Chapter 7

Low production capacity of Interleukin-10 is associated with the metabolic syndrome and type-2 diabetes

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ABSTRACT

Background It has been suggested that the metabolic syndrome and type-2 diabetes are manifestations of the inflammatory host response. This host response is orchestrated by the production of pro and anti-inflammatory cytokines that are under genetic control. We therefore hypothesized that a low production capacity of interleukin-10 (IL-10), a centrally operating cytokine with strong anti-inflammatory properties, associates with the metabolic syndrome and type-2 diabetes in old age.

Methods Five hundred-ninety-nine 85-year-old inhabitants of the city of Leiden were visited at their place of residence. Production capacity of the anti-inflammatory cytokine IL-10 was assessed in a whole blood assay whereby lipopolysaccharide was used as a stimulus. Serum concentrations of lipids, lipoproteins, glucose and HbA1c were determined, and a history of type-2 diabetes was obtained.

Results Serum concentrations of total cholesterol, LDL-cholesterol, triglycerides, glucose and HbA1c gradually decreased over strata representing higher IL-10 production capacity, whereas the concentration of HDL-cholesterol gradually increased (all p for trend <0.01). The odds ratio for type-2 diabetes was 2.7 (CI 95% 1.5-4.9) when subjects with the highest IL-10 production capacity were compared to those with the lowest IL-10 production capacity.

Conclusion These findings show that low IL-10 production capacity, i.e. a pro-inflammatory response, is associated with the metabolic syndrome and type-2 diabetes.

Introduction

The metabolic syndrome is a convergence of dyslipidemia, impaired glucose tolerance and hypertension [1]. About 70 percent of all obese adults have at least one of these major characteristics of the syndrome [2]. Since long it has been recognized that clustering of these risk factors carries an increased risk of type-2 diabetes and cardiovascular disease[3,4].

Insulin resistance has been proposed as the underlying cause for this metabolic and cardiovascular syndrome, although it's molecular basis has not yet been identified [3]. One of the biological mechanisms that may be involved is the innate immune system [5,6]. Several studies have shown that markers of inflammation, such as C-reactive protein [7], fibrinogen[8] , and pro-inflammatory cytokines such as Interleukin-6 [9-11] and Tumor Necrosis Factor-α [15-17] associate with the metabolic syndrome, type-2 diabetes and dyslipidemia. In particular, it has been suggested that TNF-α is associated with insulin resistance and type-2 diabetes, since TNF-α down-regulates the tyrosine kinase activity of the insulin receptor [5,12,13]. Infusion of anti TNF-α antibodies in patients with type-2 diabetes, however, had no effect on their insulin sensitivity [14], and raises doubt on the contribution of TNF-α to type-2 diabetes and the metabolic syndrome.

Interleukin-10 (IL-10) is a centrally operating anti-inflammatory cytokine, which plays a crucial role in the regulation of the innate immune system. It has strong de-activating properties on the inflammatory host response mediated by macrophages and lymphocytes, and potently inhibits the production of pro-inflammatory cytokines such as IL-6 and TNF-α [15-17]. IL-10 is produced by T cells, B cells, monocytes and macrophages and is under tight genetic control, with heritability estimates as high as 75 percent [18]. We therefore propose the hypothesis that low IL-10 production capacity is associated with the metabolic syndrome and type-2 diabetes. To this end, in the Leiden 85-plus Study, we have analyzed the relation between IL-10 production capacity, using a standardized whole blood assay, dyslipidemia and parameters of glucose metabolism.

Methods

Subjects

The Leiden 85-plus Study is a population-based study of inhabitants of Leiden, the Netherlands. From 1997-1999, all members of the 1912 to 1914-birth cohort (n=705) were enrolled in the month of their 85th birthday. There were no selection criteria on health or demographic characteristics. Those who were eligible for the study were informed by mail. They were then contacted by telephone, or were visited at home to ask for informed consent. When subjects were cognitively impaired, informed consent was obtained from a guardian. The Medical Ethical Committee of the Leiden University Medical Center approved the study. Subjects were visited three times at their place of residence. At these visits, face-to-face interviews were administered, and an electrocardiogram and body mass index (BMI) was obtained. All blood samples were collected early in the morning under non-fasting conditions. In addition, information on the use of medication was obtained from the subject's pharmacist.

Production capacity of Interleukin-10 and Tumor Necrosis Factor-α

Production capacity of IL-10 and TNF-a were assessed with a standardized whole blood assay [19]. The methods by which whole-blood samples were simulated with 10 ng/ml of lipopolysaccharide have been described elsewhere, including data on reproducibility [19]. In short, heparinised whole blood was diluted 2-fold with RPMI-1640. Lipopolysaccharide (endotoxin, 10 ng/ml) was used as primary stimulus. After addition of lipopolysaccharide, samples were incubated for 4 or 24 hours at 37°C and 5% CO_2. After centrifugation, the supernatants were stored at –80° C until assaying for the pro-inflammatory cytokine TNF-a in the 4-hour samples, and the anti-inflammatory cytokine IL-10 in the 24-hour samples. Production capacity of IL-10 and TNF-α were assayed using standard ELISA techniques. Unstimulated baseline samples were obtained to serve as a control for contamination. Subjects with detectable TNF-α under unstimulated conditions (TNF-α >100 pg/ml) were excluded from further analysis [19,20]. The coefficients of variation for the day-to-day variation in the whole blood stimulation ranged from 8% to 12%. The intra-individual variation was 15% for TNF-α production capacity and 19% for IL-10 production capacity [19].

All subjects were grouped in three equal strata representing decreasing IL-10 production capacity or increasing TNF-α production capacity. This was done separately for women and men, since women have lower IL-10 and TNF-α production capacity than men. The advantage of this stratification is that it intrinsically adjusts for differences in gender.

Lipids and lipoproteins

Total cholesterol and triglycerides levels were analyzed on a fully automated Hitachi 747. High density lipoprotein (HDL) was measured using a Hitachi 911. Low density lipoprotein (LDL) was estimated using the Friedenwald equation [21], whereby five subjects with a triglyceride concentration higher than 5 mmol/l were excluded.

Glucose metabolism and type-2 diabetes

HbA1c and glucose concentration were determined in serum. Subjects were classified as having type-2 diabetes when they met at least one of the following criteria; (1) history of type-2 diabetes obtained from the general practitioner, or the subject's treating physician; (2) use of sulphonylureas, biguanides or insulin, obtained from subject's pharmacist, eight subjects used insulin and were classified as having type-2 diabetes, since they were diagnosed, by there general practitioner, as having diabetes mellitus at a median age of 72 years (range 61 years to 82 years); (3) non fasting glucose of 11.1 mmol/l or higher. Ten subjects, with non fasting glucose of 11.1 mmol/l or higher, were newly diagnosed as having type-2 diabetes. However, these newly diagnosed subjects did not fulfill all the criteria of the American Diabetes Association to diagnose type-2 diabetes, since it was unknown whether these subjects had symptoms of diabetes [22].

Data analysis

The primary outcome measures were the serum concentrations of glucose, HbA1c, lipids and lipoproteins, expressed as means with corresponding 95% CI. We used the one-way ANOVA procedure to determine the p-value for trend over strata of IL-10 production capacity. Univariate odds ratios for type-2 diabetes over strata of IL-10 production capacity and the corresponding 95% confidence intervals were obtained by cross-tabulation. Multivariate odds ratios were obtained by logistic regression analysis. We tested for trend using the log-likelihood statistic with one degree of freedom.

Results

Between 1st September 1997 and 1st September 1999, 705 inhabitants of Leiden reached the age of 85 years and were eligible to participate in the study. Fourteen inhabitants died before they could be enrolled in the study. The response rate was 87 percent, i.e. a total of 599 subjects (397 women, 202 men) participated. There were no statistical significant differences between the 599 participating subjects and the source population with respect to the following characteristics, gender, marital status, socio-economical status, and mortality.

IL-10 production capacity could not be determined in seven subjects as they died before a blood sample could be drawn, while 30 subjects refused to give a blood sample. Under unstimulated conditions, nine subjects had detectable TNF-α concentrations (TNF-α >100 pg/ml) suspect for contamination of the whole blood system and were therefore excluded from the analyses[19,20] . Table 1 shows the demographic and clinical characteristics of the 553 subjects included in the present analysis. Upon stimulation with lipopolysaccharide (LPS) in whole blood samples, the mean IL-10 concentration was 945 pg/ml (CI 95% 861-1030 pg/ml) in men and 799 pg/ml (CI 95% 750-884 pg/ml) in women (Student t-test, p =0.002).

Table 1 *Clinical characteristics of the study sample*

	Subjects (n=553)
Female/Male (n)	370/183
Age (year)	85
Type-2 diabetes	
All	89 (16%)
Use of sulphonylureas or biguanides	49 (9%)
Use of insulin	8 (1%)
BMI (Mean; CI 95%; kg/m^2)	27.2 (26.8-27.7)
Use of non-steroidal anti-inflammatory drugs	152 (28%)

Interleukin-10, lipids, lipoproteins, glucose and HbA1c

We divided subjects in three equal strata dependent on their IL-10 production capacity, for men and women separately, to study the relation between IL-10 production, and lipids, lipoproteins, glucose and HbA1c (Table 2). The serum concentrations of total cholesterol, LDL-cholesterol, triglycerides, glucose and HbA1c gradually decreased over strata representing increasing IL-10 production capacity, whereas the concentration of HDL-cholesterol gradually increased (all p for trend <0.01). There was no association between IL-10 production capacity and BMI. In an additional analysis, we excluded subjects with type-2 diabetes to ascertain that our findings were not only due to the effect of type-2 diabetes on IL-10 production capacity. The trends between IL-10 production capacity, lipids, lipoproteins, glucose and HbA1c remained similar (all p for trend <0.05). The trend between IL-10 production capacity and parameters of the metabolic syndrome could also be distorted by the use of non-steroidal anti-inflammatory drugs that are often prescribed at old age. In a restricted sample of 401 subjects who did not use non-steroidal anti-inflammatory drugs, however, we could still obtain the statistical significant trends as presented in table 2. The trends as shown in table 2 also remained statistical significant when we adjusted for TNF-a using linear regression.

Table 2 *Effect of IL-10 production on lipids, lipoproteins and glucose levels*

	IL-10 production			
	Low (n=184)	Intermediate (n=185)	High (n=184)	p for trend
IL-10 pg/ml	377 (358-396)	763 (746-780)	1402 (1331-1474)	-
BMI (kg/m^2)	27.2 (26.4-27.9)	27.6 (26.8-28.4)	27.0 (26.3-27.6)	0.7
Total cholesterol (mmol/l)	5.95 (5.77-6.12)	5.67 (5.53-5.84)	5.55 (5.41-5.71)	0.001
LDL-cholesterol (mmol/l)	3.86 (3.71-4.02)	3.67 (3.53-3.80)	3.55 (3.42-3.69)	0.002
Triglycerides (mmol/l)	1.77 (1.63-1.91)	1.61 (1.48-1.74)	1.36 (1.28-1.44)	<0.001
HDL-cholesterol (mmol/l)	1.28 (1.21-1.34)	1.29 (1.23-1.34)	1.39 (1.33-1.44)	0.009
Glucose (mmol/l)	7.50 (7.09-7.91)	7.08 (6.68-7.48)	6.31 (6.00-6.61)	<0.001
HbA1c (%)	6.01 (5.83-6.19)	5.82 (5.65-6.00)	5.55 (5.46-5.65)	<0.001

Data are presented as means and 95% confidence intervals. Production capacity of IL-10 as assessed in a whole blood stimulation and were grouped in three equal strata. This was done separately for women and men, since women have lower IL-10 production than men.

In figure 1 we present the mean concentrations of triglycerides and HbA1c over strata of IL-10 production capacity as well as TNF-α production capacity, to determine whether low, intermediate and high TNF-α production capacity had an additional effect on these metabolic outcomes. In each stratum of TNF-α production capacity there was a significant decrease of triglycerides and HbA1c when IL-10 production capacity increased (p for trend in each stratum <0.05). There was no significant increase of triglycerides and HbA1c when TNF-α production increased in any of the strata of IL-10 production capacity (p for trend in each stratum >0.1). Similar trends as shown in figure 1 were obtained fortotal-total-cholesterol, HDL-cholesterol, LDL-cholesterol and glucose (data not shown).

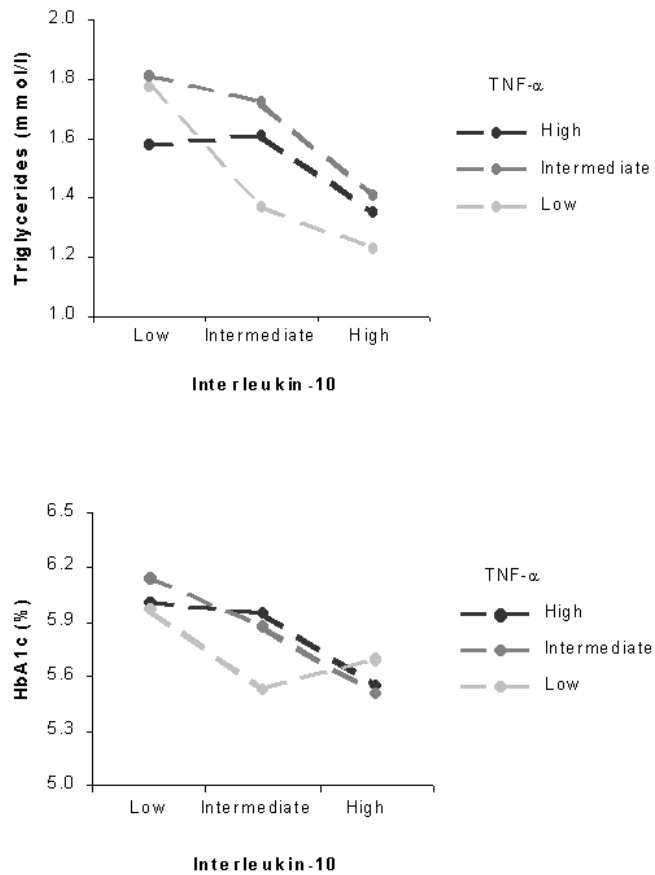

Figure 1 *Mean concentrations of triglycerides and HbA1c in strata of IL-10 and TNF-α. Trends of triglycerides and HbA1c over strata of IL-10 production, p for trend in each stratum <0.05. Trends over strata of TNF-α production, p for trend in each stratum >0.1.*

Interleukin-10 and type-2 diabetes

The proportion of subjects with type-2 diabetes gradually decreased over the strata representing a higher IL-10 production capacity (p for trend =0.001, Table 3). The odds ratio for type-2 diabetes increased to 2.7 (CI 95% 1.5-4.9) when subjects with the highest IL-10 production capacity were compared to those with the lowest IL-10 production capacity. The odds ratio for type-2 diabetes was slightly higher after adjustment for TNF-α. In an additional analysis we excluded the newly diagnosed subjects with a plasma glucose of 11.1 mmol/l or higher (n=10), since it was unknown whether these subjects had symptoms of diabetes and therefore did not fulfill all the criteria of the American Diabetes Association to diagnose diabetes mellitus[22] . The results as shown in table 3 were unaffected.

Table 3 *Odds ratios for type-2 diabetes in relation to IL-10 production*

	IL-10 production			
	Low	Intermediate	High	p for trend
Type-2 diabetes				
Present	42 (23%)	29 (16%)	18 (10%)	0.001
Absent	142 (77%)	156 (84%)	166 (90%)	
Odds ratio				
Crude	2.7 (1.5-4.9)	1.7 (0.9-3.2)	1*	0.001
Adjusted	3.4 (1.6-7.1)	1.9 (1.0-3.6)	1*	0.001

** Reference category. Adjustments for TNF-α production using logistic regression.*

Discussion

This analysis of the Leiden 85-plus Study shows that low IL-10 production capacity, i.e. a pro-inflammatory cytokine response, is associated with high plasma glucose, high HbA1c, type-2 diabetes and dyslipidemia. When production capacity of IL-10 is taken into account, production capacity of TNF-α only adds little to these metabolic parameters.

Possible mechanisms

Pro-inflammatory cytokines have earlier been associated with the development of the metabolic syndrome and type-2 diabetes. Experimental studies in humans and animals show that treatment with pro-inflammatory cytokines induces hypertriglyceridemia and insulin resistance [9,12]. TNF-α down-regulates the tyrosine kinase activity of the insulin receptor, thereby increasing insulin resistance [5,12,13]. Serum IL-6 and C-reactive protein concentrations are higher in subjects with the metabolic syndrome or type-2 diabetes compared to controls[10] . Pro-inflammatory cytokines also contribute to dyslipidemia by increasing lipolysis[7,9]. We feel that IL-10 at least partly represents the effect of an anti-inflammatory response on the metabolic syndrome and type-2 diabetes, since studies on the innate immune system suggest that IL-10 is a key regulator and a powerful suppressor of the immune response[15] . We hypothesize that high IL-10 prevents the development of the metabolic syndrome and type-2 diabetes, by limiting the effects of the inflammatory response, i.e. counter regulating the effects of pro-

inflammatory cytokines such as TNF-α and IL-6. This hypothesis is partly derived from our findings, which suggest that when production capacity of IL-10 is taken into account, production capacity of TNF -α only adds little to markers of the metabolic syndrome, such as triglycerides and HbA1c. High levels of IL-10 should theoretically cause an upregulation of tyrosine kinase activity of the insulin receptor and decrease lipolysis, by counter regulating the effects of TNF-α and IL-6[5,7,9,12,13] . Therefore, a high IL-10 production capacity could confer protection against the metabolic syndrome and type-2 diabetes, while a low IL-10 production capacity would predispose to the metabolic syndrome and type-2 diabetes.

It has been questioned why the characteristics of the metabolic syndrome are so prevalent in humans as these have such deleterious effects later in life. Evolutionary theories on aging can explain for this paradox. Critical in this understanding is that the force of selection decreases with age[23] . Therefore, pleiotropic genes that have beneficial effects early in life are favored by selection even if these genes have deleterious effects later in life[24] . Selection for genes encoding for the metabolic syndrome fit within these theories. Subjects with a pro-inflammatory cytokine response, i.e. a low IL-10 production capacity[18] and hypercholesterolemia[25,26] are relatively protected against infection early in life. In times when infant mortality from infectious disease was high, survivors are likely to have had an innate pro-inflammatory host response. The trade off for this survival benefit is the pro-inflammatory host response predisposing for the metabolic syndrome, type-2 diabetes and the development of atherosclerosis at late age.

Limitations

The cross-sectional nature of the relation between low IL-10 production capacity and the lipid and glucose metabolism is a limitation of our study. It is tempting to speculate that the association between IL-10 production capacity, the metabolic syndrome and type-2 diabetes is causal, since we have previously shown in family studies of first-degree relatives and twins that as much of 75 percent of the variance in IL-10 production capacity in humans derives from genetic factors[18]. Causality between IL-10 production capacity, the metabolic syndrome and type-2 diabetes, however, can only be determined when these associations can be confirmed in, healthy, first degree relatives of subjects with the metabolic syndrome or type-2 diabetes, or using a prospective design in younger subjects, with a long-term follow-up.

A second limitation could be that the blood samples were collected under non-fasting conditions. It is likely that we have thus underestimated the effect of IL-10 on glucose and triglyceride, since it could be argued that the relation between IL-10 production capacity, glucose and triglycerides was diluted by non-differential misclassification, i.e. misclassification of glucose and triglycerides independent on IL-10 production capacity. We found, however, that low IL-10 production capacity was associated with both high glucose and high HbA1c, suggesting that the association between IL-10 production capacity and glucose metabolism is real, since post-prandial changes in HbA1c are absent. Finally, we argue that it is a necessity to determine fasting triglycerides when absolute levels of triglycerides are assessed for

clinical purposes, i.e. to diagnose hypertriglyceridemia in individuals. However, this premise can be somewhat relaxed when we tried to elucidate the relation between inflammation, the metabolic syndrome and type-2 diabetes, in the population at large.

A final question, which could arise, is whether 85 years is a rather late age to study the association between IL-10 production capacity, the metabolic syndrome and type-2 diabetes, since subjects aged 85 years are long-term survivors from a far larger birth cohort. This does not alter the validity of the association between IL-10 production capacity, the metabolic syndrome and type-2 diabetes, as found in the oldest old. However, as with all studies, it would be valuable to demonstrate the same association in different populations, using different study designs and using younger subjects.

Conclusion

We found an association between low IL-10 production capacity, i.e. a pro-inflammatory host response, and high serum glucose, high HbA1c, type-2 diabetes and dyslipidemia. We are not aware of other studies reporting on the effect of IL-10, a strong anti-inflammatory cytokine, on these metabolic parameters. It is a challenge performing further studies that can confirm the hypothesis that low IL-10 production capacity, i.e. a pro-inflammatory response, predisposes to the metabolic syndrome and type-2 diabetes.

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