Resistin is Associated with Breach of Tolerance and Anti-nuclear Antibodies in Patients with Hepatobiliary Inflammation

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Abstract

Resistin is a cystein-rich protein, which is abundantly expressed at the site of inflammation, and acts as a regulator of the NF-kB dependent cytokine cascade. The aim of this study was to evaluate resistin levels in relation to inflammatory mediators, disease phenotype and autoantibody status in a spectrum of pathological conditions of the gastrointestinal tract. Resistin levels were measured with an ELISA in sera originated from 227 patients and 40 healthy controls (HC). 50 patients diagnosed with non-alcoholic fatty liver disease (NAFLD), 53 ulcerative colitis (UC), 51 Crohn's disease (CD), 46 autoimmune hepatitis (AIH), and 27 primary sclerosing cholangitis (PSC) were included. The sera were analyzed with respect to biochemical parameters of systemic inflammation and liver function, and to the presence of antibodies to nuclear antigens (ANA), mitochondria (AMA) and smooth muscle (SMA). Compared to HC, resistin levels were raised in AIH ($p=0.017$) and PSC ($p=0.03$); compared to NAFLD, levels were elevated in CD ($p=0.041$), AIH ($p<0.001$) and PSC ($p<0.001$). Patients with elevated levels of resistin were more often treated with corticosteroids, but no difference was found between active disease and clinical remission. Resistin levels were significantly higher in ANA positive individuals compared to ANA negative ($p=0.025$). Resistin levels were directly correlated with IL-6 ($r=0.30$, $p=0.02$) and IL-8 ($r=0.51$, $p<0.001$). Elevated levels of resistin were prominent in patients with hepatobiliary inflammation and were associated with breach of self-tolerance, *i.e.* ANA positivity. Thus, we propose that resistin may be an important marker of disease severity in autoantibody-mediated gastrointestinal inflammatory diseases.

Key words: Resistin, autoimmune hepatitis, Crohn's disease, ulcerative colitis, NAFLD, primary sclerosing cholangitis

INTRODUCTION

Inflammatory diseases of the liver and gastrointestinal tract are characterized by a dense leukocyte infiltration of stromal tissue in the liver, biliary system, or in the intestinal wall, with subsequent gradual development of fibrosis [1]. This massive leukocyte invasion, in combination with production of antibodies targeting autoantigens, forms the basis of autoimmune inflammation. Detection of antibodies targeting liver-related or non-organ specific antigens is an important diagnostic tool in the recognition of autoimmune conditions of the liver and gut and are associated with a severe course of disease and unfavorable prognosis [1]. Despite their diagnostic relevance, the pathogenetic role of autoantibodies in the induction of tissue damage remains unclear.

Several mechanisms are involved in the pathogenesis of autoimmunity, including impairment of antigen presentation, T cell regulation, and humoral control [2]. Indeed, proliferation of autoreactive T cell clones in combination with insufficient numbers of $CD4^+CD25^+$ regulatory T cells have been reported in patients with autoimmune hepatitis (AIH) [3], primary sclerosing cholangitis (PSC) and inflammatory bowel disease (IBD) [2], although the role of autoimmunity in Crohn's disease (CD) was recently questioned [4].

Resistin, also known as FIZZ3 (found in inflammatory zone 3) or ADSF (adipocyte-specific secretory factor) is a 12.5 kDa cystein-rich peptide with inter-cellular signalling activity. The cystein-rich C-terminal sequence of resistin identifies a specific signature of the resistin-like molecules (RELM) [5]. This protein family comprises four members (RELMα, RELMβ, RELMγ and resistin), which all exhibit unique tissue distribution patterns in different mammalian species [6–9]. Resistin was originally described in context with its role in carbohydrate metabolism in rodents [9]. In humans, resistin is active in inflammatory processes and can be found at places of leukocyte infiltration [10]. Resistin is secreted by mononuclear leukocytes and neutrophils following pro-inflammatory stimuli such as endotoxins, IL-6 or TNF [11]. Treatment of target cells with resistin initiates intracellular activation of the NF-kB pathway, resulting in the expression of a broad spectrum of pro-inflammatory cytokines [11].

Resistin has been studied in streptococcal infections [12] and sepsis [13], and was assumed to contribute to the pathologic inflammatory responses associated with severe infections. Recently, however, possible protective roles for RELM in persistent cervical human papillomavirus infection [14] and gastrointestinal worm infection [15] have been suggested. Resistin is also abundant in many inflammatory conditions, such as rheumatoid arthritis [10, 11], systemic lupus erythematosus [16] and primary Sjögren's syndrome [17]. Furthermore, raised levels of resistin have been reported in patients with IBD, more specifically in CD and ulcerative colitis (UC) [18–21]. Studies on autoimmune [22] and viral hepatitis [23] showed increased circulating levels of resistin in proportion to liver tissue damage assessed by aminotransferases [22], but not in relation to steatosis or stage of fibrosis [23]. One study on non-alcoholic fatty liver disease (NAFLD) has demonstrated a direct correlation between serum resistin levels and the severity of morphological changes in the liver [24].

In the present study we evaluated associations between circulating resistin levels and humoral autoimmunity in a broad spectrum of pathological conditions of the gastrointestinal tract. Our results show increased levels of resistin in patients with breach of self-tolerance. We conclude that resistin may be an important determinant in autoantibody-mediated gastrointestinal inflammatory diseases.

MATERIAL AND METHODS

Patient population

227 patients were recruited from the Division of Gastroenterology and Hepatology, Linköping University Hospital, Linköping. Clinical and demographic characteristics of the patient groups and controls are shown in Table 1. 50 patients were diagnosed with NAFLD based on the presence of biopsy-proven hepatic steatosis, self-reported alcohol consumption ≤140 g/week and no evidence of any other liver disease or medication associated with fatty infiltration of the liver as previously described [25]. 104 patients diagnosed with IBD consisted of 53 patients with UC and 51 with CD. The IBD diagnosis was based on standard clinical, endoscopic, radiological and histological findings [26]. 46 patients were diagnosed with AIH based on the revised AIH-score previously described [27, 28]. An AIH-score >17 corresponded to a definite AIH diagnosis whereas an AIH-score >11 corresponded to probable AIH. The mean score in the study group was 18.3±0.7 (range 10–26). 8/46 AIH patients (17%) had concomitant primary biliary cirrhosis (PBC), *i.e.* overlap between AIH and PBC. 27 patients were diagnosed with PSC, whereof 18 had concomitant UC, 4 AIH, 1 CD and 4 isolated PSC. The PSC diagnosis was based on standard biochemical, clinical and cholangiographic findings [29]. Onset of PSC was defined as the time of the first cholangiographic investigation consistent with a diagnosis of PSC. A group consisting of 40 apparently healthy controls (HC) served as reference. None of the control subjects had any history of autoimmune disorder or had used immunomodulatory drugs or antibiotics prior or at the time-point of blood sampling.

Serum sample collection

Venous blood samples from patients and controls were collected into heparinized tubes. Samples were centrifuged at 800*g* for 15 min, aliquoted, and stored frozen at –80°C pending analysis.

Biochemical assessment

Biochemical assessment included levels of hemoglobin, platelet and white blood cells (WBC) counts, C-reactive protein (CRP) and serum immunoglobulins (total IgM, IgG and IgA). Liver function was evaluated based on measurement of alanine aminotransferase

Table 1.

Clinical demographics and characteristics of healthy controls (HC), and patients with non-alcoholic fatty liver disease (NAFLD), ulcerative colitis (UC), Crohn's disease (CD), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC).

	PSC		**HC**		**NAFLD**	**UC**		**CD**	
	n=46		n=40		n=50	n=53		n=51	
Sex, m/f	12/34	10/30	22/5	37/13	n=27		26/27		28/23
Age, mean yrs (range)		44 (24-67)	48 (24-65)	45 (23-77)	43 (26-67)	47 (21-82)	47 (23-87)		
Active disease, n (%)	13 (28)	na	na			8 (15)		19 (37)	
ANA, n (%)	22 (48)	na	6 (22)	7 (14)		4 (8)		3 (6)	
AMA, n (%)	11 (24)	na	3 (11)	0 (0)		0 (0)		0 (0)	
SMA, n (%)	29 (63)	na	3 (11)	1 (2)		1 (2)		3 (6)	
ALT, μkat/L		na		na		0.8±0.2	0.4±0.1	1.4±0.5	0.9±1.6
AST, μkat/L		na		0.6±0.04	0.7±0.1	0.5±0.04	1.2±0.5	1.1±0.3	
Gamma-GT, μkat/L	5.3±1.9	na		1.2±0.1	na		na		2.6±0.9
Bilirubin, μM/L		na		13.2±0.9	13.7±2.2	6.3±1.2	16.0±1.4	14.9±1.9	
Immunosuppressive treatment									
Steroids, n (%)	30 (65)	7 (26)				10 (19)		17 (33)	

(ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GT) and bilirubin. In NAFLD patients, metabolic profile including body mass index (BMI), levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, homeostasis model of assessment for insulin resistence index (HOMA-IR) and thyroid hormones were determined.

Detection of autoantibodies

Antinuclear antibodies (ANA) were analyzed with indirect immunofluorescence (IF) technique using HEp-2 cells (ImmunoConcepts, Sacramento, CA, USA) as substrate. Anti-mitochondrial antibodies (AMA) were detected using rat kidney slides (BioSystems, Barcelona, Spain) and smooth muscle antibodies (SMA) were analyzed using rat stomach slides (BioSystems) as substrate. Briefly, samples were diluted 1:80 in PBS and incubated on HEp-2 cells, rat kidney or rat stomach slides for 30 minutes at room temperature. Following washing, slides were incubated with a FITC-labelled anti-IgG conjugate and analyzed in immunofluorescent light. The tested samples were compared to a serial dilution of a known positive standard. Samples with immunofluorescent staining by the initial dilution were subjected to further analysis using serial dilutions (end-point titration). Cut-off was set at dilutions ≥1:80 based on the 95th percentile of serum materials from healthy donors.

Resistin measurements

Serum resistin concentrations were determined using quantitative sandwich ELISA (R&D Systems, Abingdon, UK). Briefly, wells were coated with capture monoclonal mouse anti-human resistin antibodies (IgG_{2B} clone 184335) 0.4 µg/ml. Sera were diluted 1:5 in 1% BSA-PBS. In the detection step biotinylated polyclonal antibodies were used (200 ng/ml). Resistin levels were quantified using a standard serial dilution of recombinant resistin. Colorimetric reaction was measured following addition of HRP-substrate at a wavelength of 450 nm. Lowest detectable level was 15 pg/ml.

Measurement of cytokine levels

Levels of IL-6 and IL-8 were determined using quantitative sandwich ELISA (Quantikine HS, R&D Systems Europe Ltd., Abingdon, UK). Sera were diluted 1:25 in 1% BSA-PBS. In the detection step biotinylated polyclonal antibodies were used. Quantification of the

cytokines was performed using a serial dilution of recombinant protein and expressed in ng/ml. Colorimetric reaction was measured following addition of HRP-substrate at a wavelength of 450 nm.

Statistics

Data are expressed as mean±SEM unless indicated otherwise. The degree of association was evaluated with Spearman rank correlation test. Comparisons between groups were assessed with the Mann-Whitney *U*-test. P-values <0.05 were considered significant. All statistical analyses were performed using SPSS for Windows (version 15.0.0; SPSS Inc. (IBM), Chicago, IL, USA).

Ethics

The study protocol was approved by the Ethical Committee of the University Hospital of Linköping, and written informed consent was obtained from all the patients.

RESULTS

Serum resistin is elevated in patients with inflammatory gastrointestinal diseases

As demonstrated in Figure 1ab, resistin levels were significantly elevated in patients with inflammatory liver conditions, *i.e.* AIH (14.0±2.0 ng/ml), PSC (19.7±3.5 ng/ml) as compared to HC (7.0±0.6 ng/ml, p=0.017 and p=0.03, respectively) as well as to patients with NAFLD (6.7±0.8 ng/ml, both p<0.001). CD patients (16.9±3.0 ng/ml) showed higher resistin levels compared to NAFLD (6.7±0.8 ng/ml, p=0.041), while in patients with UC, resistin levels were similar to NAFLD (11.0±0.5 ng/ml). Resistin levels in patients with combined AIH and PBC (7.0±1.1 ng/ml, n=8) were similar to both NAFLD patients and controls.

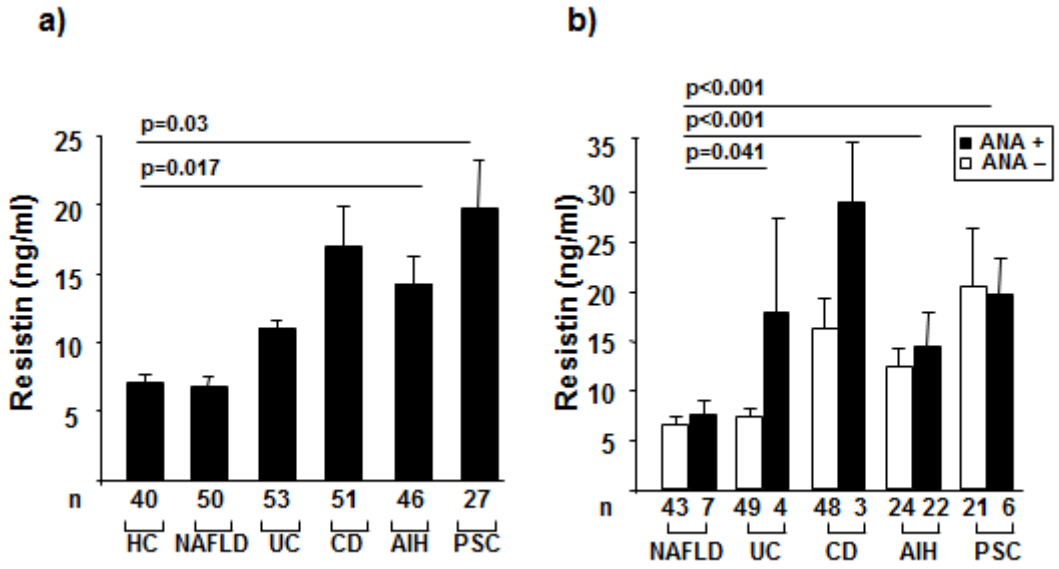

Figure 1: Serum resistin levels in patients with diseases of the gastrointestinal tract. Data from healthy controls (HC) as well as from patients with non-alcoholic fatty liver disease (NAFLD), ulcerative colitis (UC), Crohn's disease (CD), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) are shown in (A). Resistin levels in IBD patients were not significantly different from HC. Resistin levels stratified for the presence (black bars), or absence (white bars), of anti-nuclear antibodies (ANA) are demonstrated in (B). Comparisons were performed with Mann-Whitney *U*-statistics between the groups including both ANA positive and ANA negative patients. Resistin levels are given as means ± SEM.

Resistin levels were significantly higher in patients with hepatobiliary inflammation (AIH and PSC) as compared to IBD (15.6±1.8 *vs.* 12.4±1.6 ng/ml, p=0.013). Highest resistin levels were found in PSC patients, 294% above the amount in patients with NAFLD, and 140% above the levels in AIH (mean values). Resistin levels did not

correlate to markers of liver damage, *i.e.* ALT, AST, γ-GT and bilirubin. However, resistin levels were directly correlated with levels of IL-6 (r=0.30, p=0.02), IL-8 (r=0.51, p<0.001) and to IgM (r=0.31, p=0.03), but not to IgG or IgA. No correlations were found with WBC count or CRP. No difference in resistin levels was observed between patients with active disease or in clinical remission (counts for UC, CD and AIH).

Patients with diagnoses associated with elevated resistin levels (AIH, PSC, CD) were significantly more often treated with corticosteroids (AIH 65%, PSC 26%, and CD 33%) compared to patients with UC (18%) or NAFLD (0%). Patients receiving thiopurines had a tendency towards higher resistin compared to those not receiving thiopurines: In AIH, 13.7±3.5 *vs.* 12.6±1.8 ng/ml; PSC, 37.9±11.1 *vs.* 15.8±2.8 ng/ml; and UC, 8.9±2.7 *vs.* 7.9±1.1 ng/ml; significance was, however, only met in CD, 32.7±8.0 *vs.* 10.4±1.6 ng/ml (p=0.009).

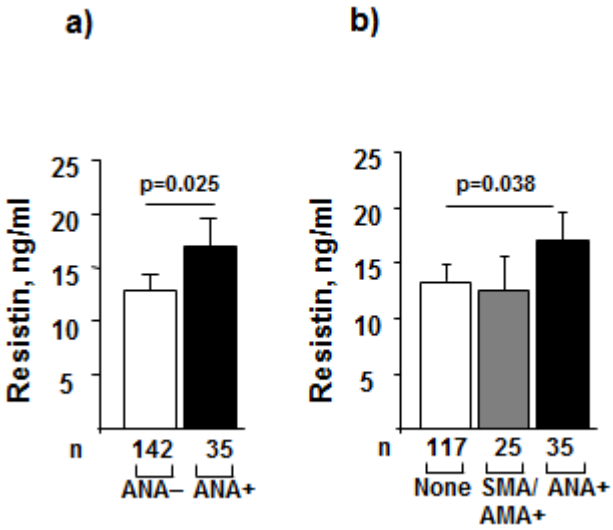

Figure 2: Serum resistin levels and autoantibodies in patients with ulcerative colitis (UC), Crohn's disease (CD), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) were included in this analysis. (A) Serum resistin levels stratified for the presence or absence of antinuclear antibodies (ANA). (B) Serum resistin levels stratified for the presence of antinuclear antibodies (ANA) and the presence of anti-mitochondrial antibodies (AMA) and/or smooth muscle antibodies (SMA). Patients included in ANA+ showed ANA alone or in combination with AMA/SMA. Patients included in AMA/SMA+ were all ANA negative, but positive for AMA and/or SMA. Comparisons were performed with Mann-Whitney *U*-statistics. Resistin levels are given as means ± SEM.

Elevated resistin levels are associated with antinuclear antibodies

Autoantibody status (ANA, AMA and SMA) in the patient groups is shown in Table 1 and resistin levels in relation to ANA in Figure 1b. In inflammatory diseases (UC, CD, AIH and PSC), resistin levels were significantly higher in ANA positive (n=35) compared to ANA negative (n=142) patients (16.9±2.7 ng/ml *vs.* 12.8±1.4 ng/ml, p=0.025; Figure 2a); where the best distinction was seen in IBD patients. As expected, the highest proportion of ANA positivity was found in AIH patients (48%) followed by PSC patients (22%), NAFLD (14%) and IBD (7%) patients. ANA titers showed no correlation to resistin levels (not shown). Within the AIH and PSC groups, resistin levels did not differ between ANA positive and negative patients. The presence of SMA was common in patients with AIH (63%), while AMA was found only in patients with overlap between AIH and PBC. No difference in resistin levels was seen in AIH patients having AMA or SMA without ANA, as compared to ANA positive and individuals without antibodies. Autoantibody status for the four inflammatory diseases combined (UC, CD, AIH and PSC) is shown in Figure 2b; and in Table 2, biochemical parameters in relation to autoantibody profile is demonstrated. ANA positive patients (n=35) had higher levels of IgG (p=0.045) and IgM (p=0.004) compared to ANA negative individuals. In addition, ANA positive patients had higher intensity of inflammation measured by WBC count (p<0.001) and platelet count (p<0.001) than ANA negative individuals. Finally, both ANA positive and AMA/SMA positive patients were treated with thiopurines more frequently than autoantibody negative patients (both comparisons p<0.001).

Resistin in NAFLD

Resistin levels in NAFLD patients (6.7±0.8 ng/ml) and HC (7.0±0.6 ng/ml) were not significantly different (Figure 1a). No correlations between the levels of resistin and BMI, blood lipids, HOMA-IR or thyroid hormones in NAFLD patients were seen; and no difference concerning resistin levels between patients with steatohepatitis and simple fatty liver was found. Resistin was measured simultaneously with liver biopsy, which made a direct comparison with liver histology possible. As demonstrated in Table 3, resistin levels showed no correlations with the intensity of fibrosis, fatty infiltration, nor to the intensity of inflammation.

Table 2.

Comparison of inflammatory parameters and therapy of patients with ulcerative colitis (UC) and Crohn's disease (CD), autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) stratified for the presence of autoantibodies. Patients positive for antinuclear antibodies (ANA) were included in the ANA+ group having either ANA alone or in combination with anti-mitochondrial antibodies (AMA) or smooth muscle antibodies (SMA). Patients included in AMA/SMA+ were ANA negative, but positive for AMA and/or SMA. Comparisons were performed with Mann-Whitney *U*-statistics. Numbers denote means ± SEM.

	ANA$^+$ n=35		**AMA/SMA$^+$** n=25	**None** n=117
Resistin, ng/ml	16.9±2.7*	12.4±3.5	13.3±1.5	
CRP, mg/L	4.2±1.1	8.4±3.4	5.4±2.2	
WBC count x10^9	6.2±0.5*	6.5±0.5*	3.9±0.3	
IL-6, ng/ml	2.3±0.9	1.5±0.5	3.7±1.6	
IL-8, ng/ml	0.4±0.1	0.8±0.4	2.1±1.1	
IgG	12.3±1.3	15.0±0.7*	8.6±1.6	
IgA	1.9±0.2	2.7±0.2	2.1±0.6	
IgM	1.6±0.2	2.5±0.5	1.3±0.2	
Thiopurine treatment, n (%)	20 (71)*	11 (44)*	11 (9)	
Steroide treatment, n (%) 3 (9)		6 (24)		15 (14)

* $p<0.05$ as compared to "None"

ANA=anti-nuclear antibodies, AMA=anti-mitochondrial antibodies, SMA=smooth muscle antibodies, CRP=C-reactive protein

Table 3.

Serum resistin levels and hepatic fibrosis demonstrated in the 50 patients with non-alcoholic fatty liver disease (NAFLD). Liver histology was scored by an independent pathologist according to the system developed by Brunt *et al* [41]. Resistin levels are given as means ± SEM.

Fibrosis stage	**n**	**Resistin, ng/ml**
F0	17	6.3±0.7
F1	14	7.8±2.6
F2	8	6.7±1.2
F3	6	5.7±0.8
F4	5	6.1±1.3
Fibrosis absent (F0)	17	6.3±0.7
Fibrosis present (F1-4)	33	6.9±1.1
No or mild fibrosis (F0-1)	31	7.0±1.2
Moderate to severe fibrosis (F2-4)	19	6.2±0.6

F0, no fibrosis; F1, expanded portal tracts and/or focally or extensively present zone 3 perisinusoidal/pericellular fibrosis; F2, focal or extensive periportal fibrosis and zone 3 perisinusoidal/pericellular fibrosis; F3, portal fibrosis with focal or extensive bridging fibrosis and zone 3 perisinusoidal/pericellular fibrosis; F4, cirrhosis.

DISCUSSION

The present study has a cross-sectional design aiming at evaluating resistin levels in a broad spectrum of pathological conditions of the gastrointestinal tract. The number of included subjects is limited, and thus, interpretation of results requires a careful approach. Our data showed no clear association between resistin levels and disease activity in UC, CD or AIH, but still – and as expected – resistin levels were higher in inflammatory diseases, being most prominent in patients with hepatobiliary inflammation (AIH and PSC), whereas levels in liver disease associated with the metabolic syndrome, *i.e.* NAFLD, did not differ from HC. By comparing resistin levels in patients with IBD and hepatobiliary conditions, we present data indicating that autoimmune processes may be an important determinant of resistin levels. Our findings are in agreement with previous reports of elevated levels of resistin in patients with IBD [18–21], showing an association between resistin levels and the degree of bowel inflammation. However, the metabolic liver condition NAFLD did not have any impact on resistin levels despite findings of extensive histopathological changes; and in contrast to the observation made by Pagano and co-workers [24], we found no correlation between resistin levels and the extent of hepatic fibrosis.

Regardless of which inflammatory disease, elevated levels of resistin were selectively associated with ANA positivity, but not with the presence of AMA and SMA. The association between resistin and autoimmune responses finds further support in its association with IgG and IgM. Lack of association between resistin levels and AMA and SMA may possibly be explained by the limited number of patients with these autoantibodies. Interestingly, levels of resistin in ANA positive patients remained elevated despite treatment with corticosteroids and immunomodulators, suggesting a stable association between resistin and the autoimmune component of inflammation. Since no difference in resistin levels was found between ANA positive and negative individuals within the AIH group, it is unlikely that resistin could aid in discriminating AIH type 1 (AIH-1), which is often ANA and/or SMA positive, from AIH-2, usually ANA negative with autoantibodies to liver kidney microsomes and/or liver cytosol [30]. However, AIH patients were significantly more often treated with corticosteroids (65%) and thiopurins (43%), compared to the other disease groups and it can not be excluded

that this could have an impact on the resistin levels and partly explain the lack of a statistical distinction between ANA positive and ANA negative AIH patients.

The knowledge of the impact of pharmaceutical treatment on resistin levels *per se* is limited. However, in rheumatoid arthritis, anti-TNF therapy results in a rapid reduction of serum resistin and the levels of resistin follow CRP levels closely [31]. In asthma, raised resistin levels are found compared to healthy subjects; and levels are associated with disease severity (frequent symptoms/exacerbations and need for continuous anti-obstructive therapy) [32]. Finally, local tissue secretion of resistin from adipose specimens was found to be significantly lower in CD patients receiving steroids compared to CD patients without steroids [20].

T cell dependent mechanisms play an essential role in the pathogenesis of AIH and PSC [3]. Support for a relation between resistin and T cell function are provided by our previous findings of increased resistin expression in the foci of T lymphocytes in patients with primary Sjögren's syndrome [17]. Furthermore, resistin induces T cell migration in functional *in vitro* studies of human CD4 T cells, supporting the concept of macrophage-induced T cell chemotaxis [33]. Resistin also decreases T cell activity via decreased antigen uptake and endocytic capacity of human dendritic cells supporting its possible relation to T cell function [34]. Additionally, two other molecules of the resistin-like family (RELMα and RELMβ) [5] has been shown to augment T cell responses, suppressing IL-4 and IFN-γ production, during chronic intestinal inflammation in mice [35]. Potential modulating effects of resistin on T and B cells, leading to autoantibody production in hepatobiliary diseases, need further investigation.

Production of resistin at the site of inflammation is considered a response of innate cells, *i.e.* macrophages [36] or neutrophils [37], to exogenous stimuli. Resistin itself is capable of inducing NF-kB dependent inflammatory responses, which are reflected by the secretion of IL-6 and IL-8 [11]. A possible regulatory function of resistin in hepatobiliary diseases finds support in the present study where moderate correlations with pro-inflammatory cytokines (IL-6 and IL-8) were found. Resistin is expressed at low levels in hepatocytes and production of resistin may be induced through NF-kB-dependent cascade in stellate cells of human liver and in a Hep G2 cell line [38]. Moreover, human

hepatic tissues respond to resistin with release of pro-inflammatory cytokines [38, 39]. Other possible origins of resistin include infiltrating mononuclear cells, peripheral blood mononuclear cells, as well as resistin produced at remote organs by infiltrating myeloid cells. Thus, excessive levels of resistin in gastrointestinal diseases may have dual roles in the pathogenesis of autoimmune hepatobiliary diseases, inhibiting CD4 T cells as well as facilitating both autoantibody and cytokine production.

The observation of the regulatory effect of resistin in carbohydrate metabolism in mice [9] initiated attempts to identify resistin-dependent mechanisms of fat deposition and insulin resistance in humans [39] and, in fact, resistin has been described as a biomarker of insulin resistance and cirrhosis related to metabolic liver disease [40]. However, the findings in our well-characterized NAFLD cohort [25] do not provide evidence for association with either diabetes, insulin resistence or the extent of hepatic fibrosis.

In conclusion, we extend the knowledge regarding resistin expression in autoimmune conditions of the gastrointestinal tract. The results show increased resistin levels in patients with breach of self-tolerance, *i.e.* ANA positivity; and diagnoses with raised resistin (AIH, PSC and CD) were significantly associated with the need of more immunosuppressive treatment in form of steroids and thiopurines (the latter only significant for CD). Our interpretation is that resistin levels are associated with disease severity rather than disease activity. Thus, we propose that resistin could be a surrogate marker for disease severity in these conditions. We admit, however, that this study is small and that the observations need confirmation. To fully answer the question on how to position resistin analysis in the clinical situation, studies with consecutive samples from patients with “active” and “non-active” disease are warranted.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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References

[1] Czaja AJ, Norman GL. Autoantibodies in the diagnosis and management of liver disease. *J Clin Gastroenterol* 2003;37:315–329

[2] Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. *Nature* 2005;435:590–597

[3] Longhi MS, Hussain MJ, Mitry RR, Arora SK, Mieli-Vergani G, Vergani D, et al. Functional study of CD4+CD25+ regulatory T cells in health and autoimmune hepatitis. *J Immunol* 2006;176:4484–4491

[4] Behr MA, Divangahi M, Lalande JD. What's in a name? The (mis)labelling of Crohn's as an autoimmune disease. *Lancet* 2010;376:202–203

[5] Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, et al. A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci U S A* 2001;98:502–506

[6] Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, et al. FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. *EMBO J* 2000;19:4046–4055

[7] Artis D, Wang ML, Keilbaugh SA, He W, Brenes M, Swain GP, et al. RELMbeta/FIZZ2 is a goblet cell-specific immune-effector molecule in the gastrointestinal tract. *Proc Natl Acad Sci U S A* 2004;101:13596–13600

[8] Nagaev I, Bokarewa M, Tarkowski A, Smith U. Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adipocytes. *PLoS One* 2006;1:e31

[9] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature* 2001;409:307–312

[10] Pang SS, Le YY. Role of resistin in inflammation and inflammation-related diseases. *Cell Mol Immunol* 2006;3:29–34

[11] Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. *J Immunol* 2005;174:5789–5795

[12] Johansson L, Linnér A, Sundén-Cullberg J, Haggar A, Herwald H, Loré K, et al. Neutrophil-derived hyperresistinemia in severe acute streptococcal infections. *J Immunol* 2009;183:4047–4054

[13] Sundén-Cullberg J, Nyström T, Lee ML, Mullins GE, Tokics L, Andersson J, et al. Pronounced elevation of resistin correlates with severity of disease in severe sepsis and septic shock. *Crit Care Med* 2007;35:1536–1542

[14] Baker R, Dauner JG, Rodriguez AC, Williams MC, Kemp TJ, Hildesheim A, et al. Increased plasma levels of adipokines and inflammatory markers in older women with persistent HPV infection. *Cytokine* 2011;53:282–285

[15] Herbert DR, Yang JQ, Hogan SP, Groschwitz K, Khodoun M, Munitz A, et al. Intestinal epithelial cell secretion of RELM-beta protects against gastrointestinal worm infection. *J Exp Med* 2009;206:2947–2957

[16] Almehed K, d'Elia HF, Bokarewa M, Carlsten H. Role of resistin as a marker of inflammation in systemic lupus erythematosus. *Arthritis Research Ther* 2008;10:R15

[17] Boström EA, d'Elia HF, Dahlgren U, Simark-Mattsson C, Hasseus B, Carlsten H, et al. Salivary resistin reflects local inflammation in Sjögren's syndrome. *J Rheumatol* 2008;35:2005–2011

[18] Karmiris K, Koutroubakis IE, Xidakis C, Polychronaki M, Voudouri T, Kouroumalis EA. Circulating levels of leptin, adiponectin, resistin, and ghrelin in inflammatory bowel disease. *Inflamm Bowel Dis* 2006;12:100–105

[19] Konrad A, Lehrke M, Schachinger V, Seibold F, Stark R, Ochsenkuhn T, et al. Resistin is an inflammatory marker of inflammatory bowel disease in humans. *Eur J Gastroenterol Hepatol* 2007;19:1070–1074

[20] Paul G, Schäffler A, Neumeier M, Fürst A, Bataillle F, Buechler C, et al. Profiling adipocytokine secretion from creeping fat in Crohn's disease. *Inflamm Bowel Dis* 2006;12:471–477

[21] Karmiris K, Koutroubakis IE. Resistin: another rising biomarker in inflammatory bowel disease? *Eur J Gastroenterol Hepatol* 2007;19:1035–37

[22] Durazzo M, Niro G, Premoli A, Morello E, Rizzotto ER, Gambino R, et al. Type 1 autoimmune hepatitis and adipokines: new markers for activity and disease progression? *J Gastroenterol* 2009;44:476–482

[23] Tiftikci A, Atug O, Yilmaz Y, Eren F, Ozdemir FT, Yapali S, et al. Serum levels of adipokines in patients with chronic HCV infection: relationship with steatosis and fibrosis. *Arch Med Res* 2009;40:294–298

[24] Pagano C, Soardo G, Pilon C, Milocco C, Basan L, Milan G, et al. Increased serum resistin in non-alcoholic fatty liver disease is related to liver disease severity and not to insulin resistance. *J Clin Endocrinol Metab* 2006;91:1081–1086

[25] Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006;44:865–873

[26] Evans JG, Acheson ED. An epidemiological study of ulcerative colitis and regional enteritis in the Oxford area. *Gut* 1965;6:311–324

[27] Werner M, Prytz H, Ohlsson B, Almer S, Björnsson E, Bergquist A, et al. Epidemiology and the initial presentation of autoimmune hepatitis in Sweden: a nationwide study. *Scand J Gastroenterol* 2008;43:1232–1240

[28] Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999;31:929–938

[29] Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. *Gastroenterology* 1980;79:200–206

[30] Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: current diagnostic and clinical challenges. *World J Gastroenterol* 2008;14:3374–3387

[31] Gonzalez-Gay MA, Garcia-Unzueta MT, Gonzalez-Juanatey C, Miranda-Filloy JA, Vazquez-Rodriguez TR, De Matias JM, et al. Anti-TNF-alpha therapy modulates resistin in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2008;26:311–316

[32] LaRochelle J, Freiler J, Dice J, Hagan L. Plasma resistin levels in asthmatics as a marker of disease state. *J Asthma* 2007;44:509–513

[33] Walcher D, Hess K, Berger R, Aleksic M, Heinz P, Bach H, et al. Resistin – a newly identified chemokine for human CD4-positive lymphocytes. *Cardiovasc Res* 2010;85:167–174

[34] Son YM, Ahn SM, Jang MS, Moon YS, Kim SH, Cho KK, et al. Immunomodulatory effect of resistin in human dendritic cells stimulated with lipoteichoic acid from Staphylococcus aureus. *Biochem Biophys Res Commun* 2008;376:599–604

[35] Nair MG, Guild KJ, Du Y, Zaph C, Yancopoulos GD, Valenzuela DM, et al. Goblet cell-derived resistin-like molecule beta augments CD4+ T cell production of IFN-gamma and infection-induced intestinal inflammation. *J Immunol* 2008;181:4709–4715

[36] Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 2003;300:472–476

[37] Boström EA, Tarkowski A, Bokarewa M. Resistin is stored in neutrophil granules being released upon challenge with inflammatory stimuli. *Biochim Biophys Acta* 2009;1793:1894–1900

[38] Bertolani C, Sancho-Bru P, Failli P, Bataller R, Aleffi S, DeFranco R, et al. Resistin as an intrahepatic cytokine: overexpression during chronic injury and induction of proinflammatory actions in hepatic stellate cells. *Am J Pathol* 2006;169:2042–2053

[39] Sheng CH, Di J, Jin Y, Zhang YC, Wu M, Sun Y, et al. Resistin is expressed in human hepatocytes and induces insulin resistance. *Endocrine* 2008;33:135–143

[40] Yagmur E, Trautwein C, Gressner AM, Tacke F. Resistin serum levels are associated with insulin resistance, disease severity, clinical complications, and prognosis in patients with chronic liver diseases. *Am J Gastroenterol* 2006;101:1244–1252

[41] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94:2467–2474