Serologic Evidence of Gut-driven Systemic Inflammation in Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective.*** Accumulating evidence links juvenile idiopathic arthritis (JIA) to nonhost factors such as gut microbes. We hypothesize that children with new-onset JIA have increased intestinal bacterial translocation and circulating lipopolysaccharide (LPS).

Methods. We studied systemic treatment-naive patients with JIA [polyarticular JIA, n = 22, oligoarticular JIA, n = 31, and spondyloarthropathies (SpA), n = 16], patients with established inflammatory bowel disease–related arthritis (IBD-RA, n = 11), and 34 healthy controls. We determined circulating IgG reactivity against LPS, LPS-binding protein (LBP), α-1-acid glycoprotein (α-1AGP), and C-reactive protein (CRP) in plasma or serum from these patients and controls. Juvenile Arthritis Disease Activity Score (JADAS-27) was calculated for patients with JIA.

Results. Circulating anticore LPS antibody concentrations in patients with polyarticular JIA ($p = 0.001$), oligoarticular JIA ($p = 0.024$), and SpA ($p = 0.001$) were significantly greater than in controls, but there were no significant intergroup differences. Circulating LBP concentrations were also significantly greater in patients with polyarticular JIA ($p = 0.001$), oligoarticular JIA ($p = 0.002$), and SpA ($p = 0.006$) than controls, as were α-1AGP concentrations ($p = 0.001$, 0.001, and 0.003, respectively). No differences were observed between controls and patients with IBD-RA in any of the assays. Circulating concentrations of LBP and α-1AGP correlated strongly with CRP concentrations ($r = 0.78$ and $r = 0.66$, respectively). Anticore LPS antibody levels and CRP ($r = 0.26$), LBP ($r = 0.24$), and α-AGP ($r = 0.22$) concentrations had weaker correlations. JADAS-27 scores correlated with LBP ($r = 0.66$) and α-1AGP concentrations ($r = 0.58$).

Conclusion. Children with polyarticular JIA, oligoarticular JIA, and SpA have evidence of increased exposure to gut bacterial products. These data reinforce the concept that the intestine is a source of immune stimulation in JIA. (First Release September 15 2017; J Rheumatol 2017;44:1624–31; doi:10.3899/jrheum. 161589)

Key Indexing Terms:
JUVENILE IDIOPATHIC ARTHRITIS ACUTE-PHASE PROTEINS
α1-ACID GLYCOPROTEIN INTESTINAL PERMEABILITY LIPOPOLYSACCHARIDE
LIPOPOLYSACCHARIDE-BINDING PROTEIN

Emerging data suggest that the clinical course of juvenile idiopathic arthritis (JIA) might be influenced by nonhost factors, including gut microbes (reviewed[1]). These lines of evidence associate microbial dysbiosis with altered gut permeability and a proinflammatory extraintestinal cascade driven by gut processes that contribute to the development of arthritis[2]. Intestinal immune activation in patients with JIA is manifest as lymphonodular hyperplasia and increased tissue γ/δ+ and cytotoxic lymphocyte populations[3,4]. Additionally, patients with JIA have defective intestinal barrier function[5,6], and many children with JIA and abdominal pain have evidence of microscopic colitis[7]. Also, exclusive enteral nutrition, which induces remission in Crohn disease, has been beneficial in patients with JIA[8], and associ-

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ations between gut microbiome alterations and JIA have been reported[9,10,11]. However, these studies have generally been performed on subjects with longstanding disease.

Lipopolysaccharide (LPS), an outer-membrane component of Gram-negative bacteria, consists of a highly conserved lipid A and a core oligosaccharide, and diverse serogroup-specific sugar (O) side chains[12]. The colon harbors an abundance of LPS-producing bacteria, and absorption of LPS from the gut may cause systemic inflammation[13]. LPS activates macrophages and neutrophils, which then synthesize proinflammatory cytokines that might initiate and/or perpetuate joint inflammation and degeneration[14]. LPS-binding protein (LBP) is upregulated by LPS and augments innate immunity to bacterial infections. LBP-bound LPS is transferred by CD14 on monocytes to the Toll-like receptor 4 (TLR-4)/myeloid differentiation factor 2 (MD-2) signaling complex, the activation of which triggers inflammation[15,16]. Indeed, LBP has been implicated in joint inflammation based on elevated intraarticular concentrations in rheumatoid arthritis[17]. There is also α-1-acid glycoprotein (α-1AGP), another acute-phase protein with immunomodulatory properties[18] that neutralizes the toxicity of LPS and enhances its clearance from the body[19]. The circulating concentration of α-1AGP is associated with disease activity in adult rheumatoid arthritis and inflammatory bowel disease (IBD)[20,21].

These findings prompted us to seek evidence of increased intestinal permeability and/or reactivity to gut bacterial contents in treatment-naive patients with JIA. Such increased permeability and exposure to gut contents might be reflected in antibodies directed against LPS and in elevated concentrations of circulating LBP. We also postulated variability in host response between children with different JIA subtypes.

MATERIALS AND METHODS

The study was approved by the institutional review board (IRB) at Washington University School of Medicine, St. Louis (IRB ID# 201408105). Patients with JIA were eligible for enrollment if they were systemic treatment-naive and had not received antibiotics for the prior month. Patients previously treated with intraarticular corticosteroids were included. JIA was defined according to the International League Against Rheumatism criteria for classifying idiopathic arthritis of childhood[22]. Sera and plasma from our pediatric rheumatology specimen bank were used as well. Patients with polyarticular JIA, oligoarticular JIA, enthesitis-related arthritis (ERA), and psoriatic arthritis (PsA) were included, but those with systemic JIA were excluded, because this entity is now widely thought to be an autoinflammatory condition[23]. Patients with ERA and PsA were considered together as spondyloarthropathies (SpA) for the purpose of this analysis. We also included patients who had IBD-related arthritis (IBD-RA) of variable duration. All patients with IBD-RA were receiving systemic treatment for IBD at the time they were studied, and their joint symptoms were assessed by a pediatric rheumatologist. Sera and plasma from healthy control subjects were obtained from our pediatric rheumatology plasma bank, as well as from normal controls obtained during studies of the pathophysiology of childhood hemolytic uremic syndrome[24], and of children undergoing colonoscopy for suspected IBD, but whose colonoscopy did not demonstrate disease. The Juvenile Arthritis Disease Activity Score (JADAS-27)[25] was calculated for patients with JIA who were prospectively recruited. All plasmas were collected in EDTA tubes, centrifuged (4°C, 15 min, 2500 g), and frozen (–80°C) in aliquots until tested.

Escherichia coli core LPS preparation and characterization. *E. coli* strain F12 (pSK+) is a TnphoA mutant of *E. coli* O157:H7 that cannot express the O157 LPS side chain[26] and therefore can serve as a source of antigen with which to measure reactivity to generic *Enterobacteriaceae* LPS. This mutant was inoculated from frozen stock into 2 l of Luria-Bertani broth containing 100 μg/ml of ampicillin and placed in a shaking incubator (37°C, overnight). Bacteria were pelleted, washed once gently with purified sterile water, and suspended in 20 ml of sterile water per 3–4 g of pellet. The suspension was heated (68°C, 10 min) in a water bath with stirring. Then an equal volume of preheated (68°C) phenol was slowly added, and the resulting suspension was stirred vigorously (68°C, 60 min), placed in an ice water bath with continuous stirring (10 min), and centrifuged (4°C, 45 min). The upper layer was aspirated and saved on ice. The organic interface and phenol layer with bacterial pellet were reextracted with an equal volume of purified water, repeating the mixing, heating, cooling, and centrifugation steps as before. The upper layers from both extractions were pooled and dialyzed against 4 l of purified water using Spectra/Por 7 dialysis membrane 1000 kD MWCO (Spectrum Laboratories) for 2 days, with 2 water changes daily, after which the dialysate was ultracentrifuged (105,000 g, 4 h). The supernatant was removed and the LPS pellet was suspended in molecular biology grade de-ionized water (Corning). Following DNase (EZ Bioresearch) and proteinase K digestion, we mixed an equal volume of 1:1 phenol/chloroform solution and then chloroform to remove residual phenol[27].

LPS was separated electrophoretically in a 10% sodium dodecyl sulfate-polyacrylamide gel[28], and visualized by silver staining (GelCode SilverSNAP Stain kit), and quantified in endotoxin units (EU/ml) by the chromogenic limulus amebocyte lysate assay (Pierce Biotechnology).

E. coli anticore LPS antibodies enzyme immunoassay. The concentration of circulating anticore LPS antibodies was determined by a laboratory-developed enzyme immunoassay (EIA). Nunc Maxisorp microplates (Thermo Scientific) were coated (4°C, overnight) with 1000 EU of LPS per well diluted in 100 μl of 0.1 M carbonate-bicarbonate buffer (pH 9.6; Sigma). The plates were washed 4 times with phosphate-buffered saline (PBS; pH 7.4) containing 0.05% Tween-20 (PBS/T) followed by incubation (room temperature, 2 h) with 100 μl of blocking solution [1% bovine serum albumin (BSA) in PBS/T], and washed again 4 times with PBS/T. The wells were then incubated with 100 μl of subject serum or plasma (1:100 diluted in 0.5% BSA in PBS/T; room temperature, 2 h). After washing 4 times with PBS/T, anti-human IgG/Fcγ-horseradish peroxidase (Jackson Laboratory), 1:10,000 in 0.5% BSA in PBS/T, was added (100 μl) to each well and incubated (room temperature, 1 h). The plates were then washed as before and incubated with 100 μl of o-phenylenediamine dihydrochloride (Sigma) substrate solution (25 min) before reaction termination with 100 μl of 2N H_2SO_4. Absorbance was measured at 490 nm with Molecular Devices VERSAmax tunable microplate reader (Conquer Scientific).

Each sample was measured in 3 independent experiments performed in duplicate on different days. For each experiment, we included as a standard the serum of a patient with polyarticular JIA with strong reactivity against core LPS. This reactivity was arbitrarily defined as 1000 EIA units.

LBP, α-1AGP, and CRP concentrations. Commercial EIA were used to determine concentrations of circulating LBP (HyCult) and α-1AGP and CRP (R&D Systems), according to the manufacturers' instructions.

Statistics. The Shapiro-Wilk test was used to determine whether the data were normally distributed. The nonparametric Levene's test was used to verify the equality of variances in the samples. The Kruskal-Wallis test was used for multiple between-group comparisons. Spearman rank-order correlations were used to determine whether age of control subjects was related to resulting values. In the 10 pairwise comparisons between groups for the variables in Table 1, we considered p values < 0.005 to be significant (i.e., 0.05 ÷ 10) after correcting for multiple comparisons. After demonstrating statistically significant differences using uncorrected p values for single

Table 1. Patient demographics.

Variables	Oligoarticular JIA, n = 31	Polyarticular JIA, n = 22	SpA, n = 16	IBD-related Arthritis, n = 11	Control Group, n = 34
Age, yrs, median (IQR)	4.0 (3.0–9.0)[1]	11.5 (9.8–14.0)	14.5 (10.5–17)	15.0 (10–16)	10.5 (6.8–15)
Sex, male:female, n (%)	11 (34):20 (65)	8 (36):14 (64)	10 (63):6 (37)	7 (64):4 (36)	19 (56):15 (44)
Race	White = 30 African American = 1	White = 20 African American = 1 African American/ Asian/White = 1	White = 13 African American = 3	White = 10 African American = 1	White = 32 Indian/Alaskan = 1 Unknown = 1
Ethnicity	Non-Hispanic = 31 Hispanic = 0	Non-Hispanic = 22 Hispanic = 0	Non-Hispanic = 16 Hispanic = 0	Non-Hispanic = 11 Hispanic = 0	Non-Hispanic = 33 Hispanic = 1
JADAS-27 score, median (IQR)	8.85 (7.3–13.6)[2]	20 (13–26.9)[3]	12.75 (9–16.9)[3]	NA	NA

[1] The age of the oligoarticular JIA group was significantly lower than the ages of all other groups (uncorrected p value for all comparisons < 0.001, with significance set at 0.005 after correction for multiple comparisons between all groups). None of the remaining comparisons were statistically significant. [2] Eight subjects evaluated. [3] Eleven subjects evaluated. JADAS: Juvenile Arthritis Disease Activity Score; JIA: juvenile idiopathic arthritis; SpA: spondyloarthropathies; IBD: inflammatory bowel disease; IQR: interquartile range; NA: not applicable.

comparisons between individual JIA subgroups (but not the IBD-RA group) and the controls for all values, we compared the assay results for the polyarticular JIA to oligoarticular JIA, polyarticular JIA to SpA, and oligoarticular JIA to SpA groups. For the 3 pairwise comparisons among the juvenile arthritis subgroups, we provide uncorrected p values, but consider p values < 0.017 (i.e., 0.05 ÷ 3) as significant after correcting for multiple comparisons. Linear relationships between the variables were measured with the Spearman's ϱ test. The family error was set to p < 0.05. SPSS 22.0 was used for these analyses. All p values were 2-tailed.

RESULTS

Samples from 114 subjects were analyzed. After correcting for multiple comparisons, the only statistically significant differences in the demographic data were between the ages of the oligoarticular JIA group and all other groups (Table 1). The clinical features of the patients with IBD-RA are provided in more detail in Supplementary Table 1 (available with the online version of this article).

We detected significant differences between the median EIA values for anticore LPS antibody concentrations (Table 2 and Figure 1) of healthy controls and polyarticular JIA ($p < 0.001$), oligoarticular JIA ($p = 0.02$), and SpA ($p = 0.001$) groups. There was not a significant difference in circulating anticore LPS antibody concentrations between the healthy controls and IBD-RA subjects. Circulating LBP concentrations (Table 2 and Figure 2) were significantly greater in the polyarticular ($p = 0.001$), oligoarticular ($p = 0.002$), and SpA ($p = 0.006$) groups than in healthy controls. The IBD-RA subjects' circulating LPB concentrations were not significantly higher than those in the controls. Circulating α-1AGP concentrations (Table 2 and Figure 3)

Table 2. Concentrations (median, interquartile range) of circulating anti-LPS, LBP, α-1AGP, and CRP in different groups. All comparisons are between individual patient groups and healthy controls for each assay. P values < 0.05 are considered significant.

Assays	Oligoarticular JIA, n = 31	Polyarticular JIA, n = 22	SpA, n = 16	IBD-related Arthritis, n = 11	Healthy Controls, n = 34
Anticore LPS, EIA units,	333	471	415	318	239
n = 112*, IQR	227–472	263–676	303–771	216–398	143–325
p value vs controls	0.024	< 0.001	0.001	0.111	NA
LBP, ng/ml, n = 112*	9.4	18.7	13.5	11.8	5.3
IQR	4.3–22.9	9.4–34.7	6.7–14.7	3.6–17.9	1.8–9.0
p value vs controls	0.002	0.001	0.006	0.108	NA
α-1AGP, µg/ml, n = 114	1203	1398	1116	759	658
IQR	820–1468	1025–1843	760–13,867	630–991	523–908
p value vs controls	0.001	0.001	0.003	0.185	NA
CRP, µg/ml, n = 107*	2.4	12.3	2.1	0.9	0.07
IQR	0.6–4.2	1.1–64.2	0.3–4.5	0–4.1	0–0.7
p value vs controls	0.001	< 0.001	0.001	0.122	NA

* For some subjects, we had insufficient serum or plasma to perform all EIA, so n for some assays does not always equal 114. LPS: lipopolysaccharide; LBP: LPS-binding protein; α-1AGP: α-1-acid glycoprotein; CRP: C-reactive protein; JIA: juvenile idiopathic arthritis; SpA: spondyloarthropathies; EIA: enzyme immunoassay; IBD: inflammatory bowel disease; NA: not applicable.

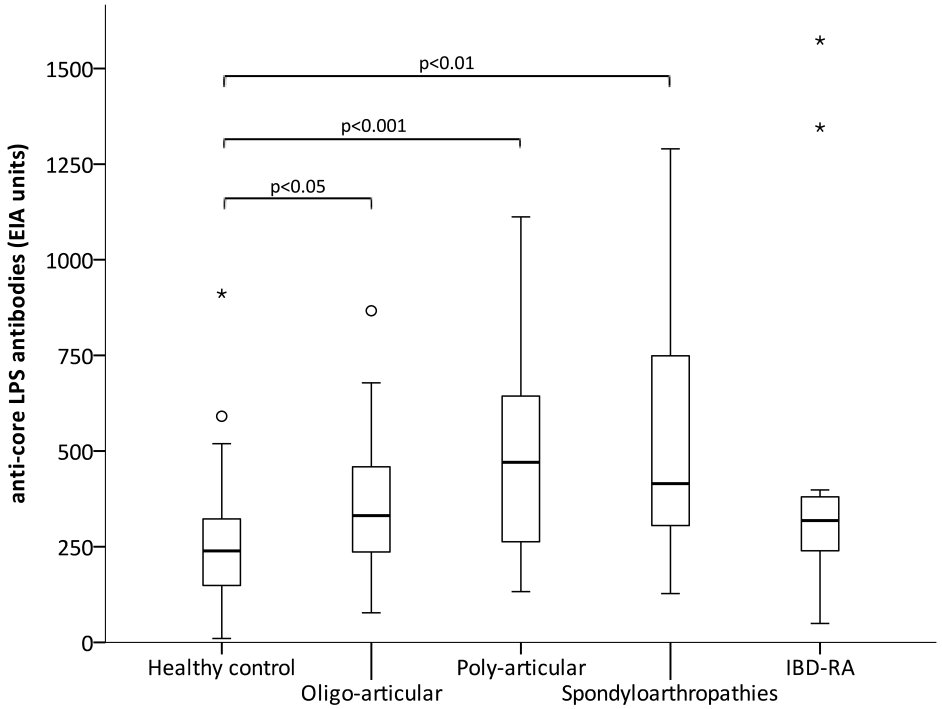

Figure 1. Circulating anticore LPS antibody concentrations across groups. * Outliers above Q3 + 1.5 IQR. Open circles are outliers between Q3 and Q3 + 1.5 IQR. IQR: interquartile range; LPS: lipopolysaccharide; EIA: enzyme immunoassay; IBD-RA: inflammatory bowel disease–related arthritis.

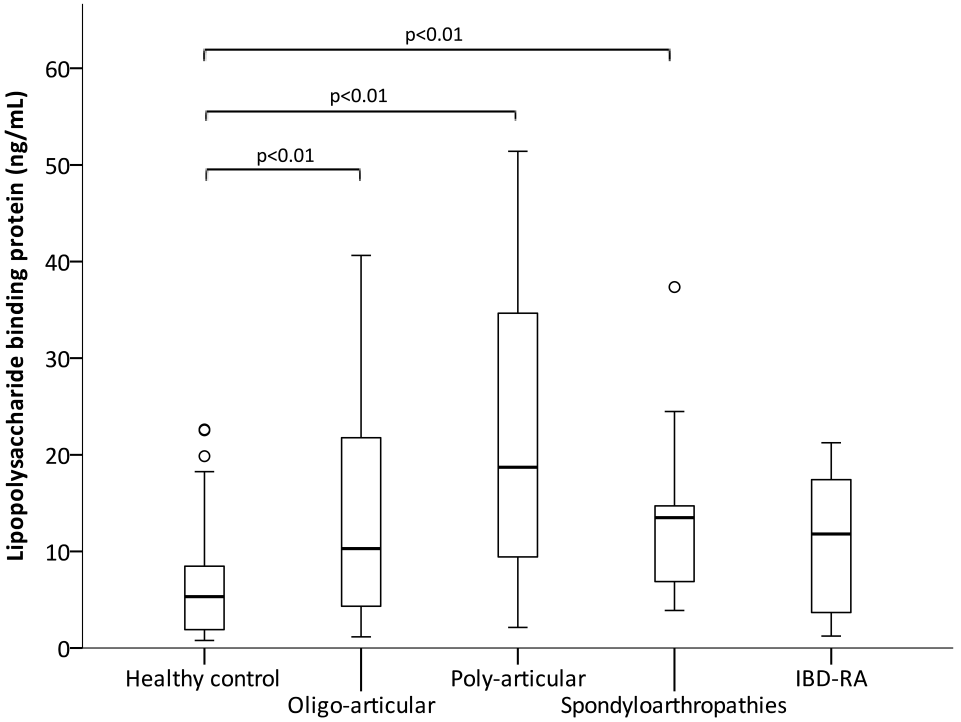

Figure 2. Circulating LBP concentrations across groups. Open circles are outliers between Q3 and Q3 + 1.5 IQR. IQR: interquartile range; IBD-RA: inflammatory bowel disease–related arthritis.

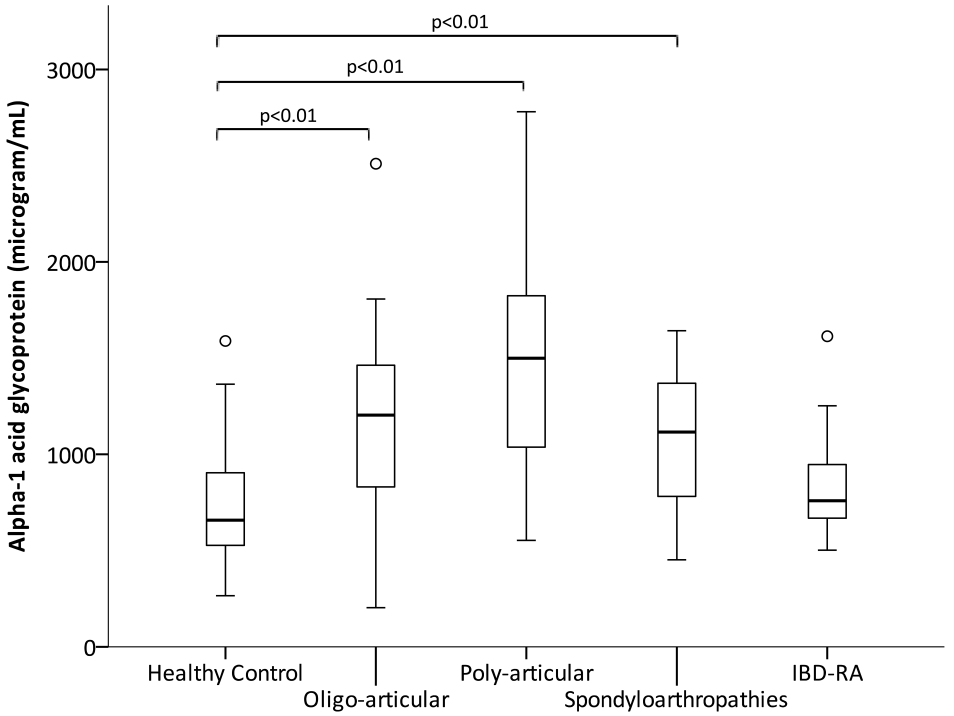

Figure 3. Circulating α-1AGP concentrations across groups. Open circles are outliers between Q3 and Q3 + 1.5 IQR. α-1AGP: α-1-acid glycoprotein; IQR: interquartile range; IBD-RA: inflammatory bowel disease–related arthritis.

were also significantly greater in all 3 JIA arthritis subgroups than in the healthy controls ($p = 0.001$ for the polyarticular and oligoarticular JIA groups; $p = 0.003$ for the SpA groups), but were not greater in the patients with IBD-RA than in the healthy controls. CRP concentrations were significantly lower in the healthy controls than in all 3 JIA disease subgroups ($p = 0.001$, $p < 0.001$, and $p = 0.001$ for oligoarticular JIA, polyarticular JIA, and SpA groups, respectively; Table 2). As with the values for anticore LPS antibodies, LBP, and α-1AGP, the CRP values did not differ between healthy controls and the IBD-RA group.

Supplementary Table 2 (available with the online version of this article) presents pairwise comparisons among the 3 JIA disease subgroups. Circulating LBP concentrations were lower in the oligoarticular group than in the polyarticular JIA group ($p = 0.02$), and circulating α-1AGP concentrations were lower in the oligoarticular JIA and the SpA groups ($p = 0.033$ and $p = 0.026$, respectively) than in the polyarticular JIA group. However, none of these differences retained statistical significance after correcting for multiple comparisons.

The concentrations of circulating CRP and α-1AGP ($r = 0.77$; $p < 0.001$), CRP and LBP ($r = 0.78$; $p < 0.001$), and LBP and α-1AGP ($r = 0.66$; $p < 0.001$) were strongly correlated. The concentrations of anticore LPS antibodies and α-1AGP ($r = 0.22$; $p = 0.02$), LBP ($r = 0.24$; $p = 0.012$), and CRP ($r = 0.26$; $p = 0.007$) had weaker correlations. The JADAS-27 score calculated for the 29 patients with JIA prospectively enrolled correlated strongly with the LBP ($r = 0.66$; $p < 0.001$) and α-1AGP ($r = 0.58$; $p = 0.001$) concentrations, but not with concentrations of circulating anticore LPS antibodies.

We were concerned that the age of the controls was at variance with the ages of the members of the oligoarticular JIA group. However, for all values determined, only the concentration of circulating antibodies to LPS changed significantly over age ($r_s = 0.5288$, $t = 3.52$, $df = 32$; $p = 0.001$). Therefore, for this variable, we compared concentrations of antibodies to LPS between 11 controls in the youngest control group tertile [median age 6.0 yrs, interquartile range (IQR) 4.0–7.0] and the 31 subjects in the oligoarticular JIA group (median age 4.0 yrs, 3.0–9.0). The median circulating antibody concentration was 124 (IQR 57.1–236.5) EIA units in the controls, compared to 333 (IQR 227–472) EIA units in the oligoarticular group ($p < 0.001$), confirming the significance of the higher value in the oligoarticular group after controlling for age effects.

DISCUSSION

The gut is a major habitat of microbes and their by-products. The seroreactivity of patients with JIA to anticore LPS, a component of Gram-negative bacterial cell walls, suggests that such organisms and their systemic absorption may explain at least part of the inappropriate immune activation in JIA.

Because the *E. coli* mutant used to produce the core LPS antigen for the EIA lacks the LPS O-side chain, we conclude that the reactivity reflects a response to generic Gram-negative LPS in which the core and lipid A moieties are conserved[29], and does not reflect exposure to *E. coli* O157:H7.

We found comparatively greater concentrations of circulating LBP and α-1AGP in treatment-naive patients with polyarticular JIA, oligoarticular JIA, and SpA than in healthy controls, suggesting systemic inflammation. Although the elevation of LBP (and of α-1AGP) could reflect acute-phase reaction, the simultaneous presence of elevated anticore LPS antibodies and LBP and α-1AGP concentrations indicates that Gram-negative bacteria or their products have influenced this upregulation for an extended interval before sampling, because antibody responses are slower to develop than acute-phase reactants. Because the gut is the largest habitat of Gram-negative bacteria in the body, this finding implicates the intestine in the induction of systemic inflammation. Our findings of elevated concentrations of circulating LBP and anticore LPS antibodies in patients with SpA are also consistent with the observation that many such patients have subclinical intestinal inflammation[30]. Also, there are recent reports of histologically determined intestinal inflammation in patients with JIA[7]. The same finding has been confirmed in patients with SpA[31], a group in which elevated levels of acute-phase reactants and anti-LPS were also observed.

Circulating LPS plausibly initiates or perpetuates systemic inflammation. LPS is the primary ligand of TLR-4, and LPS upregulates TLR-4, which in turn activates nuclear factor-κB and PI-3K pathways that exert proinflammatory activity[14]. LPS in human joint cartilage induces matrix breakdown and chondrocyte apoptosis, thereby degrading cartilage[11]. TLR-4–deficient mice are relatively resistant to LPS-induced arthritis and joint destruction[32,33]. In addition, patients with rheumatoid arthritis are sensitized to *Prevotella copri*[34] or antigens common to *Enterobacteriaceae*, including LPS[35]. Further, *Yersinia enterocolitica* O:3 LPS is detectable in the synovial fluid for extended periods in patients with post-infectious reactive arthritis[36]. However, the elevated concentrations of anticore LPS and LBP in polyarticular JIA and oligoarticular JIA are novel findings, because little published evidence exists for increased intestinal permeability or dysfunction in these disorders[5,6]. Moreover, our data are derived from treatment-naive patients, thereby lending credence to a potential role for gut bacterial content in precipitating juvenile arthritis.

Our data suggest the potential utility of α-1AGP to follow disease activity in patients with JIA. Levels of α-1AGP were elevated in all 3 groups, and interestingly, were correlated with the concentrations of LBP, CRP, and JADAS-27. Although not well studied in inflammatory arthritis, α-1AGP might play a role in the mechanisms of joint erosions and also be of value as a biomarker in predicting the potential risk of erosions[37,38,39].

CRP correlated strongly with the other 2 acute-phase proteins, namely LBP and α-1AGP, but only weakly with the anticore LPS antibodies. The fact that LBP is directly linked to LPS stimulation suggests that this acute-phase reaction is initiated by products from the intestine. Alternatively, systemic inflammation might increase intestinal permeability and secondarily increase concentrations of circulating anticore LPS antibodies and/or LBP[40]. Although we found a strong correlation between LBP and CRP concentrations in the circulation (not surprising because both proteins are acute-phase reactants), we found only a weak correlation between α-1AGP and CRP concentrations and circulating anticore LPS antibodies. This suggests that anticore LPS antibody production is independent of a systemic inflammatory state, but present chiefly in the polyarticular JIA, oligoarticular JIA, and SpA groups. JADAS-27 correlated significantly both with the LBP and α-1AGP, but not with anticore LPS antibodies, which is not surprising because both acute-phase markers are related to CRP.

Even though differences between some of the medians in pairwise comparisons between JIA subgroups were statistically significant using uncorrected p values, none of the assay values differ significantly after correcting for multiple comparisons. Hence, our data do not suggest that these markers can differentiate between oligoarticular JIA, polyarticular JIA, and SpA patients with confidence.

We acknowledge several limitations to our study. First, our databases did not permit us to identify reliably nonsteroidal antiinflammatory drug (NSAID) use prior to enrollment, and it is possible that these agents altered intestinal permeability. It will be important to account for this variable in future prospective studies that seek to corroborate our findings. Second, we used both sera and plasmas in the assays. However, plasma and serum CRP[41] and antimicrobial antibody concentrations[42,43] correlate well, and LBP concentrations from sera and plasmas are used in multiple case series (review[44]).

The patients with IBD-RA warrant several comments. First, the inability to find a significant elevation of any of the tested circulating markers in the IBD-RA group compared to healthy controls was unexpected, because LBP concentrations in Crohn disease exceed those in ulcerative colitis and in normal controls[45], and CRP is often used to indicate IBD activity. However, the central tendencies for each of these values exceeded those in the controls, suggesting some degree of immune activation in the IBD-RA group. Second, patients with IBD are admonished to avoid NSAID, and several patients had high circulating concentrations of antibodies to *E. coli* LPS, suggesting that systemic reaction to gut LPS can occur without this inciting agent, and even without active gut disease. Third, the comparative nonreactivity of this cohort might be attributed to the universal use of immunosuppressant agents. It is not clear why arthritis developed under these circumstances, but we must consider

the alternative hypothesis that the pathogenesis of IBD-RA, unlike JIA, is not related to systemic inflammation. Indeed, there is little overlap in serologic profiles between adults with IBD-RA and rheumatoid arthritis[46]. However, the small size of the IBD-RA group and the heterogeneity of immunosuppressants used limit the interpretations that can be drawn from this group.

Overall, our findings and emerging knowledge of altered intestinal microbiota in patients with JIA suggest a potential role for the gut in JIA. Although the concept of intestinal dysfunction cannot yet be generalized to all patients with JIA, those that have evidence of intestinal dysfunction as indicated by elevated anti-LPS or LBP levels could be candidates for further evaluation of their gut bacterial population. It would also be interesting to study the response of patients to bacterial antigens in the context of JIA disease monitoring. Notably, in our present study, patients with IBD-RA, all of whom were treated with immune suppressants and most of whom had quiescent gut disease, have in aggregate low anti-LPS and LBP levels. However, it is premature to use any of these markers as a treatment target in JIA. Additionally, in future studies, it will be worthwhile to obtain stool from patients to study fecal markers of gut reactivity at instructive points in the JIA disease process, and to perform sequence analysis to determine the presence and extent of potential dysbiotic gut communities, and correlate these values with markers of systemic inflammation as used in our study.

The concurrent increase in the concentrations of anticore LPS antibody and LBP in the circulation of treatment-naive patients with polyarticular JIA, oligoarticular JIA, and SpA suggest systemic exposure to gut contents. Circulating LPS increases tight junction permeability in mice[47], and a similar process is postulated in patients with IBD, where circulating LPS concentrations correlate with tissue (gut) inflammation and disease activity[45,48,49]. This exposure potentially induced a systemic inflammatory reaction in our cohorts, as evidenced by increased circulating LBP and α-1AGP concentrations in all subgroups except IBD-RA (the only group in which patients were under treatment), with the majority being in disease remission. The concentrations of the gut-related circulating acute-phase proteins (LBP and α-1AGP) correlate well with disease activity scores of patients with JIA, and therefore these molecules are worthy of further consideration in monitoring disease activity.

ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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