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Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984–2002

Abstract *Aims/hypothesis:* The chemokines monocyte chemoattractant protein-1 (MCP-1), IL-8 and interferon-γ-inducible protein-10 (IP-10) are released by adipocytes and appear to be involved in atherosclerosis. We hypothesised that these chemokines may be risk factors for the development of type 2 diabetes. *Subjects and methods:* Using a case-cohort design based on data from the population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Kooperative Gesundheitsforschung in der Region Augsburg/Cooperative Health Research in the Region of Augsburg (KORA Augsburg) study, chemokine levels at baseline were analysed in 526 individuals with and 1,695 individuals without incident type 2 diabetes. The mean follow-up time was 10.8 years. *Results:* MCP-1 was associated with type 2 diabetes, largely independently of classic risk factors, whereas various clinical and metabolic parameters as well as lifestyle factors were major confounders of the association of IL-8 and IP-10 with type 2 diabetes. Further adjustment for C-reactive protein (CRP) and IL-6 had no impact on the observed associations. The hazard ratio (HR) for subjects with systemic concentrations of all three chemokines (MCP-1, IL-8 and IP-10) above the respective median compared with those with all chemokines below or equal to the median was 1.79 (95% CI 1.18–2.72) and was comparable with the HR for elevated CRP and IL-6 together (adjusted for age, sex, survey, BMI, systolic blood pressure, total cholesterol:HDL cholesterol ratio, physical activity, alcohol intake, smoking and parental history of diabetes). *Conclusions/interpretation:* Elevated concentrations of MCP-1, IL-8 and IP-10 are associated with incident type 2 diabetes. Whereas the association of IL-8 and IP-10 with diabetes was attenuated by multivariable adjustment, high MCP-1 levels contributed to diabetes risk independently of previously described clinical, metabolic and immunological risk factors.

Keywords Chemokines · Diabetes · Inflammation · Interferon-γ-inducible protein-10 · Interleukin-8 · Monocyte chemoattractant protein-1

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Introduction

In recent years, prospective studies have demonstrated that chronic, low-grade inflammation and activation of the innate immune system is not merely a consequence of the metabolic abnormalities of type 2 diabetes, but is present years before diabetes manifestation and therefore a genuine independent risk factor for the disease [1, 2]. The type 2 diabetes-related immune activation includes upregulation of multiple acute phase proteins, cytokines and soluble adhesion molecules [3–7]. However, studies addressing the

association of several immune mediators with type 2 diabetes in the same population indicate that there is no general upregulation of systemic immune mediator expression, but rather a specific and differential activation [5, 7]. Data on IL-10 suggest that elevated expression of this cytokine in peripheral blood might even be protective against type 2 diabetes [8].

Despite the number of studies addressing this topic, the mechanistic link between immune activation and development of type 2 diabetes is still insufficiently understood, and its elucidation might require a more detailed understanding of the quality of type 2 diabetes-related inflammation. In this respect, it is remarkable that there are no data available from prospective studies on the potential role of chemokines. Members of this class of low molecular weight proteins play key roles in inflammatory processes by regulating migration and activation of many cell types [9]. Monocyte chemoattractant protein-1 (MCP-1), IL-8 and interferon-γ-induced protein-10 (IP-10) are expressed and secreted by adipocytes [10, 11 and unpublished results] and have also been reported to be involved in atherosclerosis and obesity in animal models as well as in cross-sectional clinical studies [12]. It has been postulated that chemokine expression might be an important step in the recruitment and activation of peripheral blood leucocytes in atherosclerotic lesions [12] and adipose tissue [13]. In particular elevated levels of MCP-1 and IL-8 may be associated with type 2 diabetes [14–20].

We therefore analysed serum samples from a large population-based cohort study with a mean follow-up time of more than 10 years using a case-cohort design. In addition to the characterisation of their individual contribution to type 2 diabetes risk, we aimed to investigate whether there is a joint effect of MCP-1, IL-8 and IP-10 on the risk of incident type 2 diabetes and whether the effects are independent of obesity and of other markers of inflammation (C-reactive protein [CRP] and IL-6) which have previously been shown to be associated with future type 2 diabetes.

Subjects and methods

Design of case-cohort study We designed a prospective case-cohort study [21] within the population-based Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA)/Kooperative Gesundheitsforschung in der Region Augsburg/Cooperative Health Research in the Region of Augsburg (KORA Augsburg) cohort study between 1984 and 2002. The MONICA Augsburg project was part of the multinational WHO MONICA project to estimate the prevalence and distribution of cardiovascular risk factors [22]. Three independent cross-sectional population-based surveys were performed in 1984/85 (Survey 1 [S1]), 1989/90 (Survey 2 [S2]) and 1994/95 (Survey 3 [S3]) in the city of Augsburg (Germany) and two adjacent counties. The study was approved by the local authorities, and all participants provided written informed consent. The total number of persons participating in at least one of the three surveys was 13,427 (6,725 men and 6,702 women) in the age range of 25–64 (S1) or 25–74 years (S2, S3). All subjects were prospectively followed within the KORA research frame.

Due to the low incidence of type 2 diabetes under 35 years, the present study was limited to 10,718 persons (5,382 men and 5,336 women) between 35 and 74 years at baseline. After exclusion of 1,187 subjects with missing blood samples, 509 participants with self-reported prevalent diabetes, 14 subjects with incident diabetes other than type 2 diabetes (e.g. type 1 or secondary diabetes), 30 subjects with self-reported incident diabetes where the diagnosis could not be validated, 988 subjects without follow-up information and 54 subjects with a follow-up time of less than 1 year, the source population for the present study comprised 7,936 subjects (3,894 men and 4,042 women).

For the case-cohort study a random sample of the source population, called here the subcohort, containing 1,885 subjects (1,018 men, 867 women) was selected stratifying by sex and survey. Participants with missing values for chemokines or any of the covariables used in the present analysis were excluded leading to a subcohort of 1,815 subjects (960 men, 855 women). The final stratum-specific sample sizes of this subcohort were used together with the stratum-specific sizes of the source population (n=7,936) to compute sampling fractions, and the inverse of the sampling fractions yielded the survey- and sex-specific sampling weights. The number of cases of incident type 2 diabetes until 31 December 2002 was 555 (329 men, 226 women). For 305 men and 221 women complete information on all relevant variables used in the present analysis was available. Since 74 male and 46 female cases were also part of the subcohort, the present analysis comprised a total of 2,221 participants (305 men and 221 women with incident type 2 diabetes, 886 men and 809 women without incident type 2 diabetes).

The incidence of type 2 diabetes was assessed using a written follow-up questionnaire sent to all participants of the three baseline surveys in 1997/1998 and in 2002/2003 as described previously [6]. Furthermore, all subjects who participated in S1 were invited to a follow-up examination in 1987/1988. Cases with self-reported incident diabetes were validated by a questionnaire mailed to the treating physician or by medical chart review. Only subjects for whom the treating physician clearly reported a diagnosis of type 2 diabetes or for whom a diagnosis of type 2 diabetes was mentioned in the medical records or who were taking glucose-lowering medication were classified as cases for the present analysis. The mean follow-up time (±SD) for the study population was 10.8 (±5.1) years and ranged from 1.0 to 18.2 years.

Data collection and laboratory measurements Collection of information on socio-demographic variables, smoking habits, leisure time physical activity level, alcohol consumption and parental history of diabetes and details of the standardised medical examinations including collection of a non-fasting venous blood sample have been described

extensively elsewhere [6, 23–25]. Total serum cholesterol (TC) and HDL cholesterol were measured by enzymatic methods (CHOD-PAP; Boehringer Mannheim, Germany). HDL cholesterol was precipitated with phosphotungstic acid and magnesium ions.

Blood samples were stored at −80°C prior to analysis. Serum levels of MCP-1, IL-8 and IP-10 were measured by Luminex multiplex technology using a Luminex 100 analyser (Luminex Corporation, Austin, TX, USA) based on a previously published protocol [26]. Fluorescent xMAP COOH microspheres were purchased from Luminex Corporation. Recombinant proteins were obtained from R&D Systems (Wiesbaden, Germany; MCP-1), the National Institute for Biological Standards and Controls (Potters Bar, UK; IL-8) and BD Biosciences (Heidelberg,

Germany; IP-10). Antibody pairs were purchased from R&D Systems (MCP-1, IL-8) and BD Biosciences (IP-10). CRP concentrations were measured using a high-sensitivity immunoradiometric assay (S1: men aged 45–64; S3) [27] or a high-sensitivity latex enhanced nephelometric assay on a BN II analyser (S1: men aged 35–44 and all women; S2) (Dade Behring, Marburg, Germany). Both methods gave similar results when identical samples were analysed [28]. Serum levels of IL-6 were determined by ELISA [5]. The intra- and interassay CV values of quality control test sera were as follows: MCP-1, <10 and 19.9%; IL-8, <10 and 10.9%; IP-10, <10 and 35.1%; CRP-IRMA, 4.0 and 12.0%; CRP nephelometric assay, 2.5 and 5.1%; IL-6, <10 and <10%. Cross-reactivity in the Luminex assay for the included analytes was <1%. All chemokines were stable

Table 1 Baseline demographic, clinical, immunological and lifestyle characteristics of the study participants by diabetes status at follow-up: MONICA/KORA Augsburg case-cohort study 1984–2002 (n=2,221) (see also [31])

Variable	Subjects with incident type 2 diabetes during follow-up	Subjects without incident type 2 diabetes during follow-up	p value[b]
Demographic			
Number (women/men)	221/305	809/886	–
Age (years)	56.1 (0.4)	51.7 (0.3)	<0.001
Education<12 years	82.3	75.1	<0.001
Clinical			
BMI (kg/m²)	30.2 (0.2)	26.7 (0.1)	<0.001
WHR[c]	0.925 (0.004)	0.865 (0.003)	<0.001
History of actual hypertension[d]	67.5	39.6	<0.001
History of myocardial infarction	5.1	2.1	0.003
Total:HDL cholesterol ratio	5.6 (0.1)	4.5 (0.04)	<0.001
Parental history of diabetes			<0.001
Positive	28.1	19.8	
Unknown	26.8	20.5	
Negative	45.1	59.7	
Immunological			
MCP-1 (pg/ml)[a]	211.1 (1.04)	178.8 (1.02)	<0.001
IL-8 (pg/ml)[a]	8.1 (1.03)	7.1 (1.02)	<0.001
IP-10 (pg/ml)[a]	247.7 (1.03)	215.2 (1.02)	<0.001
CRP (mg/l)[a]	2.6 (1.04)	1.4 (1.03)	<0.001
IL-6 (pg/ml)[a]	3.0 (1.04)	2.0 (1.03)	<0.001
Lifestyle			
Smoking status			0.023
Current smoker	27.0	24.1	
Former smoker	31.7	27.8	
Never smoker	41.3	48.1	
Frequency of exercise			<0.001
Active	30.2	40.6	
Inactive	69.8	59.4	
Alcohol consumption (g/day)			0.138
0	33.3	29.0	
>0–39.9 (men)/19.9 (women)	39.7	44.1	
≥40 (men)/20 (women)	27.0	26.9	
Survey			<0.001
S1	36.3	30.9	
S2	40.7	36.4	
S3	23.0	32.8	

Data are weighted percentages for categorical variables, weighted means (SEs) for normally distributed continuous variables
[a] Weighted geometric means with (antilog of standard errors of log means) for skewed continuous variables
[b] t-test for continuous variables and χ^2 test for categorical variables
[c] Only measured in participants of S2 and S3 (cases: n=332; non-cases: n=1,027)
[d] Defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or use of antihypertensive medication given that subjects were aware that they had hypertension

during long-term storage as there was no indication for lower concentrations in serum samples of S1 compared with S2 or S3 (data not shown).

Statistical analysis Means or proportions for baseline demographic and clinical characteristics were computed using the SAS procedures SURVEYREG [29] or SUR-VEYFREQ [30], which estimated SEs appropriate to the sampling scheme. Tests of differences between subjects with and without incident type 2 diabetes were based on these procedures. In the case of non-normality, tests were carried out with log-transformed variables and results were presented as geometric means with antilogs of SEs of the adjusted log means.

Weighted Pearson correlation coefficients (r) were used to describe univariate associations between markers of inflammation and continuous risk factors for diabetes and p values were obtained from weighted regression models using the SAS procedure SURVEYREG.

Cox proportional hazards analysis was used to assess the association between chemokines and incident type 2 diabetes. Due to the case-cohort design, SEs were corrected using an SAS macro with a 'sampling weight' approach developed by Barlow [21]. As in all cohort studies, the mean follow-up time for cases was shorter than for non-cases, but this difference is accounted for in the model. The weighted chemokine quartiles in the subcohort were used to classify subjects in different risk groups. In multivariable analyses we adjusted for the continuous variables age, BMI, systolic blood pressure, total:HDL cholesterol ratio, CRP, IL-6 and the categorical variables sex, survey, smoking status (never smoker, former smoker, current smoker), alcohol consumption (men 0, 0.1–39.9, ≥40 g/day; women 0, 0.1–19.9, ≥20 g/day), physical activity (inactive vs active, i.e. regular physical activity of ≥1 h/week in both summer and winter), and parental history of diabetes (negative, positive, unknown).

Moreover, Cox models were calculated to assess the joint effects of the chemokines with or without CRP and IL-6 on type 2 diabetes risk. Results are presented for each chemokine quartile (coded as dummy variables with the first quartile as the reference category) as hazard ratios (HRs) and 95% CIs. p values are based on robust variance estimates using the Barlow macro. For test for trends, median values within each quartile were assigned to the respective quartile. For all statistical analyses a p value less than 0.05 was considered to be statistically significant. All evaluations were performed with the statistical software package SAS (version 8.02 for Unix and version 9.1 for Windows; SAS Institute, Cary, NC, USA).

Results

Study population Baseline demographic, clinical, immunological and lifestyle characteristics of the study participants ($n=2,221$) are given in Table 1 (see also [31]). Briefly, individuals who developed type 2 diabetes during the follow-up period (cases) differed significantly from subjects without later onset of type 2 diabetes (non-cases) by higher age, lower level of education, higher frequency of smokers, higher BMI and WHR, higher prevalence of hypertension or previous myocardial infarction, higher total:HDL cholesterol ratio, more frequent parental history of diabetes and less physical activity. Cases exhibited highly significantly elevated systemic concentrations of the chemokines MCP-1, IL-8, IP-10, CRP and IL-6 compared with non-cases ($p<0.001$ for each mediator).

The analysis of the randomly drawn subcohort revealed that baseline concentrations of MCP-1, IL-8 and IP-10 were positively correlated with known type 2 diabetes risk factors like age, BMI and WHR (Table 2), but apart from the correlations of MCP-1 and IL-8 with WHR and of IP-

Table 2 Correlation of inflammatory markers with age and continuous type 2 diabetes risk factors in the randomly sampled subcohort: MONICA/KORA case-cohort study 1984–2002 ($n=1,815$)

	Pearson correlation coefficient[a]		
	Log MCP-1	Log IL-8	Log IP-10
Age	0.083***	0.091***	0.171***
BMI	0.078**	0.065*	0.125***
WHR[b]	0.122***	0.155***	0.091**
Systolic blood pressure	0.073**	0.054*	0.062**
Diastolic blood pressure	0.050*	0.015	0.054*
Total cholesterol	0.018	0.017	−0.023
HDL cholesterol	−0.003	−0.027	−0.079***
Log MCP-1	–	0.286***	0.225***
Log IL-8	0.286***	–	0.258***
Log IP-10	0.225***	0.258***	–
Log IL-6	0.042	0.144***	0.134***
Log CRP	0.075**	0.115***	0.194***

[a] Weighted to reflect the sampling strategy, p values were obtained from weighted regression models
[b] $n=1,081$
*$p<0.05$; **$p<0.01$; ***$p<0.001$

10 with age and BMI, correlation coefficients $r<0.1$ indicated only modest associations. However, associations were stronger between the immunological parameters: MCP-1, IL-8 and IP-10 were highly correlated with each other ($r>0.2$, $p<0.001$ for each correlation), with CRP and with IL-6 (IL-8 and IP-10 only).

Systemic levels of MCP-1, IL-8 and IP-10 and future type 2 diabetes Elevated serum concentrations of MCP-1, IL-8 and IP-10 were closely associated with higher risk for type 2 diabetes (Tables 3, 4 and 5; model 1). We performed a series of statistical adjustments (models 2–4) in order to examine the mechanism through which the three chemokines might be associated with the disease process. In the case of MCP-1 (Table 3), adjustment for baseline imbalances and for clinical, metabolic and lifestyle risk factors of type 2 diabetes (models 2 and 3) attenuated, but did not eliminate the disease association. Adjustment for the classic immunological risk factors CRP and IL-6 did not further attenuate the association of MCP-1 with type 2 diabetes (model 4). In the case of IL-8 (Table 4), the association with type 2 diabetes was weakened by adjustment for age, sex and survey (model 2) and largely lost by additional adjustment for metabolic and lifestyle factors (model 3). The association of IP-10 with type 2 diabetes was lost after adjusting for clinical and metabolic parameters (Table 5, models 2 and 3) probably reflecting the association of systemic IP-10 levels with age and BMI (Table 2).

The data summarised in Tables 3, 4 and 5 were confirmed by several sensitivity analyses. HRs were very similar when all study participants with a follow-up period of ≤3 years ($n=186$; 96 cases and 90 non-cases) or with history of myocardial infarction and/or stroke at baseline and incident myocardial infarction during the observation period ($n=193$; 76 cases and 117 non-cases) were excluded. Hence, undiagnosed cases of type 2 diabetes at baseline or cardiovascular or cerebrovascular comorbidity had most likely no confounding effect on our results. Moreover, we assessed the effect of additional adjustment for WHR in the subgroup with available WHR data ($n=1,359$; 332 cases and 1,027 non-cases) and found virtually no impact on the HRs.

Diabetes risk and combined effects of elevated immune marker concentrations Figure 1 shows the combined effect of elevated chemokine concentrations on type 2 diabetes risk (elevated levels are defined as chemokine concentrations above median level measured in the cohort random sample). When analysing pairs of chemokines and adjusting for multiple variables (model 3, see above), elevated levels of two chemokines in the same individual were always significantly associated with type 2 diabetes risk (Fig. 1a–c), whereas elevations of only one and levels below or equal to the median of the other chemokine were usually not (except from high MCP-1 vs low IP-10; Fig. 1b).

The combined effect is more apparent when MCP-1, IL-8 and IP-10 are considered simultaneously. The adjusted HR (95% CI) (model 3) for elevated levels of all three chemokines in the same subject compared with none above the respective median was 1.79 (1.18–2.72) ($p=0.007$) (Fig. 2a) and thus very similar to the adjusted HR for elevated levels of both CRP and IL-6 compared with neither mediator above the respective median (HR [95% CI] 1.82 [1.31–2.54], $p<0.001$) (Fig. 2b). The combination of MCP-1, IL-8, IP-10, CRP and IL-6 further increased the risk for type 2 diabetes. The adjusted HR (95% CI) for comparing extreme subgroups (all five immune mediators vs none above median) was 3.80 (1.87–7.71) ($p<0.001$) (Fig. 2c).

Discussion

In this population-based case-cohort study of men and women between 35 and 74 years, serum concentrations of MCP-1, IL-8 and IP-10 were significantly higher in participants who developed type 2 diabetes during follow-up compared with those who did not. MCP-1 remained associated with incident type 2 diabetes after multivariable adjustment. It is important to note that the

Table 3 Hazard ratios (HRs) of developing type 2 diabetes (T2D) comparing quartiles of MCP-1 levels ($n=1,191$ men and 1,030 women aged 35–74 years)

	Quartile of MCP-1				p value (trend)
	1	2	3	4	
Median (pg/ml)	76.12	168.12	254.77	399.79	–
Lower–upper limit	2.45–124.22	124.23–205.77	205.78–312.61	312.62–1339.81	–
n (non-T2D/T2D)	411/95	415/104	430/160	439/167	–
HR (95% CI)					
Model 1	1.0	1.12 (0.83–1.51)	1.67 (1.26–2.20)	1.77 (1.34–2.33)	<0.001
Model 2	1.0	1.16 (0.85–1.57)	1.49 (1.12–1.99)	1.53 (1.15–2.04)	0.002
Model 3	1.0	1.27 (0.90–1.81)	1.57 (1.11–2.22)	1.48 (1.05–2.09)	0.024
Model 4	1.0	1.27 (0.90–1.81)	1.57 (1.11–2.21)	1.48 (1.05–2.08)	0.026

Model 1: crude; model 2: adjusted for age, sex and survey; model 3: factors in model 2+BMI, systolic blood pressure, total:HDL cholesterol ratio, physical activity, alcohol intake, smoking status and parental history of diabetes; model 4: factors in model 3+CRP and IL-6

Table 4 Hazard ratios (HRs) of developing type 2 diabetes (T2D) comparing quartiles of IL-8 levels (*n*=1,191 men and 1,030 women aged 35–74 years)

	Quartile of IL-8				*p* value (trend)
	1	2	3	4	
Median (pg/ml)	3.86	6.00	8.17	13.16	–
Lower–upper limit	0.30–5.02	5.03–6.98	6.99–9.85	9.86–645.67	–
n (non-T2D/T2D)	395/90	421/119	425/159	454/158	–
HR (95% CI)					
Model 1	1.0	1.25 (0.92–1.68)	1.60 (1.21–2.13)	1.55 (1.17–2.07)	0.003
Model 2	1.0	1.19 (0.87–1.64)	1.52 (1.12–2.06)	1.44 (1.05–1.96)	0.026
Model 3	1.0	1.04 (0.71–1.52)	1.62 (1.14–2.29)	1.33 (0.93–1.90)	0.075
Model 4	1.0	1.04 (0.71–1.52)	1.61 (1.13–2.18)	1.32 (0.92–1.90)	0.082

Model 1: crude; model 2: adjusted for age, sex and survey; model 3: factors in model 2+BMI, systolic blood pressure, total:HDL cholesterol ratio, physical activity, alcohol intake, smoking status and parental history of diabetes; model 4: factors in model 3+CRP and IL-6

associations between chemokines and incident type 2 diabetes were independent of the inflammatory status as characterised by CRP and IL-6. To the best of our knowledge, the impact of baseline levels of MCP-1, IL-8 or IP-10 on type 2 diabetes incidence has not been reported in previous epidemiological studies. Data from several studies linked MCP-1 and IL-8 to type 2 diabetes, but in particular data on IL-8 were based on rather small case-control studies [14, 16]. Increased MCP-1 concentrations in type 2 diabetes patients were reported in a small Japanese case-control study [15], in elderly women selected from a population survey in Italy [17], in patients with prevalent cardiovascular disease [18, 20] and in the population-based Dallas Heart Study [19]. Since the association of elevated MCP-1 levels and prevalent type 2 diabetes was not seen in the Dallas Heart Study when all individuals with subclinical atherosclerosis were excluded and was also absent in the cross-sectional KORA Survey 2000 [32], it remained unclear whether there was a link between MCP-1 and type 2 diabetes and how this relationship might be modulated by the concomitant presence of cardiovascular disease. In order to minimise the confounding effect of cardiovascular and cerebrovascular conditions, we repeated our analyses without participants with a history of myocardial infarction and/or

stroke at baseline and incident myocardial infarction during the observation period. Since HRs were hardly altered, our data confirm the hypothesis that MCP-1 can be considered as risk factor for future type 2 diabetes. Since no data are available from long-term studies with multiple measurements of MCP-1, it is not known whether systemic concentrations of MCP-1 are elevated at all stages of type 2 diabetes development and during manifestation of diabetic complications and whether longitudinal variations could explain the discrepancies seen in cross-sectional and case-control studies.

The association of IL-8 with type 2 diabetes showed different characteristics from that of MCP-1. Although some of the disease association was accounted for by the correlation of IL-8 levels with parameters of obesity, it was only lost when adjusting for additional factors including physical activity, alcohol intake and smoking. A current cross-sectional analysis of a recently examined population-based sample in the same region confirms the association of lifestyle factors with systemic IL-8 levels (unpublished results). Again different is the nature of the association of IP-10 with type 2 diabetes. Here, the correlations of systemic levels with age and BMI represent important factors.

Table 5 Hazard ratios (HRs) of developing type 2 diabetes (T2D) comparing quartiles of IP-10 levels (*n*=1,191 men and 1,030 women aged 35–74 years)

	Quartile of IP-10				*p* value (trend)
	1	2	3	4	
Median (pg/ml)	99.57	179.96	284.10	499.66	–
Lower–upper limit	9.75–142.09	142.10–221.67	221.68–352.94	352.95–5100.24	–
n (non-T2D/T2D)	436/100	409/112	419/162	431/152	–
HR (95% CI)					
Model 1	1.0	1.17 (0.87–1.57)	1.64 (1.25–2.17)	1.58 (1.19–2.09)	<0.001
Model 2	1.0	1.10 (0.81–1.50)	1.48 (1.11–1.97)	1.28 (0.95–1.72)	0.095
Model 3	1.0	0.87 (0.61–1.24)	1.10 (0.80–1.53)	1.03 (0.74–1.44)	0.539
Model 4	1.0	0.87 (0.61–1.24)	1.10 (0.80–1.53)	1.03 (0.74–1.45)	0.539

Model 1: crude; model 2: adjusted for age, sex and survey; model 3: factors in model 2+BMI, systolic blood pressure, total:HDL cholesterol ratio, physical activity, alcohol intake, smoking status and parental history of diabetes; model 4: factors in model 3+CRP and IL-6
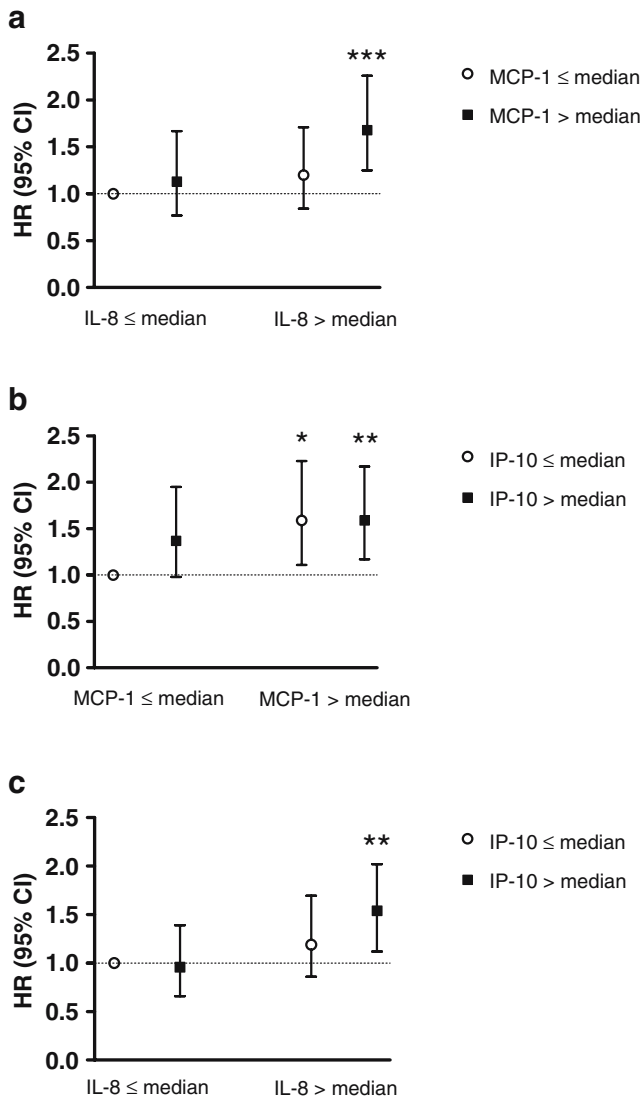
Fig. 1 Combined effect of elevated serum concentrations of pairs of MCP-1, IL-8 and IP-10 on type 2 diabetes risk: IL-8 and MCP-1 (**a**), MCP-1 and IP-10 (**b**), and IL-8 and IP-10 (**c**). Variables were stratified based on weighted median cut-off points for each chemokine using the distribution in the randomly drawn subcohort; median values were 205.8 pg/ml for MCP-1, 7.0 pg/ml for IL-8 and 221.7 pg/ml for IP-10. Hazard ratios (*HRs*) with 95% CIs were obtained after adjustment for age, sex, survey, BMI, systolic blood pressure, total:HDL cholesterol ratio, physical activity, alcohol intake, smoking status and parental history of diabetes. *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs the reference group (all immune mediator concentrations below or equal to median)

Regarding the relevance for the pathogenesis of type 2 diabetes, the present study shows that although the chemokines tested here can all be released by adipocytes, chemokine levels and BMI or WHR were only moderately correlated. In addition, the HRs shown for the joint models were obtained after multiple adjustment, which included BMI as an index of obesity. Thus, cells outside the adipose tissue or altered macrophage number and activity within the adipose tissue may contribute to the elevated circulating chemokine levels which indicate type 2 diabetes risk.
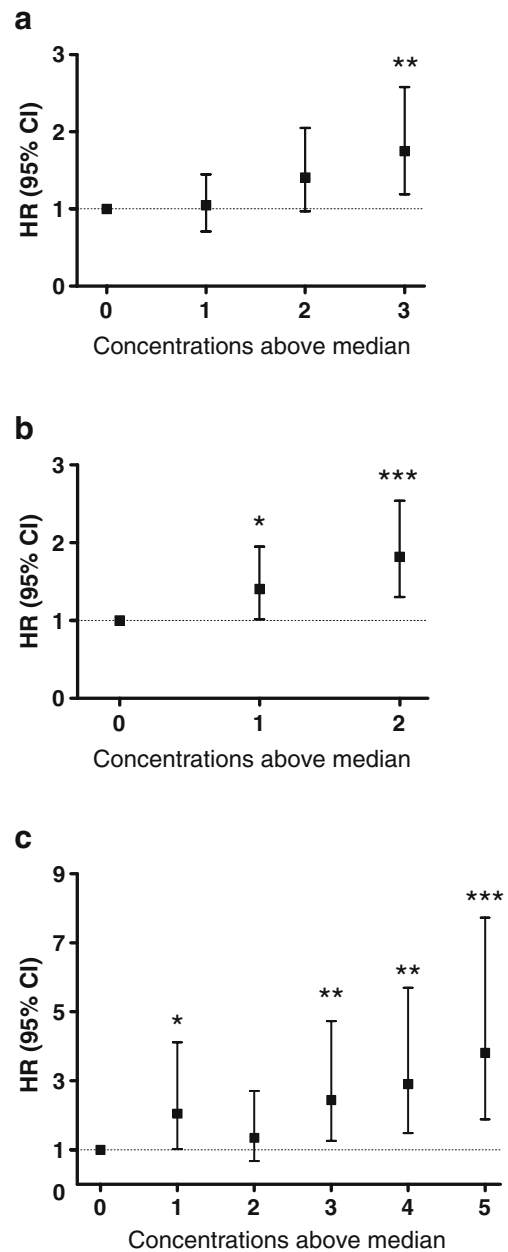
Fig. 2 Combined effect of elevated serum concentrations of MCP-1, IL-8, IP-10, CRP and IL-6 on type 2 diabetes risk: MCP-1, IL-8 and IP-10 (**a**), CRP and IL-6 (**b**), and MCP-1, IL-8, IP-10, CRP and IL-6 (**c**) Variables were stratified based on weighted median cut-off points for each mediator using the distribution in the randomly drawn subcohort; median values were 205.8 pg/ml for MCP-1, 7.0 pg/ml for IL-8, 221.7 pg/ml for IP-10, 1.4 mg/l for CRP and 2.3 pg/ml for IL-6. The numbers along the *x*-axes give the number of immune mediators in one individual whose concentrations are above the median cut-off point in the subcohort for the respective protein. Hazard ratios (*HRs*) with 95% CIs were obtained after adjustment for age, sex, survey, BMI, systolic blood pressure, total:HDL cholesterol ratio, physical activity, alcohol intake, smoking status and parental history of diabetes. *$p<0.05$; **$p<0.01$; ***$p<0.001$ vs the reference group (all immune mediator concentrations below or equal to median)

The study also demonstrates that the systemic activation of the innate immune system is a risk factor for the disease and that the association of the chemokines tested here was not confounded by CRP and IL-6. Although the individual association of the investigated chemokines with the disease risk appeared to be heterogeneous, the relevance of MCP-1 and, probably to a lesser extent of IL-8 and IP-10, was apparent by the fact that simultaneous assessment of all five immune mediators in one adjusted model yielded an increased HR (95% CI) for incident type 2 diabetes of 3.80 (1.87–7.71). Although our finding has to be interpreted with caution due to the low number of cases in the reference group, it extends data from previous studies, which have shown that the combined analysis of CRP and IL-6 [3] or of IL-1 and IL-6 [33] strengthened the association with incident type 2 diabetes.

An interesting finding of our study was the fact that chemokine concentrations above the median were associated with elevated disease risk, although there was no clear dose-response relationship. Currently, it is not possible to predict whether a linear dose-response effect or a plateau effect should be expected. We found a similar plateau-like association of incident type 2 diabetes with CRP levels in the same population as studied here [6]. This aspect was considered in the composite models that link diabetes risk with the number of immunological markers which are elevated in an individual. These models are based on a stratification according to the median so that they do not require a linear dose-response relationship of the single immune markers.

Regarding limitations of our study, it needs to be mentioned that at baseline and during follow-up, diabetes diagnosis relied on self-reported cases and not on OGTTs, so that there were probably undetected cases at both occasions. On the one hand, missing cases would most likely attenuate HRs, lead to an underestimation of the effect of risk factors and would not be causative for the significant associations that were found. In order to face the problem of misclassification at baseline, analyses were repeated after exclusion of all cases which were reported within 3 years of the baseline examination, but this had virtually no impact on the results. On the other hand, it is possible that MCP-1, IL-8 and IP-10 may be correlated with fasting glucose or insulin resistance so that adjustment for these potential confounders might have further weakened the association of the chemokines with diabetes. In order to estimate the severity of this problem, we analysed fasting concentrations of MCP-1, IL-8 and IP-10 in both normoglycaemic controls and subjects with IGT in the KORA Survey 2000, but found no statistically significant differences between the two groups [32–34].

Furthermore, we cannot exclude that BMI was not the most suitable index of obesity in our models. Since MCP-1, IL-8 and IP-10 are also produced by adipose tissue, WHR or data from bioimpedance analysis such as total fat mass might have been more informative. As these data were only available for different subsets of participants (S2 and S3 for WHR, S3 for bioimpedance), sensitivity analyses were performed using the WHR data, whereas even smaller samples sizes would have too limited power for further subanalyses. In addition, interassay CV values for IP-10 were relatively high, which might have attenuated the association between IP-10 and incident type 2 diabetes.

The present study has the advantage of being population-based with a prospective design and a long follow-up (average of 10.8 years). The study includes a large number of incident cases and appropriate controls, for whom a wide range of data on multiple metabolic and inflammatory type 2 diabetes risk factors are available. Finally, information on cardiovascular and cerebrovascular events supports the validity of the analysis since confounding effects of these conditions can be addressed by exclusion of afflicted participants.

Taken together, this study demonstrates that systemic MCP-1 concentrations were significantly associated with future type 2 diabetes in the MONICA/KORA cohort after multivariable adjustment. The association was independent of CRP and IL-6 levels. The strength of the association for the simultaneous elevation (above median) of MCP-1, IL-8 and IP-10 with type 2 diabetes was similar to the effect of combined elevation of CRP and IL-6 compared with the corresponding control groups without elevated immune mediator concentrations and could be further increased by combining all five immune mediators in a single model.

References

1. Pickup JC (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. Diabetes Care 27: 813–823
2. Kolb H, Mandrup-Poulsen T (2005) An immune origin of type 2 diabetes? Diabetologia 48:1038–1050
3. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286:327–334

4. Festa A, D'Agostino R, Tracey RP, Haffner SM (2002) Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the Insulin Resistance Atherosclerosis Study. Diabetes 51:1131–1137

5. Müller S, Martin S, Koenig W et al (2002) Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia 45:805–812

6. Thorand B, Löwel H, Schneider A et al (2003) C-reactive protein as a predictor for incident diabetes mellitus among middle-aged men: results from the MONICA Augsburg cohort study, 1984–1998. Arch Intern Med 163:93–99

7. Meigs JB, Hu FB, Rifai N, Manson JE (2004) Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA 291:1978–1986

8. van Exel E, Gussekloo J, de Craen AJ, Frölich M, Bootsma-Van Der Wiel A, Westendorp RG (2002) Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study. Diabetes 51:1088–1092

9. Gerard C, Rollins BJ (2001) Chemokines and disease. Nat Immunol 2:108–115

10. Gerhardt CC, Romero IA, Cancello R, Camoin L, Strosberg AD (2001) Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. Mol Cell Endocrinol 175:81–92

11. Bruun JM, Pedersen SB, Richelsen B (2001) Regulation of interleukin 8 production and gene expression in human adipose tissue in vitro. J Clin Endocrinol Metab 86:1267–1273

12. Charo IF, Taubman MB (2004) Chemokines in the pathogenesis of vascular disease. Circ Res 95:858–866

13. Wellen KE, Hotamisligil GS (2003) Obesity-induced inflammatory changes in adipose tissue. J Clin Invest 112:1785–1788

14. Zozulinska D, Majchrzak A, Sobieska M, Wiktorowicz K, Wierusz-Wysocka B (1999) Serum interleukin-8 level is increased in diabetic patients. Diabetologia 42:117–118

15. Nomura S, Shouzu A, Omoto S, Nishikawa M, Fukuhara S (2000) Significance of chemokines and activated platelets in patients with diabetes. Clin Exp Immunol 121:437–443

16. Esposito K, Nappo F, Giugliano F et al (2003) Cytokine milieu tends toward inflammation in type 2 diabetes. Diabetes Care 26:1647

17. Piemonti L, Calori G, Mercalli A et al (2003) Fasting plasma leptin, tumor necrosis factor-α receptor 2, and monocyte chemoattracting protein-1 concentration in a population of glucose-tolerant and glucose-intolerant women. Diabetes Care 26:2883–2889

18. de Lemos JA, Morrow DA, Sabatine MS et al (2003) Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 107:690–695

19. Deo R, Khera A, McGuire DK et al (2004) Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. J Am Coll Cardiol 44:1812–1818

20. Simeoni E, Hoffmann MM, Winkelmann BR et al (2004) Association between the A-2518G polymorphism in the monocyte chemoattractant protein-1 gene and insulin resistance and type 2 diabetes mellitus. Diabetologia 47:1574–1580

21. Barlow WE (1994) Robust variance estimation for the case-cohort design. Biometrics 50:1064–1072

22. WHO MONICA Project Principal Investigators (prepared by H. Tunstall-Pedoe) (1988) The World Health Organization MONICA Project (Monitoring of Trends and Determinants in Cardiovascular Disease): a major international collaboration. J Clin Epidemiol 34:105–114

23. Schaeffler V, Döring A, Winkler G, Keil U (1996) Trends in food consumption in a south German population from 1984/85 to 1989/90: results from the WHO MONICA project Augsburg. Ann Nutr Metab 40:129–136

24. Hense HW, Filipiak B, Döring A, Stieber J, Liese A, Keil U (1998) Ten-year trends of cardiovascular risk factors in the MONICA Augsburg Region in Southern Germany. Results from the 1984/85, 1989/90 and 1994/1995 surveys. CVD Prev 1:318–327

25. Meisinger C, Thorand B, Schneider A, Stieber J, Döring A, Löwel H (2002) Sex differences in risk factors for incident type 2 diabetes mellitus—The MONICA Augsburg cohort study. Arch Intern Med 162:82–89

26. de Jager W, te Velthuis H, Prakken BJ, Kuis W, Rijkers GT (2003) Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 10:133–139

27. Hutchinson WL, Koenig W, Fröhlich M, Sund M, Lowe GD, Pepys MB (2000) Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. Clin Chem 46:934–938

28. Khuseyinova N, Imhof A, Trischler G et al (2003) Determination of C-reactive protein: comparison of three high-sensitivity immunoassays. Clin Chem 49:1691–1695

29. SAS Institute Inc (1999) SAS/STAT user's guide, version 8. SAS, Cary, North Carolina, pp 3215–3271

30. SAS Institute Inc (2004) SAS/STAT user's guide, version 9.1. SAS, Cary North Carolina, pp 4183–4239

31. Thorand B, Kolb H, Baumert J et al (2005) Elevated levels of interleukin-18 predict the development of type 2 diabetes—Results from the MONICA/KORA Augsburg Study, 1984–2002. Diabetes 54:2932–2938

32. Herder C, Müller-Scholze S, Rating P et al (2006) Systemic monocyte chemoattractant protein-1 concentrations are independent of type 2 diabetes or parameters of obesity: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Eur J Endocrinol 154:311–317

33. Spranger J, Kroke A, Möhlig M et al (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 52:812–817

34. Herder C, Haastert B, Müller-Scholze S et al (2005) Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Diabetes 54 (Suppl. 2):S11–S17