Serum Cytokine Receptors in Ankylosing Spondylitis: Relationship to Inflammatory Markers and Endoplasmic Reticulum Aminopeptidase Polymorphisms

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ABSTRACT. ***Objective.*** Endoplasmic reticulum aminopeptidase (*ERAP)1* is associated with ankylosing spondylitis (AS) and is known to be involved in the clipping of the cytokine receptors interleukin 1 receptor II (IL-1RII), IL-6Rα, and tumor necrosis factor receptor I (TNFRI). We studied the relationship of these serum cytokine receptors and their corresponding cytokines to markers of inflammation and polymorphisms in *ERAP1* and *ERAP2* in patients with AS.

Methods. Sera from patients with AS were assayed for TNF-α, IL-1, IL-6, sTNFRI, sIL-1RII, and sIL-6Rα by ELISA. Genotyping was performed for 3 AS-associated nonsynonymous single-nucleotide polymorphisms in the *ERAP1* gene [*rs27044(C/G)*, *rs10050860(C/T)*, and *rs30187(C/T)*] and 1 in the *ERAP2* gene [*rs2549782(T/G)*]. The serum cytokine and receptor levels were compared between the different genotype groups and correlated to markers of inflammation and disease activity.

Results. Eighty patients with AS (21 women) with a mean Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) of 5.3 ± 2.4 were enrolled. There was a significant correlation of sTNFRI with C-reactive protein (CRP; $R = 0.43$, $p < 0.001$) and erythrocyte sedimentation rate (ESR; $R = 0.30$, $p = 0.01$) but not with BASDAI. Serum cytokine levels were undetectable in the majority of patients. There was no significant difference in serum cytokines or the soluble receptors between patients with the different *ERAP1*/*ERAP2* polymorphisms and their haplotypes. Similarly, there was no relationship of the polymorphisms with the serum cytokine levels nor the cytokine-receptor ratio.

Conclusion. Soluble TNFRI levels correlate with ESR and CRP in AS. The *ERAP1* and *ERAP2* polymorphisms associated with AS do not influence the serum cytokine receptor levels in patients with AS. (J Rheumatol First Release July 1 2010; doi:10.3899/jrheum.100019)

Key Indexing Terms:
ARTS-1 *ERAP1* *ERAP2* RECEPTOR SHEDDING ANKYLOSING SPONDYLITIS

The pathogenesis of ankylosing spondylitis (AS), a severe form of spondyloarthritis, remains elusive. HLA-B27, the major susceptibility gene, has an estimated attributable risk (AR) of 16%–50%[1]. The role of HLA-B27 in the pathogenesis of AS remains unknown, and recently other risk factors including non-MHC genes such as interleukin 1A (IL-1A) and IL-23R have been identified[2,3,4]. The strongest non-MHC gene associated with AS is endoplasmic reticulum aminopeptidase 1 (*ERAP1*), with an AR of 26%[3]. *ERAP2* is a closely related ERAP found in humans. We recently reported the association of a novel *ERAP1*/*ERAP2* haplotype with familial AS[5].

ERAP1 has 2 known functions: cytokine receptor shedding [tumor necrosis factor receptor I (TNFRI)[6], IL-1RII[7], and IL-6Rα[8]] and peptide trimming within the endoplasmic reticulum for MHC-I antigen presentation[9]. Reduced cytokine receptor shedding could decrease the level of soluble cytokine receptors in circulation and thereby result in altered serum cytokine bioactivity. We examined the relationship of serum cytokine receptors with disease activity and with *ERAP1* and *ERAP2* polymorphisms detected in the respective patients.

MATERIALS AND METHODS

Patients satisfying the modified New York criteria for AS and not receiving any biologic therapy were included in the study. All patients were assessed for disease activity by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP).

Genotyping was performed on peripheral blood cell DNA using allelic discrimination assays (Applied Biosystems, Foster City, CA, USA) for

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nonsynonymous single-nucleotide polymorphisms (SNP) in the *ERAP1* gene [*rs27044(C/G)*, *rs10050860(C/T)*, and *rs30187(C/T)*] and the *ERAP2* gene (*rs2549782[T/G]*).

Sera were collected from patients with AS at the time of clinical evaluation and stored at –80°C. ELISA was used to determine the serum levels of the cytokines IL-1, IL-6, and TNF-α (BD Biosciences, San Jose, CA, USA) and the soluble receptors of IL-1RII (sIL-1RII), IL-6R (sIL-6R), and TNFRI (sTNFRI; R & D Systems Inc., Minneapolis, MN, USA). The minimum detectable serum levels by the ELISA were 0.8, 2.2, and 2 pg/ml for the cytokines IL-1, IL-6, and TNF-α, and 10, 6.5, and 0.77 pg/ml for sIL-1RII, sIL-6R, and sTNFRI, respectively.

Statistical analysis. For intergroup comparisons, the Kruskal-Wallis test and the Mann-Whitney U test were used. Partial correlations and Spearman's rank correlation were used where applicable.

RESULTS

Eighty patients with AS (21 women) with a mean (± SD) age of 42.3 (± 10.6) years and a mean disease duration of 18.5 (± 11.4) years were enrolled in our study. Sixty-seven patients (83.7%) were HLA-B27-positive. The mean ESR and CRP were 18 ± 15.6 mm/h and 14.8 ± 18.8 g/dl, respectively. The mean BASDAI and Bath Ankylosing Spondylitis Functional Index (BASFI) scores were 5.3 ± 2.4 and 4.4 ± 2.6, respectively. There was no significant difference between the patients in the different genotypic subsets with regards to age, disease duration, BASDAI, ESR, or CRP.

Genotyping. The allele frequencies of *ERAP1* SNP were 12, 39, and 29 (AA, AG, GG) for *rs30187*; 37, 39, and 4 (CC, CG, and GG) for *rs27044*; and 53, 25, and 2 (CC, TC, TT) for *rs10050860*, respectively. The allele frequencies for *ERAP2 rs2549782* were 26, 36, and 17 (GG, GT, and TT, respectively).

Soluble cytokine receptor levels. The mean serum soluble cytokine receptor levels were 1.38 ± 0.42 pg/ml (sTNFRI), 11.89 ± 4.3 pg/ml (sIL-1RII), and 29.63 ± 11.8 pg/ml (sIL-6R). There was a significant positive correlation of sTNFRI with CRP ($R = 0.43$, $p < 0.001$) and ESR ($R = 0.30$, $p = 0.01$). There was no correlation of sTNFRI with BASDAI. There was also no correlation of sIL-1RII and sIL-6R with ESR, CRP, BASDAI, or BASFI (Table 1). There was a significant correlation of sIL-6R with sIL-1RII ($R = 0.40$, $p < 0.0001$) and with sTNFRI ($R = 0.28$, $p = 0.01$) but not between sTNFRI and sIL-1RII.

There was no significant difference in the soluble cytokine receptor serum levels between the different genotype groups of *ERAP1 rs30187* and *ERAP2 rs2549782* (Figure 1) nor for the other polymorphisms of *ERAP1* tested (data not shown). On haplotype analysis with dominant and recessive models there was no influence of *ERAP1*/*ERAP2* on the cytokine receptor levels.

Serum cytokine levels. Serum cytokines were detectable only in 21, 29, and 22 patients, respectively, for IL-1, IL-6, and TNF-α assays and the mean serum levels were 28.4 ± 27.1 (IL-1), 44.4 ± 71.7 (IL-6), and 104.5 ± 90.2 (TNF-α) pg/ml, respectively. There was no difference in the cytokine receptor levels between patients with and those without detectable cytokines.

DISCUSSION

This is the first study to address a possible functional consequence of the genetic polymorphisms of *ERAP1* and the *ERAP1*/*ERAP2* haplotype in AS. We found no influence of the *ERAP1* or *ERAP2* SNP on the serum levels of sTNFRI, sIL-1RII, and sIL-6R or the corresponding cytokines. We did find a significant positive correlation of sTNFRI with the inflammatory markers ESR and CRP. There was no correlation of serum cytokine receptors with BASDAI.

Our findings indicate that the functional relevance of the *ERAP1*/*ERAP2* association is unlikely to be related to cytokine receptor shedding activity. There was no difference in the serum levels of the cytokine receptors in the respective genotypic groups. Moreover, higher CRP and ESR were associated with more sTNFI, indicating more receptor shedding secondary to inflammation. The strong correlation we observed between TNFRI and CRP could indicate that TNF receptor shedding is more sensitive to inflammation than IL-1 and IL-6 receptors. The *ERAP1* knockout mouse has an abnormal peptide-MHC repertoire at the cell surface, in which some normal peptides do not get presented while some abnormal peptides are presented[10]. The *ERAP1* and *ERAP2* genes reside in the same cluster, located on chromosome 5q15[11]. Both *ERAP1* and *ERAP2* are upregulated by interferon-γ, form heterodimers, and complementarily trim peptides > 9 residues[12]. Enzymatic analysis showed that *ERAP1* preferentially hydrolyzes the leucine residues, while *ERAP2* acts on the basic residues arginine and lysine[13]. To

Table 1. Correlation of the soluble cytokine receptors with markers of inflammation.

		ESR	CRP	BASDAI	sTNFR1	sIL-1RII	sIL-6R
sTNFR1	R	0.30	0.43	0.05	1	0.17	0.28
	p	0.01	< 0.001	NS		NS	0.01
sIL-1RII	R	–0.02	0.02	0.09	0.17	1	0.40
	p	NS	NS	NS	NS		< 0.001
sIL-6R	R	0.09	0.14	0.03	0.28	0.40	1
	p	NS	NS	NS	0.01	< 0.001	

NS: not significant; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; TNF: tumor necrosis factor; IL: interleukin.

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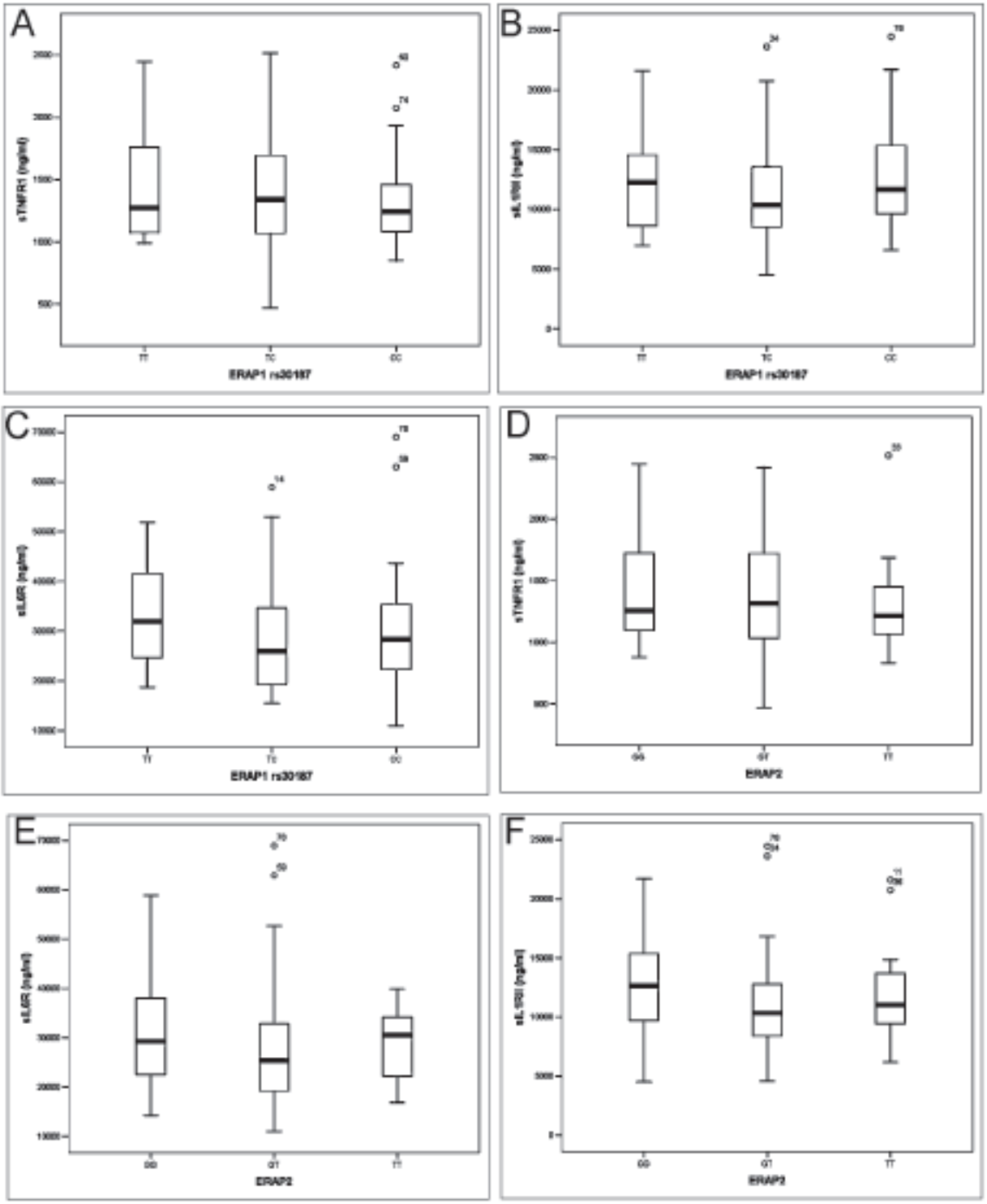

Figure 1. Levels of serum cytokine receptors in patients with different genotypes of the *rs30187* single-nucleotide polymorphism (SNP) of *ERAP1* and the *rs2549782* SNP of *ERAP2*. There was no significant difference between the genotypic groups.

date there is no report on the involvement of *ERAP2* in cytokine receptor shedding. Combined with the evidence from our study, it is more likely that the haplotype *ERAP1*/*ERAP2* is influencing the pathogenesis of AS by its peptide processing function.

We conclude that the *ERAP1*/*ERAP2* haplotype associated with AS does not influence the serum cytokine receptor levels. Further studies need to examine the peptide processing pathway and the interaction of *ERAP1*/*ERAP2* and HLA-B27.

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