. All data of the present investigation underwent quality control by a central data management unit. Data were reviewed for completeness by predefined algorithms and plausibility criteria. Data are presented as absolute numbers, percentages, and means with SDs or medians with 25th and 75th percentiles as appropriate. Fisher exact or χ^2 tests were used to test differences in categorical variables. Statistical comparisons for continuous variables were made by Mann-Whitney *U* test as well as Student *t* test as appropriate. All analyses were carried out sex specifically. For the correlation analyses, Spearman rank correlation coefficient test was used. To assess the effects of inflammation on the presence of prediabetes, diabetes, and disease-associated complications, multivariable logistic regression models were used: model 1 was adjusted for age and sex and model 2 was additionally adjusted for CVRFs (waist-to-height ratio [WHtR] as continuous variable and hypertension, smoking, dyslipidemia, and family history of myocardial infarction [MI] or stroke as dichotomous variables), cardiovascular comorbidities (coronary artery disease [CAD], MI, stroke, PAD, atrial fibrillation [AF], and chronic obstructive pulmonary disease [COPD]), arthritis, autoimmune disease, hemostatic disorders, and acute infection. Adjusted odds ratios (ORs) are given with 95% CI and *P* value. The OR is given per one interquartile range (IQR) change of the biomarkers. In this explorative analysis, the OR was used to describe the strength of an association. In general, *P* values <0.05 were considered as relevant associations. Statistical data analyses were performed using the software program R, version 3.0.2 (http://www.R-project.org).

RESULTS

Study Sample

In total, 7,584 (50.5%) men and 7,426 (49.5%) women were enrolled, median age was 55.0 years (range 35–74; IQR 46–65 years). From this study sample, 134 individuals were excluded with unspecified diabetes (0.4%; *n* = 55) or other than type 2 diabetes (0.5%; *n* = 79). From the remaining subjects (*n* = 14,876), 81.0% (*n* = 12,152) of individuals had normal HbA_{1c} concentrations. In contrast, 9.5% (*n* = 1,425) of individuals fulfilled the criteria of having prediabetes (HbA_{1c} 6.0–6.4% [42–47 mmol/mol hemoglobin]), and 8.7% (*n* = 1,299) of subjects had diabetes according to HbA_{1c} concentration ($\geq$6.5% [$\geq$48 mmol/mol hemoglobin]) or were previously diagnosed by a physician (Supplementary Fig. 1). The prevalence of prediabetes and diabetes in the sample according to decades of age and sex is provided in Supplementary Fig. 2.

With respect to disease treatment, 6.2% (*n* = 81) of individuals affected with type 2 diabetes received no or dietary treatment and 39.1% (*n* = 508) showed a noninsulin-dependent diabetes and 19.4% (*n* = 252) an insulin-dependent diabetes; no information on treatment was available for 458 subjects. The specific medicinal treatment is provided in Supplementary Table 1. The disease duration in the subgroup of individuals with diabetes was as follows: $\leq$1 year, 11.7% (*n* = 105); $>$1 to $\leq$2 years, 8.7% (*n* = 78); $>$2 to $\leq$5 years, 25.4% (*n* = 228); $>$5 to $\leq$10 years, 28.6% (*n* = 257); and $>$10 years, 25.6% (*n* = 230); no information was available in 401 individuals.

Prevalence of CVRFs and Comorbidities According to Glucose Status

Sample characteristics according to the glucose status are presented in Table 1. Compared with individuals with normoglycemia and prediabetes, subjects having type 2 diabetes were more frequently of male sex. With the exception of smoking, the prevalence of

Table 1—Characteristics of individuals according to glucose status

	Normoglycemia	Prediabetes	Diabetes
Size (*n*)	12,152	1,425	1,299
Sex (women)	50.4% (6,121)	51.6% (736)	38.3% (497)
Age (years)	53.4 ± 11.0	61.1 ± 9.0	63.0 ± 8.3
Glucose (mg/dL)	90.0 (85.0, 96.0)	97.0 (91.0, 104.0)	112.6 (99.0, 132.0)
HbA_{1c} (%)	5.40 (5.10, 5.60)	6.10 (6.00, 6.20)	6.70 (6.30, 7.20)
HbA_{1c} (mmol/mol)	36 (32, 38)	43 (42, 44)	50 (45, 55)
Anthropometry			
BMI (kg/m^2)	26.7 ± 4.6	29.3 ± 5.6	31.5 ± 5.9
Waist (cm)	92.6 ± 13.0	99.8 ± 14.1	106.7 ± 14.1
Classical CVRFs			
Hypertension	71.5% (8,687)	84.4% (1,202)	91.1% (1,184)
Smoking	19.3% (2,342)	23.6% (336)	16.2% (209)
Obesity	20.5% (2,494)	37.8% (538)	55.2% (716)
Dyslipidemia	39.2% (4,765)	59.9% (854)	76.4% (991)
Family history of MI/stroke	21.0% (2,552)	26.5% (377)	27.5% (357)
Comorbidities			
CAD	3.0% (355)	7.6% (106)	14.0% (173)
MI	2.0% (238)	5.4% (77)	9.6% (123)
Stroke	1.4% (168)	3.0% (42)	4.9% (63)
PAD	2.6% (316)	5.1% (72)	8.4% (107)
AF	2.3% (274)	4.0% (56)	5.9% (75)
CHF	1.0% (121)	2.2% (31)	3.4% (44)
COPD	4.5% (541)	6.7% (95)	7.7% (100)

Participants having type 1 diabetes, other types, or an unspecified type were excluded from the cohort (*n* = 134). With the exception of smoking (*P* = 0.4), the prevalence of the depicted parameters increased significantly from individuals with normoglycemia to prediabetes and to diabetes ($P < 0.001$). Data are presented as relative and absolute numbers (sex, hypertension, smoking, obesity, dyslipidemia, family history of MI/stroke, CAD, MI, stroke, PAD, AF, CHF, COPD), mean and standard deviation (age, BMI, waist), median and interquartile range (glucose, HbA_{1c}).

CVRFs increased from individuals with normoglycemia to those with prediabetes and to those with diabetes, respectively. Subjects with prediabetes had the highest prevalence of smoking compared with subjects with diabetes or normoglycemia.

With respect to cardiovascular comorbidities, individuals with prediabetes and diabetes showed a higher prevalence of CAD, MI, stroke, PAD, AF, congestive heart failure (CHF), and COPD. Again, the frequencies of comorbidities increased gradually from normoglycemic subjects to those with prediabetes and, finally, subjects with diabetes. Overall, 19.8% (273 of 1,379) of individuals with prediabetes had CVD, i.e., the individuals had at least one of the following: CAD, MI, stroke, PAD, AF, CHF, or COPD. With more detail, 69.6% (n = 190) of subjects had one, 22.3% (n = 61) had two, and 8.1% (n = 22) had three or more CVDs; no data were available for 46 subjects. Subjects with diabetes were more often affected with CVD, i.e., 30.5% (378 of 963) of subjects with diabetes reported at least one CVD; with more detail, 62.7% (n = 237) had one, 23.5% (n = 89) had two, and 13.8% (n = 52) had three or more CVDs, with no data available in 61 subjects. With respect to the diabetes subsample, 14.5% (n = 189) of the subjects had neuropathy, 14.5% (n = 189) had macroalbuminuria, 4.9% (n = 64) had proteinuria, 4.3% (n = 56) had retinopathy, 0.8% (n = 11) had blindness, 0.7% (n = 9) had foot amputation, and 0.7% (n = 9) had a need for dialysis.

Distribution of Inflammatory and Immune Biomarkers

Concentrations of inflammatory and immune biomarkers compared between normoglycemic subjects, individuals with prediabetes and diabetes, and their differences are presented in Table 2. Biomarkers were distinguishable in four different subgroups:

1. Gradual increase from normoglycemic via subjects with prediabetes to diabetes (WBC, granulocyte, and monocyte count and IL-1RA, IL-18, and fibrinogen)
2. Increase with subclinical disease and approximately stable levels despite progression to diabetic disease (lymphocytes and CRP)
3. Comparable levels between individuals with normoglycemia and

Table 2—Differences in concentration of cells and proteins reflecting inflammation and immune system between normoglycemia, prediabetes, and diabetes

	Normoglycemia	Prediabetes	Diabetes	*P*			
				For trend	(Normoglycemia vs. prediabetes)	(Normoglycemia vs. diabetes)	(Prediabetes vs. diabetes)
Size (n)	12,152	1,425	1,299				
WBCs (10^9/L)	6.80 (5.76, 8.12)	7.30 (6.09, 8.60)	7.60 (6.36, 8.90)	<0.0001	<0.0001	<0.0001	<0.0001
Granulocytes (10^9/L)	4.24 (3.41, 5.29)	4.53 (3.59, 5.55)	4.74 (3.85, 5.81)	<0.0001	<0.0001	<0.0001	<0.0001
Lymphocytes (10^9/L)	1.78 (1.45, 2.19)	1.89 (1.51, 2.35)	1.89 (1.49, 2.35)	<0.0001	<0.0001	<0.0001	0.53
Monocytes (10^9/L)	0.40 (0.32, 0.48)	0.42 (0.34, 0.53)	0.46 (0.37, 0.56)	<0.0001	<0.0001	<0.0001	<0.0001
Platelets (10^9/L)	269 (230, 312)	270 (235, 325)	262 (218, 308)	0.55	0.0013	<0.0001	<0.0001
IL-1RA (pg/mL)	310.7 (233, 408)	350.8 (271, 485)	404.9 (298, 545)	<0.0001	<0.0001	<0.0001	<0.0001
IL-18 (pg/mL)	222 (178, 285)	233 (181, 298)	260 (204, 334)	<0.0001	0.05	<0.0001	<0.0001
CRP (mg/L)	1.40 (0.50, 2.90)	2.30 (1.20, 4.40)	2.40 (1.30, 5.10)	<0.0001	<0.0001	<0.0001	0.0078
Neopterin (pmol/L)	5.40 (4.70, 6.30)	5.40 (4.70, 6.30)	5.90 (5.10, 7.37)	<0.0001	0.76	<0.0001	<0.0001
Albumin (g/L)	42 (40, 44)	41 (39, 43)	41 (39, 43)	<0.0001	<0.0001	<0.0001	0.34
Fibrinogen (mg/dL)	315 (273, 366)	345 (298, 410)	359 (308, 417)	<0.0001	<0.0001	<0.0001	0.00038
Hematocrit (%)	41.9 (39.7, 44.2)	42.2 (39.9, 44.4)	41.9 (39.6, 44.3)	0.57	0.098	0.43	0.08

Participants having type 1 diabetes, other types, or not specified were excluded (n = 134). Data are presented as median (Q1, Q3).

prediabetes and strong increase from prediabetes to diabetes (neopterin)
4. No influence on levels by prediabetes and diabetes (hematocrit)

The relative change between individuals with diabetes and prediabetes and subjects with normal HbA_{1c} concentration as well as sex-specific relative changes of biomarker concentrations are presented in Supplementary Fig. 3. The largest relative differences in the subsample with diabetes were observed for CRP, IL-1RA, and fibrinogen, whereas the relative increase in CRP levels was the highest in both males (~60%) and females (~90%). With respect to individuals with prediabetes, the strongest increase was seen for CRP as well, both in men (~50%) and women (~70%). The other biomarkers displayed minor relative changes when subjects with prediabetes were compared with subjects with diabetes. Correlation analyses between biomarkers and between biomarkers and CVRFs indicate that there is no impact of diabetes and prediabetes on correlations (see Supplementary Data).

Association of Biomarkers With Disease Status

The concentrations of inflammatory and immune biomarkers depending on the presence of CVD within the cohort with prediabetes and the cohort with diabetes are presented in Supplementary Table 2. As expected, the majority of biomarkers varied clearly between individuals with prediabetes with (n = 273) and without CVD (n = 1,106) and individuals with diabetes with (n = 378) and without CVD (n = 860). Using disease duration and the treatment form as surrogate markers for disease severity within the diabetes subcohort, an association between the prevalence of CVD and disease severity was observed with an increasing prevalence of CVD with longer type 2 diabetes duration and more intense treatment (Supplementary Table 3). Similar to these data, several inflammatory and immune biomarkers increased with disease severity. With respect to intensity of treatment, WBCs, granulocytes, monocytes, CRP, IL-1RA, IL-18, neopterin, albumin, and fibrinogen concentrations clearly increased from subjects with no/dietary treatment via noninsulin-dependent diabetes to insulin-dependent diabetes. Focusing on differences between noninsulin-dependent diabetes and insulin-dependent diabetes, a substantial increase was present for WBCs, granulocytes, monocytes, CRP, albumin, neopterin, fibrinogen, and hematocrit (Supplementary Table 4). Of note, the concentration of granulocytes increased with a longer disease duration (Supplementary Table 5).

In multivariable logistic regression models for the presence of diabetes and prediabetes, the concentrations of almost all biomarkers were strongly associated under adjustment for sex and age (Fig. 1, model 1). Especially for WBCs, granulocytes, monocytes, IL-1RA, IL-18, and fibrinogen, the association increased from prediabetes to diabetes. No or minor differences were seen for lymphocytes, CRP, and albumin. Interestingly, neopterin showed an inverse association with prediabetes but, in contrast, a weakly positive association with diabetes. The multivariable logistic regression model was adjusted for traditional CVRFs (WHtR, hypertension, smoking, dyslipidemia, and family history of MI or stroke), cardiovascular comorbidities and arthritis, hemostatic disorders, autoimmune disease, and

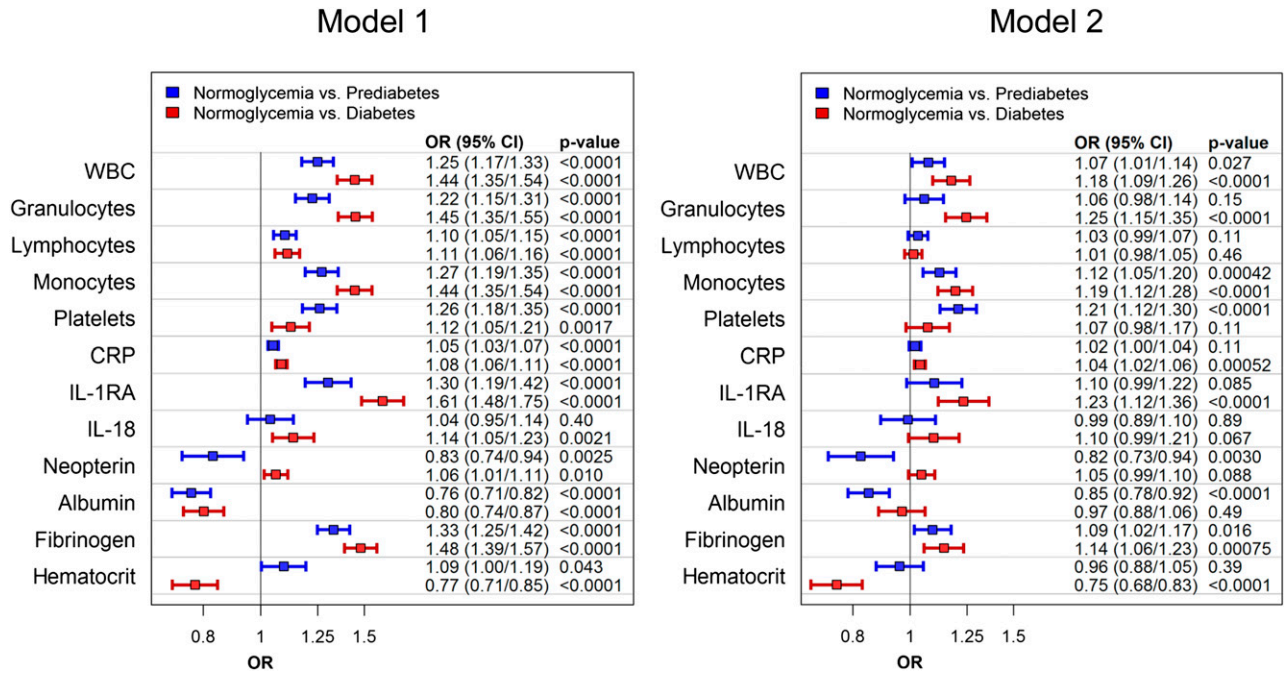

Figure 1—Impact of inflammatory and immune biomarkers on the presence of prediabetes and diabetes. A logistic model with diabetes or prediabetes vs. normoglycemia as dependent variable was calculated for each biomarker. Model 1 was adjusted for sex and age. Model 2 was adjusted additionally for CVRFs, including WHtR, hypertension, smoking, dyslipidemia, and comorbidities including CAD, MI, stroke, PAD, AF, and COPD as well as arthritis, hemostatic disorder, autoimmune disease, and acute infection.

acute infection. Here, elevated WBC, granulocyte, and monocyte count; CRP, IL-1RA, and fibrinogen concentrations; and hematocrit (with inverse association) were still independently associated with the presence of type 2 diabetes (Fig. 1, model 2). Platelets and, with an inverse association, albumin and neopterin showed strong associations only with prediabetes, but not with diabetes, whereas granulocytes, CRP, IL-1RA, and hematocrit were strongly associated with diabetes but showed no associations with the presence of prediabetes. A further comparison between subjects with prediabetes and diabetes in models 1 and 2 is presented in Supplementary Fig. 4.

Of note, only marginal differences were observed when comparing regression models with adjustment for CVRFs only to models adjusting for CVRFs and comorbidities. This suggests CVRFs as significant confounders, whereas comorbidities do not affect the association between inflammatory and immune markers and the diabetic disease (data not presented). Of note, the socioeconomic status did not affect the association between diabetes and biomarkers.

Biomarkers of Inflammation and Immunity and Disease-Specific Complications

Finally, the role of inflammatory and immune biomarkers for the prevalence of disease-associated complications was evaluated in the subgroup with type 2 diabetes. Regression models for the four most frequent complications, i.e., neuropathy (14.5%; *n* = 189), macroalbuminuria (14.5%; *n* = 189), nephropathy (4.9%, *n* = 64), and retinopathy (4.3%, *n* = 59), were analyzed (Fig. 2). After adjustment for sex and age, CVRF, comorbidities, acute infection, arthritis, hemostatic disorders, and autoimmune disease, higher concentrations of WBCs in general and granulocytes in particular were associated with the prevalence of retinopathy and elevated lymphocytes and CRP concentrations with the prevalence of neuropathy. Further, higher hematocrit values were associated with a lower prevalence of proteinuria. The sample size for foot amputation, dialysis, and blindness was too low for further analysis.

CONCLUSIONS

The current study represents one of the largest single-center population-based cohort studies (*n* = 15,010) profiling the inflammatory and immune response in subjects with prediabetes, diabetes, and its disease-specific complications. Besides the worsening of the cardiovascular risk profile, a clear alteration of the inflammatory and immune response was observed depending on the glucose status. Interestingly, biomarkers showed varying profiles with disease progression (i.e., normoglycemia vs. prediabetes vs. diabetes): *1*) an increase with subclinical disease (i.e., prediabetes) and stable levels despite progression to overt type 2 diabetes (lymphocytes and CRP), *2*) comparable levels for normoglycemia and prediabetes with an increase in type 2 diabetes for neopterin, *3*) a continuous increase with worsening of endogenous glycemic control (WBCs, granulocytes, monocytes, IL-1RA, IL-18, and fibrinogen), and *4*) biomarkers where concentrations

	Model 1				Model 2			
	Proteinuria	Macroalbuminuria	Neuropathy	Retinopathy	Proteinuria	Macroalbuminuria	Neuropathy	Retinopathy
WBC	1.16 (0.88,1.53) 0.30	1.42 (1.11,1.81) **0.0049**	1.16 (0.98,1.39) 0.091	1.55 (1.18,2.03) **0.0014**	1.09 (0.79,1.50) 0.58	1.30 (0.96,1.76) 0.087	1.05 (0.84,1.31) 0.69	1.54 (1.11,2.13) **0.0097**
Granulocytes	1.13 (0.87,1.47) 0.35	1.41 (1.12,1.77) **0.0035**	1.10 (0.92,1.30) 0.29	1.45 (1.13,1.85) **0.0031**	1.06 (0.78,1.42) 0.72	1.31 (0.99,1.72) 0.056	0.97 (0.79,1.21) 0.81	1.42 (1.06,1.91) **0.019**
Lymphocytes	1.08 (0.81,1.44) 0.61	1.03 (0.81,1.32) 0.82	1.22 (1.02,1.46) **0.031**	1.24 (0.91,1.69) 0.18	1.11 (0.79,1.56) 0.55	1.01 (0.73,1.39) 0.96	1.25 (1.00,1.56) **0.047**	1.26 (0.87,1.81) 0.23
Monocytes	1.11 (0.84,1.46) 0.45	1.23 (0.97,1.57) 0.092	1.16 (0.98,1.37) 0.086	1.29 (1.00,1.67) 0.054	1.13 (0.83,1.54) 0.45	1.06 (0.78,1.44) 0.70	1.04 (0.84,1.29) 0.73	1.25 (0.91,1.71) 0.17
Platelets	0.92 (0.68,1.24) 0.59	1.12 (0.86,1.44) 0.40	0.94 (0.78,1.14) 0.52	1.06 (0.79,1.42) 0.70	0.92 (0.66,1.29) 0.63	1.06 (0.80,1.41) 0.69	0.87 (0.70,1.09) 0.23	1.10 (0.80,1.52) 0.56
CRP	1.01 (0.95,1.07) 0.76	1.03 (0.98,1.07) 0.27	1.08 (1.02,1.13) **0.0051**	1.01 (0.95,1.07) 0.77	0.95 (0.82,1.09) 0.45	1.03 (0.96,1.11) 0.43	1.07 (1.02,1.12) **0.0070**	0.98 (0.89,1.08) 0.70
IL-1RA	0.90 (0.63,1.28) 0.55	1.16 (0.85,1.59) 0.35	1.26 (1.02,1.54) **0.028**	1.00 (0.71,1.41) 1.00	0.73 (0.45,1.17) 0.19	1.13 (0.74,1.71) 0.57	1.32 (0.98,1.78) 0.064	0.84 (0.51,1.37) 0.49
IL-18	0.94 (0.61,1.43) 0.76	1.25 (0.85,1.84) 0.26	1.05 (0.80,1.37) 0.75	1.10 (0.77,1.56) 0.61	0.71 (0.40,1.25) 0.23	1.33 (0.82,2.15) 0.25	1.25 (0.90,1.74) 0.18	1.31 (0.88,1.96) 0.18
Neopterin	1.18 (0.98,1.42) 0.073	1.10 (0.87,1.38) 0.43	1.10 (0.94,1.28) 0.22	1.09 (0.95,1.25) 0.20	1.12 (0.92,1.37) 0.27	1.24 (0.86,1.79) 0.24	1.12 (0.88,1.41) 0.36	1.10 (0.89,1.37) 0.36
Albumin	0.81 (0.59,1.12) 0.20	0.63 (0.47,0.85) **0.0024**	0.80 (0.65,0.98) **0.028**	1.04 (0.74,1.44) 0.84	0.94 (0.65,1.37) 0.76	0.82 (0.57,1.16) 0.25	0.93 (0.72,1.20) 0.58	1.35 (0.91,2.01) 0.14
Fibrinogen	1.27 (1.02,1.59) **0.036**	1.42 (1.18,1.71) **0.00021**	1.24 (1.07,1.44) **0.0053**	1.17 (0.92,1.48) 0.20	1.17 (0.89,1.55) 0.27	1.29 (1.00,1.66) 0.050	1.11 (0.91,1.36) 0.29	0.97 (0.70,1.34) 0.85
Hematocrit	0.70 (0.50,0.98) **0.035**	1.32 (0.96,1.82) 0.085	0.74 (0.60,0.91) **0.0052**	0.81 (0.57,1.14) 0.22	0.65 (0.45,0.95) **0.025**	1.31 (0.92,1.87) 0.13	0.87 (0.68,1.11) 0.25	0.89 (0.60,1.32) 0.56

Figure 2—Inflammatory and immune biomarkers and presence of disease-associated complications. Logistic regression analysis with the presence of complications as dependent variable. As in Fig. 1, model 1 was adjusted for sex and age and model 2 additionally for CVRFs, including WHtR, hypertension, smoking, dyslipidemia, and comorbidities including CAD, MI, stroke, PAD, AF, and COPD as well as acute infection, arthritis, hemostatic disorder, and autoimmune disease. Boldface text indicates ORs with a *P* value <0.05 and are highlighted in red (for OR <1) or blue (for OR >1).

were not influenced by the presence of prediabetes or diabetes (albumin and hematocrit).

The current study underlines that type 2 diabetes comes along with an ongoing cytokine-mediated acute phase response initiated by the innate and, as novel data suggest, the adaptive immune system (5,8,10,11). Obesity and β-cell stress have been demonstrated to be involved in increased expression of proinflammatory cytokines in the liver and adipose tissue (3,28). These cytokines, including TNF-α, IL-6, and IL-1β, may promote insulin resistance in the tissues, where they are produced and further affect more distant sites via circulation such as vessel walls, skeletal and cardiac muscle, kidney, and circulating leukocytes, respectively (8). Further, new data suggest that cytokines such as IL-1R, IL-1β, IL-6, or TNF-α contribute to an islet cell autoimmunity in type 2 diabetes through activation of T cells, B cells, and macrophages, increased expression of β-cell antigens, and subsequent β-cell apoptosis (5).

In the present project, two proteins from the IL-1 superfamily have been investigated. IL-1RA is an anti-inflammatory cytokine that inhibits the effect of the proinflammatory IL-1β, an important cytokine in the context of type 2 diabetes due to its relation to insulin resistance and β-cell dysfunction (25). In contrast, IL-18 is a potent proinflammatory cytokine that plays a central role in the inflammatory cascade. In this study, IL-1RA was more strongly increased in subjects with prediabetes versus subjects with normoglycemia than IL-18. Yet, the concentration of both cytokines substantially increased further from the precursor to the manifest disease. In several studies, elevated IL-1RA concentrations were reported to discriminate between individuals who finally developed type 2 diabetes compared with lower values in individuals who remained diabetes free (9,14,25,29,30). Therefore, not only proinflammatory but also anti-inflammatory markers, even though not sufficient to prevent further diabetes development, are elevated in individuals with prediabetes and diabetes and may reflect the body's response to counterbalance increased IL-1β activity (25).

WBCs, including lymphocytes, monocytes, and granulocytes, are essential in the innate and adaptive immune response and influenced by stress, infection, and inflammation. In this study, WBCs, granulocytes, and monocytes gradually increased from normoglycemic subjects to subjects with diabetes, whereas the lymphocyte concentration immediately increased with the subclinical disease and was stable despite the disease progression. Interestingly, in the model adjusted for CVRFs and diseases, granulocytes and monocytes showed a stronger association for diabetes than prediabetes compared with normoglycemia in the sense of a dose relationship. Of note, neopterin, a product of interferon-γ–activated monocytes/macrophages and a sensitive indicator of cell-mediated immune activation (31), was inversely associated with the subclinical disease in the multivariable linear regression model but positively associated with the prevalence of the established disease. The reason for this is still unknown.

Moreover, WBCs, monocytes, and granulocytes also increased with disease severity, i.e., there was a gradient from individuals with insulin-dependent diabetes to noninsulin-dependent diabetes to dietary or no treatment. Thereby, >90% of the granulocytes are neutrophils, which play an important role in the early stages of inflammatory responses (32). A recent publication in mice presented evidence that neutrophils are involved in insulin resistance through a secreted elastase (32). This elastase appears to cause a reduction of insulin signaling, enhancement of glucose production, and derangement of the lipid metabolism, all of them contributing to an increased cellular insulin resistance (32). Further, granulocyte aggregation is enhanced in subjects with diabetes influenced by the intense metabolic disturbances (33). Activated granulocytes can also trigger vascular damage, which might contribute to the development of atherosclerosis (33).

In addition to cytokines and immune cells, acute-phase proteins were elevated as well in this study. CRP is one of the best-investigated epidemiological biomarkers for prediabetes, diabetes, and type 2 diabetes–associated CVD (8,14,17,34) and plays a crucial role in natural host defense (11). Immunoregulatory functions of CRP include enhancement of leukocyte reactivity, complement fixation, and modulation of platelet activation (11,35,36). In the current study, CRP was only weakly associated with prediabetes and diabetes prevalence after adjusting for other CVRFs and comorbidities, although CRP was the marker with the highest relative change in these individuals. CRP strongly increased from normoglycemic subjects to subjects with prediabetes (1.4 vs. 2.3 mg/L), whereas only a small increase was observed between subjects with prediabetes and diabetes (2.3 vs. 2.4 mg/L), reflecting a very early activation of the immune system. Fibrinogen, another acute-phase protein participating in the systemic response to inflammation, contributes to blood viscosity, platelet aggregation, and fibrin formation; modulates coagulation activation and fibrinolysis; and may enhance plaque progression (37,38). Higher concentrations of fibrinogen in subjects with diabetes can therefore possibly contribute to atherosclerosis in the advanced stage of disease. Similar to CRP, fibrinogen strongly increased in subjects with normal HbA_{1c} concentrations to subjects with subclinical disease (315 vs. 345 mg/L) and only weakly increased from subclinical to clinical disease (345 vs. 359 mg/L). In contrast to CRP, it was strongly associated with prediabetes and diabetes independently from CVRF and CVD and may therefore point to an important role in the pathophysiology of type 2 diabetes.

Inflammatory processes are also determinants for the presence of diabetes complications (15). Rectified associations were found in the present work between elevated CRP/lymphocyte concentrations and neuropathy and WBC count/granulocytes and retinopathy, whereas lower hematocrit levels were correlated with proteinuria. Lower levels of hemoglobin, hematocrit, and erythrocytes have been reported to be associated with impaired glomerular filtration rate in patients with type 1 diabetes in the absence of nephropathy compared with subjects with normal glomerular filtration rate (39). Recently, hematocrit, serum urea concentration, and sex have been found useful in evaluating renal function in patients with diabetic nephropathy as part of the HUGE formula (40). The current data confirm the association of hematocrit level with renal function and indicate

an independent association of subclinical inflammation with the development of diabetes complications.

Limitations of this study are as follows: *1*) data on diabetes and CVD and disease-associated complications were assessed based on medical diagnosis recorded from the interview with study participants and/or medical records, implying a potential misclassification; *2*) 384 subjects were newly diagnosed as having diabetes within the baseline examination by elevated HbA_{1c} (≥6.5% [≥48 mmol/mol hemoglobin]) concentrations (these subjects were classified as having type 2 diabetes, which is the most probable type of disease in higher age, without additional tests to confirm the diagnosis); *3*) the time between diabetes diagnosis and time of examination as surrogate for disease duration might include a potential misclassification; and *4*) this investigation provides only an indirect estimate for the dynamics of the inflammatory and immune system in diabetes as the association is presented for representative subgroups of individuals with normoglycemia, prediabetes, and diabetes. This study cannot report a causal interrelation between diabetes, inflammation, and immunity.

In summary, we demonstrated the variation of the inflammatory and immune biomarker profile with the development and progression of type 2 diabetes in a large and population-representative sample including 15,010 subjects. The biomarkers of inflammation and immunity show varying dynamics with the advancing disease, enabling differentiation of the early preclinical and clinical phases of the disease (i.e., prediabetes), diabetes complications, and disease progression by intensity of medical treatment.

Funding. The GHS is funded through the government of Rhineland-Palatinate (Stiftung Rheinland-Pfalz für Innovation, contract AZ 961-386261/733), the research programs Wissen schafft Zukunft and Center for Translational Vascular Biology of the Johannes Gutenberg-University of Mainz, and its contract with Boehringer Ingelheim and Philips Medical Systems, including an unrestricted grant for the GHS. V.G., M.P.-N., and P.S.W. are funded by the Federal Ministry of Education and Research (BMBF 01EO1003). S.B., T.Z., T.M., and P.S.W. are principal investigators of the German Center for Cardiovascular Research.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. V.G. and P.S.W. conceived, designed, and performed the experiments; analyzed the data; wrote the manuscript; and provided results discussion and critical review. V.H.S., M.P.-N., R.B.S., T.G.J.A., R.L., and S.B. conceived and designed the experiments. T.Z. and K.J.L. conceived, designed, and performed the experiments. A.S. and D.L.-R. analyzed the data. C.J. conceived and designed the experiments, analyzed the data, and provided results discussion and critical review. J.W. and E.S. provided results discussion and critical review. T.M. conceived and designed the experiments and provided results discussion and critical review. The authors are responsible for the contents of this publication. P.S.W. 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.

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