Interleukin-1 receptor antagonist is associated with both lipid metabolism and inflammation in rheumatoid arthritis

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ABSTRACT

Background. *There is a relationship between cardiovascular morbidity, inflammatory activity, and changes in the lipid profile in rheumatoid arthritis (RA), although the mechanisms are not fully elaborated. Recent knowledge that white adipose tissue (WAT) is a producer of immunologically and metabolically active substances gives another perspective to study.*

Objective. *To evaluate the relationship between interleukin-1 receptor antagonist (IL-1Ra) and variables associated with WAT and inflammation in RA.*

Methods. *Anthropometric, inflammatory and metabolic variables were assessed in 23 women with RA and 23 matched controls. Spearman, partial correlation and factor analyses were performed.*

Results. *Inflammatory markers were increased in patients. In both groups, IL-1Ra correlated with leptin independent of age and BMI. IL-1Ra also correlated with haptoglobin and apolipoprotein (Apo) B in patients and with soluble TNF receptor (sTNFR) 1 in controls. In factor analysis, three latent factors were identified among patients. The first loaded on IL-1Ra, leptin, BMI, ApoB and body fat content (BF%), the second loaded on IL1-Ra and sTNF-receptors and the third showed inverse loadings on ApoA-I together with loadings on ESR, haptoglobin, orosomucoid, BF% and BMI.*

Conclusion. *IL-1Ra was associated with markers of inflammation and with fat-related factors in RA patients, suggesting a dualistic relationship of IL-1Ra in RA. IL-1Ra correlated independently with leptin in both patients and controls, indicating a relationship between inflammation and leptin.*

Introduction

A premature development of atherosclerosis and an increased cardiovascular mortality in patients with rheumatoid arthritis (RA) has been reported in a series of studies. In RA patients with active inflammatory disease decreased levels of lipids, cholesterol and triglycerides, but increased levels of lipoprotein (a) have been found compared with reference populations (1, 2). The level of inflammation predicts cardiovascular disease (3), and total cholesterol, low density lipoprotein (LDL) and LDL/high density lipoprotein (HDL) ratio were associated with manifestations of premature atherosclerosis identified by ultrasound measurements, *i.e.*, intima-media wall thickness and plaques, in RA patients (4).

Recent studies have shown white adipose tissue (WAT) to be an important source of interleukin-1 receptor antagonist (IL-1Ra), in obesity and models of inflammation (5). WAT acts not only as storage depot for excessive energy, but also as a producer of cytokines (*e.g.*, TNF, IL-6, IL-1β) and other proteins (*e.g.*, leptin, adiponectin) with ability to affect different metabolic and immunological processes. These WAT-derived adipokines have been suggested to be important links between obesity and atherosclerosis (6).

With this background, the aim of this study was to evaluate the relationship between IL-1Ra and factors associated with WAT and inflammation in patients with RA.

Materials and methods

This cross-sectional study constituted of 23 females with RA [according to the 1987 American College of Rheumatology criteria, (7)] drawn from an inception cohort previously described in full (3). The mean age (± SD) of the patients was 54.0 ± 7.2 years and the disease duration between 20 and 24 years. Controls were 23 age-matched females (mean age (± SD) of 52.3 ± 6.4 years), randomly assembled from within the age cohort of the population register from the same region. The patients were assessed clinically and disease activity score for 28-joint count (DAS28) was calculated (8).

Sampled sera were stored at -80ºC until analysed. Erythrocyte sedimentation rate (ESR, mm/h, whole blood), haptoglobin (g/L) and orosomucoid (g/L) were done by routine methods. Apolipoprotein A-I (ApoA-I, mg/L) and apolipoprotein B (ApoB, mg/L) were measured by an immunological turbidimetric method (DakoCytomation Denmark A/S, Glostrup, Denmark). Leptin (ng/mL) was analysed with double-antibody

radioimmunoassays (Linco Res., St Louis, MO, USA). IL-6 (pg/L), IL-1Ra (pg/L), sTNFR1 (pg/L) and sTNFR2 (pg/L) were analysed by quantitative sandwich enzyme linked immunoassay technique A (R&D Systems Inc., Minneapolis, IL, USA). Body fat content, *i.e.*, percentage of body fat (BF%), and body water content, *i.e.*, percentage of body water (W%) was measured by bioimpedance.

The significance of differences between the patients and matched controls were tested using the Wilcoxon Signed Ranks Test. Bivariate correlation coefficients were calculated using Spearman's rank correlation method. The partial correlations procedure was used to describe possible linear relationships between variables when controlling for age and BMI or sTNFR2. *P*-values less than 0.05 were considered significant and two-tailed significance test was used. Factor analysis, with Maximum Likelihood as extraction method and Varimax rotation and Kaiser normalization, was used to identify patterns of correlations within the observed variables. Thus, factor analysis generated a model for interpretation of the relationships in observations by examining the contribution of each variable in explaining one or more unobservable, latent factors. Calculations were performed using the SPSS for Windows 11.5.1 (SPSS inc., Chicago, Illinois, USA). One outlier, and her matched control, who had an leptin level eight times higher than the median value for the group, was excluded from the parametric (factor analysis, linear regression and partial correlation) analyses.

Results

Serum levels of IL-6, sTNFR1, sTNFR2, IL-1Ra and other markers of inflammation were significantly higher in patients compared with controls (Table I). In both patients and controls there was a correlation between IL-1Ra and anthropometric data on one hand, and between IL-1Ra and leptin on the other (Table II). There were also correlations between IL-1Ra and other markers of inflammation: haptoglobin in patients and sTNFR1 in controls. When adjusted for age and BMI the correlation between IL-1Ra and leptin persisted and an association with sTNFR2 was found in the patient group (Table II). After adjustments for age and sTNFR2 the correlations remained in patients. In addition, a correlation with ApoB was seen in patients. Associations between IL-1Ra and anthropometric data and leptin, respectively, remained in the control group (Table II). Factor analysis yielded 3 latent factors in the patients. These had an eigenvalue > 1.7, accounting for 74% of the total variation between these variables (Table III). A latent factor showed large loadings on IL-1Ra, leptin, BMI, ApoB

Table I. Clinical, anthropometric and laboratory characteristics of 23 female patients with established RA and matched controls.

	Patients (n = 23)		Controls (n = 23)		
	Median	IQ range	Median	IQ range	*p*-value
BMI (cm²/kg)	24.5	3.7	25.0	5.1	0.7
Fat (% body tissue)	36.4	4.9	33.8	7.3	0.2
Water (% body tissue)	45.1	5.5	46.9	8.2	0.2
Haptoglobin (g/L)	1.39	1.00	1.04	0.57	0.03
IL-1Ra (pg/mL)	603	325	273	159	< 0.001
IL-6 (pg/mL)	8.50	27.20	≤ 3.12†	0.00	< 0.001
Leptin (ng/mL)	13.7	19.9	12.1	13.1	0.9
Orosomucoid (g/L)	0.91	0.40	0.63	0.26	< 0.001
ESR (mm/h)	20	32	6	6	0.002
sTNFR1 (pg/mL)	1183	403	920	241	< 0.001
sTNFR2 (pg/mL)	2628	1230	1808	621	< 0.001
Apolipoprotein A-I (mg/L)	1507	399	1451	387	0.9
Apolipoprotein B (mg/L)	1136	343	1190	258	0.6
DAS28*	3.58	1.98	NA	NA	-

NA: not assessed.
*Disease activity score for 28-joint count according to Prevoo *et al*. 1995 (8).
†All except 3 below cut-off: 3.12.

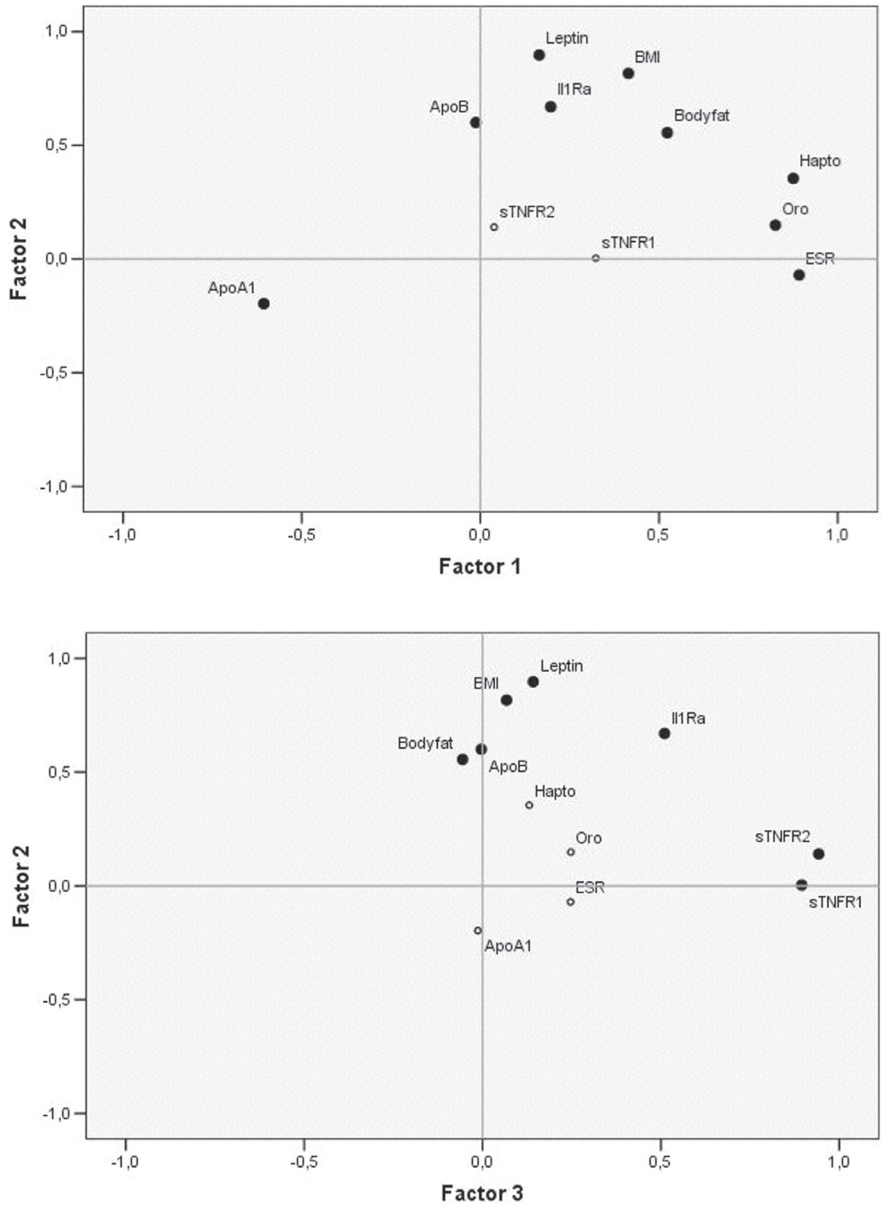
Table II. Correlation coefficients between IL-1Ra and variables. Unadjusted (Spearman's rank correlation) and adjusted (partial correlations) for age and BMI/sTNFR2 in patients with RA and matched controls.

Adjusted for:			Age, BMI		Age, sTNFR2	
	Patients (n = 23)	Controls (n = 23)	Patients (n = 22)	Controls (n = 22)	Patients (n = 22)	Controls (n = 22)
Age	0.42*	0.14	-	-	-	-
BMI	0.56**	0.55**	-	-	0.61**	0.63**
Fat (% body tissue)	0.48*	0.54**	0.02	-0.16	0.46*	0.58**
Water (% body tissue)	-0.50*	-0.52**	0.00	0.22	-0.50*	-0.56**
Leptin	0.71***	0.58**	0.48*	0.63**	0.74***	0.79***
Haptoglobin	0.56**	0.14	0.08	0.09	0.45*	0.27
IL-6	0.35	-	-0.05	-	0.03	-
Orosomucoid	0.30	0.39	0.04	0.12	0.31	0.41
ESR	0.33	0.36	-0.08	0.21	0.07	0.40
sTNFR1	0.31	0.46*	0.26	0.32	-0.15	0.24
sTNFR2	0.35	0.35	0.46*	0.22	-	-
Apolipoprotein A-I	-0.32	-0.20	0.02	-0.08	-0.26	-0.07
Apolipoprotein B	0.15	0.28	0.13	0.39	0.52*	0.34

*$p < 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$.

Table III. Factor analysis with analysis of latent factors for IL-1Ra, sTNFR1, sTNFR2, haptoglobin, orosomucoid, leptin, ApoA-I, ApoB, ESR, BF% and BMI in 22 patients with RA. Only factor loadings <-0.40 or > 0.40 are presented.

Factor	1	2	3
IL-1Ra		0.669	0.510
sTNFR1			0.895
sTNFR2			0.942
ESR	0.892		
Haptoglobin	0.876		
Orosomucoid	0.826		
Leptin		0.897	
Fat (% body tissue)	0.523	0.555	
BMI	0.414	0.816	
Apolipoprotein A-I	-0.606		
Apolipoprotein B		0.600	



Figs 1a. and 1b. Factor plots on latent factors derived in factor analysis from 22 patients with RA. Factor loadings <-0.4 or > 0.4 are showed with filled circles. Details on factor loadings are showed in Table III. **Fig. 1a.** Factor plot of factor 1 and 2. The factor loading of ApoA1 is opposite to the loadings of the other variables, depicted in figure. **Fig. 1b.** Factor plot of factor 2 and 3.

and some on BF% (Table III). A similar cluster was found in controls except for a lack of loadings on ApoB (data not shown). In patients, another latent factor showed loadings on IL1-Ra, together with large loadings on sTNF-receptors (Table III). Inverse loadings on ApoA-I was seen with one latent factor, which also showed loadings on ESR, haptoglobin, orosomucoid, BF% and BMI (Table III). No equivalent to these two latter factors was found in the control group (data not shown), but a second factor in controls loaded on sTNF-receptors and inversely on ApoA-I. The two other latent factors found in controls showed loadings on inflammatory markers *e.g.*, one cluster assembled ESR, haptoglobin and orosomucoid, and the other ESR and ApoB (data not shown).

Discussion

In this study, IL-1Ra associated with both metabolic (BMI, BF%, leptin, ApoB) and inflammatory factors (haptoglobin, sTNFR1, sTNFR2) in RA. It implicates a dualism in the role of IL-1Ra, illustrated in the results of both the factor analysis and correlation analyses. In RA we, and others, have shown an inverse relationship between inflammation and lipid levels (1, 2). In this study, an interesting association between IL-1Ra and ApoB in RA patients was revealed. ApoB is a major component of LDL, intermediate-density lipoprotein (IDL) and very low-density lipoprotein (VLDL) and ApoA-I is a component of HDL. Measurement of apolipoproteins has been suggested as a substitute for, or to complement, analysis of lipid pattern as indicators of cardiovascular risk (9). When the correlations were adjusted for sTNFR2, the association between IL-1Ra and metabolic factors remained in both patients and controls. In factor analysis a cluster with IL-1Ra, BMI, BF% and leptin was derived for both patients and controls, in patients also including ApoB. This was the only one with obvious loadings on IL-1Ra among the latent components in controls. Our interpretation of these results is that adipose tissue is also a significant source of IL-1Ra in non-obese subjects, and that production by adipose tissue contributes to the serum levels of IL-1Ra in RA.

In contrast to ApoB, ApoA-I assembled inversely with the acute-phase reactants, orosomucoid, haptoglobin, and ESR, as well as BMI and BF%, in patients. In inflammation, modulations of the HDL particles, such as replacement of ApoA-I, are suggested to have negative impact on the atheroprotective properties of HDL (10). ApoA-I inhibits production of TNF and IL-1 by hindrance of cellular contact between stimulated T-cells and monocytes (11). This is consistent with the negative association between ApoA-I and indicators of TNF activity found in controls. It has been suggested that a low ApoA-I concentration, maintained by chronic inflammation, could favour cytokine production creating a vicious circle (12). In this study, no differences in ApoA-I serum levels between patients and controls was found despite the higher inflammatory activity seen in patients. The result of the factor analysis, however, indicates a negative relationship between inflammation and

ApoA-I, even if this did not include the measured cytokine activity measured by sTNFR. One must bear in mind that measurements of levels of cytokine receptors and receptor antagonists might not truly reflect the activity in the cytokine systems.

Consistent with earlier studies, no difference was found between groups for circulating leptin levels (13, 14). A correlation between IL-1Ra and leptin, as previously reported in human obesity (15) was found; this correlation remained when controlling for BMI or sTNFR2.

The association between IL-1Ra and the inflammatory factors in RA was expected and part of the elevation of the IL-1Ra serum levels in patients is likely to be due to an increased production by activated monocytic cells.

In summary, IL-1Ra levels were associated with inflammatory markers and with ApoB serum levels in patients with RA. Furthermore, IL-1Ra associated with leptin in both patients and controls, indicating a relationship between inflammation and leptin.

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References

1. RANTAPÄÄ-DAHLQVIST S, WÅLLBERG-JONSSON S, DAHLEN G: Lipoprotein (a), lipids, and lipoproteins in patients with rheumatoid arthritis. *Ann Rheum Dis* 1991; 50: 366-8.
2. PARK YB, LEE SK, LEE WK *et al*.: Lipid profiles in untreated patients with rheumatoid arthritis. *J Rheumatol* 1999; 26: 1701-4.
3. WÅLLBERG-JONSSON S, JOHANSSON H, ÖHMAN ML, RANTAPÄÄ-DAHLQVIST S: Extent of inflammation predicts cardiovascular disease and overall mortality in seropositive rheumatoid arthritis. A retrospective cohort study from disease onset. *J Rheumatol* 1999; 26: 2562-71.
4. WÅLLBERG JONSSON S, BACKMAN C, JOHNSON O *et al*.: Increased prevalence of atherosclerosis in patients with medium term rheumatoid arthritis. *J Rheumatol* 2001; 28: 2597-602.
5. JUGE-AUBRY CE, SOMM E, GIUSTI V *et al.:* Adipose tissue is a major source of interleukin-1 receptor antagonist: upregulation in obesity and inflammation. *Diabetes* 2003; 52: 1104-10.
6. LAU DC, DHILLON B, YAN H, SZMITKO PE, VERMA S: Adipokines: molecular links between obesity and atherosclerosis. *Am J Physiol Heart Circ Physiol* 2005; 288: H2031-41.
7. ARNETT FC, EDWORTHY SM, BLOCH DA *et al.:* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315-24.
8. PREVOO MLL, VAN 'T HOF MA, KUPER HH, VAN LEEUWEN MA, VAN DE PUTTE LBA, VAN RIEL PLCM: Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995; 38: 44-8.
9. WALLDIUS G, JUNGNER I: Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. *J Intern Med* 2004; 255: 188-205.
10. ROHRER L, HERSBERGER M, VON ECKARDSTEIN A: High density lipoproteins in the intersection of diabetes mellitus, inflammation and cardiovascular disease. *Curr Opin Lipidol* 2004; 15: 269-78.
11. HYKA N, DAYER JM, MODOUX C *et al.:* Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 2001; 97: 2381-9.
12. BURGER D, DAYER JM: High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev* 2002; 1: 111-7.
13. ANDERS HJ, RIHL M, HEUFELDER A, LOCH O, SCHATTENKIRCHNER M: Leptin serum levels are not correlated with disease activity in patients with rheumatoid arthritis. *Metabolism* 1999; 48: 745-8.
14. POPA C, NETEA MG, RADSTAKE TR, VAN RIEL PL, BARRERA P, VAN DER MEER JW: Markers of inflammation are negatively correlated with serum leptin in rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 1195-8.
15. MEIER CA, BOBBIONI E, GABAY C, ASSIMACOPOULOS-JEANNET F, GOLAY A, DAYER JM: IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? *J Clin Endocrinol Metab* 2002; 87: 1184-8.