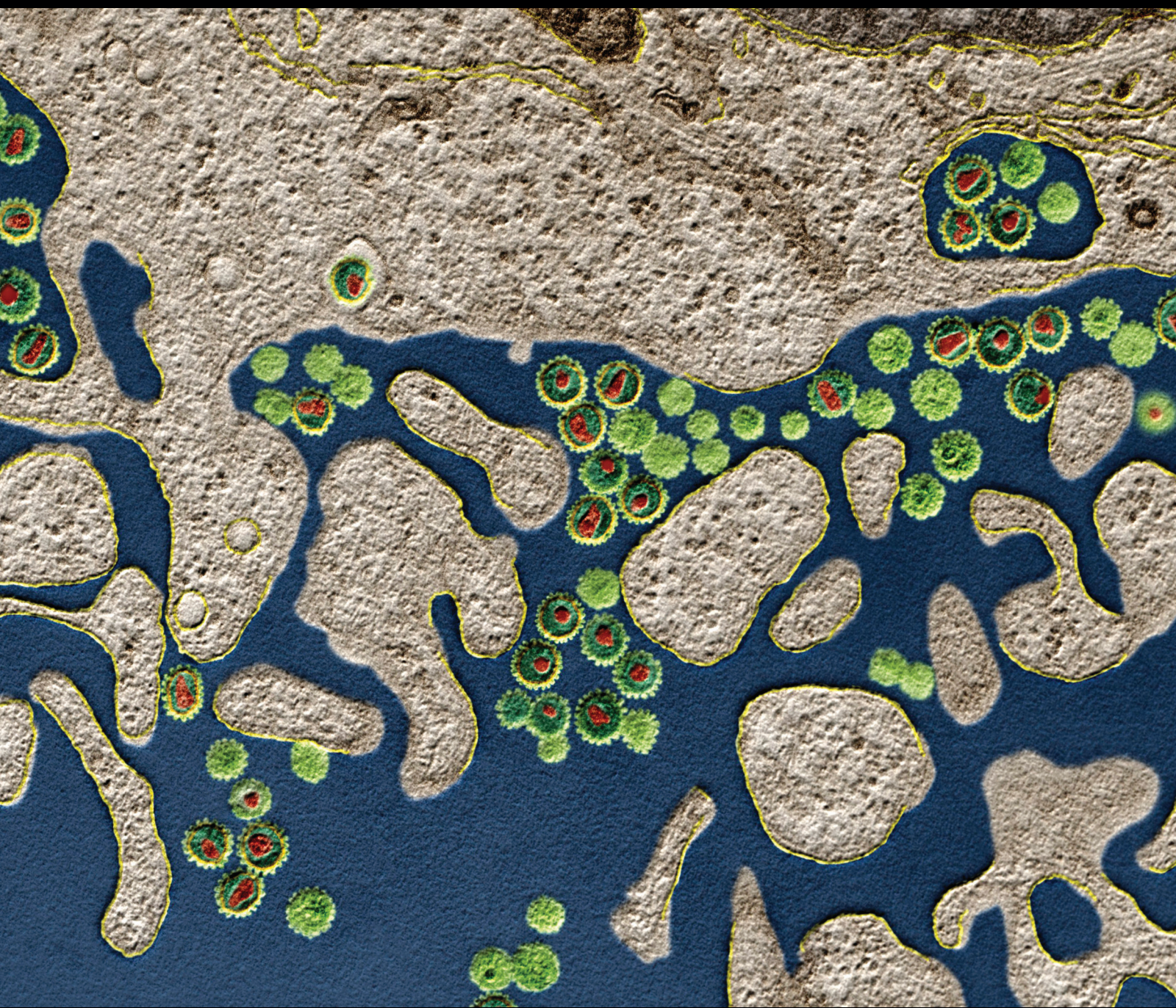
Journal of Immunology Research

Inflammation and Dysmetabolism in Systemic Autoimmune Diseases

Lead Guest Editor: Antonella Afeltra
Guest Editors: Antonio Abbate, Gabriele Valentini, and Roberto Giacomelli



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Editorial Board

Contents

Inflammation and Dysmetabolism in Systemic Autoimmune Diseases
Antonella Afeltra, Antonio Abbate, Gabriele Valentini, and Roberto Giacomelli
Editorial (2 pages), Article ID 5438287, Volume 2019 (2019)

The Association of Olfactory Dysfunction, Frailty, and Mortality Is Mediated by Inflammation: Results from the InCHIANTI Study
Alice Laudisio, Luca Navarini, Domenico Paolo Emanuele Margiotta, Davide Onofrio Fontana, Irene Chiarella, Daniele Spitaleri, Stefania Bandinelli, Antonella Gemma, Luigi Ferrucci, and Raffaele Antonelli Incalzi
Research Article (8 pages), Article ID 3128231, Volume 2019 (2019)

Fn14 Deficiency Ameliorates Anti-dsDNA IgG-Induced Glomerular Damage in SCID Mice
Jiawen Wu, Xiaoyun Min, Li Wang, Jie Yang, Ping Wang, Xingyin Liu, and Yumin Xia
Research Article (12 pages), Article ID 1256379, Volume 2018 (2019)

Low Serum IGF-1 in Boys with Recent Onset of Juvenile Idiopathic Arthritis
Anna-Carin Lundell, Malin Erlandsson, Maria Bokarewa, Hille Liivamägi, Karin Uibo, Sirje Tarraste, Tiina Rebane, Tiina Talvik, Chris Pruunsild, and Rille Pullerits
Research Article (10 pages), Article ID 3856897, Volume 2018 (2019)

Adipocytokines in Rheumatoid Arthritis: The Hidden Link between Inflammation and Cardiometabolic Comorbidities
Piero Ruscitti, Paola Di Benedetto, Onorina Berardicurti, Vasiliki Liakouli, Francesco Carubbi, Paola Cipriani, and Roberto Giacomelli
Review Article (10 pages), Article ID 8410182, Volume 2018 (2019)

Role of the Specialized Proresolving Mediator Resolvin D1 in Systemic Lupus Erythematosus: Preliminary Results
Luca Navarini, Tiziana Bisogno, Domenico Paolo Emanuele Margiotta, Alessandra Piccoli, Silvia Angeletti, Alice Laudisio, Massimo Ciccozzi, Antonella Afeltra, and Mauro Maccarrone
Research Article (6 pages), Article ID 5264195, Volume 2018 (2019)

Lipid and Bile Acid Dysmetabolism in Crohn's Disease
Koji Uchiyama, Hisashi Kishi, Wataru Komatsu, Masanori Nagao, Shuji Ohhira, and Gen Kobashi
Review Article (6 pages), Article ID 7270486, Volume 2018 (2019)

Biological Properties and the Role of IL-25 in Disease Pathogenesis
Yuwan Liu, Zewei Shao, Guoqiang Shangguan, Qingli Bie, and Bin Zhang
Review Article (8 pages), Article ID 6519465, Volume 2018 (2019)

Hindawi
Journal of Immunology Research
Volume 2019, Article ID 5438287, 2 pages
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Editorial

Inflammation and Dysmetabolism in Systemic Autoimmune Diseases

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Despite advances in pathogenic and clinical knowledge, rheumatic diseases are still burdened by high morbidity, accrual of irreversible organ damage with development of disability, and increased mortality [1–4]. In the few last decades, interest in the metabolic aspects of rheumatic diseases has gradually increased. The impact of dysmetabolism on rheumatic diseases is complex and extends from pathogenesis to clinical manifestations and potential therapeutic targets.

Metabolic syndrome (MeS) is a cluster of metabolic disorders that includes visceral adipose tissue accumulation, insulin-resistance, alteration in blood cholesterol components and apolipoproteins, and systemic inflammation [5, 6]. The incidence and prevalence of metabolic syndrome is increased in several systemic autoimmune diseases with possible impact on cardiovascular complication and damage accrual [7–9]. One of the possible links between metabolism, MeS, and inflammation is adipokines, a group of cytokines mainly produced by adipose tissue. Consistent literature data clearly demonstrated the involvement of adipokines in autoimmunity and several systemic autoimmune diseases. In this issue, P. Ruscitti et al. deeply reviewed the role of adipokines in the atherogenesis and MeS development in patients with rheumatoid arthritis.

Interleukin-6 (IL-6) is the prototype of a molecular link between inflammation, autoimmunity, metabolism, and adipose tissue [5]. In this issue, A. Laudisio et al. analyzed the impact of olfactory dysfunction on frailty and mortality of elderly patients and demonstrated that this relation could be mediated by IL-6.

Particularly interesting are the implications of Western diet in rheumatic diseases. Polyunsaturated fatty acids (PUFAs) are members of the family of fatty acids, with a wide spectrum of immunological functions: n-6 PUFAs have predominantly proinflammatory features, while n-3 PUFAs seem to exert anti-inflammatory and proresolving properties. We recently reviewed the literature on PUFA in rheumatoid arthritis, showing that n-3-PUFA supplementation could represent an interesting therapeutic option [10]. D-Series resolvins are a product of the metabolism of n-3 PUFA. Crescent data demonstrated the involvement of D-series resolvins and, in particular, resolvin-D1 in immune homeostasis. In general, resolvin-D1 seems to downregulate the production of proinflammatory cytokines from T helper 1 and T helper 17 lymphocytes and to promote the differentiation of T regulatory cells. However, only few data are available on the role of resolvins in systemic autoimmune diseases. In a paper published in this special issue, L. Navarini et al. demonstrated a marked reduction of resolvin-D1 levels in patients affected by Systemic Lupus Erythematosus (SLE) compared to the general population, especially in association with low complement levels. These findings suggest a specific role of bioactive lipids in SLE [11].

Another relevant topic in the field of relation between inflammation and metabolism is represented by bile acids. Bile acids play a pivotal role in intestinal absorption of fatty

acids and in delivery of fatty acids to the apical membrane of enterocytes. K. Uchiyama et al. presented in this special issue a review of the available evidences on the implication of dietary lipids and fatty acids malabsorption in Crohn disease.

Overall, crescent data demonstrated the involvement of metabolism in several aspects of systemic autoimmune diseases with interesting implication for disease prevention, optimization of disease management, and drug development.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue.

Antonella Afeltra
Antonio Abbate
Gabriele Valentini
Roberto Giacomelli

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Research Article

The Association of Olfactory Dysfunction, Frailty, and Mortality Is Mediated by Inflammation: Results from the InCHIANTI Study

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Background. Olfactory dysfunction might unveil the association between ageing and frailty, as it is associated with declining cognitive function, depression, reduced physical performance, reduced dietary intake, and mortality; all these conditions are characterized by increased levels of inflammatory parameters. The present study is aimed at evaluating the association between olfactory dysfunction, frailty, and mortality and whether such association might be mediated by inflammation. *Methods*. We analysed data of 1035 participants aged 65+ enrolled in the "InCHIANTI" study. Olfactory function was tested by the recognition of the smells of coffee, mint, and air. Olfactory dysfunction was defined as lack of recognition of at least two smells. Considering the items "shrinking," "exhaustion," "sedentariness," "slowness," and "weakness" included in the Fried definition, frailty was defined as the presence of at least three criteria, prefrailty of one or two, and robustness of none. Serum interleukin-6 (IL-6) was measured in duplicate by high-sensitivity enzyme-linked immunosorbent assays. Logistic regression was adopted to assess the association of frailty with olfactory function, as well as with the increasing number of olfactory deficits. Cox regression was used to test the association between olfactory dysfunction and 9-year survival. *Results*. Olfactory dysfunction was associated with frailty, after adjusting (OR 1.94, 95% CI = 1.07-3.51; $P = .028$); analysis of the interaction term indicated that the association varied according to interleukin-6 levels (P for interaction = .005). Increasing levels of olfactory dysfunction were associated with increasing probability of being frail. Also, olfactory dysfunction was associated with reduced survival (HR 1.52, 95% CI = 1.16-1.98; $P = .002$); this association varied according to the presence of frailty (P for interaction = .017) and prefrailty status (P for interaction = .046), as well as increased interleukin-6 levels (P for interaction = .011). *Conclusions*. Impairment of olfactory function might represent a marker of frailty, prefrailty, and consequently reduced survival in an advanced age. Inflammation might represent the possible link between these conditions.

1. Introduction

Due to its prevalence rates exceeding 50% among individuals aged 65-80 years and reaching 80% above the age of 80, olfaction dysfunction is considered a very common problem in older populations [1]. This sensory deficit has important implications for safety, nutrition, quality of life, and social relationships [2]. Olfactory impairment is partially age-related and reflects either central neurodegenerative mechanisms or peripheral cumulative damage of olfactory receptors [1]. In fact, the olfactory system is the only sense which depends upon stem cell turnover, and the olfactory nerve is the only cranial nerve directly exposed to the environment [1].

Frailty is an age-related condition of increased vulnerability, associated with higher risk of several adverse outcomes,

including mortality [3]. Among different criteria proposed to define frailty, the frailty phenotype proposed by Fried and colleagues is among the most commonly adopted [4]; also, prefrailty status has been associated with reduced survival, as compared with robustness [3]. Indeed, frailty can be attenuated and even reversed, so that this syndrome has to be considered a dynamic process, mainly for subjects in their intermediate stage [5]. In an Italian cohort of elderly people, although most participants tended to retain their baseline frailty status, more than one-third of the sample experienced a transition (with either improvement or worsening) in their frailty status over a four-year follow-up [6].

It has been documented that sensory perception, including smell perception, is associated with several components of frailty [7]. On the other hand, it has been acknowledged that both frailty and olfactory loss are associated with reduced survival [3, 8]. Furthermore, both olfactory impairment and frailty are characterized by subclinical inflammation, which could partially explain the adverse outcomes associated with these two conditions.

Olfactory impairment, but not hearing or visual impairment, has been associated with decreased survival in older subjects [9]. However, to our knowledge, neither the association of olfactory impairment with prefrailty nor the impact of frailty phenotypes on the association between olfactory dysfunction and mortality has been so far investigated.

The aim of this study was to assess in an older population the association, if any, of olfactory dysfunction with frailty and mortality and whether such an association might be mediated by frailty status.

2. Methods

2.1. Study Design and Participants. The present study is based upon the data from the "Invecchiare in Chianti" study, a prospective population-based study of older persons in Tuscany, Italy, that is aimed at identifying risk factors for late-life disability [10]. The Italian National Research Council on Aging Ethical Committee ratified the study protocol, and participants provided written consent to participate.

Analyses for this study included all 1035 subjects aged 65+.

2.2. Frailty. Frailty was defined according to the Fried criteria [4]: unintended weight loss, self-reported exhaustion, muscle weakness, slowness, and sedentariness. Weight loss was defined as self-reported unintentional weight loss > 4.5 kg within the past year. Exhaustion was defined as a response of "occasionally," "often," or "always" to the statement "I felt that everything was an effort." Muscle weakness was defined as grip strength in the lowest quintile, stratified by sex and BMI quartiles. Grip strength was measured by a handheld dynamometer (Nicholas Muscle Tester, Sammons Preston Inc.). Slowness was defined as the time to walk 4.57 meters or 15 ft (the mean of 2 repetitions) in the slowest quintile, stratified by sex and height. Sedentariness was defined as either complete inactivity or spending <1 h/wk performing low-intensity activities. "Frailty" was defined as the presence of at least three criteria, "prefrailty" of one or two criteria, and "robustness" of none.

This syndrome is thought to emerge from multisystem dysregulation that is common in older adults and characterized by increased vulnerability to stressors and increased risk of disease, disability, and death. Also, frailty is linked to multimorbidity and inflammation.

2.3. Mortality. Data on 9-year mortality were collected using the data from the Mortality General Registry maintained by the Tuscany Region, as well as death certificates delivered immediately after death to the registry office of the municipality of residence.

2.4. Olfactory Function. Olfactory function was self-reported and explored during the medical visit according to the questions: "Does he/she recognize mint?", "does he/she recognize coffee?", and "does he/she recognize air?". Olfactory dysfunction was defined when at least two smells were not recognized. Increasing levels of olfactory impairment (0 to 3 smell losses) were also considered.

2.5. Inflammation. Blood samples were drawn in the morning after a 12-hour overnight fast and resting period. Aliquots of serum were stored at −80°C. Serum interleukin-6 (IL-6) was measured in duplicate by high-sensitivity enzyme-linked immunosorbent assays (ELISAs; kits from BioSource, Camarillo, CA) with a sensitivity of 0.1 pg/mL and an intra-assay coefficient of variations less than 6%.

2.6. Covariates. Data on dietary intake were collected by the questionnaire created for the European Prospective Investigation into Cancer and Nutrition (EPIC) study [11]. Adjudicated disease diagnoses were based on self-reported history, clinical documentation, and medication use, as well as standardized criteria derived from the Women's Health and Aging Study protocol [12]. Comorbidity was quantified using the Charlson Comorbidity Index score [13]. All drugs assumed by participants were coded according to the Anatomical Therapeutic Chemical codes [14]. Functional ability was estimated using Katz's activities of daily living [15], depressive symptoms by the original 20-item version of the Center for Epidemiological Studies Depression Scale (CES-D) [16], and cognitive performance by the Mini Mental State Examination [17]. Blood samples were obtained from participants after 12-hour fasting and after resting for at least 15 minutes. Aliquots of serum were stored at –80°C and were not thawed until analysis. Interleukin-6 concentrations were determined by high-sensitivity ELISA using commercial kits (Human Ultrasensitive, BioSource International Inc., Camarillo, CA, USA). Glomerular filtration rate was estimated using the Cockcroft-Gault equation.

2.7. Statistical Analyses. Data were recorded using dedicated software. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS for Mac version 20.0, 2011, SPSS Inc., Chicago, IL); differences were considered significant at the $P < .050$ level.

Table 1: Characteristics of 1035 participants according to olfactory dysfunction.

	Presence of olfactory dysfunction ($n = 590$)	Absence of olfactory dysfunction ($n = 445$)	P
Demographics & lifestyle habits, n (%), mean (SD), or median (IQR)			
Age (years)	76 (8)	73 (7)	<.001
Sex (female)	315 (53)	262 (59)	.088
Education (years)	5 (3)	6 (3)	.003
Living alone	241 (41)	164 (37)	.199
Smoking (former and current)	253 (43)	170 (38)	.142
Dietary intake			
Alcohol (g/day/kg)	0.11 (0–0.29)	0.10 (0–0.31)	.594
Total protein intake (g/day/kg)	1.12 (0.33)	1.13 (0.31)	.657
Total lipid intake (g/day/kg)	0.96 (0.31)	0.98 (0.31)	.310
Available carbohydrate intake (g/day/kg)	3.73 (1.17)	3.72 (1.29)	.913
Fibre (g/day/kg)	0.29 (0.08)	0.29 (0.08)	.551
Energy intake (kcal/day/kg)	28.60 (8.25)	28.71 (8.50)	.829
Comorbid conditions, n (%) or median (IQR)			
Diabetes	67 (11)	47 (11)	.764
Heart failure	39 (7)	20 (4)	.176
Chronic pulmonary disease	52 (9)	37 (8)	.823
Parkinson's disease	15 (2)	12 (3)	.999
Stroke	37 (6)	23 (5)	.503
Hip fracture	19 (3)	19 (4)	.406
Peripheral arterial disease	80 (14)	41 (9)	.032
Malignancy	28 (5)	36 (8)	.036
Frailty phenotype	85 (14)	26 (6)	<.001
Charlson Comorbidity Index	1 (0-2)	1 (0-1)	.489
Medications, n (%), mean (SD), or median (IQR)			
Neuroleptics	18 (3)	15 (3)	.859
Selective serotonin reuptake inhibitors	13 (2)	3 (1)	.072
ACE inhibitors	92 (16)	49 (11)	.035
Antiplatelets	73 (12)	40 (9)	.088
Anticoagulants	8 (1)	5 (1)	.787
Benzodiazepines	112 (19)	61 (14)	.029
Loop diuretics	53 (9)	32 (7)	.306
Corticosteroids	8 (1)	10 (2)	.339
Biohumoral, physical, and cognitive parameters, n (%) or mean ± SD			
Glomerular filtration rate (mL/min)	62.6 (19.3)	68.1 (19.2)	<.001
Total serum proteins (g/dL)	7.2 (0.4)	7.1 (0.5)	.308
Interleukin 6 (pg/mL)	1.49 (0.84-2.32)	1.44 (0.88-2.27)	.582
Hemoglobin (g/dL)	13.6 (1.4)	13.8 (1.4)	.112
CES-D	13 (9)	12 (8)	.035
Mini Mental State Examination	24.3 (4.3)	24.7 (5.5)	.160
Katz's activities of daily living	5 (1)	4 (2)	.107
Body mass index (kg/m^2)	27.3 (4.0)	27.6 (4.2)	.183

Data of continuous variables are presented as mean values ± standard deviation or medians and interquartile ranges. Normally distributed variables according to olfactory dysfunction, as well as to mortality, were assessed by the analysis of variance (ANOVA) or the nonparametric Mann–Whitney U test if appropriate. The two-tailed Fisher exact test was used for dichotomous variables.

Multivariable logistic regression was used to evaluate the association of the frailty phenotype with age, sex, and all those variables which differed significantly in univariate analysis, including olfactory dysfunction.

The fully adjusted model was also adopted to evaluate the association of increasing levels of olfactory dysfunction with frailty. Also, the analysis of the interaction terms

Table 2: Characteristics of 1035 participants according to the presence of frailty.

	Positive for frailty ($n = 111$)	Negative for frailty ($n = 924$)	P
Demographics & lifestyle habits, n (%), mean (SD), or median (IQR)			
Age (years)	81 (7)	74 (7)	<.001
Sex (female)	70 (63)	507 (55)	.106
Education (years)	4 (3)	5 (3)	<.001
Living alone	68 (61)	337 (36)	<.001
Smoking (former and current)	38 (34)	385 (42)	.153
Dietary intake			
Alcohol (g/day/kg)	0.04 (0–0.25)	0.11 (0–0.31)	.021
Total protein intake (g/day/kg)	1.04 (0.30)	1.12 (0.32)	.009
Total lipid intake (g/day/kg)	0.87 (0.28)	0.97 (0.31)	.002
Available carbohydrates intake (g/day/kg)	3.41 (1.11)	3.76 (1.23)	.008
Fibre (g/day/kg)	0.27 (0.09)	0.30 (0.09)	.006
Energy intake (kcal/day/kg)	26.0 (7.5)	29.0 (8.4)	.001
Comorbid conditions, n (%) or median (IQR)			
Diabetes	16 (14)	98 (11)	.259
Heart failure	19 (17)	40 (4)	<.001
Chronic pulmonary disease	18 (16)	71 (8)	.006
Parkinson's disease	8 (7)	19 (2)	.002
Stroke	16 (14)	44 (5)	<.001
Hip fracture	11 (10)	27 (3)	.001
Peripheral arterial disease	23 (21)	98 (11)	.004
Malignancy	8 (7)	56 (6)	.675
Olfactory dysfunction	85 (77)	505 (55)	<.001
Charlson Comorbidity Index	1 (1-2)	0 (0-1)	<.001
Medications, n (%), mean (SD), or median (IQR)			
Neuroleptics	10 (9)	23 (5)	.001
Selective serotonin reuptake inhibitors	7 (6)	9 (1)	.001
ACE inhibitors	24 (22)	117 (13)	.013
Antiplatelets	18 (16)	95 (10)	.075
Anticoagulants	2 (2)	11 (1)	.641
Benzodiazepines	32 (29)	141 (15)	.001
Loop diuretics	21 (19)	64 (7)	<.001
Corticosteroids	4 (4)	14 (1)	.118
Biohumoral, physical, and cognitive parameters, n (%) or mean ± SD			
Glomerular filtration rate (mL/min)	54.8 (20.5)	66.0 (19.0)	<.001
Total serum proteins (g/dL)	7.2 (0.6)	7.1 (0.4)	.838
Interleukin 6 (pg/mL)	2.21 (1.35 – 4.09)	1.40 (0.83–2.07)	<.001
Hemoglobin (g/dL)	13.1 (1.6)	13.8 (1.3)	<.001
CES-D	20 (9)	12 (8)	<.001
Mini Mental State Examination	21 (6)	25 (4)	<.001
Katz's activities of daily living	6 (0-1)	6 (0–0)	<.001
Body mass index (kg/m^2)	27.9 (5.1)	27.4 (4.0)	.289

"olfactory dysfunction*interleukin-6" was performed to assess whether the association of frailty with olfactory dysfunction varied according to inflammation.

In addition, to evaluate the whole spectrum of the frailty phenotype, the same summary model was analysed in multinomial logistic regression having robustness, prefrailty, and frailty as the dependent variables.

Also, Cox proportional hazard regression analysis was used to estimate the association of mortality with age, sex, and all those variables which differed significantly in univariate analysis, including olfactory dysfunction. Eventually, in Cox regression, the analysis of the interaction terms "olfactory dysfunction*frailty," "olfactory dysfunction*prefrailty," and "olfactory dysfunction*

Table 3: Characteristics of 1035 participants according to survival status.

	Dead ($n = 393$)	Alive ($n = 642$)	P
Demographics & lifestyle habits, n (%), mean (SD), or median (IQR)			
Age (years)	80 (7)	72 (5)	<.001
Sex (female)	200 (51)	377 (59)	.014
Education (years)	5 (3)	6 (3)	<.001
Living alone	206 (52)	199 (31)	<.001
Smoking (former and current)	172 (44)	251 (39)	.152
Dietary intake			
Alcohol (g/day/kg)	0.10 (0–0.26)	0.10 (0–0.32)	.021
Total protein intake (g/day/kg)	1.14 (0.32)	1.11 (0.32)	.262
Total lipid intake (g/day/kg)	0.97 (0.30)	0.96 (0.32)	.582
Available carbohydrate intake (g/day/kg)	3.82 (1.26)	3.67 (1.20)	.066
Fibre (g/day/kg)	0.29 (0.09)	0.29 (0.08)	.654
Energy intake (kcal/day/kg)	29.08 (8.33)	28.40 (8.37)	.227
Comorbid conditions, n (%) or median (IQR)			
Diabetes	52 (13)	62 (10)	.082
Heart failure	46 (12)	13 (2)	<.001
Chronic pulmonary disease	63 (16)	26 (4)	<.001
Parkinson's disease	21 (5)	6 (1)	<.001
Stroke	44 (11)	16 (2)	<.001
Hip fracture	24 (6)	14 (2)	.002
Peripheral arterial disease	86 (22)	35 (5)	<.001
Malignancy	28 (7)	36 (6)	.353
Olfactory dysfunction	249 (63)	341 (53)	.001
Frailty	90 (23)	21 (3)	<.001
Charlson Comorbidity index	1 (0-2)	0 (0-1)	<.001
Medications, n (%), mean (SD), or median (IQR)			
Neuroleptics	17 (4)	16 (2)	.143
Selective serotonin reuptake inhibitors	11 (3)	5 (1)	.017
ACE inhibitors	72 (18)	69 (11)	.001
Antiplatelets	65 (16)	48 (7)	<.001
Anticoagulants	11 (3)	2 (1)	.001
Benzodiazepines	81 (21)	92 (14)	.010
Loop diuretics	55 (14)	30 (5)	<.001
Corticosteroids	11 (3)	7 (1)	.050
Biohumoral, physical, and cognitive parameters, n (%) or mean ± SD			
Glomerular filtration rate (mL/min)	56.4 (19.5)	69.5 (17.8)	<.001
Total serum proteins (g/dL)	7.1 (0.5)	7.1 (0.4)	.680
Interleukin 6 (pg/mL)	1.89 (1.14–3.31)	1.22 (0.78–1.84)	<.001
Hemoglobin (g/dL)	13.4 (1.6)	13.9 (1.2)	<.001
CES-D	14 (9)	12 (9)	<.001
Mini Mental State Examination	22 (6)	26 (3)	<.001
Katz's activities of daily living	5 (1)	6 (0)	<.001
Body mass index (kg/m^2)	27.0 (4.3)	27.7 (4.0)	.016

interleukin-6," was performed to assess whether the association between reduced survival and olfactory dysfunction varied according to the presence of frailty, prefrailty, and inflammatory status.

3. Results

The main characteristics of 1035 participants according to olfactory dysfunction are depicted in Table 1. Frailty was

Table 4: Association (odds ratios (OR) and 95% confidence intervals (CI)) of frailty with the variables of interest, including olfactory dysfunction, according to the logistic regression model. All the covariates were entered simultaneously into the regression model.

	OR	95% CI	*P*
Age (each year)	1.15	1.09-1.21	<.001
Sex (female)	.92	.52-1.64	.778
Education (years)	.95	.86-1.05	.306
Malignancy	1.72	.65-4.57	.279
Peripheral arterial disease	2.46	1.29-4.69	.006
ACE inhibitors	1.65	.87-3.12	.122
Benzodiazepines	1.15	.63-2.10	.653
Glomerular filtration rate (mL/min)	1.02	1.01-1.04	.018
CES-D	1.11	1.07-1.14	.000
Olfactory dysfunction	1.94	1.07-3.51	.028

diagnosed in 111 (11%) subjects, prefrailty in 420 (41%) participants, and robustness in 504 (48%). The main characteristics of subjects according to frailty are shown in Table 2.

Over the 9-year follow-up, 393 (38%) subjects died. The main characteristics of participants according to survival are depicted in Table 3.

Olfactory dysfunction was reported by 590/1035 (57%) participants; specifically, lack of recognition of one smell was recorded in 190 (18%) subjects, two smells in 243 (23%), and three smells in 347 (33%). In particular, failure to recognize air was found in 638 (62%) subjects, failure to recognize mint was found in 574 (55%), and failure to recognize coffee was found in 505 (49%).

In multivariable logistic regression, olfactory dysfunction was associated with increased probability of being frail (OR 1.94, 95% CI = 1.07-3.51; $P = .028$), after adjusting (Table 4). Analysis of the interaction term indicated that the association of frailty with olfactory dysfunction varied according to interleukin-6 levels (P for interaction = .005).

Also, increasing levels of olfactory dysfunction were associated with increasing probability of frailty (P for trend = .021).

Both frailty (OR 2.60, 95% CI = 1.39-4.85) and prefrailty (OR 1.59, 95% CI = 1.17-2.16) were associated with olfactory dysfunction in multinomial logistic having robustness as the reference.

According to Cox regression analysis, olfactory dysfunction was associated with reduced survival (HR 1.52, 95% CI = 1.16-1.98; $P = .002$), after adjusting (Figure 1); analysis of the interaction term indicated that this association varied according to the presence of frailty ($P = .017$), prefrailty ($P = .046$), and increased interleukin-6 levels (P for interaction = .011).

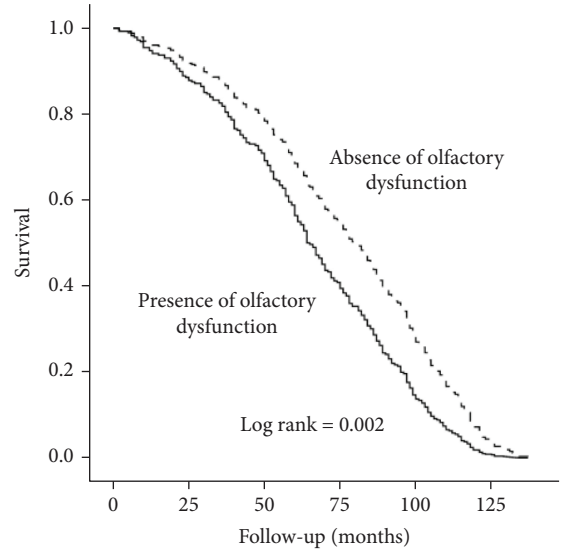

Figure 1: Nine-year survival curves of participants stratified for olfactory dysfunction. The model was simultaneously adjusted for age, sex, education level, glomerular filtration rate, hemoglobin levels, CES-D, Mini Mental State Examination, ADLs, diagnosis of malignancy, peripheral arterial disease, use of ACE inhibitors and benzodiazepines, and frailty.

4. Discussion

Results of the present study indicate that in older subjects, olfactory dysfunction is associated not only with frailty, but even with prefrailty. This association seems to be mediated by subclinical inflammation.

This association was independent of several confounders, including comorbid conditions, medication use, and lifestyle habits; this finding indicates that olfactory dysfunction might represent an early marker of increased risk for adverse outcomes. In fact, in this population, olfactory dysfunction represented a risk factor for reduced survival, and both frailty and prefrailty seemed to mediate this association.

Several factors might explain the association of olfactory dysfunction with frailty and mortality.

Olfactory dysfunction is among the earliest findings which predict the development of mild cognitive impairment [18]. Also, olfactory dysfunction heralds several neurodegenerative disorders, including Parkinson's disease, which is a paradigm of frailty [2]; of notice, olfactory loss has been included as a marker of prodromal Parkinson's disease by the Movement Disorders Society [19]. Among patients with Parkinson's disease, the severity of olfactory dysfunction seems to correlate with the severity of ensuing dementia [2].

In experimental as well as human models, olfactory dysfunction has been linked with the expression of the apolipoprotein e4 allele and of tau protein and amyloid-β deposits [20, 21]; all these findings, in turn, are associated with several adverse clinical outcomes, including cardiovascular diseases and Alzheimer's disease [20]. Olfactory dysfunction has also been associated with depression, probably due to the damage of the hippocampal pathways [22]. In turn, late-life depression has been associated with increased risk of dementia [23].

Systemic diseases, such as diabetes, iron deficiency, and autoimmune diseases, might cause central and peripheral olfactory dysfunction and disrupt the peripheral olfactory pathways [24, 25]. The olfactory deficit represents a preclinical marker of alpha-synucleinopathies and a risk factor for

delirium [2, 26]. Also, impairment in olfactory function has been related to the intake of macro- and micronutrients and directly affects food intake behaviour [27]. Eventually, olfactory dysfunction represents a risk factor for reduced survival [8]. Even better, olfactory dysfunction is the only sense which has been associated with mortality, when compared with hearing or visual impairment [9]. With special regard to the frailty components, olfactory function is associated with mobility, balance, fine motor function, and manual dexterity and independent of cognitive function, with challenging upper- and lower-extremity motor function tasks [28]. Also, olfactory loss represents a risk factor for weight loss, while aerobic exercise might preserve olfactory function in selected populations, such as patients with Parkinson's disease [2, 29].

Furthermore, our finding of a potential role of IL-6 serum levels in the association between olfactory loss and frailty is of interest. Increased IL-6 levels have been found in serum and nasal mucus of hyposmic patients [30]. On the other hand, increased IL-6 serum levels have also been associated with frailty, as well as mortality, in older populations [31, 32]. Thus, inflammation represents a common pathophysiological pathway that links hyposmia, frailty, and mortality in the elderly.

In this study, olfactory dysfunction was self-assessed. Self-assessed tools for evaluating olfactory function might underestimate the dysfunction, as compared with objective evaluation. Nevertheless, this would represent a conservative bias, which further supports our findings. Also, regarding the association of olfactory dysfunction with frailty, due to its cross-sectional design, this study does not allow establishing any cause-effect relationship. Nonetheless, this study enrolled a representative community-dwelling population, with high participation rate and with extensive information on risk factors, comorbid conditions, and objective parameters.

In conclusion, olfactory loss represents a correlate of frailty and even of prefrailty; this association seems to affect the role of olfactory dysfunction as a predictor of mortality in older populations. Thus, olfaction seems worth testing in geriatric practice for both clinical and epidemiological purposes.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

The Italian National Research Council on Aging Ethical Committee ratified the study protocol, and informed consent was obtained from all individual participants included in the study. The present study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Disclosure

None of the sponsoring institutions interfered with the design, methods, subject recruitment, data collections, analysis, and preparation of the paper.

Conflicts of Interest

The authors declare that they have no conflict of interest in this study.

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Research Article

Fn14 Deficiency Ameliorates Anti-dsDNA IgG-Induced Glomerular Damage in SCID Mice

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Many studies have demonstrated that anti-dsDNA IgG is closely associated with lupus nephritis. Recently, it was found that activation of the fibroblast growth factor-inducible 14 (Fn14) signaling pathway damages glomerular filtration barrier in MRL/lpr lupus-prone mice. However, MRL/lpr mice have high titers of serum autoantibodies other than anti-dsDNA IgG. The aim of this study was to further explore the effect of Fn14 deficiency on anti-dsDNA IgG-induced glomerular damage in severe combined immunodeficiency (SCID) mice that have no endogenous IgG. Fn14 deficiency was generated in SCID mice. The murine hybridoma cells producing control IgG or anti-dsDNA IgG were intraperitoneally injected into mice. In two weeks, the urine, serum, and kidney tissue samples were harvested from mice at sacrifice. It showed that the injection of anti-dsDNA IgG, but not control IgG hybridoma cells, induced proteinuria and glomerular damage in SCID mice. Between the wild-type (WT) and knockout (KO) mice injected with anti-dsDNA IgG hybridoma cells, the latter showed a decrease in both proteinuria and glomerular IgG deposition. The histopathological changes, inflammatory cell infiltration, and proinflammatory cytokine production were also attenuated in the kidneys of the Fn14-KO mice upon anti-dsDNA IgG injection. Therefore, Fn14 deficiency effectively protects SCID mice from anti-dsDNA IgG-induced glomerular damage.

1. Introduction

Lupus nephritis (LN) is one of the most common complications occurring in the internal organs of patients with systemic lupus erythematosus (SLE). Although the precise mechanism of LN remains unclear, many studies strongly suggested that anti-double-stranded (ds) DNA IgG is pivotal in the pathogenesis of LN [1–3]. Serum levels of anti-dsDNA IgG increase in patients with LN and fluctuate with flares of lupus disease [4]. Anti-dsDNA IgG binds to circulating nuclear antigens and forms immune complexes, which further deposit in the glomerular basement membrane. Alternatively, anti-dsDNA IgG recognizes multiple renal self-antigens through cross-reaction, such as histone, heparan sulfate, laminin, collagen IV, alpha-actinin, and annexin II [3, 5–9]. Such cross-reaction between anti-dsDNA IgG and renal components leads to direct immune complex formation in local tissues or even IgG penetration into renal resident cells [6, 7]. SCID mice that receive intraperitoneal injection of anti-dsDNA IgG hybridoma cells exhibit renal anti-dsDNA IgG deposition, urine albumin, and microstructural changes in the glomeruli [5]. Anti-dsDNA IgG induces fibronectin secretion of renal tubular epithelial cells and myofibroblast-like phenotype of mesangial cells [10, 11], which are early-stage events of renal fibrosis, a histopathologic feature associated with poor outcomes of LN

[12]. Additionally, anti-dsDNA IgG contributes to renal fibrosis through selective inhibition of the suppressor of cytokine signaling 1 signals [13]. Inhibition of anti-dsDNA IgG production may ameliorate renal damage in LN [2, 14]. Obviously, anti-dsDNA IgG is nephritogenic and participates in the pathophysiological processes of LN.

Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) is a key regulator of proinflammatory cytokines and chemokines. TWEAK acts in the local tissues through engaging its sole receptor fibroblast growth factor-inducible 14 (Fn14). The expression of Fn14 is relatively lower in normal cells, but increases significantly in local tissues under inflammatory conditions [15–19]. The TWEAK/Fn14 signaling pathway is involved in various autoimmune disorders, such as polymyositis or dermatomyositis, systemic sclerosis, bullous pemphigoid, and psoriasis [17, 18, 20, 21]. Moreover, TWEAK/Fn14 signals play an important role in the pathogenesis of lupus erythematosus. TWEAK/Fn14 activation contributes to the damage of the skin, kidney, and brain [22–24]. Fn14 is upregulated in the glomeruli of mice with acute kidney injury, spontaneous LN, or antibody-mediated nephritis, and Fn14 deficiency or neutralizing anti-TWEAK antibody attenuates renal injuries in these models [25–27]. TWEAK/Fn14 interaction can modulate gene expression, cytokine production, and cell cycle (proliferation or apoptosis) of renal resident cells [23, 26, 27]. Therefore, TWEAK/Fn14 activation is prominent in tissue injuries of SLE, including LN.

An interesting phenomenon is that Fn14 deficiency attenuates LN in the MRL/lpr lupus-prone mice, but not affecting serum titers of anti-dsDNA antibodies [27]. Fn14 deficiency significantly reduces proteinuria, glomerular and tubulointerstitial diseases, leukocyte infiltration, and even glomerular IgG deposition in these mice. Nevertheless, the serum levels of total IgG and anti-dsDNA IgG as well as the numbers and distribution of splenocytes are comparable between the Fn14-deficient and wild-type (WT) mice [27]. Moreover, both *in vivo* and *in vitro* experiments showed that Fn14 deficiency preserves the integrity of the renal filtration barrier [27]. These findings suggest that TWEAK/Fn14 activation is pivotal in the pathogenesis of LN.

However, current studies only provide indirect evidence indicating different roles of anti-dsDNA IgG and TWEAK/Fn14 signals in the progression of LN, and there are no results interpreting the relationship between them in the kidneys. Moreover, MRL/lpr mice have high titers of serum autoantibodies other than anti-dsDNA IgG, which may also contribute to the progression of LN [28]. Therefore, the role of TWEAK/Fn14 signals in the nephritogenicity of anti-dsDNA IgG should be elucidated exclusively in the model with anti-dsDNA IgG. The purpose of this study was aimed at revealing the effect of Fn14 deficiency on glomerular injuries of mice that only generate anti-dsDNA IgG.

2. Materials and Methods

2.1. Generation of Fn14-Deficient Mice. Fn14 deficiency was generated in severe combined immunodeficiency (SCID) mice (NOD.CB17-Prkdcscid/NcrCrl strain; Charles River Laboratories, Wilmington, MA, USA) by using a clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) 9 approach [29]. In brief, Cas9 mRNA and small guide RNA were synthesized *in vitro* and then injected into one-cell stage embryos of SCID mice. The F0 generation mice were identified for genotypes by polymerase chain reaction (PCR) and hybridized with other SCID mice. Finally, the Fn14−/− homozygote mice were selected for the next reproduction. Both CRISPR/Cas9 engineering and reproductive experiments were conducted in Shanghai Biomodel Organism Science & Technology Development Company (Shanghai, China). The strategy for CRISPR/Cas9 engineering is detailed in Figure S1.

2.2. Injection of Hybridoma Cells. Murine hybridoma cells of anti-dsDNA IgG (WJ31 clone, IgG2a) or control IgG (WJ77 clone, IgG2a) were purchased from Jieqing Biotech (Wuhan, China) [13]. The Fn14-knockout (KO) and WT mice aged 8 weeks received priming of pristine, followed by intraperitoneal injection of 1×10^7 hybridoma cells per mouse [5]. There were eight mice in each group. Urine, blood, and kidney tissue samples were collected 2 weeks after the injection. The levels of serum IgG (total IgG or IgG isotypes) were measured by enzyme-linked immunosorbent assay as described [27]. Animal experiments were approved by the hospital research ethics committee.

2.3. Immunohistochemistry and Immunofluorescence. Immunohistochemistry was performed as described previously [13]. Paraffin sections were incubated with rabbit primary antibodies to Fn14, Ki-67, platelet-derived growth factor subunit B (PDGFB), CD3, Iba-1, or phospho-epidermal growth factor receptor (pEGFR) (2 μg/ml; Abcam, Cambridge, MA, USA). The secondary antibody was polymer-horseradish peroxidase-conjugated goat anti-rabbit IgG (1 μg/ml; DAKO, Glostrup, Denmark). 3,3′-Diaminobenzidine chromogen substrate (DAKO) was used for color development. Some sections were routinely stained with trichrome or hematoxylin and eosin. By following histological scoring systems [27], the stains were scored by two renal pathologists blinded to the mouse grouping. The pathological changes (by hematoxylin and eosin staining), fibrotic changes (by trichrome or PDGFB staining), and inflammatory cell infiltration (by CD3 or Iba-1 staining) in glomeruli were each scored from 0 to 4 (0, absent; 1, mild; 2, mild–moderate; 3, moderate; and 4, severe) by two pathologists blind to the grouping. Glomerular proliferation was quantitated by counting the number of Ki-67-positive cells in 20 glomeruli of each section.

IgG deposition was detected by immunofluorescence in frozen sections [13]. Sections were incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG isotypes (2 μg/ml; Southern Biotech, Birmingham, AL, USA). A digital fluorescent microscope (Carl Zeiss, Jena, Germany) was used for viewing fluorescence in the sections.

2.4. Transmission Electron Microscopy and Immunogold Staining. As described previously [5], kidney tissue sections were incubated in saturated sodium metaperiodate solution. After blocking with 1% bovine serum albumin in

phosphate-buffered saline, sections were incubated with gold-labeled donkey anti-mouse IgG (Electron Microscopy Sciences, Hatfield, PA, USA). Finally, sections were postfixed in 2% glutaraldehyde solution and examined under an electron microscope (JEOL, Peabody, MA, USA).

2.5. Real-Time Quantitative PCR. Total RNA was extracted from fresh kidney tissues of mice and processed for cDNA by reverse transcription. SYBR Green Master Mix (Invitrogen, Grand Island, NY, USA) was used as a fluorescent dye. PCR was carried out on the 7900HT Fast PCR System (Applied Biosystems, Carlsbad, CA, USA). The sequences of primers (Jieqing Biotech) are detailed in Table S1.

2.6. Western Blotting. Fresh tissues were extracted for protein lysates with the addition of protease inhibitor cocktail (Thermo Scientific, Waltham, MA, USA). Proteins were transferred onto polyvinylidene difluoride membranes, followed by incubation with rabbit IgG targeting Fn14 or pEGFR (2 μg/ml; Abcam). Biotinylated goat anti-rabbit IgG (Southern Biotech) was the secondary antibody (2 μg/ml). After incubation with horseradish peroxidase-streptavidin, an ECL solution kit (Thermo Scientific, Waltham, MA, USA) was used for signal development. The intensities of bands were quantitated by ImageJ 1.61u software (National Institutes of Health, Bethesda, MD, USA) and normalized to the values of β-actin bands accordingly.

2.7. Measurement of Proteinuria, Urine Creatinine and Monocyte Chemotactic Protein 1 (MCP-1), and Blood Urea Nitrogen (BUN). The levels of urine albumin were determined by an enzyme-linked immunosorbent assay kit (Bethyl Laboratories, Montgomery, TX, USA). Urine creatinine and BUN were determined by using commercial kits (BioAssay Systems Inc., Hayward, CA, USA). MCP-1 was determined with a commercial enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA). The protocols were provided by the manufacturers.

2.8. Statistical Analysis. All data were expressed as means ± standard error of the mean. The STATA 10.0 software package (StataCorp, College Station, TX) was used for analyzing these data. Analysis of variance was used for comparing more than two groups. A two-tailed unpaired Student t-test was then used for comparison of the two groups. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Fn14 Deficiency Significantly Reduces Proteinuria in SCID Mice. The expression levels of Fn14 were determined in the kidneys of SCID mice. By Western blotting and real-time PCR, the WT mice exhibited higher protein and mRNA expression of Fn14 when compared with the KO mice (Figure 1). Moreover, both immunohistochemistry and real-time PCR revealed that the anti-dsDNA IgG hybridoma cell-injected WT mice had higher Fn14 expression in glomeruli when compared with the KO mice (Figures 1(a) and 1(b)). There were no significant differences in Fn14 expression between the WT and KO mice that received control IgG hybridoma cells or those that had no injection. The renal mRNA expression of TWEAK was comparable between the anti-dsDNA IgG-injected WT mice and KO mice although both of them had higher levels than the two control groups accordingly (Figure 1(d)). Furthermore, renal function was evaluated in these mice, revealing that the anti-dsDNA IgG hybridoma cell-injected WT mice had higher levels of urine albumin, serum creatinine, and BUN than the other mice, and these levels decreased significantly in the KO mice (Figures 1(d)–1(f)).

3.2. Glomerular IgG Deposition Decreases in Fn14-Deficient SCID Mice. Because TWEAK/Fn14 activation affects renal IgG deposition in lupus-prone mice [27], we further examined IgG deposition in the glomeruli of these mice. By immunofluorescence, the anti-dsDNA IgG hybridoma cell-injected WT mice had the strongest fluorescent intensity in glomeruli (Figures 2(a) and 2(b)). Also, IgG deposits were detected by immunogold staining and transmission electron microscopy, showing that IgG deposition was the most prominent in the glomerular basement membrane of the anti-dsDNA IgG hybridoma cell-injected WT mice (Figure 2(c)). Fn14 deficiency partially reduced both fluorescence and gold particles of anti-dsDNA IgG in glomeruli. There were no differences in IgG deposition between the blank and the control IgG groups (Figure 2). Interestingly, the serum levels of total IgG or anti-dsDNA IgG were comparable between the WT and KO mice that were injected with either control IgG or anti-dsDNA IgG hybridoma cells or as blank controls (Figure S2). Serum IgG1, IgG2b, and IgG3 were not detectable in these mice (data not shown). By immunofluorescence, we also found no deposition of these three isotypes in glomeruli (data not shown).

3.3. Histopathological Changes Are Attenuated in Fn14-Deficient Kidneys. To elucidate the effect of Fn14 deficiency on microstructure of the glomeruli, we evaluated the histopathological changes in these mice. The hematoxylin and eosin-stained kidney sections were scored, showing that the anti-dsDNA IgG hybridoma cell-injected WT mice had the most severe damage in glomeruli (Figures 3(a) and 3(b)). Such histopathological changes were attenuated in the anti-dsDNA IgG hybridoma cell-injected KO mice, which still had a higher score than the KO mice receiving no IgG injection (Figures 3(a) and 3(b)). Glomerular proliferation also reflects the inflammatory severity in LN [26, 27]. Hence, Ki-67 staining was performed with kidney sections. The results showed that the Ki-67-positive cells lessened in the KO mice when compared with the WT mice, both were injected anti-dsDNA IgG hybridoma cells (Figures 3(c) and 3(d)).

Renal fibrosis is one of the histopathologic features associated with poor outcomes of LN [12] and correlates with the nephritogenicity of anti-dsDNA IgG [13]. We examined glomerular fibrosis in SCID mice by trichrome staining, which showed that the score of tissue fibrosis was the highest in the anti-dsDNA IgG hybridoma cell-injected WT mice but decreased partially in the KO mice

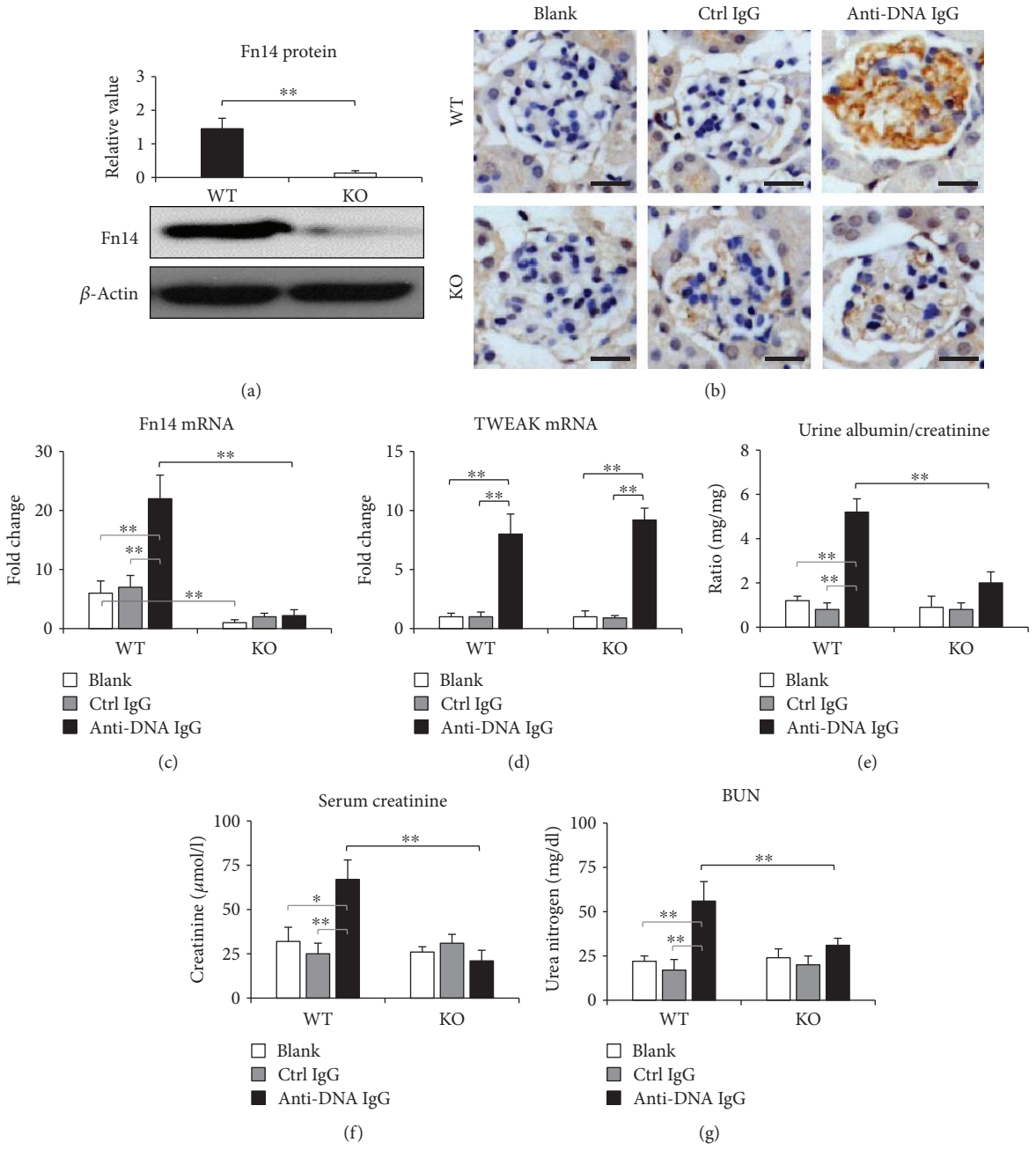

Figure 1: Fn14 deficiency reduces proteinuria in SCID mice. (a) Fn14 protein was determined in the kidneys by Western blotting. (b) IgG deposition was detected in glomeruli by immunohistochemistry. (c) The mRNA expression levels of Fn14 were determined in the kidneys by real-time PCR. (d) Similarly, the mRNA expression levels of TWEAK were also determined. (e–g) The levels of urine albumin, serum creatinine, and blood urea nitrogen (BUN) were determined in mice at sacrifice. There were eight mice in each group. Representative images are shown. Fn14: fibroblast growth factor-inducible 14; TWEAK: tumor necrosis factor-like weak inducer of apoptosis. Scale bar = 50 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$.

receiving the same treatment (Figures 4(a) and 4(b)). Similarly, the mRNA expression levels of transforming growth factor-β (TGF-β), PDGFB, connective tissue growth factor (CTGF), fibronectin 1, and collagen 1A1 increased in the WT mice but decreased in the KO mice upon injection of anti-dsDNA IgG hybridoma cells (Figure 4(c)). The alteration of PDGFB expression was further confirmed in kidneys by immunohistochemistry (Figures 4(d) and 4(e)).

3.4. Less Inflammatory Infiltrate in the Kidneys of Fn14-Deficient Mice. Glomerular infiltration of macrophages was evaluated by immunohistochemical staining for CD3- or Iba-1-positive cells. The results showed that injection of anti-dsDNA IgG induced prominent infiltration of these cells in the glomeruli or periglomerular regions of WT mice (Figure 5). However, Fn14 KO in the anti-dsDNA IgG hybridoma cell-injected mice led to remarkable amelioration

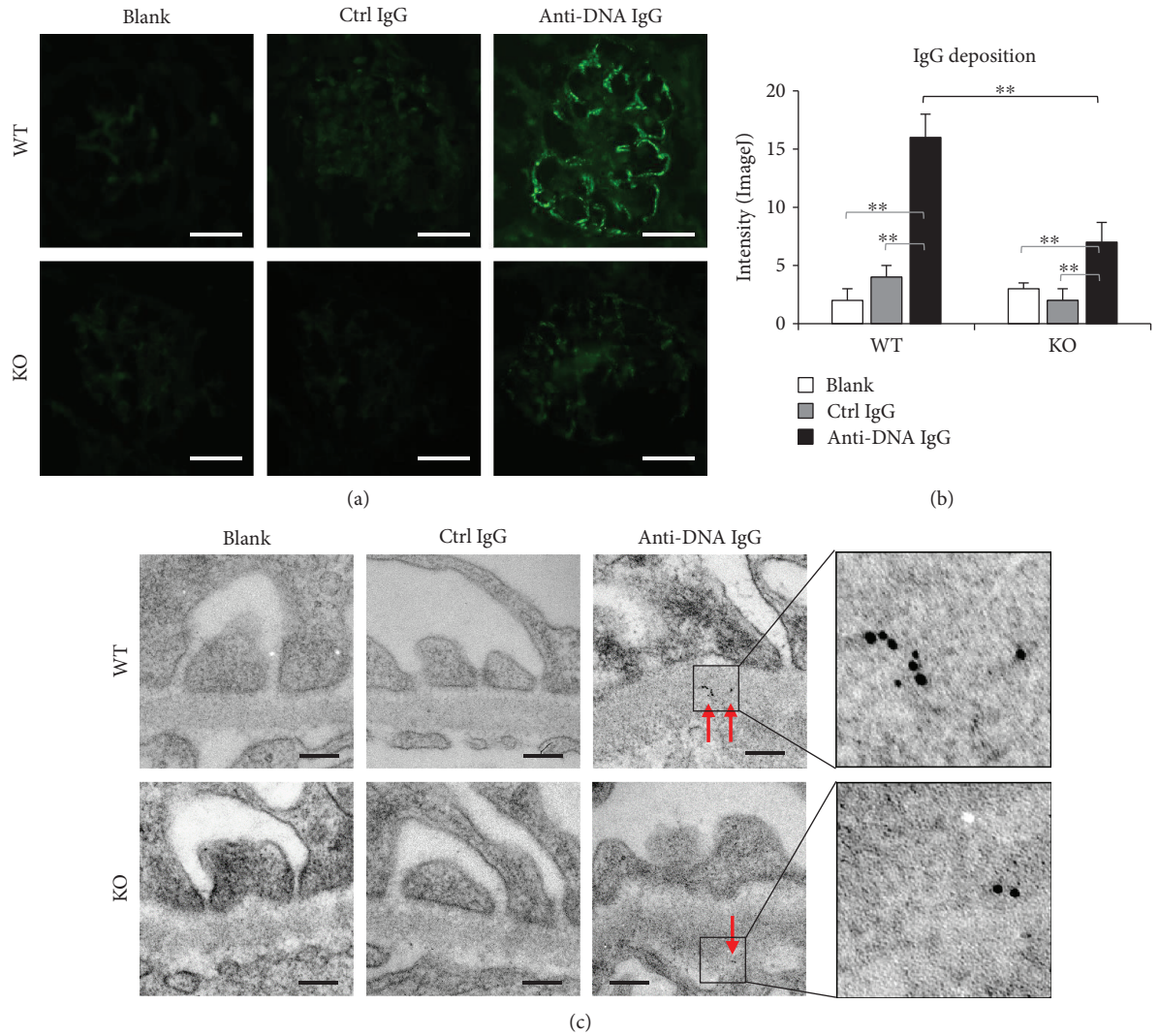

Figure 2: Glomerular IgG deposition is attenuated in Fn14-deficient SCID mice. (a) IgG deposition was detected in glomeruli by immunofluorescence. Scale bar = 50 μm. (b) Fluorescent intensities of glomeruli were quantitated by ImageJ software. (c) By immunogold staining and transmission electron microscopy, IgG deposits were detected in glomeruli. Gold particles were indicated by red arrows. Scale bar = 200 nm. There were eight mice in each group. Representative images are shown. $^{**}p < 0.01$.

of inflammatory cell infiltration (Figure 5). By semiquantitative scoring, the anti-dsDNA IgG hybridoma cell-injected WT mice showed significant increase in CD3- or Iba-1-positive cells, which was tempered upon Fn14 deficiency (Figures 5(b) and 5(d)).

3.5. Proinflammatory Cytokines Are Downregulated in Fn14-Deficient Kidneys. Proinflammatory cytokines in the kidneys were assessed by real-time PCR. It showed that the mRNA and protein expression levels of regulated on activation, normal T cell expressed and secreted (RANTES), MCP-1, and interferon gamma-induced protein 10 (IP-10) were higher in the WT mice when compared with the KO mice, both of which received injection of anti-dsDNA IgG hybridoma cells (Figures 6(a)–6(c)). In accordance, urine levels of MCP-1 were lower in the KO mice (Figure 6(d)).

Interestingly, EGFR, a transmembrane protein that can be transactivated by TWEAK and contributes to renal disease progression especially fibrosis [30], was also less expressed in the kidneys of the KO mice after injection of anti-dsDNA IgG hybridoma cells (Figures 6(a)–6(c)). There were no significant differences in both proinflammatory cytokines and EGFR between the blank and control IgG hybridoma cell-injected mice. By immunohistochemistry and semiquantitative scoring, the anti-dsDNA IgG hybridoma cell-injected WT mice showed significant increase in glomerular pEGFR expression, which was attenuated upon Fn14 deficiency (Figures 6(e) and 6(f)).

4. Discussion

In this study, we demonstrated that anti-dsDNA IgG upregulates TWEAK and Fn14 expression in kidneys and induces prominent glomerular damage in the WT SCID mice. However, Fn14 deficiency significantly reduces proteinuria as well as glomerular IgG deposition in anti-

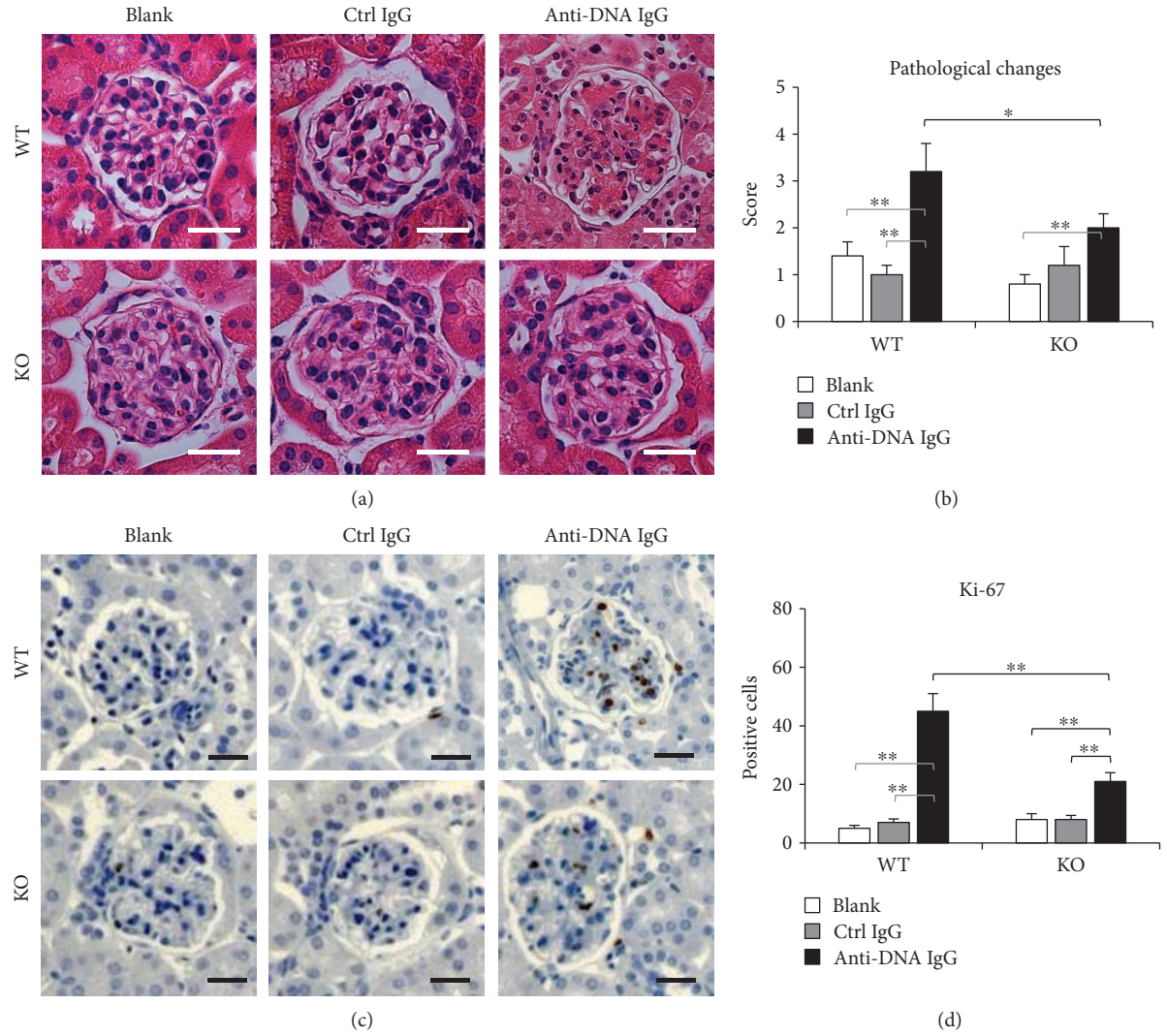

Figure 3: Histopathological changes are ameliorated in Fn14-deficient glomeruli. (a) Kidney sections were stained by hematoxylin and eosin. (b) The hematoxylin and eosin-stained sections were scored for glomerular damage. (c) By immunohistochemistry, Ki-67-positive cells were detected in glomeruli. (d) The Ki-67-positive cells were counted in glomerular areas. There were eight mice in each group. Representative images are shown. Fn14: fibroblast growth factor-inducible 14. Scale bar = 50 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$.

dsDNA IgG hybridoma cell-injected mice. Accordingly, the histopathological changes and inflammatory cell infiltration are attenuated in the kidneys of these Fn14-deficient mice. Furthermore, the expression of proinflammatory cytokines and EGFR is downregulated in Fn14-deficient kidneys. Therefore, Fn14 deficiency effectively ameliorates anti-dsDNA IgG-induced glomerular damage in SCID mice.

Previous studies demonstrated that intraperitoneal injection of anti-dsDNA IgG hybridoma cells induces LN-like renal damage in SCID mice, and Fn14 deficiency attenuates LN in MRL/lpr mice without affecting serum IgG levels [5, 27]. Our results confirmed such nephritogenicity of anti-dsDNA IgG as well as the protective effect of Fn14 deficiency in SCID mice. Because MRL/lpr mice have multiple autoantibodies in sera, which may contribute to renal damage [27], Fn14 deficiency was generated in SCID mice, which have no detectable IgG because of V(D)J recombination impairment [5]. It showed that anti-dsDNA IgG upregulates both Fn14 and TWEAK expression in the kidneys of the WT mice. The downstream proinflammatory cytokines, including RANTES, MCP-1, and IP-10, also increase in these kidneys. These results supported the finding that TWEAK/Fn14 signals activate upon anti-dsDNA IgG deposition in the glomeruli. On the other hand, the Fn14-KO mice showed less glomerular IgG deposition though their serum IgG levels remained the same as those of the WT mice after injection of anti-dsDNA IgG hybridoma cells. Hence, renal deposition of anti-dsDNA IgG correlates closely with local TWEAK/Fn14 activation. There were no differences in circulating the anti-dsDNA IgG level between the two strains though the KO mice had less glomerular IgG deposition. Such discrepancy might be due to persistent production of IgG by intraperitoneal hybridoma cells, which could conceal an actual difference.

Although SCID mice are characterized by the absence of functional T cells and B cells, their kidneys may be infiltrated

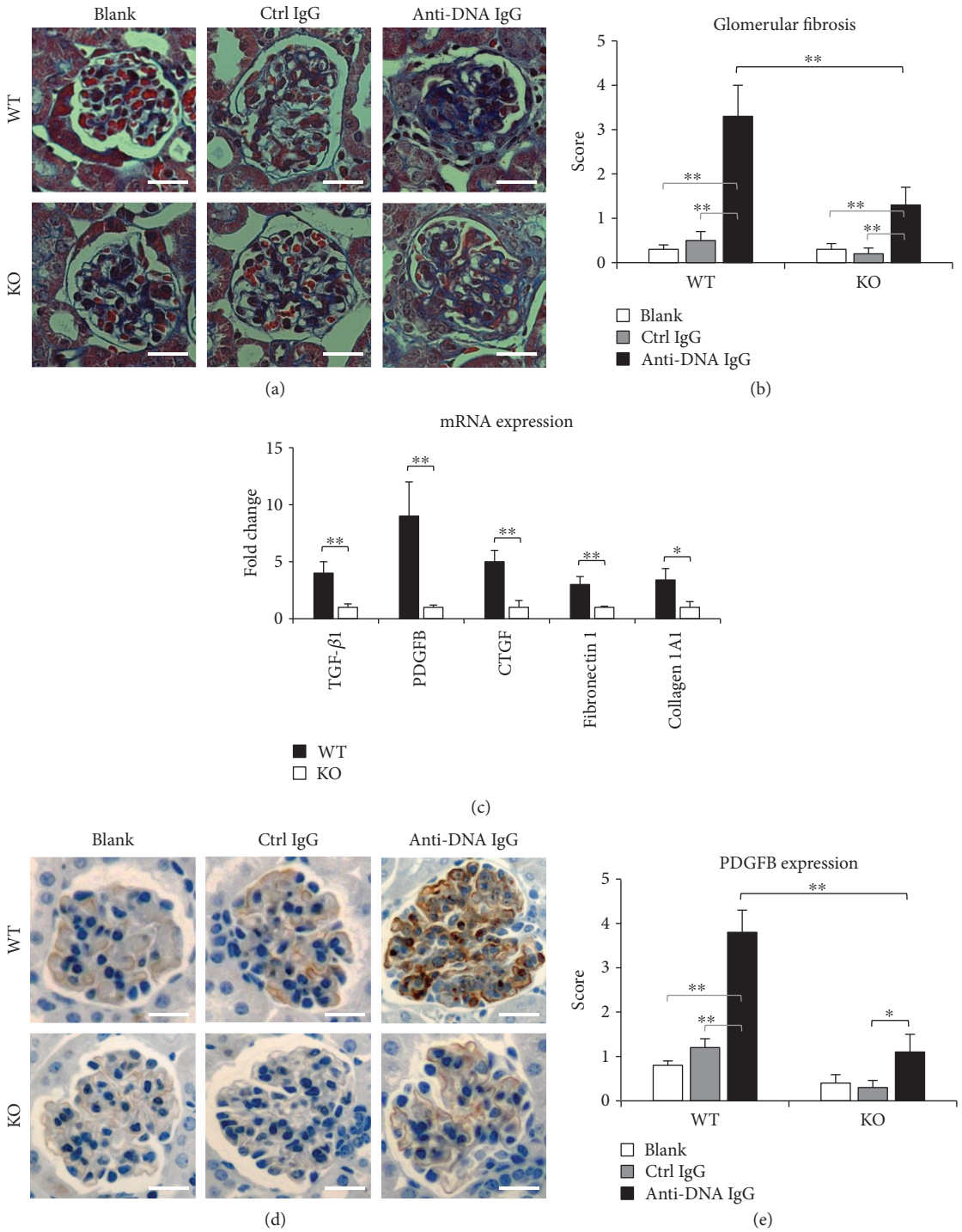

Figure 4: Renal fibrosis is reduced in Fn14-deficient kidneys. (a) Kidney sections were stained by trichrome. (b) The trichrome-stained sections were scored for glomerular fibrosis. (c) By real-time PCR, the mRNA levels of transforming growth factor-β (TGF-β), platelet-derived growth factor subunit B (PDGFB), connective tissue growth factor (CTGF), fibronectin 1, and collagen 1A1 were determined in kidneys of anti-dsDNA IgG hybridoma cell-injected mice. (d) By immunohistochemistry, PDGFB expression was detected in glomeruli. (e) The PDGFB-stained sections were scored semiquantitatively. There were eight mice in each group. Representative images are shown. Fn14: fibroblast growth factor-inducible 14. Scale bar = 50 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$.

by macrophages under inflammation [31]. In fact, macrophages are one of the main resources of soluble TWEAK in inflammatory tissues [18]. In this study, macrophages (Iba-1-positive) infiltrated the kidneys of SCID mice after injection of anti-dsDNA IgG hybridoma cells, accompanied by an increase in TWEAK mRNA expression. Anti-dsDNA IgG injection induced less glomerular infiltration of T cells (CD3-positive) in Fn14-KO mice, confirming an inhibitive

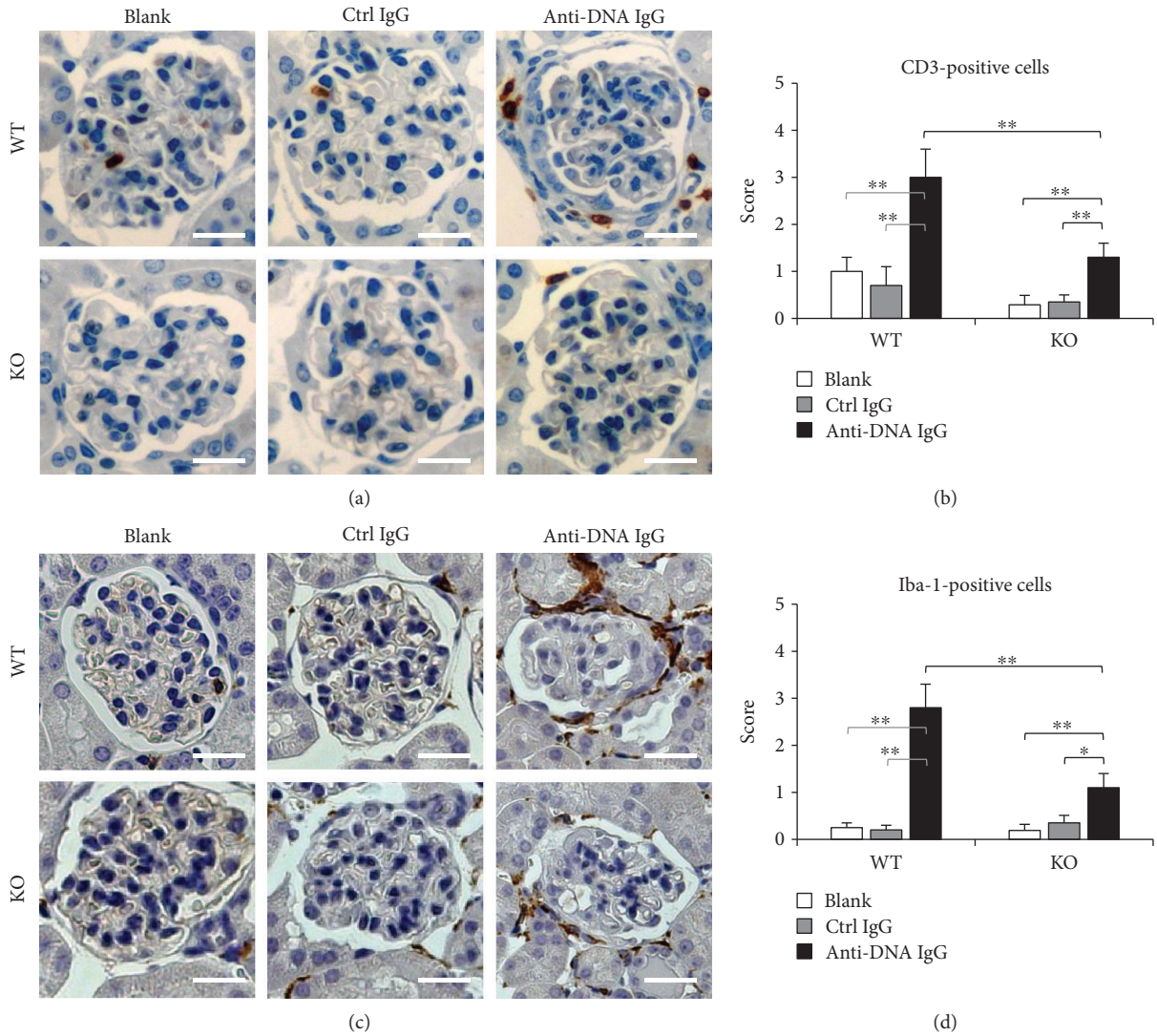

Figure 5: Infiltration of inflammatory cells decreases in glomeruli of Fn14-deficient mice. SCID mice were intraperitoneally injected with hybridoma cells producing control IgG or anti-dsDNA IgG. (a) By immunohistochemistry, CD3-positive cells were detected in glomeruli. (b) The CD3-stained sections were scored by semiquantitative systems. (c) Iba-1-positive cells were also detected by immunohistochemistry. (d) The Iba-1-stained sections were scored semiquantitatively. There were eight mice in each group. Representative images are shown. Fn14: fibroblast growth factor-inducible 14. Scale bar = 50 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$.

effect of Fn14 deficiency on T cell recruitment. The expression of Fn14 was significantly elevated in the kidneys of SCID mice upon anti-dsDNA IgG injection. Accordingly, the TWEAK/Fn14 signaling-regulated downstream cytokines, including RANTES, MCP-1, and IP-10, increase in these kidneys. Obviously, immunodeficiency in SCID mice does not block the TWEAK/Fn14 pathway, which is actually activated in anti-dsDNA IgG-induced renal damage.

Renal fibrosis is one of the final outcomes of patients with LN [12]. It has been known that anti-dsDNA IgG participates in the processes of renal fibrosis through blocking the suppressor of cytokine signaling 1 signals and inducing fibronectin secretion or myofibroblast-like phenotype of renal resident cells [10, 11, 13]. TWEAK/Fn14 signaling is also deeply involved in the inflammation-related fibrosis of tissues, including the liver, heart, lung, and kidney [23, 32–35]. TWEAK/Fn14 activation promotes kidney fibrosis involving Ras-dependent proliferation of renal fibroblasts [36]. So, anti-dsDNA IgG deposition and TWEAK/Fn14 activation may cooperate in facilitating renal fibrosis of LN. Our results showed that glomerular fibrosis is apparent in SCID mice after injection of anti-dsDNA IgG hybridoma cells. Moreover, the mRNA expression levels of fibrotic markers, such as TGF-β, PDGFB, CTGF, fibronectin 1, and collagen 1A1, increase significantly in kidneys of these mice. However, both glomerular fibrotic scores and fibrotic marker expression decrease upon Fn14 deficiency. These findings not only support the finding that anti-dsDNA IgG contributes to renal fibrosis of LN but also affirm that TWEAK/Fn14 inhibition blocks such effect of anti-dsDNA IgG on kidneys.

RANTES, MCP-1, and IP-10 are TWEAK-induced cytokines that trigger cellular and tissular inflammatory responses [16, 17, 22, 24, 26]. These cytokines are

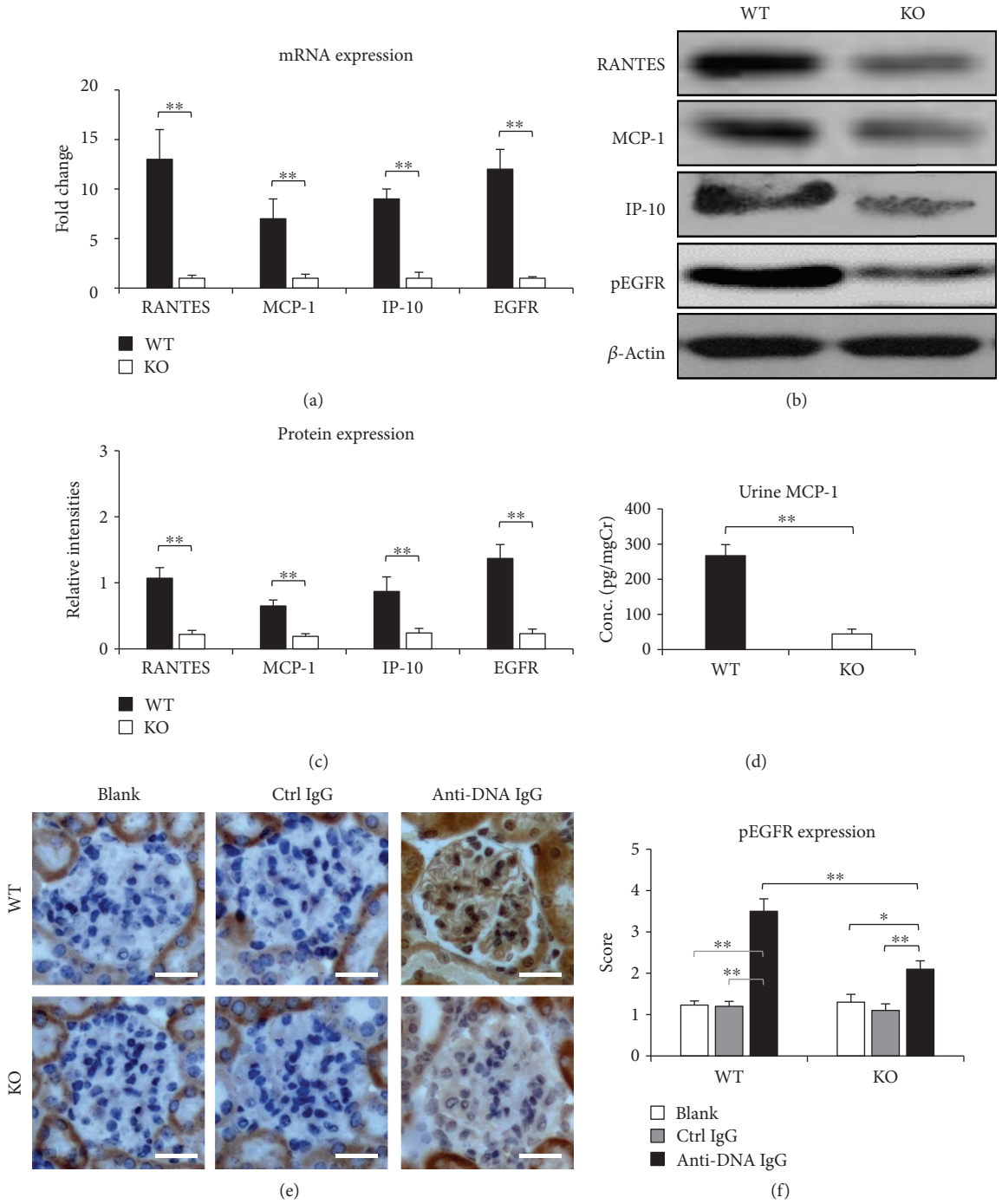

Figure 6: Proinflammatory cytokines and pEGFR are downregulated in Fn14-deficient kidneys. Both WT and KO mice were injected with anti-dsDNA IgG hybridoma cells. (a) By real-time PCR, the mRNA levels of regulated on activation, normal T cell expressed and secreted (RANTES), monocyte chemotactic protein 1 (MCP-1), interferon gamma-induced protein 10 (IP-10), and epidermal growth factor receptor (EGFR) were determined in kidneys. (b) By Western blotting, the proteins of RANTES, MCP-1, IP-10, and phospho-EGFR (pEGFR) were determined in the kidney lysates. (c) The intensities of blots were measured by ImageJ software. (d) Urine MCP-1 levels were determined in different groups. (e) Glomerular pEGFR expression was also detected by immunohistochemistry. (f) The pEGFR-stained sections were scored semiquantitatively. There were eight mice in each group. Representative images are shown. Fn14: fibroblast growth factor-inducible 14. Scale bar = 50 μm. $^{*}p < 0.05$ and $^{**}p < 0.01$.

upregulated in kidneys with inflammation and fibrosis and attract inflammatory cells, such as monocytes, T lymphocytes, and neutrophils [37, 38]. Monitoring of these cytokines may help follow up the progression of renal diseases [39, 40]. Our results showed that the mRNA expression levels of RANTES, MCP-1, and IP-10 were higher in the WT mice when compared with the Fn14-KO mice, both of which received injection of anti-dsDNA IgG

hybridoma cells. Also, urine MCP-1 level decreased in the KO mice. Therefore, TWEAK/Fn14 activation is more prominent in the WT mice, and Fn14 deficiency protects mice from anti-dsDNA IgG-induced renal inflammation. We speculate that Fn14 inhibition induces less production of these cytokines, thus maintaining the integrity of the glomerular filtration barrier. The rest of the findings such as histological changes as well as inflammatory cell infiltration are secondary to both TWEAK/Fn14 inhibition and decreased IgG deposition.

EGFR is a transmembrane protein that activates through engaging its ligands including epidermal growth factor and transforming growth factor-α [41]. Recent studies demonstrated that Fn14 upregulation correlates with EGFR phosphorylation (activation) in tumor cells [42, 43]. Moreover, TWEAK/Fn14 interaction can directly induce phosphorylation of EGFR, which mediates TWEAK-induced proinflammatory factor upregulation and inflammatory cell infiltration in kidneys [30]. Furthermore, TWEAK/Fn14 signaling induces cell proliferation and renal fibrosis through activating the EGFR pathway [30, 44]. In this study, we found that renal expression of EGFR correlates positively with TWEAK/Fn14 activation in SCID mice. This phenomenon also reflects that the TWEAK/Fn14 pathway mediates renal inflammation and fibrosis in anti-dsDNA IgG-treated mice.

5. Conclusions

Based on our findings, we conclude that the TWEAK and Fn14 upregulation and glomerular damage are prominent in anti-dsDNA IgG hybridoma cell-injected SCID mice. Fn14 deficiency effectively protects SCID mice from renal damage through reducing glomerular IgG deposition and local inflammatory responses. In future studies, exogenous inhibitory approaches should be developed to suppress the TWEAK/Fn14 pathway in a murine model of LN.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Contributions

Jiawen Wu and Xiaoyun Min contributed equally to this paper.

Acknowledgments

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Supplementary Materials

Table S1: sequences of primers. Figure S1: the strategy for generating Fn14 knockout mice by the CRISPR/Cas9 method. Figure S2: the serum levels of IgG are comparable between the WT and KO mice. *(Supplementary Materials)*

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Research Article

Low Serum IGF-1 in Boys with Recent Onset of Juvenile Idiopathic Arthritis

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Background. Liver-derived insulin-like growth factor-1 (IGF-1) contributes bone formation. Decreased IGF-1 levels are common in juvenile idiopathic arthritis (JIA), but whether IGF-1 is related to sex and differ during the pathogenic progress of JIA is unknown. *Objective*. The aim of this study was to examine IGF-1 levels in boys and girls with newly diagnosed JIA, with established JIA and in controls. *Methods*. The study group included 131 patients from the Estonian population-based prevalence JIA study. Blood samples were obtained from 27 boys and 38 girls with early JIA (≤1 month from the diagnosis), 29 boys and 36 girls with established JIA (mean disease duration 18 months), and from 47 age- and sex-matched controls. *Results*. IGF-1 levels in boys were significantly decreased in early JIA compared to male controls, while IGF-1 levels in girls were comparable between JIA and controls. In early JIA, IGF-1 levels were 12-fold lower in boys relative to girls. In controls, IGF-1 levels correlated with both age and height, while these correlations were lost in boys with early JIA. *Conclusion*. We report a sex-dependent deficiency in serum IGF-1 in boys with early JIA, which argues for sex-related differences in biological mechanisms involved in the disease pathogenesis.

1. Introduction

The term juvenile idiopathic arthritis (JIA) comprises a number of chronic inflammatory disorders with onset before 16 years of age with symptoms presenting for longer than six weeks. JIA is the most common rheumatic disease in children, with a prevalence from 3.8 to 400 per 100,000 in the European population [1]. There are seven clinical categories defined in the JIA spectrum according to the criteria from the International League of Associations for Rheumatology (ILAR) [2]. Distinct distributions regarding age at onset and sex of the child, as well as the course of the disease and outcome, vary between the different categories [3, 4]. The pathogenesis of JIA is currently unknown, but is thought to be due to a combination of environmental triggers and specific immunogenic factors [5].

Impaired linear growth is a commonly encountered complication in children with JIA, which can result in short final height [6, 7]. The etiology of growth retardation in JIA is not fully elucidated, but elevated levels of proinflammatory cytokines, delayed onset of puberty, malnutrition, and long-term glucocorticoid therapy have been implicated [6, 8, 9]. The growth hormone (GH)/insulin-like factor-1 (IGF-1) axis is a main regulator of linear growth [7], and the major part of circulating IGF-1 levels is liver derived [10]. In healthy children, circulating IGF-1 levels increase with age in pre- and early puberty, while in late puberty, this relationship is negative [11]. Therefore, normal IGF-1 levels vary in different age

Table 1: Clinical and demographic characteristics of boys and girls with early JIA (≤1 month from diagnosis) and established disease (>1 month from diagnosis).

	Early JIA all ($n = 65$)	Establ JIA all ($n = 66$)	Early JIA boys ($n = 27$)	Early JIA girls ($n = 38$)	Establ JIA boys ($n = 29$)	Establ JIA girls ($n = 37$)
Age at inclusion, years (mean ± SD)	9.7 ± 4.0	10.8 ± 4.6	9.3 ± 3.8	10.0 ± 4.2	10.8 ± 4.6	10.7 ± 4.6
Age at diagnosis, years (mean ± SD)	9.7 ± 4.0	9.4 ± 4.3	9.3 ± 3.8	10.0 ± 4.2	9.3 ± 4.1	9.1 ± 4.6
Disease duration, months (mean ± SD)	0.06 ± 0.2	17.9 ± 14.0***	0.0 ± 0	0.1 ± 0.3	19.2 ± 13.7	16.9 ± 14.4
DMARDs, *n* (%)[$]	9 of 60 (15)	26/35 (74)***	4/26 (15)	5/34 (15)	14/16 (87)	12/19 (63)
Steroids, *n* (%)[$]	5 of 60 (8)	6/35 (17)	2/26 (8)	3/34 (9)	1/16 (6)	5/19 (26)
ANA-positive, *n* (%) [$]	16 of 50 (32)	6/21 (29)	3/19 (16)	13/31 (42)	5/11 (45)	1/10 (10)
HLAB27-positive, *n* (%) [$]	18 of 62 (29)	20/63 (32)	10/26 (38)	8/36 (22)	8/28 (29)	11/34 (32)
RF-positive, *n* (%)	2 of 51 (4)	3/29 (10)	0/21 (0)	2/30 (7)	1/15 (7)	2/14 (14)
SJC, *n* (median, IQR)	1 (1–3)	1 (0–2.5)	2 (1–4)	1 (0.25–2.75)	1.5 (0–3.5)	1 (0–2.5)
TJC, *n* (median, IQR)	1 (0–1)	1 (0–1.75)	1 (0–1)	0.5 (0–1)	0 (0–0)	0 (0–2)
ESR at diagnosis, mm/h (median, IQR)	15 (7–29)	11 (6–25)	13 (8–32)	15 (7–28)	15 (7–26)	7 (5–21)
CRP mg/l, median (IQR) [#]	39 (30–63)	48 (18–115)	42 (26–59)	38 (32–128)	80 (18–130)	33 (22–133)
Neutrophils at diag, % (median, IQR)	53 (46–62)	60 (49–67)	51 (46–66)	54 (44–62)	61 (48–69)	59 (48–66)
JIA categories, *n* (%)						
(i) Oligoarthritis, persistent	16 (25)	33 (50)**	7 (26)	9 (24)	18 (62)	15 (41)
(ii) Oligoarthritis, extended	11 (17)	8 (12)	3 (11)	8 (21)	2 (7)	6 (16)
(iii) Polyarthritis, RF-positive	2 (3)	3 (5)	0	2 (5)	1 (3)	2 (5)
(iv) Polyarthritis, RF-negative	15 (23)	16 (24)	5 (19)	10 (26)	4 (14)	12 (32)
(v) Enthesitis-related	8 (12)	1 (2)*	6 (22)	2 (5)	1 (3)	0
(vi) Systemic	4 (6)	3 (5)	1 (4)	3 (8)	3 (10)	0
(vii) Psoriatic	4 (6)	0 (0)	2 (7)	2 (5)	0	0
(viii) Other	5 (8)	1 (2) †	3 (11)	2 (5)	0	2 (5) †

IQR: interquartile range. [$]Data for ANA, HLAB27, and treatment not available for all children in the cohort. [#]Median CRP values shown for children with increased CRP. †Category missing for one patient. Statistical comparisons between all early JIA vs. all established JIA: $^{***}P < 0.0001$, $^{**}P < 0.005$, and $^{*}P < 0.05$.

groups for boys and girls [11]. Several studies have demonstrated decreased IGF-1 levels in children with JIA compared to healthy controls [12–15]. However, as the JIA disease duration varied greatly and the numbers of JIA patients included in these studies were relatively low, sex-related differences were not examined. Thus, it remains to be elucidated if there are distinctions in IGF-1 levels between boys and girls with JIA and if IGF-1 levels differ in early disease compared to established JIA. To address these gaps in knowledge, we here examined circulating levels of IGF-1 separately in boys and girls who were newly diagnosed with JIA (≤1 month from JIA diagnosis), who had established JIA with a mean disease duration of 18 months, and in age- and sex-matched controls.

2. Materials and Methods

2.1. Patients and Healthy Controls. The patient group comprised 131 Estonian children diagnosed with JIA during the population-based prevalence and incidence studies in the Children's Clinic, Tartu University Hospital and Tallinn Children's Hospital during 1995–2000 [16–18]. The inclusion criteria to the study were as follows: children under the age of 16 years who had (a) arthritis of unknown cause in at least one joint for at least 6 weeks or (b) inflammatory back pain and enthesitis, or (c) spiking fever together with other symptoms suggestive of systemic arthritis. Arthritis was defined as a swollen joint or two of the following symptoms: (a) limitation of movement, (b) warmth/redness, and (c) pain on active or passive movements. All patients were classified according to the ILAR revised criteria for JIA into the following categories: persistent or extended oligoarthritis, rheumatoid factor- (RF-) positive polyarthritis, RF-negative polyarthritis, systemic arthritis, psoriatic arthritis, and enthesitis-related arthritis [2]. Children who did not fulfill any or fulfilled the criteria for more than one subtype were categorized as "other arthritis." Children presenting with other explanations for arthritis, such as infection, trauma, and systemic connective tissue disease, were excluded. Children included at their visit to pediatric rheumatologist with no previous JIA diagnosis and ≤1 month from diagnosis were defined as patients with early JIA. The majority were treatment naïve for steroids (92%) and DMARDs (85%) (Table 1), or the drug was just initiated during the first weeks before inclusion. Children who were diagnosed with JIA more than one month ago and had received treatment longer than 1 month were defined as established JIA. The median symptom duration (the interval between time when the first symptoms were noticed and the time when the diagnosis was made by the pediatric rheumatologist) for

early JIA group was 6 months (IQR 1–12): respectively, 5 months (1–12) for boys and 6 months (1.5–12) for girls. The baseline characteristics of early and established JIA patients including the total number of patients and the number of patients with available data are presented in Table 1. The control group comprised of 47 sex- and age-matched children with no arthritis or any other inflammatory autoimmune conditions. The study was approved by the Ethics Committee of Tartu University (173/T-5 and 200T-1) in accordance with the Declaration of Helsinki, and informed consent for participation in the study was obtained from all parents and/or children.

2.2. Clinical and Laboratory Assessment. Clinical examinations by a pediatric rheumatologist at the tertiary hospitals were performed for all children and disease activity variables, i.e., erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count with differential, swollen and tender joints, joints with limited range of motion, and joints painful in passive and/or active movements were recorded. CRP and rheumatoid factor (RF) were measured using turbidimetry with normal range 0–5 mg/l and <14 IU/ml, respectively. Anti-nuclear antibodies (ANA) were assessed by indirect immunofluorescence (IIF) using rat kidney-liver-stomach sections and considered positive if a titer $\geq 1:10$ on two or more occasions at least 8 weeks apart were detected in the blood. If ANA was assessed by IIF using Hep-2 cells as a substrate, a titer $\geq 1:100$ on two or more occasions was considered positive. The cut-off levels for both methods were established according to common laboratory routines by analyzing the healthy local population.

2.3. Determination of IGF-1 in Serum. Blood samples were obtained from cubital vein into the tubes without additive. Collected blood samples were centrifuged within 4 h at 1400g for 10 min, immediately aliquoted, and stored at −80°C until assayed. Previous studies have shown that IGF-1 levels are relatively stable up to more than 20 years if properly stored at −80°C and if repeated freeze-thaw cycles are avoided [19, 20]. The serum levels of IGF-1 were determined using a specific sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the instructions of the manufacturer. The minimum detectable concentration after taking into account the dilution factors and standard curve was 0.3 ng/ml.

2.4. Statistical Analysis. The D'Agostino and Pearson omnibus normality test were used to assess if the data were normally distributed (GraphPad Prism, San Diego, USA). Kruskal-Wallis test followed by Dunn's multiple comparison test were used to compare IGF-1 levels between early JIA, established JIA, and controls. The effect of sex and disease on IGF-1 levels was analyzed using two-way ANOVA with the factors sex (P_{sex}), disease (P_{disease}), and their interaction ($P_{\text{sex}*\text{disease}}$) followed by Tukey's multiple comparisons test. Two-tailed Spearman rank correlation test was used to assess correlations between two variables. A p value < 0.05 was regarded as being statistically significant ($^{*}p < 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$). Fisher's exact probability test was used to assess differences between groups with regard to disease characteristics. Fisher's r-to-z transformation analysis was used to compare correlation coefficients. Linear regression analysis was used to investigate the relationship between two variables (age and height in relation to IGF-1 levels). Multivariate factor analysis orthogonal projection to latent structures (OPLS), as previously described in detail [21], was implemented to investigate the relationship between IGF-1 serum levels (Y-variable) and disease activity/inflammation variables (X-variables) among girls and boys with early JIA (SIMCA-P+ software; Umetrics, Umeå, Sweden).

3. Results

3.1. Patient Characteristics. Blood samples were obtained from 131 JIA patients (mean ± SD age 10.2 ± 4.3 years, 43% boys) and from 47 sex- and age-matched controls (mean age 9.3 ± 4.1 years, 43% boys). Of the children with JIA, 65 (50%) were diagnosed with JIA at entry into the study and classified as early JIA (≤1 month since the diagnosis), whereas 66 (50%) were classified as established JIA patients (mean disease duration 17.9 ± 14.0 months). There was a lower prevalence of persistent oligoarthritis ($P = 0.004$) and a higher prevalence of enthesitis-related arthritis ($P = 0.02$) in the early JIA group compared to established JIA. Otherwise, these two groups were similar with regard to disease characteristics and inflammatory parameters at diagnosis. There were no sex-related differences in disease characteristics, including clinical categories of JIA and inflammatory parameters among the patients with early or established JIA (Table 1). The vast majority of children who were classified as early JIA patients were treatment naïve for DMARDs (85%) and steroids (92%). In the early JIA group, all children with systemic disease (1 boy, 3 girls) and 1 boy with polyarthritis initiated glucocorticoid treatment at diagnosis (8% of boys and 9% of girls). In addition, 4 boys with early JIA (15%) and 5 girls with early JIA (15%) had received hydroxychloroquine treatment for about one month before the inclusion to the study. In the established JIA group, 87% of boys and 63% of the girls received DMARDs, mainly hydroxychloroquine only or in combination with azathioprine ($n = 2$) or methotrexate ($n = 2$). None of the patients had received biologic therapy at inclusion with biologic anticytokine drugs.

3.2. Boys with Early JIA Present with Decreased IGF-1 Levels. First, we examined the levels of serum IGF-1 levels in all early JIA patients, established JIA patients, and in controls. As shown in Figure 1(a), there were significantly lower IGF-1 levels in the group of early JIA patients, but not established JIA patients, compared to controls. To examine the effect of sex and disease on IGF-1 levels, a two-way ANOVA was conducted. There was a sex- and disease-related difference in IGF-1 levels ($F_{(1,171)} = 7.4$, $P_{\text{sex}} = 0.007$; $F_{(2,171)} = 5.2$, $P_{\text{disease}} = 0.007$). However, no significant interaction between sex and disease was detected ($F_{(2,171)} = 2.3$, $P_{\text{sex}*\text{disease}} = 0.1$). IGF-1 levels were significantly lower in boys with early JIA compared to age-matched controls, while girls displayed similar IGF-1 levels between the three groups (Figure 1(a)). The

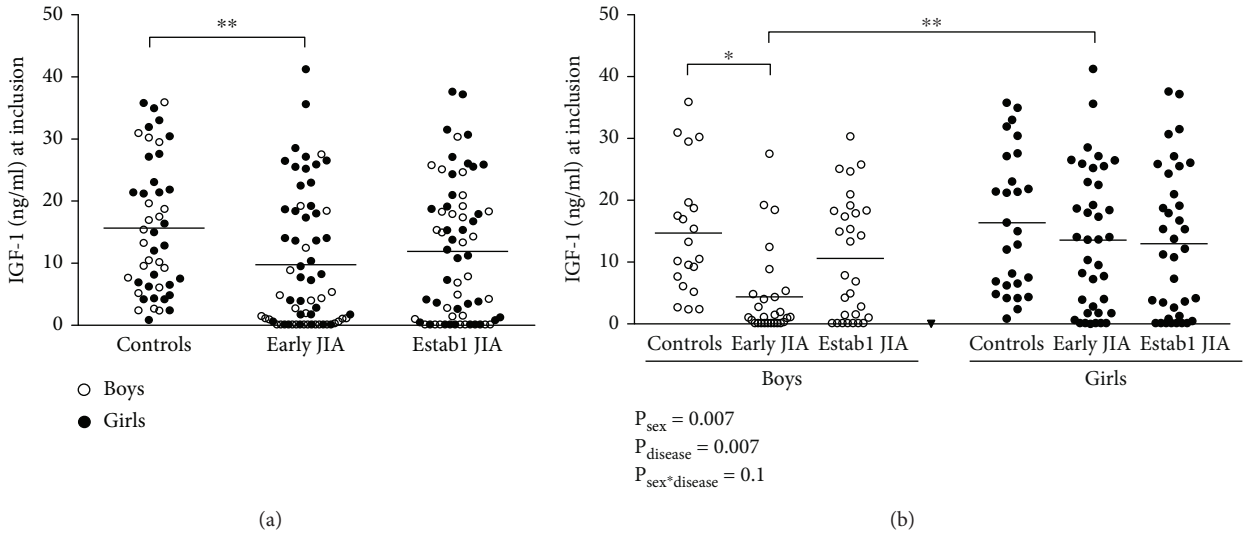

Figure 1: (a) IGF-1 serum levels among all control children ($n = 47$), all patients with early JIA ($n = 65$), and all patients with established JIA ($n = 66$). (b) IGF-1 serum levels among boys and girls with early JIA ($n = 27$ and $n = 38$, respectively), established JIA ($n = 29$ and $n = 36$, respectively), and in age-matched controls ($n = 20$ and $n = 27$, respectively). Horizontal bars indicate means. Data were analyzed using a two-way ANOVA with the factors sex, disease, and their interaction followed by Tukey's multiple comparisons test. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$.

IGF-1 levels were 12-fold lower in boys compared to girls with early JIA, but no such sex-related differences were observed among controls or patients with established JIA.

3.3. The IGF-1 System Is Disturbed in Early JIA. Age could be a potential confounding factor for the observed differences in IGF-1 levels among boys with or without early JIA. In multiple regression analysis, age contributed independently to the IGF-1 levels in both boys and girls ($P \leq 0.0001$ for both sexes). However, as the median age at inclusion of boys with early JIA did not differ significantly from the other two groups (Figure 2(a)), decreased IGF-1 in boys with early JIA cannot solely be explained by age.

Next, we examined how IGF-1 levels were related to age in the different subgroups. Age correlated strongly to IGF-1 in serum among male controls, but not in boys with early JIA ($r = 0.83$ vs. $r = 0.33$, respectively) (Figure 2(b)). Both the correlation coefficients and the regression slopes differed significantly for these two independent groups ($P = 0.009$ and $P = 0.006$, respectively). Similarly to boys, IGF-1 levels correlated strongly to age in female controls (Figure 2(c)). In contrast to boys, there was a moderate correlation between age and IGF-1 in girls with early JIA (Figure 2(c)). Accordingly, the correlation coefficients ($r = 0.69$ vs $r = 0.49$, $P = 0.23$) and the regression slopes ($P = 0.23$) were similar in controls and early JIA girls. In established JIA, there was a strong correlation between age and serum IGF-1 levels in both sexes (Figures 2(d) and 2(e)).

There were no significant differences in height between the groups of children, either among boys or girls (Figure 3(a)), but boys with established JIA had tendency towards shorter body height. Similar to age, height correlated strongly to serum IGF-1 in male controls ($r = 0.79$), but not in those with early JIA (Figure 3(b)), and the correlation coefficients differed significantly ($P = 0.03$) between the groups as well as the regression slopes ($P = 0.003$) (Figure 3(b)). Among girls, height correlated to serum IGF-1 in controls and there was a trend for correlation also in early JIA (Figure 3(c)). Neither the correlation coefficients ($P = 0.5$) nor the regression slopes ($P = 0.7$) differed between control group of girls and in girls with early JIA. In established JIA, there was a significant correlation between height and serum IGF-1 levels in girls but not in boys (Figures 3(d) and 3(e)). Overall, these results indicate a disturbance in the IGF-1 system in relation to age and anthropometrics in the early JIA, especially among boys.

3.4. IGF-1 Levels in Relation to Clinical Disease Activity. We next investigated whether serum IGF-1 was associated with disease-related variables in early JIA by the use of multivariate factor analysis. As shown in the OPLS loading column plot in Figure 4(a), higher IGF-1 among boys were positively related to higher CRP and correlated significantly to platelet counts (Figure 4(b)), ESR, and proportion of neutrophils. Among girls, higher IGF-1 levels correlated negatively with platelet counts, swollen joint counts, and tender joint counts (Figures 4(c) and 4(d)). The presence of RF, ANA, and HLA-B27 were not related to IGF-1 levels in either boys or girls (Figures 4(a) and 4(b)).

4. Discussion

Decreased serum IGF-1 levels are a common feature among children with JIA. The main novel findings observed in the present study were (a) boys with early JIA have almost 12-fold lower serum IGF-1 levels compared to age-matched girls with early JIA and (b) boys with early JIA have significantly lower IGF-1 levels relative to age-matched male controls.

One possible explanation for lower IGF-1 levels among boys compared to girls with early JIA could be differences

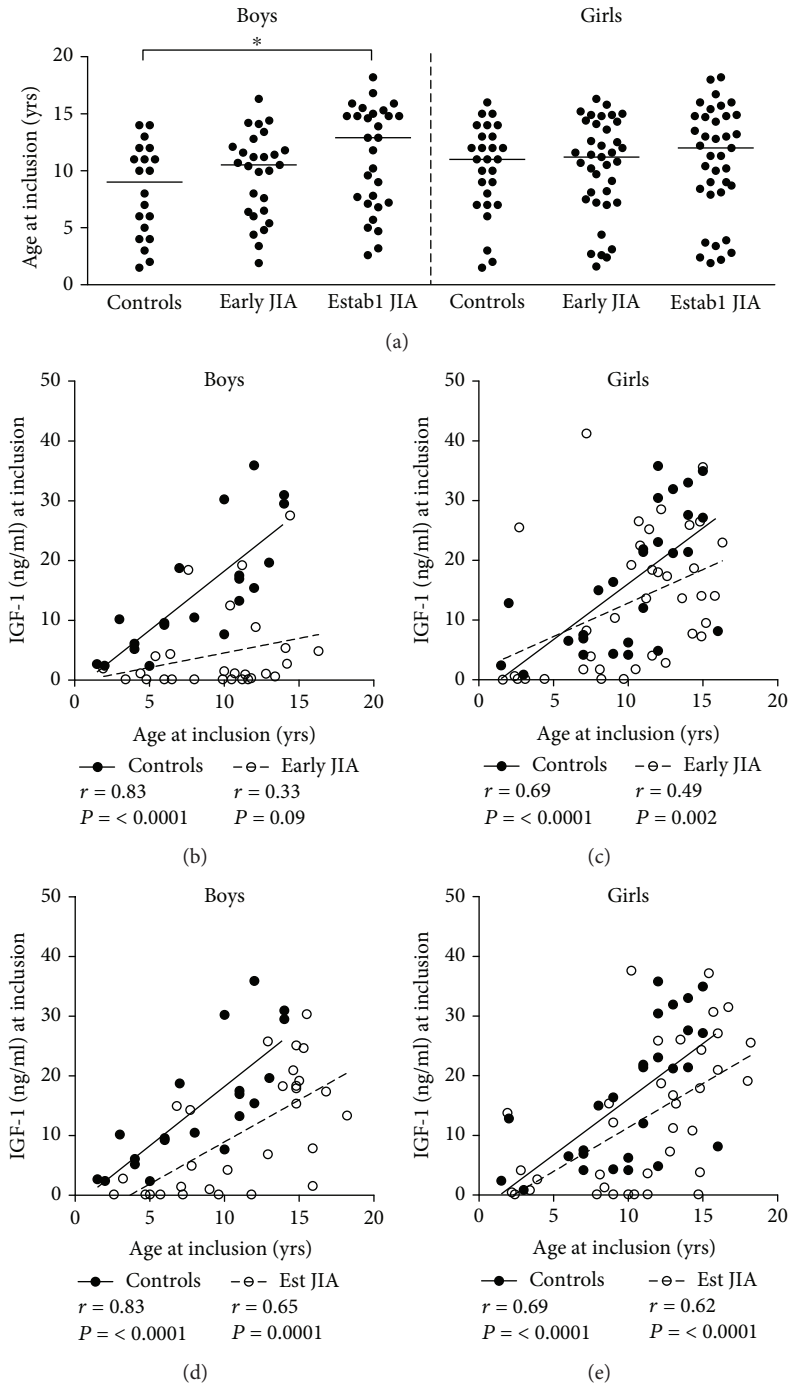

Figure 2: (a) Age of boys and girls at inclusion to the study. The horizontal bars indicate the median. (b, c) Correlations between serum IGF-1 levels and age in control groups of boys and girls, respectively (solid symbols), and in boys and girls with early JIA (open symbols). (d, e) Correlations between serum IGF-1 levels and age in control groups of boys and girls, respectively (solid symbols), and between boys and girls with established JIA (open symbols) (Spearman rank correlation test). (b–e) The regression lines are presented in the correlations plots. $^{*}P \leq 0.05$ (Kruskal-Wallis test followed by Dunn's multiple comparison test).

in their pubertal age. On average, boys usually begin puberty at an older age than girls. In healthy children, circulating IGF-1 levels are positively related to age in prepuberty (mean age: 9.4 years for boys and 8.5 years for girls) and early puberty (mean age: 13.3 years for boys and 12.1 years for girls), while the relationship between these two factors is negative in late puberty [11]. The majority of the boys in our early JIA group were in prepuberty whereas the girls were already in early or midpuberty [11]. Increased estrogen levels could be expected in our early/midpuberty study girls as compared to their prepubertal period. Rising estrogen (estradiol) levels transiently increase growth hormone (GH) concentrations, which in turn lead to increase in IGF-1 levels. Additionally, serum IGF-1 levels have been found to increase

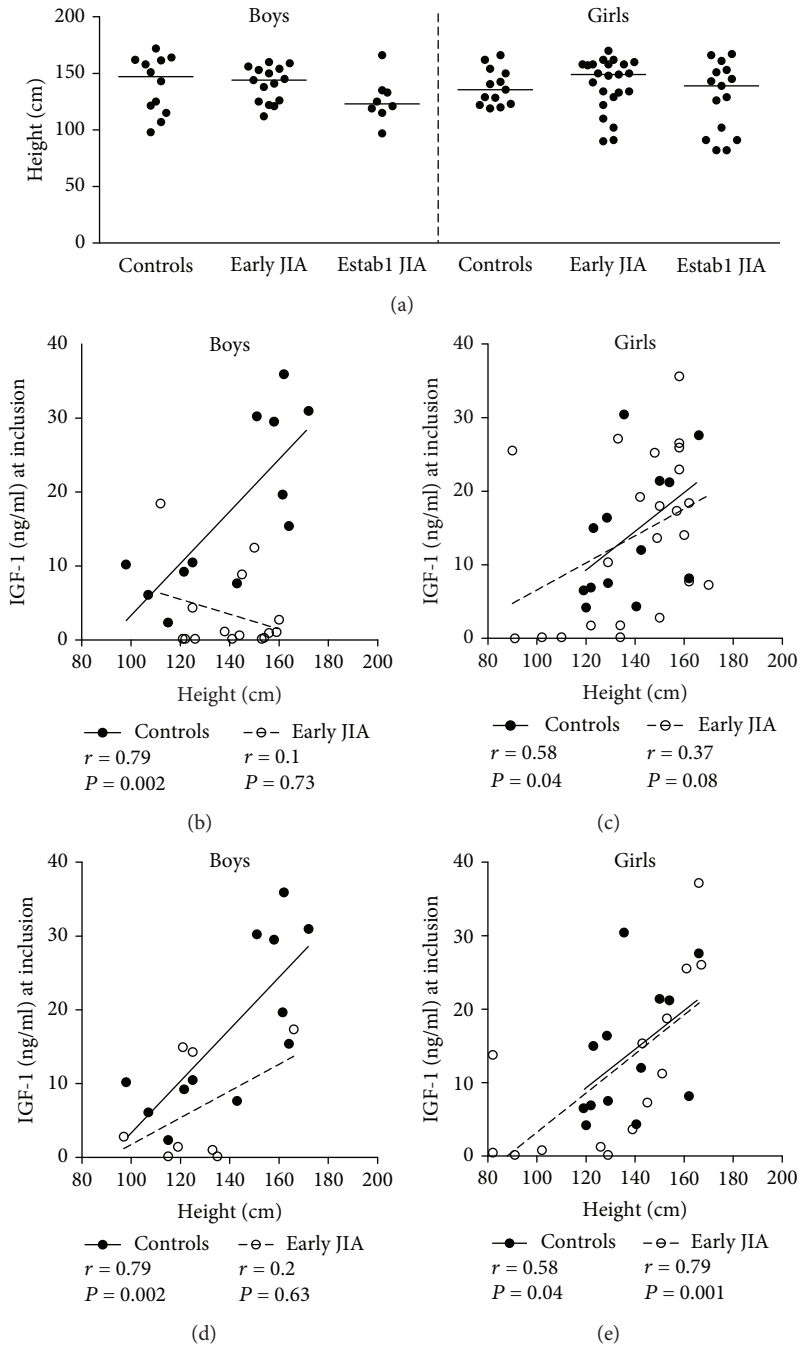

Figure 3: (a) Height of boys and girls at inclusion to the study. The horizontal bars indicate the median. (b, c) Correlations between serum IGF-1 levels and height in control groups of boys and girls, respectively (solid symbols), and in boys and girls with early JIA (open symbols). (d–e) Correlations between serum IGF-1 levels and height in control groups of boys and girls, respectively (solid symbols), and between boys and girls with established JIA (open symbols) (Spearman rank correlation test). (b–e) The regression lines are presented in the correlation plots.

in parallel with the transient rise in insulin resistance that occurs during pubertal development [22, 23].

In line with our findings, low serum IGF-1 levels have previously been reported in several studies of JIA [12–15], but IGF-1 levels in the normal range have also been found [24]. However, these studies consisted of relatively small JIA cohorts, most of them including less than 25 children, and male and female patients were pooled. The disease duration for the included JIA patients varied considerably from a few months up to several years, children with different treatments were analyzed together, and early JIA was not specifically investigated. An important strength with our JIA cohort is the relatively large number of included patients, which enabled separate analyses of IGF-1 levels in boys and girls as well as distinctions between early and established JIA.

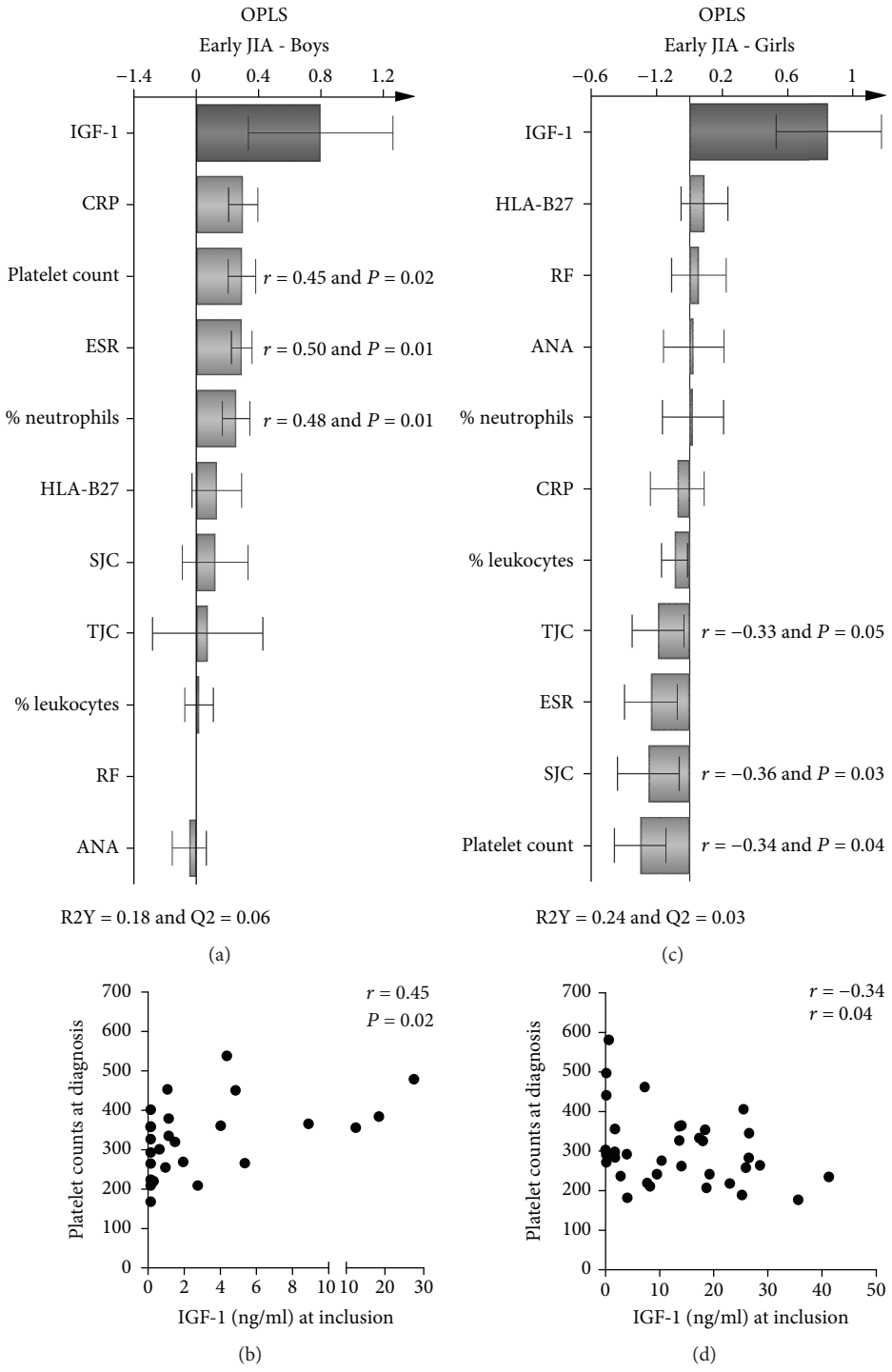

Figure 4: Serum IGF-1 levels in relation to disease activity/inflammation variables among boys and girls with early JIA. Multivariate OPLS loading column plots displaying the associations between Y, i.e., IGF-1 serum levels, and X-variables, i.e., clinical disease activity/inflammation variables, among (a) boys and (c) girls with early JIA. X-variables with bars projected in the same direction as Y are positively associated, whereas parameters in the opposite direction are inversely related to Y. The larger the bar and smaller the error bar, the stronger and more certain is the contribution to the model. Correlations between serum IGF-1 levels and platelet counts in boys (b) and in girls (d). (b, d) Spearman's rank correlation test.

We also observed that boys with early JIA displayed significantly lower IGF-1 serum levels compared to age-matched male controls. One possible explanation for this finding could be delayed onset of puberty compared to healthy children (reviewed in [7]). In this study, samples from population-based prevalence and incidence cohorts of JIA were utilized and since these studies had other epidemiological aims, pubertal development data were unfortunately

not recorded. This is an important limitation of our study since pubertal development influences serum IGF-1 levels and therefore may affect comparison between age-matched controls and children with JIA as well as between sexes. However, this does not change our intriguing observation that IGF-1 levels in early JIA boys were significantly lower.

In children with systemic and polyarticular JIA, growth failure is a common feature [1, 7, 25–27]. Several studies also demonstrate that slow growing children with JIA present with significantly lower serum IGF-1 levels compared to those in healthy controls [13, 14, 28]. IGF-1 deficiency could have severe clinical consequences in children and lead to growth failure resulting in short adult height [29, 30]. In the present study, age and height correlated strongly to serum IGF-1 levels in the control groups, but not in boys with early JIA. Our findings could indicate that there is a potential dysregulation of IGF-1 production in the pathogenesis of early JIA among boys. However, our results should be interpreted with some caution since all data regarding height and weight were not available.

Several studies have demonstrated interaction between IGF-1 and proinflammatory cytokines, which are commonly elevated in JIA patients [8, 31, 32]. Inflammation-related cytokines, including TNF-α, IL-1β, and IL-6, have been shown to dysregulate IGF-1 downstream intracellular signaling in chondrocytes [33–35]. Moreover, elevated IL-6 serum levels were associated with low circulating IGF-1 levels and growth delay in a transgenic mouse model [36], and serum IL-6 was inversely correlated with IGF-1 levels in children with systemic JIA [32, 36, 37]. Also, systemic JIA patients who were treated with anti-IL-6 receptor antibody (tocilizumab) experienced a catch-up in growth and an increase in serum IGF-1 levels [38]. In the present population-based JIA cohort, the majority of the established JIA children received nonbiological antirheumatic treatment with DMARDs, while most early JIA patients were treatment-naïve, which could explain normalized IGF-1 levels in established disease.

Few studies have examined serum IGF-1 levels in relation to inflammation- and disease activity-related variables in JIA. In a JIA cohort including all categories, but systemic JIA, serum IGF-1 levels correlated inversely to CRP [24]. In another cohort including systemic JIA patients only, there was also a negative association, although not statistically significant, between IGF-1 levels and CRP [38]. In both of these cohorts, boys and girls were analyzed together. In the present study, we observed an opposite pattern of association with disease activity measurements between male and female patients with early JIA. In boys, higher IGF-1 levels were related positively to inflammation-related markers, whereas they were either negatively associated or unrelated in girls. The underlying mechanisms for these sex-based discrepancies are unclear.

5. Conclusions

We here report for the first time that boys, but not girls, with early JIA present with decreased IGF-1 levels. Despite accumulating evidence that support sex-related differences in immune responses and in the prevalence of autoimmune diseases [39], a majority of JIA studies do not analyze data by sex. Based on our results, there is a need for further investigation of sex-based differences in biological mechanisms involved in the very early phase of JIA process in larger cohorts.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors do not have any conflicts of interest.

Authors' Contributions

ACL analyzed and interpreted all data, designed all figures, and wrote the paper. CP, MB, ME, and RP were involved in analysis and interpretation of the data and revising the manuscript critically for important intellectual content. ME and RP performed experiments. HL, KU, ST, TR, TT, and CP were involved in collecting samples and clinical data and revising the manuscript critically for important intellectual content. CP and RP designed the study and continuously supervised all aspects of the work. All authors read and approved the final manuscript.

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Review Article

Adipocytokines in Rheumatoid Arthritis: The Hidden Link between Inflammation and Cardiometabolic Comorbidities

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Rheumatoid arthritis is a chronic autoimmune disease affecting typically synovial joints and leading to progressive articular damage, disability, and reduced quality of life. Despite better recent therapeutic strategies improving long-term outcomes, RA is associated with a high rate of comorbidities, infections, malignancies, and cardiovascular disease (CVD). Remarkably, some well-known pathogenic proinflammatory mediators in RA, such as interleukin-1β (IL-1β) and tumor necrosis factor (TNF), may play a pivotal role in the development of CVD. Interestingly, different preclinical and clinical studies have suggested that biologic agents commonly used to treat RA patients may be effective in improving CVD. In this context, the contribution of adipocytokines has been suggested. Adipocytokines are pleiotropic molecules, mainly released by white adipose tissue and immune cells. Adipocytokines modulate the function of different tissues and cells, and in addition to energy homeostasis and metabolism, amplify inflammation, immune response, and tissue damage. Adipocytokines may contribute to the proinflammatory state in RA patients and development of bone damage. Furthermore, they could be associated with the occurrence of CVD. In this study, we reviewed available evidence about adipocytokines in RA, because of their involvement in disease activity, associated CVD, and possible biomarkers of prognosis and treatment outcome and because of their potential as a possible new therapeutic target.

1. Introduction

Rheumatoid arthritis is a chronic autoimmune disease affecting typically synovial joints and leading to progressive articular damage, disability, and reduced quality of life [1–4]. RA is associated with an increased rate of comorbidities, including infections, malignancies, and cardiovascular disease (CVD), leading to the excess of mortality experienced by these patients [5–7]. Remarkably, a close association between RA and accelerated atherosclerosis has been highlighted, due to the interaction between traditional cardiovascular (CV) risk factors and proinflammatory pathways [8–11]. Furthermore, the fact that traditional CV risk factors are underdiagnosed and undertreated may increase the atherosclerotic process [12, 13]. In addition, some well-known pathogenic proinflammatory mediators in RA, such as interleukin-1β (IL-1β) and tumor necrosis factor (TNF), may play a pivotal role in the development of CVD [14–16]. In fact, common pathogenic inflammatory pathways between the atherosclerotic process and rheumatic diseases have been shown [16–18]. Different reports have suggested that biologic DMARDs, commonly used to treat RA patients, may be effective in improving CV comorbidities [19, 20]. In this context, the contribution of adipocytokines has been suggested [21]. Adipocytokines are pleiotropic molecules, mainly released by white adipose tissue and by immune cells [21, 22]. Adipocytokines modulate the function of different tissues and cells, amplifying inflammation, immune response, and tissue damage [21]. During RA, adipocytokines could contribute to the proinflammatory state, develop bone damage, and accelerate concomitant atherosclerosis [22–25].

In this study, we reviewed available evidence about adipocytokines in RA, because of their involvement in

disease activity, associated CVD, and possible biomarkers of prognosis and treatment outcome and because of their potential as possible new therapeutic targets.

2. Methods

We designed a narrative review aimed at providing an overview about leptin, adiponectin, resistin, and visfatin in RA, because of their involvement in disease activity, associated cardiometabolic diseases, and possible biomarkers of prognosis and treatment outcome and because of their potential as possible new therapeutic targets. We performed an analysis of available evidence linking the same molecule to joint damage and cardiometabolic comorbidities, in order to discuss previous studies but also to provide a rationale for further researches. MEDLINE (via PubMed) was searched and the bibliography of relevant articles was also hand searched for identification of other potentially suitable studies.

3. Adipocytokines in RA: Generality, Pathogenic Mechanisms, and Changing Pattern to Treatment

3.1. Leptin. Leptin is a 16 kDa nonglycosylated adipocytokine with a long-helix structure and it is one of the most common adipocyte-derived molecules [26]. Leptin shows different biological actions deriving from an activation of OB-Rb long-form isoform receptors, which are encoded by the diabetes (db) gene [27]. Acting on hypothalamic nuclei, leptin decreases food intake and increases energy consumption, via induction of anorexigenic factors and suppression of orexigenic neuropeptides [28]. Furthermore, leptin is involved in both innate and adaptive immune responses being its production influenced by proinflammatory mediators [21–23]. Specifically, this adipocytokine exerts proinflammatory activities upregulating the production of TNF, IL-6, IL-1β, and IL-12, which, in turn, increase the expression of leptin in adipose tissue [21, 28, 29]. Leptin modulates the activity of innate immune cells by (i) enhancing the phagocytic activity of monocytes/macrophages; (ii) stimulating chemotaxis and release of reactive oxygen species by neutrophils; and (iii) promoting NK cell differentiation, proliferation, activation, and cytotoxicity [27, 30–32]. Concerning the effects on adaptive immune cells, leptin is able to (i) stimulate proliferation of naïve T lymphocytes and activate B cells; (ii) shift the T-cell cytokine production towards a Th1 phenotype, increasing the production of IFN-γ and IL-2; and (iii) induce regulatory T-cell anergy and T-cell receptor-reduced responsiveness [33–35]. As shown by a recent meta-analysis, circulating leptin levels are significantly higher in RA patients compared with controls [36]. Furthermore, it has been reported that obese RA patients showed an increased production of leptin according to ACPA positivity, thus suggesting that leptin could favour the humoral response against citrullinated proteins [37]. In addition, Olam et al. assessed the ratio between serum leptin levels and the synovial fluid [38]. Synovial/serum leptin ratio was significantly higher in RA patients and correlated with disease duration, disease activity, proinflammatory cytokines, and acute phase reactants [38]. However, conflicting results are also available in the literature and future studies are needed to elucidate the pathogenic role of leptin in RA [39, 40]. In fact, although this adipocytokine is considered to be proinflammatory, it has also been reported to be associated with reduced radiographic joint damage and this effect could be related to the anabolic effects of leptin [39, 40].

Recently, many studies assessed the effects of biologic DMARDs on leptin in RA, considering a relevant issue the changing pattern of this molecule after treatments [41–43]. RA patients treated by TNFi were investigated for leptin levels, assessing serum levels before and after such treatment [42, 43]. Interestingly, leptin levels did not change, suggesting that the beneficial effect of TNFi therapy on CVD outcomes in RA could not be mediated by a reduction of leptin [44, 45]. In fact, no significant modification was observed assessing leptin levels during therapy with adalimumab, etanercept, and infliximab [41–45]. However, these studies should be cautiously interpreted because the number of enrolled patients was relatively small.

3.2. Adiponectin. Adiponectin is a 244-residue protein, also known as GBP28, apM1, Acrp30, or AdipoQ, and it is mainly synthesised by adipose tissue [46]. This adipocytokine increases fatty acid oxidation and glucose uptake in the muscle and reduces the synthesis of glucose in the liver, acting via 2 receptors, AdipoR1 and AdipoR2, found in skeletal muscle and liver, respectively [47]. Ablation of the adiponectin gene has a dramatic effect in knockout mice on a high-fat/high-sucrose diet, inducing insulin resistance and lipid accumulation in muscles [46, 47]. Mirroring animal models, adiponectin levels are lower in obese patients and higher in patients losing weight [48, 49]. On the contrary, adiponectin and its receptors increase during physical activities [50]. Furthermore, the secretion of adiponectin is inhibited by proinflammatory cytokines, suggesting that inflammation may contribute to hypoadiponectinemia in insulin resistance and obesity [51].

In rheumatic diseases, adiponectin could act as a proinflammatory mediator in joints and it could be involved in matrix degradation [52, 53]. During RA, adiponectin and AdipoR1 expressions were higher in the synovial fluids and synovial tissues of patients compared with those of controls [54]. In this study, many cells derived from RA synovial fluids and tissues, including synovial fibroblasts, showed adiponectin, adipoR1, and adipoR2. Interestingly, the addition of adiponectin to cultures of synovial fibroblasts increased the production of proinflammatory cytokines, such as IL-6 and IL-8 [54]. The stimulation with adiponectin also contributed to the production of metalloproteinases, such as MMP-1 and MMP-13, by RA synovial fibroblasts [55]. Furthermore, adiponectin could synergise with IL-1β thus increasing the production of proinflammatory mediators by RA synovial fibroblasts [56, 57]. Adiponectin aggravated bone erosions by promoting osteopontin production in RA synovial tissue, suggesting that adiponectin induced the expression of osteopontin,

which in turn recruited osteoclasts [58]. Recently, the effects of adiponectin were assessed on adipose mesenchymal stem cells (ASCs) derived from the infrapatellar fat pad of RA patients [59]. ASCs were stimulated with both low molecular weight (LMW) and high/middle molecular weight (HMW/MMW) adiponectin isoforms. The authors observed that the secretion of proinflammatory mediators was upregulated by HMW/MMW adiponectin, but not by LMW adiponectin. In addition, they observed that the stimulation with HMW/MMW adiponectin reduced the proproliferative effects of ASC-derived soluble factors on RA synovial fibroblasts [59]. Taking together these results, it is possible to suggest a proinflammatory and joint destructive role of adiponectin in RA [55–59].

3.3. Visfatin. Visfatin is a protein of 471 amino acids and 52 kDa, and it is produced by the liver, bone marrow, muscle, macrophages, and visceral adipose tissue [60, 61]. This adipocytokine is increased in obesity [61]. Visfatin is regulated by proinflammatory cytokines and, in turn, it induces chemotaxis and the production of inflammatory cytokines, such as IL-1β, IL-6, and TNF, in lymphocytes from obese patients, suggesting involvement in the obesity proinflammatory *milieu* [62]. Furthermore, the proinflammatory actions of visfatin have been observed in experimental models of arthritis, in which the high levels of visfatin were proposed to modulate the proinflammatory process and the joint destruction [63, 64].

During RA, serum visfatin levels were higher in patients and correlated with radiographic joint damage [65–67]. Despite the association with radiographic outcome, the correlation with disease activity has shown conflicting results. In fact, the association with disease activity reported in some studies has been not confirmed in others [66–68]. The relatively small sample size and different experimental conditions could partially explain these results. Similarly, the analysis of results derived from clinical studies evaluating the changing pattern of visfatin after treatment with TNFi showed conflicting results. Serum visfatin levels were analysed in RA patients, who were differently treated (i) after 16 weeks of adalimumab treatment, (ii) after 2 weeks of high-dose prednisolone, and (iii) after 22 weeks of treatment with a combination regimen with tapered high-dose prednisolone and synthetic DMARD. Treatment with adalimumab was associated with a reduction in visfatin levels, whereas in other groups of patients, opposing effects on visfatin levels were observed [42]. On the contrary, other authors showed that visfatin levels did not change after the administration of infliximab [68].

3.4. Resistin. Resistin is a 12.5 kDa protein included in the resistin-like molecule (RELM) family, and it is mainly produced by nonadipocyte resident inflammatory cells, mainly macrophages [69–71]. Resistin increases with obesity and promotes insulin resistance, suggesting a possible link between obesity and diabetes [72–74].

Although a significant difference was not found in serum resistin levels between patients and controls, a pathogenic role for resistin has been suggested in RA [75, 76]. In fact, the intra-articular injection of recombinant resistin in the knee joints of murine models induced arthritis and increased the production of several proinflammatory cytokines, such as an increased expression of several proinflammatory cytokines including IL-1β, IL-6, IL-12, and TNF [76]. Furthermore, higher levels of this adipocytokine were observed in synovial fluid samples from RA patients and were correlated with disease activity and joint damage [77]. These data could suggest the production and the contribution of resistin in the inflamed joint, despite the lack of correlation with inflammatory markers in peripheral blood [76, 77].

Concerning the changing pattern after treatment, TNFi reduced serum resistin levels [42, 78]. After the administration of infliximab, the serum resistin levels significantly decreased in RA patients. In this cohort, resistin levels also correlated with inflammatory markers thus suggesting a possible role in the RA inflammatory process [78].

4. Adipocytokines and Cardiometabolic Diseases in RA

RA patients characteristically experience an increased risk of CVD derived from the synergy between traditional CV risk factors and inflammation [7–10]. In this context, the role of adipocytokines has been suggested as a possible link between adiposity, inflammation, and cardiometabolic diseases (Figure 1) [79, 80]. A previous study was performed to evaluate whether adipocytokines could affect insulin resistance and coronary atherosclerosis in RA patients [81]. In this study, the authors assessed the coronary calcium score, homeostatic model assessment for insulin resistance (HOMA-IR), and serum adipocytokine (leptin, adiponectin, resistin, and visfatin) levels in 169 RA patients. To date, high leptin levels correlated with HOMA-IR, even after adjustment for possible clinical confounders, age, gender, BMI, traditional CV risk factors, and inflammatory mediators. On the contrary, visfatin, adiponectin, and resistin showed no association with the HOMA-IR index. No association was retrieved between the coronary calcium score and assessed adipocytokines [81]. More recently, adipocytokines were further investigated as a link between inflammation, insulin resistance, and atherosclerosis in RA, being associated with pathogenic mechanisms of these diseases (Figure 2) [82]. A study evaluated HOMA-IR, intima-media thickness (IMT), carotid artery (CCA) resistive index (RI), and carotid plaques in 192 RA patients. These data were correlated with levels of adiponectin, leptin, and resistin. The authors observed that leptin and leptin : adiponectin (L : A) ratio were correlated with HOMA-IR and with CCA-RI after adjustment for CV risk factors, suggesting a possible independent role of leptin in predicting CVD in RA [82]. Although these correlations were not observed in another experience [83], it is possible to speculate that leptin is associated with insulin resistance in RA. Multiple lines of evidence showed the influence of leptin in the metabolism of glucose and pathogenesis of insulin resistance and diabetes [84, 85]. Insulin resistance in diabetic leptin

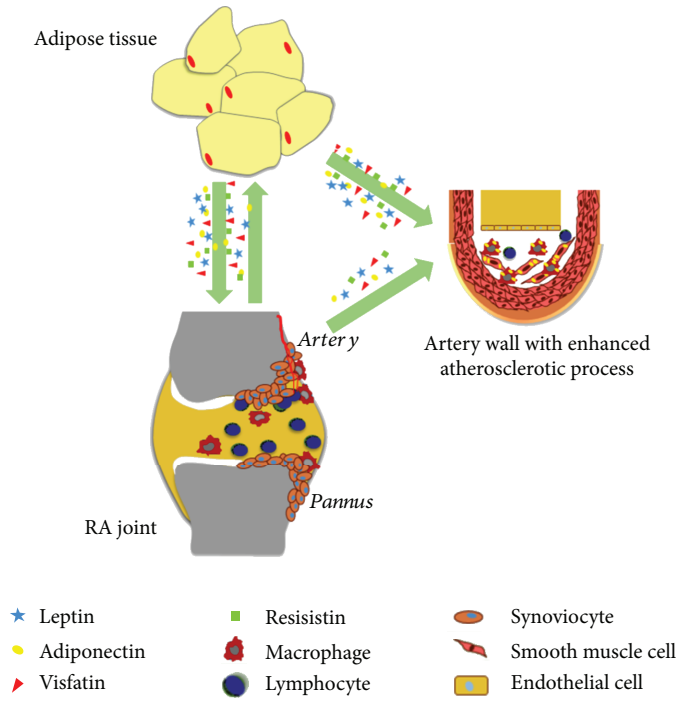

Figure 1: Schematic role of adipocytokines on the relationship among adipose tissue, rheumatoid arthritis, and atherosclerotic process.

receptor-deficient or genetic leptin-deficient animal models could not be fully attributed for their obesity and hyperphagia; the restriction in caloric intake failed to improve or recover the sensitivity of insulin in these models [86]. Furthermore, leptin administration in these models reduced plasma insulin and blood glucose levels [87]. In addition, leptin could influence glucose metabolism via the modulation of glucagon by α-cells of pancreas [88]. Furthermore, leptin could provide a functional link between obesity and CVD [88]. The link between fat mass and atherogenesis is confirmed by the findings in animal models of obesity [89, 90]. Leptin levels were associated with endothelial dysfunction proatherogenic actions, enhancing oxidative stress in endothelial cells, smooth muscle cell proliferation, and vascular calcification [90].

Concerning adiponectin, the correlation between total and HMW adiponectin concentrations, cardiometabolic risk, and surrogate markers of enhanced early atherogenesis was performed in 210 RA patients [91]. Total and HMW adiponectin concentrations correlated with high systolic, diastolic, and mean blood pressure and HDL cholesterol concentrations, low total HDL cholesterol ratios and triglyceride concentrations, and triglyceride-HDL cholesterol ratios and glucose concentrations [91]. These results mirrored what was observed in a lipoatrophy mouse model with adiponectin deficiency [92]. In these models, the replacement of adiponectin improved insulin resistance, fatty acid oxidation, and energy consumption, leading to a reduction of triglyceride levels in muscle and liver tissue [92, 93]. Furthermore, wild-type mice which received a high-fat diet showed a reduction in adiponectin levels and the replacement of adiponectin improved this diet-induced hypertriglyceridemia [94, 95]. To date, the possible role of adiponectin in modulating the homeostasis of blood pressure has been suggested [96]. In a cross-sectional study assessing patients with high blood pressure, high serum adiponectin levels were correlated with low procollagen type I carboxy-terminal propeptide circulating levels, a molecule reported to be associated with the arterial stiffening process [97]. Furthermore, adiponectin showed the ability to increase the gene expression and to activate the endothelial nitric oxide synthase by activation of AMPK [98]. Finally, it has been reported that adiponectin inhibited the deleterious effect of the renin-angiotensin system on the vascular system [99].

The potential impact of visfatin was assessed on CVD in 232 RA patients [100]. Visfatin concentrations were related to increased diastolic blood pressure and presence of diabetes [100]. In this context, it has been reported that visfatin could represent a proinflammatory cytokine influenced by insulin and/or insulin sensitivity via the NF-κB and JNK pathways [101, 102]. The role of visfatin was investigated in the impairment of the insulin pathway by TNF activity in adipocytes. In that study, the authors showed that visfatin was involved in TNF-mediated insulin resistance in adipocytes, via the NAD(+)/Sirt1/PTP1B pathway [103]. Furthermore, heterozygous mice with a mutation in the visfatin gene had higher levels of plasma glucose, impaired glucose tolerance, and reduced glucose-stimulated insulin secretion when compared with controls [104]. In addition, high visfatin levels could mediate vascular damage by inducing the expression of adhesion molecules via oxidative stress-dependent NF-κB activation,

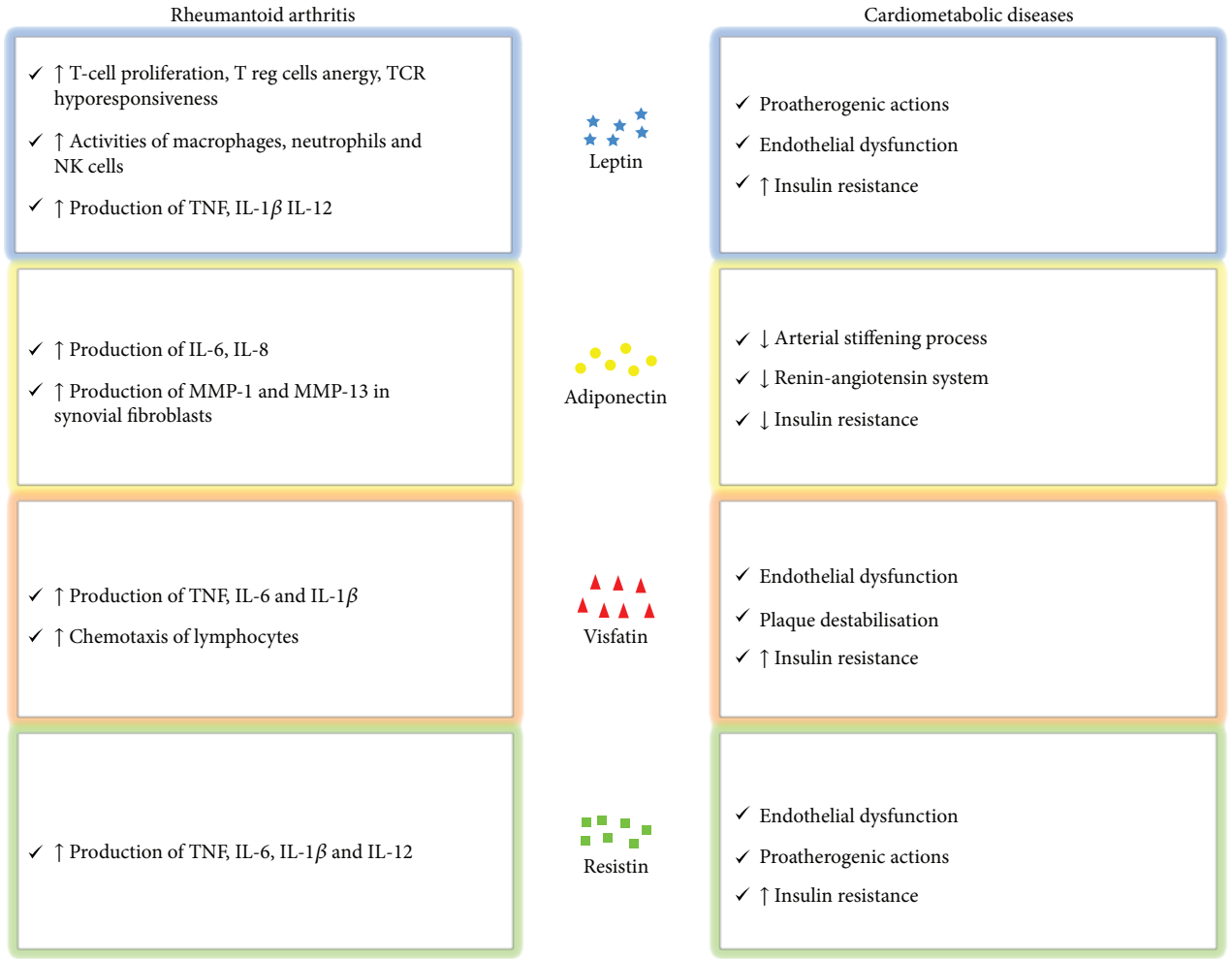

Figure 2: Pathogenic mechanisms of adipocytokines in rheumatoid arthritis and cardiometabolic diseases. Abbreviations: T Reg cells—T regulatory cells; TCR—T-cell receptor; NK cells—natural killer cells; TNF—tumor necrosis factor; IL—interleukin; MMP—metalloproteinase.

thus leading to endothelial inflammation and plaque destabilisation [105]. However, conflicting results are available concerning the role of visfatin [106, 107], thus future studies are needed to entirely clarify its role in cardiometabolic diseases.

Finally, the role of resistin has been proposed in cardiometabolic diseases. Of note, a certain degree of cross-talk between resistin and other adipokines has been reported [108, 109]. In fact, the expression on endothelial cells of VCAM-1 and ICAM-1 by resistin is counteracted by adiponectin [108]. A further link between leptin and resistin has also been proposed, and the expression of resistin was shown to be suppressed by leptin administration in animal models with subsequently decreased glucose and insulin levels [109]. In addition, the pathogenic role of resistin in atherogenesis has been proposed [110]. The secretion of resistin from atheroma-derived macrophages was suggested because of the colocalization of resistin and CD68 in the staining of human aneurysms and the higher mRNA resistin expression in cultured macrophages than in controls [111].

5. Adipocytokines as Future Possible Therapeutic Targets

In the last decades, long-term outcomes of RA have remarkably improved by using synthetic and biological DMARDs [112–114] and, presently, multiple lines of evidence assessed the best therapeutic strategy of concomitant diseases [115, 116]. In this context, it has been proposed that the inhibition of some cytokines may extend beyond the inflamed joints thus targeting, at the same time, associated comorbidities and improving the management of these patients [115–117]. Taking together these observations, it could be possible to speculate whether targeting adipocytokines may be effective in RA and comorbidities. Presently, antagonists of leptin have been developed to treat metabolic disorders. It should be tested if they could also have anti-inflammatory activities *in vivo* [118–120]. Interestingly, a monoclonal antibody against the leptin receptor was shown to block human TNF production by monocytes acting as an antagonist [121]. Recently, an orally active adiponectin receptor agonist improved insulin

resistance and glucose intolerance in mice [122]. Considering that adiponectin showed anti-inflammatory properties, it could be speculated that adiponectin or adiponectin receptor agonists could be promising targets for the development of therapeutic drugs to treat insulin-resistant states and possible inflammatory states [123].

6. Conclusions

RA is a chronic autoimmune disease with increased mortality, due mainly to CVD. Adipocytokines are shown to be of importance in the pathogenesis of RA and associated comorbidities. Future studies are needed to identify the new mechanisms of action of adipocytokines and to elucidate if these molecules could be new possible therapeutic targets, thus improving the management of RA patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Research Article

Role of the Specialized Proresolving Mediator Resolvin D1 in Systemic Lupus Erythematosus: Preliminary Results

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Objective. Systemic lupus erythematosus (SLE) is an autoimmune systemic disease and its pathogenesis has not yet been completely clarified. Patients with SLE show a deranged lipid metabolism, which can contribute to the immunopathogenesis of the disease and to the accelerated atherosclerosis. Resolvin D1 (RvD1), a product of the metabolism of the omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA), acts as a specialized proresolving mediator which can contribute in restoring the homeostasis in inflamed tissues. The aim of the present pilot study is to evaluate plasma levels of RvD1 in patients with SLE and healthy subjects, investigating its potential role as a biomarker of SLE and assessing its relationship with disease activity and laboratory parameters. *Methods*. Thirty patients with SLE and thirty age- and sex-matched healthy subjects (HSs) have been consecutively recruited at Campus Bio-Medico University Hospital. RvD1 plasma levels were measured by ELISA according to the manufacturer's protocol (Cayman Chemical Co.). RvD1 levels were compared using Mann–Whitney test. Discriminatory ability for SLE has been evaluated by the area under the ROC curve. *Results*. Lower levels of RvD1, 45.6 (35.5–57.4) pg/ml, in patients with SLE have been found compared to HSs, 65.1 (39.43–87.95) pg/ml ($p = 0.0043$). The area under the ROC curve (AUC) for RvD1 was 0.71 (95% CI: 0.578–0.82) and the threshold value of RvD1 for the classification of SLE was <58.4 pg/ml, sensitivity 80% (95% CI: 61.4–92.3), and specificity 63.3% (95% CI: 43.9–80.1), likelihood ratio 2.2 (95% CI: 1.3–3.6). *Conclusions*. The present preliminary study allows hypothesizing a dysregulation of RvD1 in patients with SLE, confirming the emerging role of bioactive lipids in this disease.

1. Introduction

Systemic lupus erythematosus is an autoimmune systemic disease which can involve virtually every organ or apparatus [1]. Despite intense research efforts, the pathogenesis of SLE is not completely understood [2] and many unmet needs still remain in the diagnosis, management, and prognosis [3]. In SLE patients, accelerated atherosclerosis and increased risk of cardiovascular disease (CVD) have been observed [4, 5]. SLE patients are also characterized by an altered lipid metabolism [6], with increased triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, and

apolipoprotein B (ApoB) levels, as well as reduced high-density lipoprotein (HDL) cholesterol levels [6]. Oxidation of LDL (oxLDL), which occurs in the early stages of atherosclerosis and can induce inflammation and formation of anti-oxLDL autoantibodies [7], is increased in patients with SLE, and this phenomenon is associated with CVD parameters and renal involvement [5, 8, 9]. Notably, omega-6 polyunsaturated fatty acid (FA) elevations have been observed in SLE [10], and recently, altered metabolism of the endocannabinoid 2-arachidonoylglycerol (2-AG) has been demonstrated in patients with SLE, who show higher plasma levels of this molecule compared to healthy subjects [11]. On the other hand, the role of omega-3 polyunsaturated FAs still remains elusive. Of note, the dietary supplementation of the omega-3 polyunsaturated FAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) provides improvement of SLE manifestations in 5 of 7 studies [12–16], while one study failed to demonstrate beneficial effects [17] and another one showed an initial improvement followed by loss of effectiveness [18] (see [19] for a recent review).

Omega-3 polyunsaturated FA can be metabolized in inflamed tissue leading to specialized proresolving mediators (SPMs), which prevent further polymorphonuclear cell (PMN) infiltration, induce efferocytosis of apoptotic bodies in macrophages, and promote tissue repair and healing [20–22]. D-series resolvins (RvDs) are derived from DHA and include RvD1-RvD6, while E-series resolvins (RvEs) are derived from EPA and include RvE1-RvE3 [20, 23]. In vivo, the biosynthesis of RvDs, including RvD1, requires a first step involving 15-lipoxygenase (15-LOX) or aspirin-triggered cyclooxygenase-2 (COX-2) and a second step involving 5-LOX [24, 25]. RvD1 exerts its biological functions through interaction with G-protein-coupled receptor 32 (GPR32) or lipoxin A4 receptor/formyl peptide receptor 2 (ALX/FPR2). In addition to the effects on innate immunity, RvD1 is able to support the humoral response that increases IgM and IgG production [26], also reducing IgE secretion [27]. Overall, the output of proinflammatory cytokines from T helper (Th) and cytotoxic lymphocytes and the differentiation of Th1 and Th17 are reduced, and the differentiation of T regulatory (Treg) cells is enhanced [28]. As yet, little is known about the role of RvD1 in rheumatic diseases. For instance, RvD1 shows proresolving features and protects the cartilage from injury in a mouse model of arthritis [29]. In vitro, RvD1 also reduces inflammatory mediators and oxidative stress in osteoarthritis [30].

The present pilot study is aimed at evaluating plasma levels of RvD1 in patients with SLE and healthy subjects, investigating its potential role as a biomarker of SLE and assessing its relationship with disease activity and laboratory parameters.

2. Materials and Methods

2.1. Study Population and Clinical Assessment. Thirty patients with SLE, classified according to the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria [31], were consecutively enrolled from outpatient lupus clinic of the Campus Bio-Medico Università Hospital of Rome. As for the control group, thirty age- and sex-matched healthy subjects (HSs) without chronic diseases and not taking any medication were also enrolled using a "friend of the same age" referral strategy. The study was conducted in compliance with International Conference on Harmonization Good Clinical Practice guidelines and the Declaration of Helsinki. In the SLE cohort, the inclusion criteria included serological disease activity: anti double-strand DNA (anti-dsDNA) positivity and/or low plasma of complement component 3 (C3) and/or C4 with or without extractable nuclear antigen antibodies (anti-ENA), anti-phospholipids, and hypergammaglobulinemia [32]. At enrollment, SLE treatment with low-medium dose glucocorticoids (prednisone <25 mg/day), immunosuppressants (such as methotrexate, cyclosporine, azathioprine, and mycophenolate mofetil), was not an exclusion criterion. In both SLE and HS cohorts, the exclusion criteria included past or present biological therapy (such as rituximab, tocilizumab, or belimumab), glucocorticoids bolus in the previous year, cyclophosphamide treatment in the previous year, cancer at enrollment or in the previous 5 years, infectious diseases at enrollment or in the previous 2 months, and current pregnancy. At enrollment, antinuclear antibodies (ANA), anti-dsDNA, erythrosedimentation rate (ESR), and C-reactive protein (CRP) C3 and C4 levels have been assessed with conventional laboratory tests. In the SLE cohort, disease activity has been measured with the Safety of Estrogens in Lupus Erythematosus National Assessment-SLE Disease Activity Index (SELENA-SLEDAI) and the British Isles Lupus Activity Group (BILAG), while organ damage has been evaluated with the SLICC/American College of Rheumatology (ACR) Damage Index (SDI). At enrollment, a blood sample has been taken and plasma was separated.

2.2. Quantification of RvD1. RvD1 plasma levels were measured by ELISA according to the manufacturer's protocol (Cayman Chemical Co., Ann Arbor, MI), as validated elsewhere [33].

2.3. Statistical Analysis. Data were expressed as median (25th–75th percentile). RvD1 levels between patients with SLE and HSs as well as demographic and laboratory parameters have been compared using Mann–Whitney U test. Fisher's exact test has been used to analyze contingency tables. Receiver operating characteristic (ROC) analysis was used to define the ability of RvD1 to differentiate patients with SLE and HSs; the optimum cutoff value has been identified from the highest Youden's index. Pretest odds, posttest odds, and posttest probability were calculated. Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, Ca, USA) and MedCalc 11.6.1.0 (Belgium).

3. Results

Demographic features and clinical and laboratory characteristics of the patients with SLE and HSs are shown in Table 1.

Patients with SLE showed lower levels of RvD1, 45.6 (35.5–57.4) pg/ml, compared to HSs, 65.1 (39.43–87.95) pg/mL ($p = 0.0043$), as reported in Figure 1. Notably, in the SLE

Table 1: Patients' characteristics at enrollment.

	SLE ($n = 30$)	HSs ($n = 30$)	p
Age (years)	39 (35–46.25)	40.5 (35–46.5)	ns
Sex (F/M)	29/1	29/1	ns
Disease duration (months)	64 (31–99)	NA	
Antiphospholipid syndrome (*N*)	9	0	0.0019
Anti-dsDNA positivity (*N*)	18	0	<0.0001
Hypcomplementemia C3 (*N*)	19	0	<0.0001
C3 (g/l)	0.69 (0.34–0.8)	1.01 (0.96–1.2)	0.0002
Hypocomplementemia C4 (*N*)	10	0	0.0008
C4 (g/l)	0.078 (0.03–0.115)	0.15 (0.1–0.225)	0.0039
No prednisone (*N*)	4	30	<0.0001
Prednisone ≤5 mg (*N*)	13	0	0.0003
Prednisone >5 mg	13	0	0.0003
Immunosuppressants (*N*)	18	NA	
Hydroxychloroquine (*N*)	19	NA	
Low dose aspirin (*N*)	8	0	0.0046
SELENA-SLEDAI	4 (2–6.75)	NA	
BILAG A	4	NA	
BILAG B	9	NA	
SDI	0.917 (0–1.04)	NA	

NA: not applicable; ns: not significant.

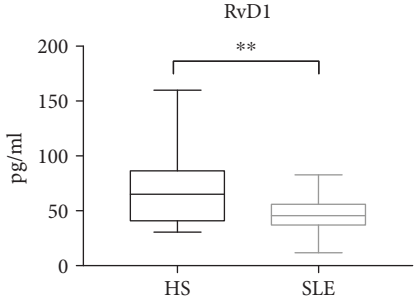

Figure 1: Levels of resolvin D1 (RvD1) in plasma from SLE patients ($n = 30$) and matched HSs ($n = 30$). Data are expressed as pg/ml, median (horizontal bar) with 25th and 75th percentile (boxes), and minimum and maximum (bars) ($^{**}p = 0.0043$).

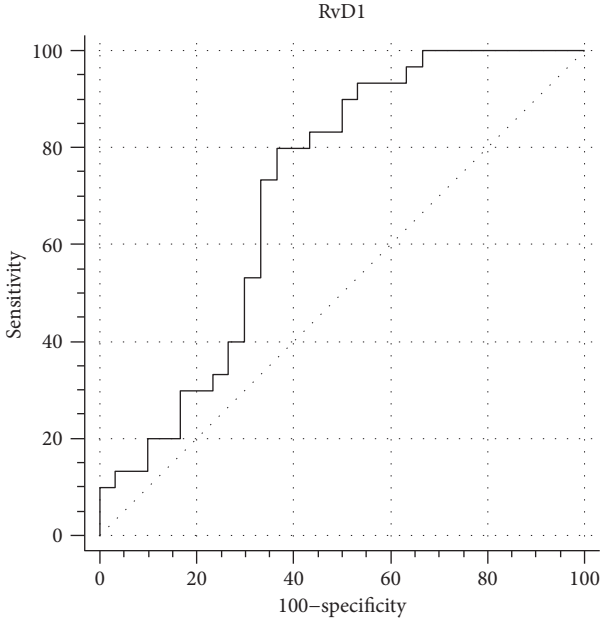

Figure 2: ROC curve for RvD1. Area under the curve (AUC) value is 0.71 (95% CI: 0.58 to 0.82).

cohort, no difference in RvD1 levels has been demonstrated between patients taking low-dose aspirin or not ($p = 0.11$).

The area under the ROC curve (AUC) was 0.71 (95% CI: 0.578–0.82), showing a fair discriminatory ability as reported in Figure 2. Based on ROC and AUC analysis, the threshold value of RvD1 for the classification of SLE was <58.4 pg/ml, sensitivity 80% (95% CI: 61.4–92.3), and specificity 63.3% (95% CI: 43.9–80.1), and likelihood ratio 2.2 (95% CI: 1.3–3.6). Based on the cutoff value of 58.4 pg/ml, the odds ratio of SLE was 0.1522 (95% CI: 0.0488–0.4742), $p = 0.0012$.

In the SLE cohort, no relation has been found between RvD1 plasma levels and disease activity scores, SDI, or disease duration. Likewise, no relation has been demonstrated between RvD1 plasma levels and pharmacological therapies. Nevertheless, SLE patients with low C4 levels (<0.1 g/l) also had lower RvD1 plasma levels, 36.05 pg/ml (29.55–42.45), compared to patients with normal (>0.1 g/l) C4 levels of 52.2 pg/ml (43.4–61.8), $p = 0.0087$, as reported in Figure 3. In the SLE cohort, no significant difference in RvD1 plasma levels has been found among patients with low (<0.9 g/l) and normal (>0.9 g/l) C3 levels.

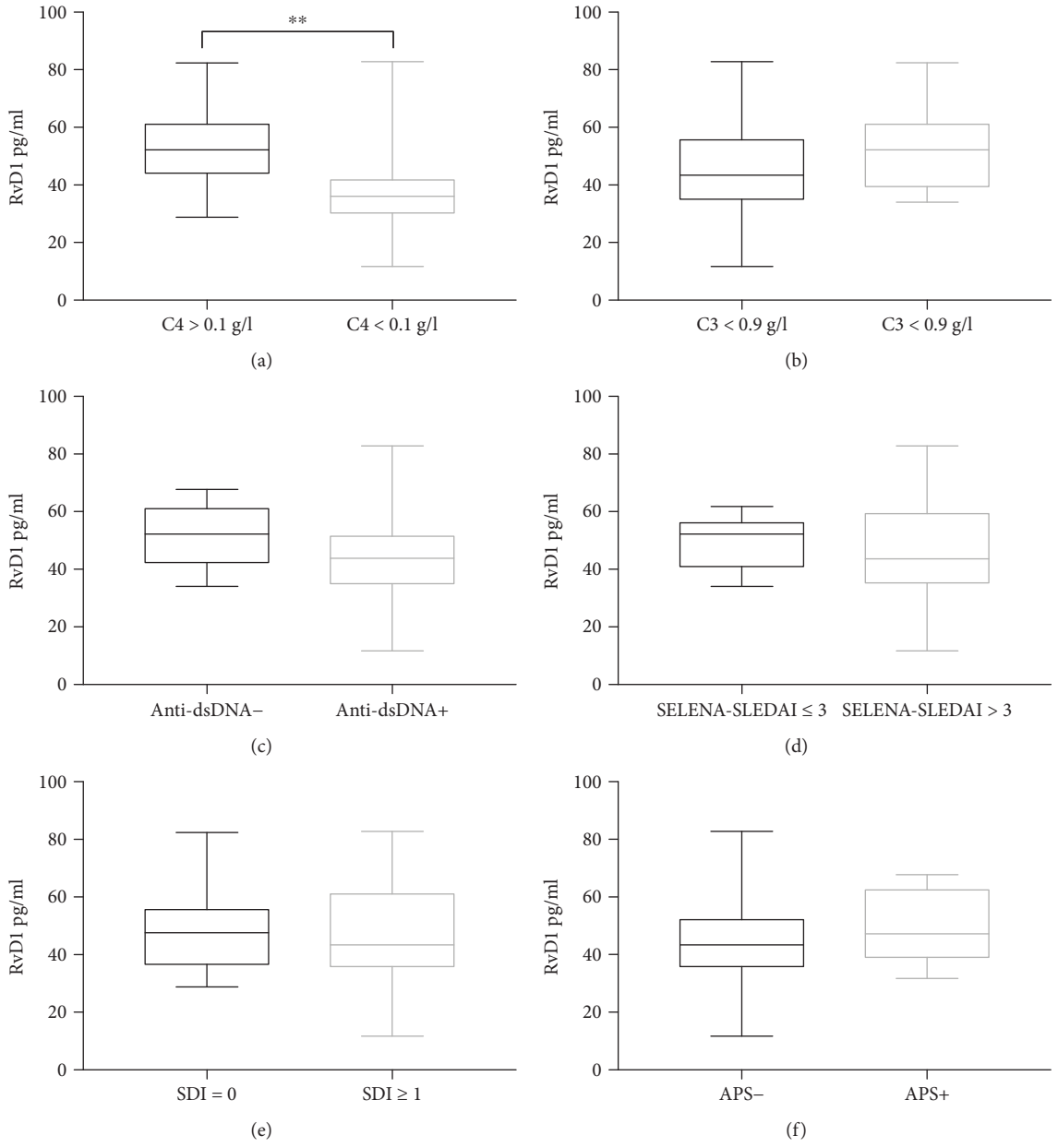

Figure 3: (a) Levels of resolvin D1 (RvD1) in plasma from SLE patients with low C4 levels (<0.1 g/l) ($N = 10$) and SLE patients with normal C4 levels (>0.1 g/l) ($N = 20$). (b) Levels of resolvin D1 (RvD1) in plasma from SLE patients with low C3 levels (<0.9 g/l) ($N = 19$) and SLE patients with normal C4 levels (>0.9 g/l) ($N = 11$). (c) Levels of resolvin D1 (RvD1) in plasma from anti-dsDNA antibody-positive SLE patients ($N = 18$) and anti-dsDNA antibody-negative SLE patients ($N = 12$). (d) Levels of resolvin D1 (RvD1) in plasma from SLE patients with SELENA-SLEDAI ≤ 3 ($N = 10$) and SLE patients with SELENA-SLEDAI > 3 ($N = 20$). (e) Levels of resolvin D1 (RvD1) in plasma from SLE patients with SDI = 0 ($N = 15$) and SLE patients with SDI ≥ 1 ($N = 15$). (f) Levels of resolvin D1 (RvD1) in plasma from SLE patients without antiphospholipid syndrome (APS) ($N = 21$) and SLE patients with APS ($N = 9$). Data are expressed as pg/ml, median (horizontal bar) with 25th and 75th percentile (boxes), and minimum and maximum (bars) (** $p = 0.0087$).

4. Discussion

SLE is a complex autoimmune multisystemic disease, with a huge impact on quality of life and development of organ damage [34, 35]. Despite having a large number of studies clarify many aspects of the pathogenesis of this disease, a comprehensive understanding of the immunological phenomena underlying the clinical manifestations of SLE still remains a challenge [36, 37].

Bioactive lipids seem to be a main actor in inflammation, and they could play a pivotal role in immunopathogenesis on many inflammatory diseases [22, 38, 39]. At present, despite SPMs representing key mediators in rheumatic diseases, data are still scarce and their potential from a therapeutic point of view has not yet been adequately addressed [22].

For the first time, our study demonstrates lower levels of RvD1 in plasma of SLE patients compared to HSs. Moreover, the analysis of the ROC curve showed a fair ability of RvD1 to

discriminate SLE patients from HSs, providing a preliminary cutoff value of 58.4 pg/ml. Recently, Barden and coworkers demonstrated higher plasma levels of RvD1 in patients with arthritis compared to healthy subjects; therefore, the role of RvD1 in SLE and arthritis appears to be different [40]. In our cohort, no relation between RvD1 plasma levels and disease activity has been found. However, we demonstrated lower levels of RvD1 in patients with low plasma levels of C4 (but not of C3). This finding supports the hypothesis that RvD1 could affect complement cascade activation, which is a well-established pathogenetic feature of SLE [41].

Several weaknesses of this study should be considered. The sample size was relatively small, and further studies on a larger number of patients are required to better evaluate the role of RvD1 in SLE, especially its potential role as a biomarker. Furthermore, this study did not schedule a follow-up of patients and therefore could not ascertain the role RvD1 in predicting changes in disease activity, damage accrual, or laboratory parameters over time. Moreover, no information about omega-3 polyunsaturated FAs was available. In the present study, we cannot exclude that the difference of RvD1 plasma concentrations between patients with SLE and HSs may partly reflect the use of glucocorticoids or immunosuppressants.

In conclusion, this study allows hypothesizing a dysregulation of RvD1 in SLE and confirms the emerging role of bioactive lipids in this disease.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

Antonella Afeltra and Mauro Maccarrone are equally senior authors.

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Review Article

Lipid and Bile Acid Dysmetabolism in Crohn's Disease

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Crohn's disease is one of the systemic autoimmune diseases. It commonly affects the small intestine and colon but may involve any portion of the gastrointestinal tract from the mouth to the anus. The most affected area by Crohn's disease is the distal part of the small intestine, in which the bile acid molecules are most efficiently reabsorbed. Bile acids form mixed micelles together with fatty acids, which function as a transport vehicle to deliver fatty acids to the apical membrane of enterocytes for absorption. Therefore, if the terminal ileum is impaired, bile acid malabsorption may occur, which may cause congenital diarrhoea in Crohn's disease. Similarly, the impairment of the terminal ileum also induces fatty acid malabsorption, which may influence the role of fatty acids in Crohn's disease. In contrast, a recent study reported that multidrug resistance protein 1 (MDR1) regulated effector T-cell function in the ileum from bile acid-driven oxidative stress and MDR1 loss of function in a subset of patients with Crohn's disease. However, the role of consumption of fatty acids in Crohn's disease remains to be fully elucidated. This review is aimed at providing an overview of some recent developments in research of Crohn's disease from comprehensive perspective with a focus on the connection between disease location and behaviour, lipid diets, and bile acid malabsorption.

1. Introduction

Crohn's disease is one of the main disease phenotypes of inflammatory bowel disease. It is often characterised by systemic symptoms and discontinuous lesions with inflammation that can involve the full thickness of the affected portion of the bowel from mucosa to serosa. The small intestine and the colon are the most affected areas, but any portion of the gastrointestinal tract from the mouth to the anus can be involved.

Crohn's disease is considered to be a multifactorial disease with both genetic and acquired factors in its aetiology. Genetic studies highlighted the importance of the intestinal immune system, including dysregulation of intestinal $CD4^+$ T-cell subgroups [1, 2]. Meanwhile, as discussed in the European Crohn's and Colitis Organisation's Epidemiological Committee study [3], the "westernised" lifestyle, such as an increased consumption of refined sugar, fatty acids, and fast food and a reduced consumption of fruits, vegetables, and fibres, was deemed to link to the development of Crohn's disease. In parallel, the first choice of therapy in children with Crohn's disease is exclusive enteral nutrition, which is an induction therapy [4]. Enteral nutrition is one of the established remission–induction therapies for both children and adults with Crohn's disease in Japanese population [5]. However, it is not a common treatment in adults with Crohn's disease in western countries [6], because no studies on its significant efficacy were found in systematic reviews [7]. Interestingly, a recent review showed an evidence to support a possible role of exclusive enteral nutrition in a newly diagnosed adult with Crohn's disease with ileal involvement [6].

This review is aimed at providing an overview of some recent developments in research concerning the molecular pathways of lipid consumptions, which may connect immunological and nutritional studies in Crohn's disease and epidemiological studies on disease location, phenotype, and diet.

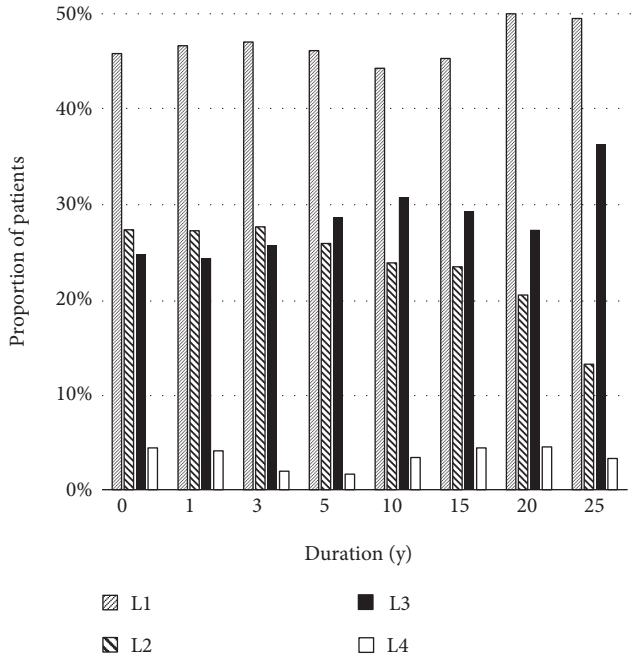

Figure 1: Disease location at diagnosis and at elapsed years among patients with Crohn's disease in a study of the University Hospital of Liège in Belgium [11].

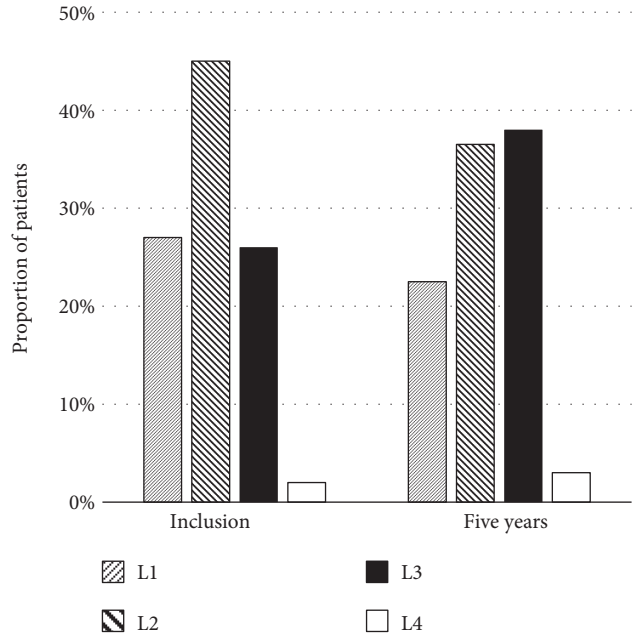

Figure 2: Disease location at recruitment and after 5 years among patients with Crohn's disease in a study in southeastern Norway [12].

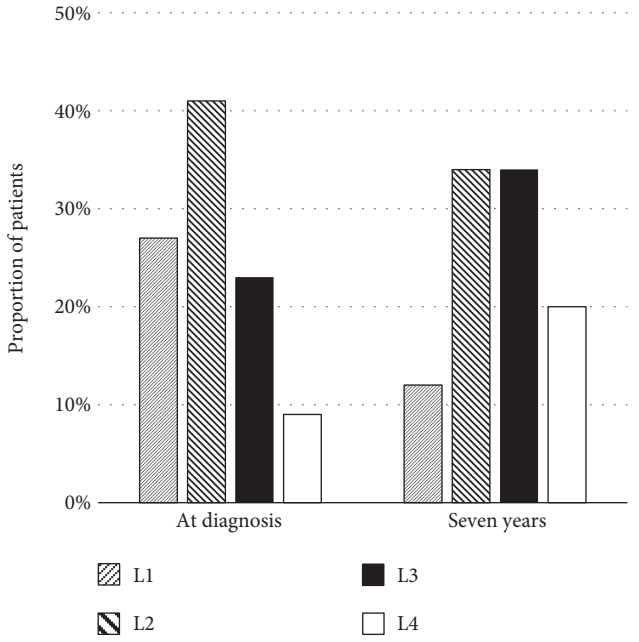

Figure 3: Disease location at diagnosis and after 7 years among patients with Crohn's disease in a study of a Danish cohort [10].

2. Location and Phenotype of Crohn's Disease

Generally, the terminal ileum and colon are the commonest locations affected in Crohn's disease, of which approximately 50% of patients have involvement [8, 9]. About 30% of the patients have only small bowel involvement, and the remaining 20% of the patients have isolated colonic involvement [8]. Changes in disease location are likely to occur over time in about 5–24% of patients with the disease [10–12]. In the Montreal classification, the disease location was defined as follows: L1, terminal ileum; L2, colon; L3, ileocolon; and L4, upper gastrointestinal tract [13]. Figures 1–3 show three studies, which reported disease location changes [10–12]. Their results suggest that only ileal or colonic involvement tends to occur at disease onset. However, as time progresses, the disease location extends to the other organs; especially both the ileum and colon (L3) will be involved. In the study of a Danish cohort [10], biologics, infliximab or adalimumab, showed protective effects on changes in disease location. Infliximab and adalimumab are anti-tumour necrosis factor-α (TNF-α) antibody drugs. The result of the above study may suggest that inflammatory cytokines, such as TNF-α, play an important role in the expansion of the disease location.

The phenotype of Crohn's disease was defined as B1, nonstricturing and nonpenetrating (inflammation); B2, stricturing; and B3, penetrating in the Montreal classification [13]. At diagnosis, the predominance of inflammatory behaviour (B1) is the most prevalent phenotype in patients with Crohn's disease [14]. However, dramatic changes in the proportion of disease behaviour were reported [14–16]. Usually, inflammation (B1) evolves to stricturing (B2) or penetrating (B3). This suggests that inflammation influences factors that may cause the exacerbation of Crohn's disease.

3. Epidemiology of Diet and Crohn's Disease

Several large longitudinal studies have reported dietary information prior to the onset of Crohn's disease. An association was found between long-term intakes of dietary fibre

[17] and fish [18] and high intake of zinc [19] and lower risk of Crohn's disease in the Nurses' Health Study (NHS). Several systematic reviews also reported dietary factors that were related to the development of Crohn's disease. A late meta-analysis of 12 studies revealed that 570 patients with Crohn's disease had a significantly higher odds ratio of vitamin D deficiency than 778 controls [20]. A recent systematic review of 19 studies reported that high intakes of total fats, total polyunsaturated fatty acids (PUFAs), n-6 PUFAs, and meat were associated with an increased risk of Crohn's disease [21].

Several potential mechanisms, which could explain an inverse association with fibre, zinc, and vitamin D, were proposed. Short-chain fatty acids, mainly acetate, propionate, and butyrate, are produced in the large bowel by anaerobic bacterial fermentation of fibre [22, 23]. Butyrate was reported to decrease inflammatory cytokine expression, such as TNF-α, via inhibition of nuclear factor-kappa B (NF-κB) activation [22, 24]. TNF-α has an effect on activation of T helper-1 (Th1) immune responses [24, 25]. Therefore, fibre consumption may regulate Th1 immune responses in Crohn's disease. In a recent case–control study using the metagenomic shotgun sequencing technique, changes in gut microbiota were confirmed to be associated with the prevalence of Crohn's disease, and short-term exclusive enteral nutrition elicited limited impact on the overall composition of the microbiota in patients with Crohn's disease [26]. In the study, of the 49 patients with Crohn's disease, 35 patients (71%) had colonic involvements [26]. Fibre metabolised by intestinal bacteria and its absorption in the large bowel are required; therefore, fibre intake may work protectively on patients whose disease location is limited in the ileum (L1). Consequently, colonic involvement might cause the limited impact of exclusive enteral nutrition in the study and might also relate to the effectiveness of nutritional therapy to a newly diagnosed adult patient with Crohn's disease with ileal involvement [6]. Zinc plays an essential role in the function of the immune system and modulates the function of innate immune cells, including macrophages and neutrophils [19]. Zinc signals impact on NF-κB activation; however, these findings have raised controversies [27]. Zinc deficiency induces intestinal membrane damage and inflammatory cell infiltration; conversely, zinc consumption maintains the membrane barrier integrity and prevents from massive neutrophil infiltration [28]. Vitamin D has effects on the regulation of the innate and adaptive immune systems, including Th1/Th17 T-cells and inflammatory cytokine decreases [29, 30].

In contrast, the role of fish and fatty acid consumptions still remains to be fully elucidated. A few studies, including a cohort study in the NHS II [18], reported the inverse correlation between fish intake and risk of Crohn's disease [31]. However, a Japanese case–control study indicated the consumption of fish was positively associated with Crohn's disease risk [32]. The effect of marine n-3 PUFAs on fish is a proposed potential mechanism to explain the inverse association between intake of fish and Crohn's diseases [18, 31]. Meanwhile, a clinical randomised controlled trial reported that treatment with n-3 PUFAs was not effective in the prevention of relapse in Crohn's disease [33]. A case–control study in children reported positive but nonsignificant associations between most fats and fatty acids and the risk of Crohn's disease. Moreover, they reported that n-3 PUFAs were negatively associated with Crohn's disease [34]. Interestingly, the other case–control study in children revealed diet–gene interactions between the dietary ratio of n-6 to n-3 PUFAs and single nucleotide polymorphisms (SNPs) in cytochrome P450 family 4 subfamily F member 3 (CYP4F3) or fatty acid desaturase 2 (FADS2), which are PUFA metabolic genes [35]. Specifically, the guanine and cytosine alleles of 2 SNPs, rs1290617 and rs1290620, in CYP4F3, are associated with high plasma levels of docosapentaenoic acid, which is one of the n-3 PUFAs. In the subjects who have those alleles, the ratio of n-6 to n-3 PUFAs was associated with higher odds ratio of Crohn's disease [35]. Equivalently, but except for SNP rs17831757, the high ratio of n-6 to n-3 PUFAs increased the odds ratio of Crohn's disease in the subjects who have the alleles of 3 SNPs in FADS2, which are associated with high plasma levels of n-3 PUFAs and low plasma levels of n-6 PUFA [35]. In contrast, the diet–gene interaction model has also been a controversial topic. A recent nested case–control study reported that no association was found between the ratio of n-6 to n-3 PUFAs and the risk of Crohn's disease under consideration of SNPs at CYP4F3 and FADS2 loci [36].

Oxidised n-3 PUFAs have anti-inflammatory effects, which may result in n-3 PUFAs acting as a ligand of peroxisome proliferator-activated receptor alpha (PPARα) [37, 38]. This effect was observed with only oxidised n-3 PUFA, not unoxidised ones, in in vitro human umbilical vein endothelial cell experiments, which resulted from inhibitory effects on NF-κB activation through a PPARα-dependent pathway [38]. As mentioned above, zinc has inhibitory effects on NF-κB activation. Indeed, a case–control study reported that zinc supplementation modulated docosahexaenoic acid levels in the red blood cell phospholipids [39]. Therefore, the dietary status of zinc may be a possible confounding factor in the relation between n-3 PUFAs and the development of Crohn's disease. Incidentally, an opposite result in mice experiments was reported [40]. In this report, consumption of oxidised n-3 PUFAs resulted in accumulation of 4-hydroxy-2-hexenal (4-HHE), an oxidised n-3 PUFA end product, in blood after its intestinal absorption and triggered oxidative stress and inflammation in the upper intestine. A previous study of 4-hydroxy-2-alkenals, 4-HHE, and 4-hydroxy-2-nonenal concentrations in a Korean foodstuff reported that the average daily exposure to 4-HHE was 1.6 μg/day [41]. This report concluded that the value might not pose a significant risk for human health; however, excessive consumption of 4-HHE might increase the risk of Crohn's disease. No sufficient data is available concerning the safe level of 4-HHE; therefore, this may provide solutions to the problem regarding the controversial results in fish consumption.

Dietary fat is an important source of concentrated energy, together with other nutrients, and fatty acids are an important reservoir of stored energy [42]. They are stored as triacylglycerols in the body, which are principally from

two sources, animal fats and vegetable oils [42]. The small intestine is a vital organ for triacylglycerol homeostasis; therefore, a better understanding of the mechanisms of intestinal fatty acid absorption is necessary to consider the role of fatty acids and meet consumption in Crohn's disease.

4. Malabsorption of Fatty Acids and Bile Acids in Crohn's Disease

Pancreatic lipase is essential in the digestion of dietary fats. Biliary bile acids form mixed micelles together with fatty acids, which function as a transport vehicle to deliver fatty acids to the apical membrane of enterocytes for absorption [42]. CD36 is one of the fatty acid transporters identified in the small intestine [42]. Despite in vitro experimental results with biopsy specimens from damaged and nondamaged colonic mucosa of 12 patients with inflammatory bowel disease, the number of CD36-positive cells was significantly lower in the damaged mucosa than in the nondamaged mucosa [43]. Even if n-3 PUFAs have anti-inflammatory effects, ineffective absorption of n-3 PUFAs can reduce the effects. Consequently, disease location and behaviour could have influenced on the dietary effect of fatty acids. This may be one possible reason why the controversial results in consumption of the various fatty acids, including n-3, were observed.

Bile acids are formed from cholesterol in the liver and secreted into bile [44]. Bile acids are concentrated in the gallbladder during the fasting state and secreted in the duodenum after stimulation by food [44]. Two primary bile acids, cholic acid and chenodeoxycholic acid, are synthesised in the liver, and gut microbiota produces secondary bile acids through two enzymatic reactions [45]. Most of the bile acids remain in the gut lumen until they reach the terminal ileum [44]. Bile acid uptake into the enterocyte occurs principally in the terminal ileum via the apical sodium-dependent bile acid transporter (ASBT) [46]. Reabsorbed bile acids enter hepatic portal circulation [46]. Bile acids are recycled with almost perfect yield (approximately 95%) [47], and their malabsorption can cause congenital diarrhoea, steatorrhoea, and reduced plasma cholesterol levels.

A recent case–control study suggested that bile acid malabsorption occurred in patients with Crohn's disease [46]. In the study, mRNA expression levels of ASBT, breast cancer-related protein (BCRP), sulfotransferase family 2A member 1 (SULT2A1), and fibroblast growth factor 19 (FGF-19) were significantly lower in inflamed regions in patients with active Crohn's ileitis than in controls. BCRP is a drug efflux transporter of the adenosine triphosphate-binding cassette (ABC) transporter family, which works in the opposite direction of a bile acid uptake transporter, ASBT. Meanwhile, SULT2A1 metabolises bile acids for protecting enterocytes from accumulation of bile acids in potentially harmful concentrations, and FGF-19 mediates the negative feedback regulation of hepatic bile acid synthesis between the gut and liver [46]. Therefore, bile acid transport and metabolism are reduced; increased bile acid concentrations appear associated with enhanced mucosal permeability and structural changes. Additionally, hepatic bile acid synthesis is enhanced due to missing FGF-19 signalling; intraluminal concentrations of bile acids may induce the onset of diarrhoea in Crohn's disease. As a result, bile acid malabsorption would lead to difficulty in revealing the positive effects of any kinds of foods by epidemiological studies.

The different aspect of bile acid malabsorption is seen in the relation to the $CD4^+$ T effector (Teff) cell function in the ileum. The elevation of Teff cytokine expression in tissue is significantly associated with inflammatory bowel diseases [48, 49]. A recent study reported that multidrug resistance protein 1 (MDR1) expressing Teff plays a key role in mucosal homeostasis in the ileum [49]. MDR1 is one of the ABC transporter families. Therefore, MDR1 prevents bile acids from driving oxidative stress to intestinal T-cells [49], which may cause dysregulation of intestinal T-cells induced in Crohn's disease [1, 2]. Further studies paying more attention to bile acid handling in Crohn's disease not only in dietary dysmetabolism but also in immunological aspects are warranted.

5. Conclusions

The role of consumption of fatty acids in Crohn's disease remains to be discussed. This review provides an overview of the recent developments in the molecular pathways of lipid consumptions, which may connect immunological and nutritional studies in Crohn's disease, and epidemiological studies on disease location, phenotype, and diet. Data suggests that disease location, phenotype, and intestinal microbiota may be possible confounding factors. Additionally, bile acid may play a key role in the pathogenesis and/or exacerbation of Crohn's disease through bile acid malabsorption or dysregulation of negative MDR1-expressed Teff. Further comprehensive studies on diet and immunology may be warranted.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Review Article

Biological Properties and the Role of IL-25 in Disease Pathogenesis

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The interleukin- (IL-) 17 superfamily, a T cell-derived cytokine, consists of 6 ligands (IL-17A–IL-17F) and 5 receptors (IL-17RA–IL-17RE). IL-17A, a prototype member of this family, is involved in the pathogenesis of allergies, autoimmune diseases, allograft transplantations, and malignancies. By contrast, IL-17B is reported to be closely related to certain diseases, particularly tumors such as breast cancer, gastric cancer, and pancreatic cancer. Recently, the biological function of IL-17E (also called IL-25) in disease, particularly airway diseases, has attracted the attention of researchers. However, studies on IL-25 are scant. In this review, we detail the structural characteristics, expression patterns, responder cells, biological properties, and role of IL-25 in disease pathogenesis.

1. Introduction

Cytokines are a class of small molecular proteins with broad biological activity. They are synthesized and secreted by immune cells (monocytes, macrophages, T cells, B cells, and natural killer (NK) cells) and nonimmune cells (endothelial cells, epidermal cells, and fibroblasts). Cytokines can regulate innate and adaptive immunities, blood-cell production, cell growth, adult pluripotent stem cells (APSC), pluripotent cells, and damaged tissue repair. The interleukin- (IL-) 17 family is a recently discovered group of cytokines that share homology in amino acid sequences and have highly conserved cysteine residues [1]. The IL-17 family and its receptors, which share minimal homology with other cytokines or known proteins, have been recognized as a distinct cytokine-receptor family and are crucial for normal host immune responses; this family is associated with many human pathogeneses, including those of inflammation and cancer [2–4].

2. Structural Characteristics of IL-25

IL-25, also named IL-17E, was first reported by Lee et al. [5] as a new member of the IL-17 family. Shortly after, Fort et al. reported that IL-25 is a cytokine produced by type 2 helper T (Th2) cells with structural similarity with IL-17 [6]. IL-25 was discovered after a BLAST search of the NCBI expression sequence tag (EST) database. A sequence of EST with a significant homology to IL-17 was discovered, and the IL-25 gene was finally cloned through reverse genetics. The IL-25 gene is located on chromosome 14 (14q11.2); it is 3987 base pairs (bp) in length and contains a 483 bp open reading frame, encoding a 161-amino acid hydrophobic signal peptide. The final products include a hydrophobic signal peptide consisting of 16 amino acids and a mature protein composed of 145 amino acids [7]. The IL-25 gene has two types of alternative splicing mRNA products that encode two subtypes (subtypes 1 and 2). The mRNA of both subtypes contains two exons; subtype 2 is less of an internal fragment than subtype 1 for a shorter N end. The mRNA of subtype 1 encodes a protein composed of 177 amino acids, and the mRNA of subtype 2 encodes a protein with 161 amino acids. Both subtypes have the same carboxy-terminal end composed of 159 amino acids. So far, no studies have reported differences in the physiological function of the two subtypes [8]. The murine IL-25 gene is located on chromosome 7, measures 985 bp in length, and encodes a protein composed of 169 amino acids. The

human and mouse IL-25 genes share 80% homology. The IL-25 proteins of the human and mouse have a potential N-glycosylation site and a conserved cysteine sequence, which is composed of 10 cysteine residues in humans and 11 cysteine residues in mice [6].

3. Expression Patterns of IL-25

Fort et al. reported that IL-25 is a cytokine produced by Th2 cells, which are one of the earliest recognized sources of IL-25 [6]. Subsequently, the bone marrow-derived mast cells [9], alveolar epithelial cells [10], and alveolar macrophages were identified as other sources of IL-25 [11]. Later, the IL-25 expression was identified in the central nervous system [12] and the bronchial submucosa in asthmatic patients [13]. Sonobe et al. proved that IL-25 can be produced by brain capillary endothelial cells (BCECs) [14]. Gregory et al. reported the expression of IL-25 in varying degrees in allergic diseases [15]. A recent study has shown that mesenchymal stem cells derived from the placenta and bone marrow also secrete IL-25 [16]. A series of experimental studies have found that IL-25 is widely distributed and can be expressed in various cells, tissues, and systems.

4. IL-25 Responder Cells

The effects of IL-25 are mediated by the IL-25 receptors that are composed of two subunits, IL-17RA and IL-17RB [5]. Terashima et al. reported that NKT cells are target cells of IL-25 [17]. Stock et al. further proved that IL-17RB is highly expressed on a subset of inactive and activated CD4(+) invariant NKT (iNKT) cells [18]. Subsequently, type 2 myeloid cells, Th9 cells, basophils, eosinophils, mast cells, and endothelial cells [19] were identified as target cells of IL-25 in the course of allergic inflammation [20–22]. Recently, Yang et al. reported that macrophages carry IL-17RB [23], and Hongjia et al. proved that dendritic cells carry IL-17RB as well [24].

5. Biological Activities of IL-25

IL-25 can induce and enhance Th2-type immune responses and plays an important role in some allergic diseases. However, how IL-25 regulates the Th2 immune response is not fully understood. Some studies have suggested that IL-25 enhances the expression of Th2-type cytokines and induces Th2-type immune responses mainly through two mechanisms: (i) high doses of IL-25 can induce inherent lymphoid type 2 cells (ILC2s) to produce IL-4, IL-5, IL-13, and other cytokines and (ii) low doses of IL-25 can induce Th cells to differentiate into Th2 cells with the participation of cluster of differentiation 4 ($CD4^+$) T cells and increase the expression of Th2-type cytokines [25, 26].

In addition to promoting Th2-type immune responses, IL-25 can also inhibit the immune responses mediated by Th1/Th17. Kleinschek et al. found that IL-25 in knockout mice were highly susceptible to autoimmune encephalomyelitis and rapid deterioration [12]. Through a study of patients with inflammatory bowel disease, Caruso et al. found that IL-25 can inhibit IL-12 production, reduce inflammation mediated by Th1, and inhibit Th17 immune responses by inducing IL-23 production [27].

6. Role of IL-25 in Asthma

Bronchial asthma is a chronic inflammatory disease of the airways, which is caused by various cells (e.g., eosinophils, mast cells, T lymphocytes, neutrophils, smooth muscle cells, and airway epithelial cells) and cellular components. The pathogenesis of asthma is related to Th2 cells, ILC2s, Th2 cytokines secreted by Th2 cells and ILC2s, and epithelial cell factors [28]; however, the pathogenesis of asthma has not yet been fully clarified. IL-25 is associated with bronchospasm after aspirin challenge, possibly via mechanisms other than altered LTC4 and PGD2 production [29]. Blockade of the IL-25 receptor (IL-25R) reduced many rhinovirus-induced exacerbation-specific responses, including type 2 cytokine expression, mucus production, and recruitment of eosinophils, neutrophils, basophils, and T and non-T type 2 cells [30, 31]. The release of IL-25 has been found to increase when the airway epithelium has been damaged, and this plays an important role in allergic diseases represented by bronchial asthma [2, 32, 33]. Similarly, Wang et al. found that IL-25 promoted the accumulation of inducible costimulator (ICOS) and T1/ST2 on nuocytes, further inducing the proinflammatory Th2 cells, and promoted Th2 cytokine responses in ovalbumin-induced airway inflammation [34]. Eosinophils are considered a typical marker of bronchial asthma airway inflammation [35]. IL-25 through immune reactivity localize with eosinophils [19]. Wong et al. suggested that IL-25 can activate eosinophils in allergic inflammation, while levels of IL-4, IL-5, eosinophil chemokines, and IgE increased [36]. The IL-25/IL-25R axis plays a crucial role in promoting the recruitment and proinflammatory function of eosinophils in allergic asthma [37]. It also plays an important role in the recruitment of eosinophils, airway mucus oversecretion, and airway remodeling in the airway of mice [2, 6, 38, 39]. Corrigan et al. found that IL-25 contributes to angiogenesis, at least partly by increasing endothelial cell VEGF/VEGF receptor expression through PI3K/Akt and Erk/MAPK pathways [40]. IL-25 can also mediate bronchial smooth muscle hyperplasia and collagen deposition around the airway [15], which further supports the idea that IL-25 promotes airway remodeling. IL-25 and its receptor IL-17RB are considered as targets for innate and adaptive immune responses in chronic allergic airway disease [41]. Specific immunotherapy reduced asthmatic Th2 cytokine levels and the production of IL-25 and alleviated oxidative stress and cell apoptosis in the lung tissue of an asthma mouse model [42]. Lipopolysaccharide and ovalbumin (OVA) induced the production of IL-25 in bronchial epithelial cells in vitro via the activation of MAPK p38 and JNK [34]. Zhang et al. reported that the coblockade of IL-13 and IL-25 with sIL-13R and sIL-25R was more effective than either agent alone at decreasing inflammatory cell infiltration, airway hyperresponsiveness, and airway remodeling, including mucus production, extracellular collagen deposition, smooth muscle cell hyperplasia, and angiogenesis in mice exposed to OVA [43]. Bronchial

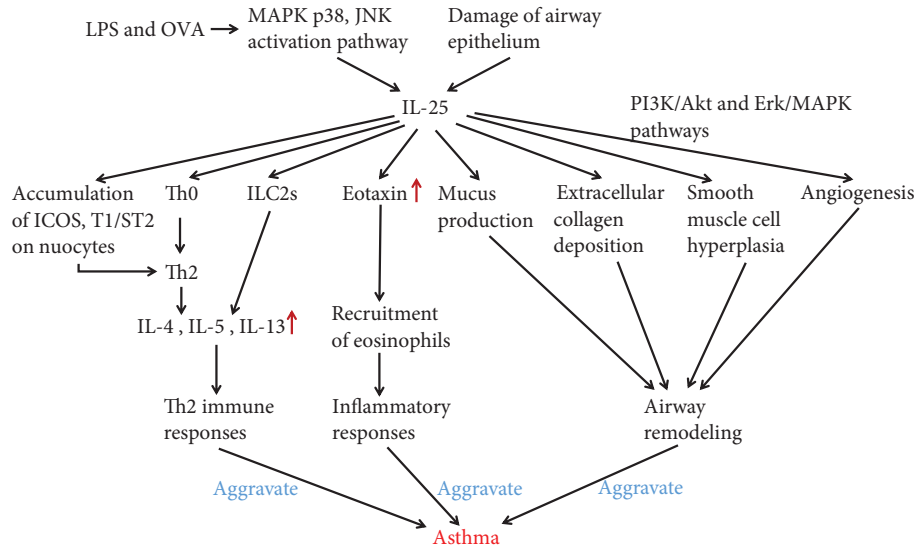

Figure 1: The potential mechanism of IL-25 in asthma. LPS and OVA induce the production of IL-25 in bronchial epithelial cells via activating MAPK p38 and JNK. Damage of airway epithelium induces the production of IL-25 in bronchial epithelial cells as well. IL-25 can enhance the Th2-type immune responses, stimulate ILC2s, promote the accumulation of inducible costimulator (ICOS) and T1/ST2 on nuocytes, or induce naïve T cells differentiated into Th2 cells to produce IL-4, IL-5, IL-13, or other cytokines. IL-25 increases chemokines and promotes the recruitment of eosinophils and inflammation. IL-25 can also promote airway remodeling by mediating mucus secretion, extracellular collagen deposition, smooth muscle cell proliferation, and angiogenesis. All of the above can aggravate asthma.

mucosal vascular remodeling refers to structural changes such as loss of epithelial integrity. Chronic exposure of the airways to IL-25 alone is sufficient to cause functionally relevant airway remodeling, with the corollary that targeting of IL-25 may attenuate bronchial remodeling and fibrosis in human asthmatics [44]. To target IL-17Rb$^+$CD4$^+$NKT cells for the treatment of allergic asthma, IL-25 is considered to be a novel therapeutic approach [17]. In conclusion, IL-25 plays a key role in the pathogenesis of bronchial asthma, and the regulation of IL-25 production is expected to become a new direction for the treatment of bronchial asthma (Figure 1).

7. Role of IL-25 in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by erosive and symmetrical arthritis. The basic pathological changes of RA include synovitis, pannus formation, and gradual joint cartilage and bone destruction, which may eventually lead to joint malformation and loss of function. The exact pathogenesis of RA is unknown, but it is classified as an immune-mediated inflammatory disorder. Studies have shown that RA inflammation is dominated by Th1 cell immunity and that there is an imbalance of Th1 cell polarization. The inflammatory damage caused by this immune imbalance is closely related to Th17 cells and IL-17 family members [45]. Some researchers have found that the expression of IL-25 in articular cartilage inhibits the synthesis of articular cartilage matrix, stimulates the release of nitric oxide (NO), and stimulates the production of IL-6, which is related to the occurrence of arthritis. Recent studies [10, 27, 46] suggest that IL-25 has dual immune-regulatory effects: it can upregulate Th2-mediated immune responses and also downregulate Th1 and Th17 cell-mediated immune

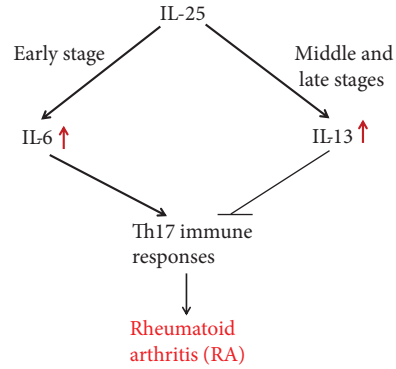

Figure 2: The mechanism of IL-25 involvement in rheumatoid arthritis. IL-25 stimulates the production of IL-6 at the early stage of RA to promote Th17 immune responses. IL-25 also suppresses Th17 immune responses in an IL-13-dependent manner at the middle and late stages of RA.

responses. Moreover, collagen-induced arthritis (CIA) in a mouse model showed high expression of IL-25 and IL-17 in the early stage of diseases [47]. In a recent study, Liu et al. found that IL-25 can alleviate CIA development in mice through suppression of Th17-type immune responses in an IL-13-dependent manner [48]. In conclusion, IL-25 may be involved in the immune and inflammatory responses of RA and has considerable value in the treatment of RA (Figure 2).

8. Role of IL-25 in Allergic Rhinitis

Allergic rhinitis (AR) is considered a nasal inflammation mediated by Th2-type cytokines, characterized by the aggregation of nasal mucosal eosinophils and mast cell and increased

serum antigen-specific IgE levels [49]. It is a common and recurrent disease in the ear-nose-throat department that severely affects the quality of life of the patients. In recent years, the imbalance of Th1/Th2 cytokines has been discovered to play an important role in the pathogenesis of AR: Th2 cytokines increase while Th1 cytokines decrease, breaking the balance between them, which is the basis of AR. The pathogenesis of AR and bronchial asthma is very similar; both are Th2-type immune hyperactivity reactions and diseases essentially caused by Th2-type hyperimmune reaction. Grossman [50] introduced the concept of "the same airway, the same disease." Casale and Dykewicz have found that AR and bronchial asthma are highly similar in their etiology, pathogenesis, and treatment, further proving this concept [51]. Hence, we speculate that the role of IL-25 in bronchial asthma patients is also applicable in the ARAs and that eosinophils are associated with allergic diseases, such as AR and bronchial asthma [52]. IL-25 can promote the expression of Th2 cytokines and accumulation of eosinophils [6, 26], inhibit the apoptosis of eosinophils, enhance adhesion between eosinophils and epithelial cells, release cytokines and chemokines by stimulating eosinophils, and thus promote allergy [2, 6, 36, 38, 39, 45, 53, 54]. These studies have confirmed that IL-25 is involved in the pathogenesis of allergic rhinitis and that it plays a crucial role in the occurrence and development of the disease. The expression of IL-25 in the nasal mucosa and the concentration of IL-25 in the serum are positively correlated with the severity of AR, which can be used to judge the severity of allergic rhinitis. Therefore, inhibitors related to IL-25 may be a new target for the treatment of allergic rhinitis.

9. Role of IL-25 in Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is an idiopathic intestinal inflammatory disease involving the ileum, rectum, and/or colon. The clinical manifestations are diarrhea, abdominal pain, and even persistent loose stools. The most common forms of the disease are ulcerative colitis (UC) and Crohn's disease (CD). The etiology and pathogenesis of IBD are not completely clear, and recent studies suggest that IBD is caused by the interaction of several factors, for example, environmental, genetic, and immune factors. Its pathogenesis is related to the regulation of the intestinal mucosal immune barrier to the inflammatory response of the intestinal antigen, and Th1/Th17-type reaction mediated by IL-12/23 is one of the key factors [55]. Commensal-dependent expression of IL-25 by intestinal epithelial cells limits the expansion of Th17 cells in the intestine by inhibiting expression of macrophage-derived IL-23 [46]. Kleinschek et al. found that IL-25 knockout mice are highly susceptible to autoimmune encephalomyelitis, which was associated with increased expression of IL-23; IL-17, interferon γ, tumor necrosis factor α, and other proinflammatory factors infiltrate the central nervous system [12]. Caruso et al. used IL-25 to stimulate $CD4^+$ cells in the intestinal mucosal tissue of CD patients, which lead to decreased synthesis of IL-23 and IL-12, similar to the levels in the peripheral blood [27]. Su et al. found that

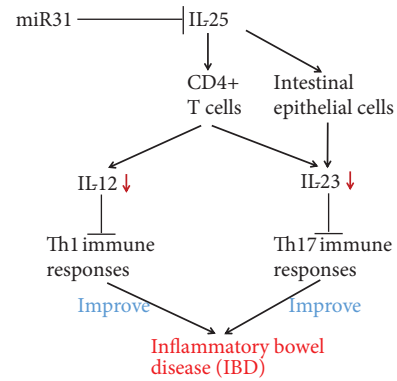

Figure 3: The potential mechanism of IL-25 in IBD. IL-25 stimulates $CD4^+$ T cells to reduce synthesis of IL-12 and inhibit Th1 immune responses. IL-25 also stimulates $CD4^+$ T cells and intestinal epithelial cells to decrease IL-23 to inhibit Th17 immune responses, thus improving inflammatory bowel diseases.

the level of IL-25 in the intestinal mucosa and serum of patients with active IBD was significantly lower than that of the control group and was negatively correlated with the degree of IBD activity and the level of C-reactive protein [56]. A recent study by Shi et al. showed that the expression of IL-25 was negatively correlated with microRNA-31 in rats with CD and patients with UC. Luciferase test results showed that miR-31 could bind to the untranslated region of IL-25 mRNA 3′ and directly regulate the expression of IL-25. The content changes of microRNA-31 in CD rats can affect the Th1/Th17 pathway mediated by IL-12/23 in the intestinal mucosa and consequently improve or aggravate colitis [57]. If IL-25 in CD mice was cleared or the content of IL-25 in the colon was decreased, the treatment effects of miR-31 inhibitors on colitis were significantly decreased. The results suggest that IL-25 is an important anti-inflammatory factor in the pathogenesis of IBD and a possible target to inhibit the Th1/Th17 inflammatory pathways, which are mediated by IL-12/IL-23. In the future, IL-25 inhibitors may be a new therapy for the treatment of IBD with potential quality of life benefits for patients with IBD (Figure 3).

10. Role of IL-25 in Skin Diseases

Urticaria is a localized edema caused by a temporary increase in vascular permeability of the skin and mucous membrane. Chronic urticaria (CU) is defined as skin lesions that recur for more than 6 weeks with attacks occurring at least 2 times per week. The pathogenesis of this disease is not clear. However, several studies have demonstrated that the imbalance of Th1/Th2 and Th2-mediated immune response is dominant in the pathogenesis of CU [58–60]. As explained earlier, IL-25 can increase Th2 cytokines via two mechanisms, resulting in enhanced Th2-type immune response. Therefore, IL-25 may be involved in the pathogenesis of CU.

Psoriasis is a polygenic inflammatory dermatosis that can be induced by certain environmental factors [61]. It is typically characterized by scaly erythema or plaque that can be limited or widely distributed. The exact etiology

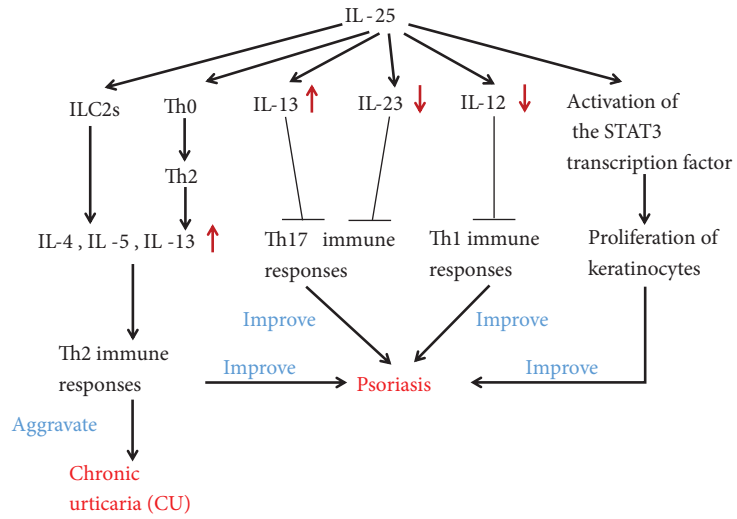

Figure 4: The role of IL-25 in skin diseases. IL-25 can aggravate CU by enhancing the Th2-type immune responses. IL-25 stimulates the proliferation of keratinocytes via activation of the STAT3 transcription factor, inhibits the production of IL-12 to reduce Th1 immune responses, increases the production of IL-13, and decreases the production of IL-23 to inhibit the Th17 immune responses. Thus, IL-25 can improve psoriasis.

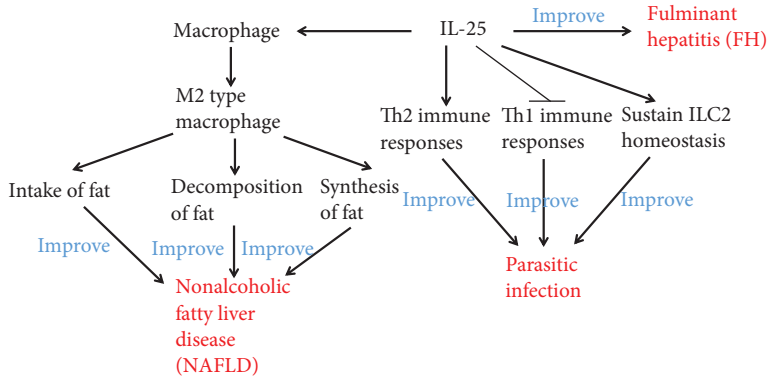

Figure 5: The relationship between IL-25 and other diseases. IL-25 can activate macrophages, transform macrophages into M2 type, enhance its intake of fat, promote fat decomposition, inhibit fat synthesis, and significantly improve NAFLD. IL-25 enhances Th2 immune responses, inhibits Th1 immune responses, sustains ILC2 homeostasis, and consequently improves parasitic infection. IL-25 is associated with FH as well.

and pathogenesis of psoriasis are not yet clear, but a series of studies have shown that Th1/Th2 imbalance and Th17 cells comediate this autoimmune disease [62, 63]. IL-25 is well known to regulate allergic responses and type 2 immunity. Caruso et al. found that IL-25 levels in the peripheral blood of patients with psoriasis vulgaris were significantly reduced as compared to normal people [27]. Thus, IL-25 may play a certain inhibitory role in the pathogenesis of psoriasis vulgaris. Recently, Xu et al. have shown that via IL-17RB expression in keratinocytes, IL-25 stimulated the proliferation of keratinocytes and induced the production of inflammatory cytokines and chemokines, via activation of the STAT3 transcription factor [64]. Thus, the IL-17-induced autoregulatory circuit in keratinocytes is proved to be mediated by IL-25, and this circuit could be targeted in the treatment of psoriasis patients (Figure 4).

11. Relationship between IL-25 and Other Diseases

Bernal et al. found that the expression of IL-25 in the peripheral blood of patients with nonalcoholic fatty liver disease (NAFLD) is significantly decreased and is negatively correlated with body mass index (BMI) [65]. In vitro and in vivo studies have further confirmed that IL-25 can activate macrophages, transform macrophages into M2 type, enhance its intake of fat, promote fat decomposition, inhibit fat synthesis, and significantly improve NAFLD. Moreover, fulminant hepatitis (FH) is a liver disease characterized by massive destruction of hepatocytes and severe impairment of liver functions [65]. In a FH animal model study, Sarra et al. found that the IL-25 content in the liver of FH mice was significantly reduced and the intervention of IL-25 before drug

induction could prevent the occurrence of FH, suggesting that IL-25 might have therapeutic effects on FH [66].

Owyang et al. found that IL-25 not only enhanced Th2 immune response but also inhibited the secretion of Th1-type cytokines to inhibit gastrointestinal inflammation induced by parasite in a mouse model of whipworm infection [67]. Fallon et al. found that IL-25 knockout mice had delayed secretion of Th2 cytokines and could not effectively expel the nematode, further confirming that IL-25 played an important role in parasitic infection [68]. Parasitic helminths and allergens induce a type 2 immune response leading to profound changes in tissue physiology, including hyperplasia of mucus-secreting goblet cells and smooth muscle cell's hypercontractility. Tuft cells express IL-25, sustain ILC2 homeostasis, and regulate type 2 immune responses in mice [69, 70] (Figure 5).

12. Conclusion

As one of the members of the IL-17 family, IL-25 is distinctly different from other family members in molecular structure and biological functions. The current studies show that IL-25 not only plays an important role in the regulation of type 2 immune responses and inflammatory, skin, and autoimmune diseases but also has a certain role in the treatment of tumors. Therefore, IL-25 provides new exploratory direction and is expected to become a new treatment target for these diseases. However, further research is needed to confirm these results.

Conflicts of Interest

The authors declare no competing financial interests.

Authors' Contributions

Yuwan Liu and Zewei Shao contributed equally to this work.

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