Elevated levels of the anti-inflammatory interleukin-1 receptor antagonist (IL-1Ra) precede the onset of type 2 diabetes (Whitehall II Study)

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Objective -- Interleukin-1 receptor antagonist (IL-1Ra), a natural inhibitor of IL-1β, improved beta-cell function and glycemic control in patients with type 2 diabetes. The aim of this study was to investigate whether baseline systemic levels of IL-1Ra are associated with incident type 2 diabetes during more than 10 years of follow-up.

Research design and methods – We measured serum IL-1Ra concentrations in a nested case-control study (181 cases; 376 age-, sex- and BMI-matched normoglycemic controls) within the Whitehall II cohort (UK).

Results -- IL-1Ra concentrations were higher in cases ($P=0.0006$) and associated with incident type 2 diabetes (OR[95%CI] for 1-SD increase of IL-1Ra: 1.48 [1.21-1.80]). This association remained significant after adjustment for multiple potential confounders, but was attenuated by adjusting for 2-hr glucose.

Conclusions -- Our findings indicate that individuals who will develop type 2 diabetes are characterized by a complex immune activation that also includes upregulation of the anti-inflammatory cytokine L-1Ra.
Systemic concentrations of several acute-phase proteins, cytokines and chemokines are elevated in individuals who will subsequently develop type 2 diabetes compared to individuals who remain disease-free (1-3). Immune mediators like interleukin (IL)-6 and monocyte chemoattractant protein (MCP)-1 have been shown to interfere with insulin signaling in fat, liver and muscle cells (1,4), while in particular the pro-inflammatory cytokine IL-1β inhibits beta-cell function and promotes beta-cell apoptosis (5). Therefore, low-grade inflammation may contribute to diabetes development both by inducing insulin resistance and reducing insulin secretion.

The importance of IL-1β and IL-1Ra was emphasized by a randomised, double-blind, clinical trial. IL-1Ra improved beta-cell function and glycemic control in patients with type 2 diabetes (6). It is thus tempting to speculate that high circulating IL-1Ra concentrations could indicate decreased risk of type 2 diabetes in a similar way as increased adiponectin levels are associated with lower incidence of type 2 diabetes (7). Systemic IL-1Ra levels are increased in patients with obesity, impaired glucose tolerance and the metabolic syndrome in cross-sectional studies (8-10). Longitudinal data on the relationship between IL-1Ra and the risk of type 2 diabetes are not available. Therefore, the aim of the current study was to investigate whether systemic levels of IL-1Ra are associated with incident T2D in a nested case-control study within the prospective Whitehall II cohort study.

**RESEARCH DESIGN AND METHODS**

We present results from a nested case-control study within the Whitehall II cohort, which was established in 1985 and included 10,308 civil servants aged 35-55 years (11). Study phase 3 (1991-1994) was the first phase where glucose tolerance was assessed by a 75g oral glucose tolerance test (OGTT) and is the baseline for the current study (n=7537). Participants were followed through postal questionnaires at 2.5-year intervals (phases 4-8) and further clinical examinations (including an OGTT) in 1997-1999 (phase 5) and 2003-2004 (phase 7) (12). Individuals without type 2 diabetes at baseline and with incident type 2 diabetes during the follow-up period of 11.5±3.0 years served as cases (n=181). Controls (n=376) with normal glucose tolerance (NGT) at baseline and during follow-up were frequency-matched to cases for age (5-year bands), sex and BMI (5-kg/m² bands).

Further details on selection criteria for this nested case-control study and information on the collection of anthropometric, metabolic, socioeconomic and immunological variables and on statistical analysis are given in the extended Methods section in an online-only appendix which is available at http://care.diabetesjournals.org.

**RESULTS**

Characteristics of cases and controls for this study are shown in Table A1 of the Online-Only Appendix. The comparison of the included and excluded diabetes cases revealed only few significant differences: selected cases were less likely to be women, smokers, more likely to be ex-smokers, and had a marginally lower BMI. Other comparisons between the two groups were not significant (data not shown). Characteristics of controls in our selection mainly reflected the selection criteria (NGT throughout the study; matching for age, sex and BMI to cases): they were slightly older, had a higher BMI, waist circumference (WC) and diastolic blood pressure, but lower fasting and 2-hr glucose compared to the rest of the cohort who were non-diabetic at baseline and follow-up (data not shown).
IL-1Ra concentrations at baseline (median [25th; 75th percentiles]) were higher in cases (232.8 [180.7; 342.2] pg/ml) than in controls (207.6 [159.3; 274.8] pg/ml) ($P=0.0006$). A 1-SD increase of IL-1Ra (157.7 pg/ml) was associated with incident type 2 diabetes in models that adjusted for multiple potential confounders including age, sex, WC, cardiovascular risk factors, socioeconomic status, proinflammatory mediators and fasting glycemia (Table 1). Further inclusion of BMI had virtually no impact on ORs (model 2 + BMI: OR [95%CI]=1.41 [1.13; 1.77]; $P=0.0027$). However, addition of 2-hr glucose led to reduced effects sizes and loss of statistical significance (Table 1).

CONCLUSIONS

Elevated levels of IL-1Ra were associated with increased risk of developing type 2 diabetes in this nested case-control study. We found a slight attenuation of this association when adjusting for WC which is consistent with IL-1Ra production in adipose tissue (13). It is remarkable that the association was stable to adjustment for a range of further potential confounders including fasting glucose and insulin. However, adjustment for 2-hr glucose attenuated the association. This finding could be interpreted in a way that increased IL-1Ra levels are a reaction to, and not cause of, early postprandial hyperglycemia before the onset of diabetes. Studies with measurements of glycemic markers and IL-1Ra at multiple time-points and analysis of their trajectories will be needed to clarify this point.

Our findings expand observations from cross-sectional studies that reported elevated levels of IL-1Ra in the circulation of individuals with obesity and insulin resistance (8-10). Thus, individuals who will develop type 2 diabetes are not only characterized by an upregulation of pro-inflammatory immune mediators (1-3), but also by the upregulation of at least one anti-inflammatory immune marker. Since animal studies and a recent clinical trial indicated that administration of IL-1Ra attenuate subclinical inflammation, support beta-cell function/insulin secretion and may also improve insulin sensitivity (6, 14), it is tempting to speculate that elevated IL-1Ra levels in individuals at risk of type 2 diabetes may be an attempt to counteract the pro-inflammatory effects of IL-1β and to preserve insulin secretion and insulin sensitivity – an effort that eventually fails. However, our data cannot rule out the alternative interpretation that IL-1Ra has additional metabolic effects beyond the inhibition of IL-1β that could lead to insulin resistance and type 2 diabetes.

As potential limitation of the study, it should be mentioned that point estimates and confidence intervals are derived from non-weighted data from a nested case-control study and therefore their statistical inference may be restricted and not the best available estimate within the context of the original cohort. Thus, further studies will be needed to support our hypothesis that individuals with high risk of type 2 diabetes are characterized by the presence of an early compensatory, anti-inflammatory response that precedes the development of the disease. This hypothesis could be tested by the analysis of further, mainly anti-inflammatory immune mediators such as IL-10 or transforming growth factor-β in additional cohorts.

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Table 1. Association between circulating concentrations of IL-1Ra and incident diabetes (OR [95% CI] given for a 1-SD increase of IL-1Ra concentrations)

<table>
<thead>
<tr>
<th>Model</th>
<th>Covariables</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age, sex</td>
<td>1.48</td>
<td>0.0001</td>
</tr>
<tr>
<td>2</td>
<td>Age, sex, waist circumference</td>
<td>1.39</td>
<td>0.0038</td>
</tr>
<tr>
<td>3</td>
<td>Model 2 + cardiovascular risk factors (cholesterol, fasting triglycerides, systolic blood pressure, smoking*, physical activity†, anti-hypertensive medication, lipid-lowering medication)</td>
<td>1.34</td>
<td>0.021</td>
</tr>
<tr>
<td>4</td>
<td>Model 2 + socioeconomic status (employment grade‡)</td>
<td>1.39</td>
<td>0.0059</td>
</tr>
<tr>
<td>5</td>
<td>Model 2 + proinflammatory mediators (CRP, IL-6)</td>
<td>1.35</td>
<td>0.013</td>
</tr>
<tr>
<td>6</td>
<td>Model 2 + fasting glycemia (fasting glucose, fasting insulin)</td>
<td>1.39</td>
<td>0.024</td>
</tr>
<tr>
<td>7</td>
<td>Model 2 + 2-hr glucose</td>
<td>1.24</td>
<td>0.17</td>
</tr>
<tr>
<td>8</td>
<td>Age, sex, waist circumference + all covariables from models 3-6</td>
<td>1.43</td>
<td>0.034</td>
</tr>
<tr>
<td>9</td>
<td>Age, sex, waist circumference + all covariables from models 3-7</td>
<td>1.20</td>
<td>0.32</td>
</tr>
</tbody>
</table>

IL-1Ra, triglycerides, CRP, IL-6 and insulin entered the models as ln-transformed variables.
*Smoking is coded in 3 classes (never smoked, former smoker, current smoker); †physical activity is coded in 3 classes (none/mild, moderate, vigorous); ‡employment grade is coded in 6 classes running from 1 (highest grade) to 6 (lowest grade).