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Acute experimental endotoxemia induces visceral hypersensitivity and altered pain evaluation in healthy humans

Sven Benson [a], Joswin Kattoor [a], Alexander Wegner [b], Florian Hammes [b], Daniel Reidick [a], Jan-Sebastian Grigoleit [a], Harald Engler [a], Reiner Oberbeck [b], Manfred Schedlowski [a], Sigrid Elsenbruch [a,*]

[a] Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
[b] Department of Trauma Surgery, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

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ABSTRACT

Growing evidence suggests that systemic immune activation plays a role in the pathophysiology of pain in functional bowel disorders. By implementing a randomized crossover study with an injection of endotoxin or saline, we aimed to test the hypothesis that endotoxin-induced systemic inflammation increases visceral pain sensitivity in humans. Eleven healthy men (mean ± standard error of the mean age 26.6 ± 1.1 years) received an intravenous injection of either lipopolysaccharide (LPS; 0.4 ng/kg) or saline on 2 otherwise identical study days. Blood samples were collected 15 min before and 1, 2, 3, 4, and 6 h after injection to characterize changes in immune parameters including proinflammatory cytokines. Rectal sensory and pain thresholds and subjective pain ratings were assessed with barostat rectal distensions 2 h after injection. LPS administration induced an acute inflammatory response indicated by transient increases in tumor necrosis factor alpha, interleukin 6, and body temperature (all $P < .001$). The LPS-induced immune activation increased sensitivity to rectal distensions as reflected by significantly decreased visceral sensory and pain thresholds (both $P < .05$) compared to saline control. Visceral stimuli were rated as more unpleasant ($P < .05$) and inducing increased urge to defecate ($P < .01$). Pain thresholds correlated with interleukin 6 at +1 h ($r = 0.60$, $P < .05$) and +3 h ($r = 0.67$, $P < .05$) within the LPS condition. This report is novel in that it demonstrates that a transient systemic immune activation results in decreased visceral sensory and pain thresholds and altered subjective pain ratings. Our results support the relevance of inflammatory processes in the pathophysiology of visceral hyperalgesia and underscore the need for studies to further elucidate immune-to-brain communication pathways in gastrointestinal disorders.

1. Introduction

Visceral or abdominal pain is a common symptom of great clinical significance in many areas of medicine [16,28]. Chronic or recurrent abdominal pain constitutes a defining symptom of many functional gastrointestinal disorders, such as irritable bowel syndrome (IBS) [28]. In addition, pain occurs in a significant proportion of patients with inflammatory bowel diseases (IBD), not only during phases of disease exacerbation but also during clinical remission [7], with a detrimental effect on quality of life [40]. The pathophysiology of chronic abdominal pain is undoubtedly complex involving biological as well as psychological mechanisms. The putative role of the immune system in the pathophysiology of human pain and especially in hyperalgesia is increasingly acknowledged within neurogastroenterology as well as in the context of somatic and neuropathic pain [47,48]. Strong support for the relevance of inflammatory processes in chronic abdominal pain comes from findings in postinfectious IBS patients [10,16,34,44]. Although the primary infectious process is local—that is, within the gut mucosa—inflammatory markers are also increased systemically, probably through changes in gut permeability [8,45]. Furthermore, IBS patients without a history of an infection show a low-grade activation of the innate immune system, including increases in systemic inflammatory markers [2,16,23]. Levels of proinflammatory cytokines determined in the serum of IBS patients have been found to be associated with IBS symptom score [13], and lipopolysaccharide (LPS)-stimulated production of proinflammatory cytokines by peripheral blood mononuclear cells was significantly increased in IBS patients with diarrhea-associated pain [30]. In patients with

* Corresponding author. Address: Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, Hufelandstr. 55, 45122 Essen, Germany. Tel.: +49 201 723 4502; fax: +49 201 723 5948.
E-mail address: sigrid.elsenbruch@uk-essen.de (S. Elsenbruch).

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IBD in clinical remission, the occurrence of IBS-like symptoms including abdominal pain has been attributed to low-grade inflammation [27]. Altogether, observations in clinical populations have supported the notion of an overlap (or a possible continuum) between IBS and IBD, and have drawn attention to the putative relevance of not only mucosal but also systemic immune system alterations and disturbed immune-to-brain communication in the pathophysiology of visceral hypersensitivity [6,45]. However, these findings in humans are largely of correlative nature, and it has been difficult to establish cause-and-effect relationships.

Experimental administration of lipopolysaccharide (LPS), the major component of the outer membrane of gram-negative bacteria, constitutes an established model to induce a transient systemic immune activation characterized by elevated levels of circulating proinflammatory cytokines [eg, tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6], as well as behavioral changes that are collectively termed sickness behavior [3]. Sickness behavior includes reduced exploration, increased anxiety, cognitive dysfunction, and social withdrawal in rodents [11,19,33,42], as well as depressed mood and cognitive impairments in humans [12,29]. In addition, hyperalgesia is viewed as an integral part of sickness behavior, aiming at restricting behavior [48]. In animal models, intraperitoneal administration of bacterial endotoxin led to pain facilitation for different somatic noxious stimuli [48] and reportedly lowered rectal pain thresholds [9]. In humans, LPS administration resulted in increased intestinal permeability [25], but to date, to our knowledge, effects on visceral pain sensitivity have not been investigated in humans. Therefore, we tested the hypothesis that endotoxin-induced systemic inflammation increases visceral pain sensitivity in humans by implementing a randomized crossover study with an injection of LPS or saline.

2. Methods

2.1. Recruitment and characterization of subjects

Healthy men were recruited by a local advertisement in the surrounding community. The in-depth screening process consisted of a physical examination and a personal interview conducted by a physician (AW or FH), completion of standardized questionnaires, and repeated laboratory analyses of blood samples (ie, complete blood cell count, liver enzymes, renal parameters, electrolytes, coagulation factors, C-reactive protein) before and up to 1 week after completion of the study. General exclusion criteria included age <18 years and >45 years, body mass index of <18 or $\geqslant$30 kg/m^2, any concurrent medical condition, including neurological, psychiatric, cardiovascular, immunological, endocrine, and gastrointestinal conditions, history of allergies, current use of prescription and nonprescription medications, smoking, and regular high alcohol use (>4 drinks per week). All participants were evaluated digitally for anal tissue damage (eg, painful hemorrhoids) that may interfere with balloon placement. Frequency and severity of gastrointestinal complaints suggestive of any functional or organic gastrointestinal condition were assessed with a standardized questionnaire. Beck Depression Inventory (BDI) [24] scores exceeding the cutoff indicating mild to moderate depressive symptoms (ie, BDI score of >11) were also exclusionary. Additional safety measures included a physical examination and normal blood cell counts 6 h after injection as a precondition for subjects being allowed to leave the laboratory. Further, participants were not allowed to drive a vehicle on the days of the study, and they underwent follow-up examinations including laboratory analysis of C-reactive protein levels 24 h after each session and 7 days after the final session. The study protocol was approved by the local ethics committee. All subjects provided written informed consent and were paid for their participation.

2.2. Study protocol

This randomized, crossover study was composed of 2 identical study days, on which subjects received an intravenous injection of either lipopolysaccharide (LPS; 0.4 ng of *Escherichia coli* endotoxin per kilogram of body weight dissolved in sterile water) or the same volume of saline (placebo). Endotoxin (reference standard endotoxin, lot G3E069; United States Pharmacopeia, Rockville, MD) was prepared for human use as previously described [22]; it had been subjected to a microbial safety testing routine approved by the German Federal Agency for Sera and Vaccines (Paul Ehrlich Institute, Langen, Germany). Subjects and the investigator involved in pain assessments were blinded to the study condition (ie, LPS or saline).

On both study days, an intravenous catheter was placed in an antecubital forearm vein for intermittent blood collection and drug application. Subjects were injected between 9 AM and 10 AM. Visceral sensory and pain threshold assessments and pain evaluations were initiated 2 h after injection, when proinflammatory cytokines have been shown to peak in a previous study from our group with an identical dose of LPS [22]. Blood samples were collected in EDTA-treated tubes at baseline (15 min before injection) and at 1, 2, 3, 4, and 6 h after injection, and were immediately centrifuged. Plasma was stored at −80°C until analysis. Body temperature, and blood pressure and heart rate were then measured with an auricular thermometer and a blood pressure cuff, respectively. State anxiety was assessed with the state version of the State-Trait Anxiety Inventory (STAI) [43]. Subjects were discharged 6 h after injection if white blood cell counts returned to normal range. All subjects received a medical assessment 1 week after the experimental sessions.

2.3. Assessment of visceral pain sensitivity

Two aspects of visceral pain sensitivity were assessed by applying rectal distensions, carried out with a pressure-controlled barostat system (modified Isobar 3 device; G&J Electronics, Toronto, Ontario, Canada). First, sensory and rectal pain thresholds were determined by a double-random staircase distension protocol with random pressure increments of 2–10 mm Hg as previously described [17,18,39] to test the hypothesis that LPS-induced systemic immune activation reduces rectal sensory and pain thresholds. Each pressure was maintained for 10 s; subjects were then prompted to rate the sensation as follows: 1 = no perception, 2 = doubtful perception, 3 = sure perception, 4 = little discomfort, 5 = severe discomfort, still tolerable, 6 = pain, not tolerable. The sensory threshold was defined as the pressure when the rating changed from 2 to 3; the pain threshold as the pressure at which the rating changed from 5 to 6. For ethical reasons, maximal distension pressure was set at 50 mm Hg.

Second, subjects were asked to rate stimuli of predefined distension pressures on visual analog scales (VAS) to assess possible effects of LPS on the subjective evaluation of visceral stimuli. To this end, a total of 9 distensions were delivered at 3 intensities (ie, 3 distensions of each: liminal = 10 mm Hg, supraliminal = 25 mm Hg, unpleasant = 40 mm Hg), each lasting 20 s, in a pseudorandomized order. Subjects rated each of the 9 stimuli on 100-mm VAS regarding perceived painfulness, unpleasantness, and urge to defecate. The ends of the VAS were defined as 0 (“none”) to 100 (“very much”). This assessment was designed to complement and extend the quantification of rectal thresholds assessed with the double-random staircase distension protocol.

2.4. Plasma cytokines

Plasma concentrations of TNF-α, IL-6, and IL-10 were quantified by using multiplexed bead-based assays (Bio-Plex cytokine assays;

Bio-Rad Laboratories, Munich, Germany) as previously described [22]. Samples were prepared according to the manufacturer's instructions and were analyzed on a triple-laser FACSCanto II flow cytometer by FACS-Diva software (BD Immunocytometry Systems, Heidelberg, Germany). Absolute cytokine levels were calculated on the basis of the mean fluorescence intensity of cytokine standard dilutions with a 4 Parameter Logistics (4PL) curve model by GraphPad Prism 5 (GraphPad Software, La Jolla, CA). The detection limits of the assays were 0.2 pg/mL (IL-6), 0.1 pg/mL (IL-10), and 1.8 pg/mL (TNF-α), respectively.

2.5. Questionnaires

State anxiety was assessed by the state version of the STAI (STAI-S) [43]. Briefly, the STAI-S consists of 20 four-point Likert-scaled items, with a sum score ranging from 20 to 80. Higher scores indicate increased state anxiety [43]. The STAI-S was filled by the subjects during both conditions at baseline as well as 1, 2 (ie, before the rectal distension protocol), 3, 4, and 6 h after injection. Chronic stress during the past 3 months was assessed with the 12-item screening scale of the German Trier Chronic Stress Inventory (TICS) [41], with higher scores indicating more pronounced stress. The BDI [24] was used as screening tool to exclude depression on the basis of the published cutoff score for mild to moderate depressive symptoms (ie, BDI score of >11). Chronic stress (TICS) and depression (BDI) were assessed during the screening procedure.

2.6. Statistical analyses

Responses in psychological and physiological variables after LPS vs saline administration were analyzed with 1-tailed paired *t* tests and repeated measures analysis of variance (ANOVA) with the factors time and condition (ie, LPS vs saline). Only significant interactions (time × condition) are presented unless indicated otherwise. For significant ANOVA treatment or interaction effects, post hoc paired *t* tests comparing LPS vs saline condition at specific time points were computed. Correlations between pain variables and cytokine responses were computed as Pearson's *r*. All variables were tested for normal distribution by Kolmogorov–Smirnov tests. For nonnormally distributed variables (ie, TNF-α, IL-6, IL-10), logarithmic transformations were applied before data analysis. The α level was set at 0.05. All data are presented as mean and standard error of the mean (SEM).

3. Results

3.1. Sociodemographic and psychological characteristics

Twelve healthy men participated in the study. One subject was excluded from analysis because he rated the maximal visceral distension pressure (ie, 50 mm Hg) as only "little discomfort." Mean ± standard error of the mean (SEM) age of the remaining subjects was 26.6 ± 1.1 years, and mean body mass index was 23.7 ± 0.9 kg/m^2. All subjects completed high school (ie, German "Abitur"), 54.5% (n = 6) were living in a partnership, and all rated their overall health as either good or very good. Mean chronic stress (TICS 50.1 ± 2.7) and depression (BDI 2.6 ± 0.7) scores were low and well within the normal range.

3.2. Plasma cytokines and body temperature

Endotoxin administration induced transient systemic increases in pro- and anti-inflammatory cytokines and a moderate elevation in body temperature (Fig. 1A–D). Plasma levels of the proinflammatory cytokines IL-6 (F = 35.3, P < .001) and TNF-α (F = 13.9, P < .001) as well as of the anti-inflammatory cytokine IL-10 (F = 9.9, P < .001) were significantly increased in the LPS compared to placebo condition (all interaction effects). Additionally, a slight but significant increase in body temperature with a maximum of 37.2 ± 0.13°C (vs 36.6 ± 0.09°C in the saline condition) at 4 h after injection (F = 4.6, P = .002; interaction effect) was observed after LPS administration.

3.3. State anxiety

State anxiety significantly increased immediately before the rectal distensions (F = 4.4, P = .004, time effect) (Fig. 1E), but

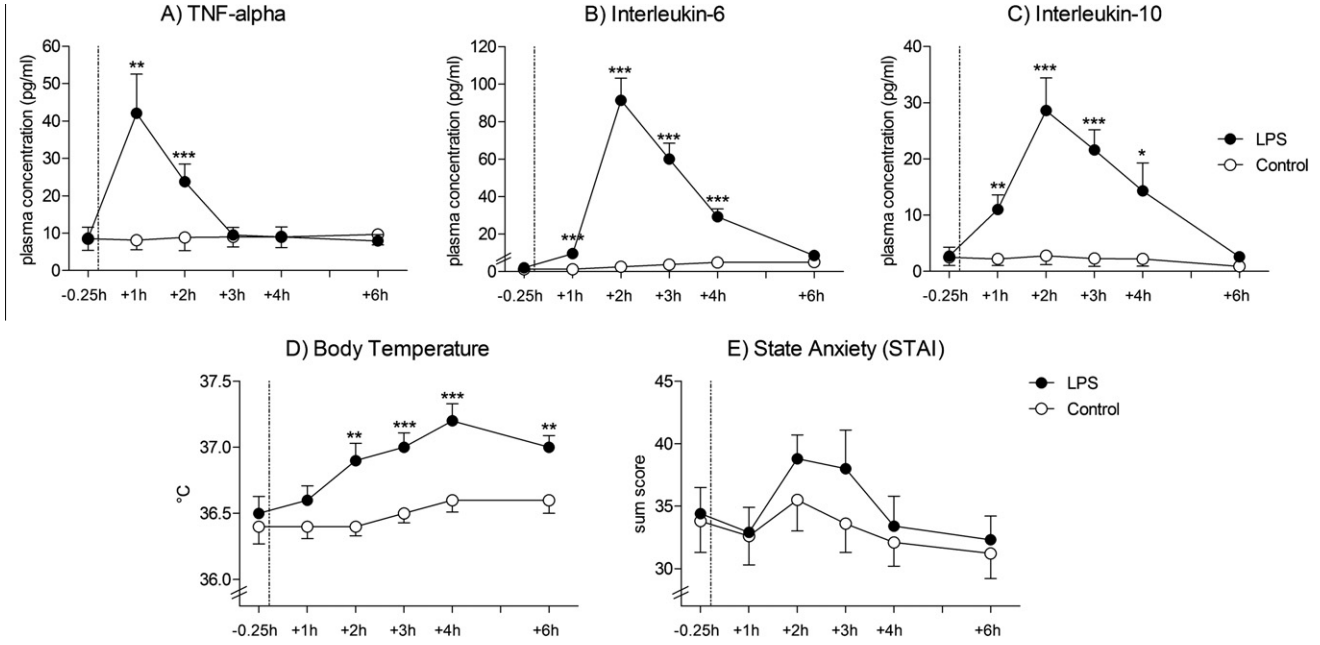

Fig. 1. TNF-α (A), IL-6 (B), IL-10 (C), body temperature (D), and state anxiety (E) for endotoxin (LPS) and saline (control) conditions. LPS administration induced transient systemic increases in TNF-α, IL-6, IL-10, and body temperature (all P < .001, interaction effects). STAI scores were significantly increased immediately before the rectal distensions (P < .01, time effect), but neither a significant condition effect nor a significant interaction was found. Asterisks indicate results of post hoc computed paired *t* tests (*P < .05, **P < .01, ***P < .001).

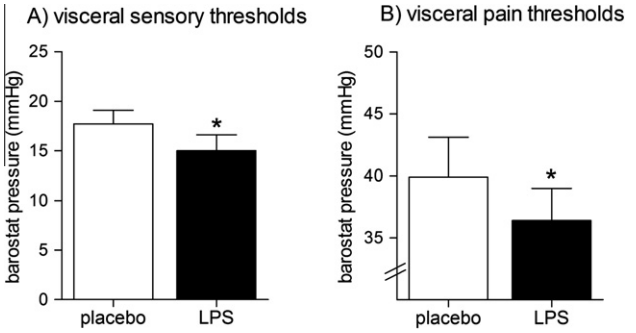

Fig. 2. Visceral sensory thresholds (A) and visceral pain thresholds (B) were assessed 2 h after LPS and saline administration. The LPS-induced immune activation was associated with a significantly decreased rectal sensory and pain threshold (*$P < .05$, paired t test).

neither a significant condition effect nor a significant interaction was found, supporting the notion that other effects of LPS were not the result of the effects on stress or anxiety.

3.4. Rectal sensory and pain thresholds and evaluation of predefined distension stimuli

The LPS-induced immune activation was associated with a significantly decreased rectal sensory ($t = -1.9$, $P = .045$) and pain threshold ($t = -2.3$, $P = .005$) (Fig. 2).

Regarding ratings of predefined distension pressures (ie, liminal = 10 mm Hg, supraliminal = 25 mm Hg, unpleasant = 40 mm Hg) on VAS, we unexpectedly encountered a marked proportion of individuals who demonstrated pain thresholds below 40 mm Hg (ie, $n = 6$ in the LPS condition; $n = 4$ in the placebo condition). Hence, for obvious ethical reasons, unpleasant stimuli could not be delivered at 40 mm Hg in these individuals. Instead, the individual pain threshold was then utilized for the 3 unpleasant stimuli to maintain a standardized number of total distensions in each subject. Given the relatively larger number of cases, this condition was excluded from further analysis. Interestingly, in the LPS condition, liminal stimuli (ie, 10 mm Hg) were rated as significantly more unpleasant ($t = 2.3$, $P = .021$) and inducing a significantly increased urge to defecate ($t = 3.4$, $P = .004$) (Fig. 3A). These differences remained significant in analyses of covariance controlling for the difference in pain thresholds between conditions (data not shown). No significant effects of LPS were observed for supraliminal stimuli (Fig. 3B).

3.5. Correlation analyses

Under LPS treatment, visceral pain thresholds were significantly correlated with IL-6 at +1 h ($r = 0.60$, $P = .035$) and +3 h ($r = 0.67$, $P = .038$). Visceral sensory thresholds were negatively associated with IL-10 at +1 h ($r = -0.73$, $P = .018$) after LPS administration. For the remaining measurement points, no significant correlations between visceral sensory and/or pain thresholds and cytokine levels were observed.

4. Discussion

The association between immune activation and visceral pain is increasingly documented by correlative findings in clinical populations. Here, we present the first experimental study in healthy humans testing the hypothesis that a systemic, endotoxin-induced immune activation increases visceral pain sensitivity.

Administration of endotoxin (lipopolysaccharide, LPS) is an established model to induce a transient systemic immune activation characterized by elevated levels of circulating cytokines. After bolus injection of LPS (0.4 ng/kg body weight), we observed significantly decreased rectal sensory and pain thresholds and altered subjective pain evaluation of liminal distensions when compared to placebo. These findings confirm and extend previous results on the putative role of systemic immune activation in the pathophysiology of visceral hypersensitivity, and support that the acute endotoxemia model may constitute a safe and valid model to study the role of immune-to-brain communication and possibly the effectiveness of drugs targeting systemic immune markers in human visceral pain.

Administration of LPS induced a transient systemic inflammation characterized by an increased release of proinflammatory cytokines and a moderate elevation in body temperature. This is in line with previous studies from our group [22] and other groups by providing similar or higher doses of endotoxin [14,15,29,36,37]. We observed decreased visceral sensory and pain thresholds after LPS administration when compared to placebo, consistent with visceral hypersensitivity. Hence, during transient systemic immune activation, painful stimuli become more painful (ie, visceral hyperalgesia), and the sensitivity to for nonpainful stimuli is increased. In addition, stimulus ratings of predefined stimulus intensities were significantly affected for stimuli of low (liminal) but not of moderate (supraliminal) intensity. Interestingly, we found no evidence to support temporal summation—that is, ratings of repeated predefined stimuli did not change across stimuli within a given subject. Whereas the visceral thresholds primarily reflect discriminatory-sensory qualities of visceral perception, the ratings of predefined visceral stimuli quantify the experience of aversive stimuli, including pain, with respect to affective-motivational components, especially discomfort. We have previously shown that it is critically important to differentiate these aspects because we could show that patients with IBS showed unaltered rectal pain thresholds (based on a double-random staircase distension protocol, as used

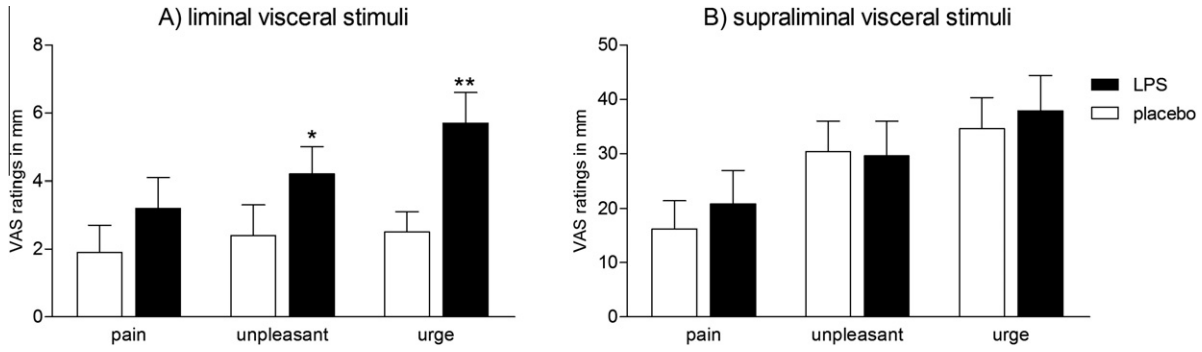

Fig. 3. Predefined distension pressures, liminal (10 mm Hg) (A) and supraliminal (25 mm Hg) (B), were evaluated on 100-mm VAS for pain, unpleasantness, and inducing urge to defecate. Liminal stimuli were rated as significantly more unpleasant and inducing increased urge to defecate (*$P < .05$, **$P < .01$).

in this study) and at the same time significantly increased pain and discomfort ratings of predefined stimulus intensities [18].

Together, the results of our present study indicate that endotoxin-induced systemic immune activation in healthy subjects may have 2 effects: on the one hand, sensory threshold are decreased; that is, sensory-discriminatory qualities of visceral sensation are enhanced, and consequently, visceral stimuli are perceived "earlier." On the other hand, the evaluation of these very low-intensity stimuli is also altered with respect to affective-motivational components (ie, more discomfort). Hence, transient systemic immune activation may turn stimuli that are normally not perceived at all into consciously perceivable stimuli, which is relevant in the context of clinical conditions involving recurrent abdominal pain [32].

Our findings extend data from experimental animals demonstrating an increased visceral sensitivity in rats after intraperitoneal injection of LPS [9] and findings of increased sensitivity to somatic noxious stimuli after LPS administration [48]. In humans, neither visceral nor somatic pain stimuli have been tested in response to endotoxin. However, these experimental results provide strong support for the role of systemic immune mechanisms in visceral pain perception, in the pathophysiology of visceral hyperalgesia in IBS, and possibly for recurrent pain during apparent clinical remission in patients with IBD. An increasing body of literature documents that inflammatory processes both in IBS and IBD patients are not limited to the gut. Local inflammation of the intestine may result in endotoxemia and a systemic immune activation [1,20,45,46,51], mediated, for example, by an increased permeability of the intestinal mucosa [46,51]. Indeed, systemic bacterial endotoxin [8,35] and increased levels of circulating proinflammatory cytokines [38] have been detected in IBD patients. Further, associations between endotoxin levels and disease activity in IBD patients have been reported [21,35]. Similarly, systemic cytokine levels and symptom scores (including pain ratings) were associated in IBS patients [13,30]. Moreover, the neutralization of the proinflammatory cytokine IL-6 was shown to improve symptoms in Crohn disease [26]. Together, these findings suggest that systemic immune activation may be involved in the pathophysiology of gastrointestinal systems, accompanied by abdominal pain, which is now further underscored by our finding of a significant correlation between LPS-induced IL-6 and pain thresholds.

The effects of systemic immune activation on visceral pain thresholds and subjective pain evaluation could be mediated by several possible pathways, including central and peripheral mechanisms. At the level of the central nervous system, proinflammatory cytokines can reportedly affect neural processing of ascending visceral input by directly targeting central receptors via active transport or indirectly by crossing at circumventricular organs or binding to receptors in blood vessels that course through the brain [31,49,50]. Further, signals to the brain could be carried by the subdiaphragmatic vagus because sensory vagal nerve neurons are activated by pathogenic agents [28] and subdiaphragmatic vagotomy blocks endotoxin-induced hyperalgesia [50], or via the release of proinflammatory cytokines from activated glia cells [16,48]. In the periphery, proinflammatory cytokines may act directly on receptors found on enteric neurons and may contribute to the pathophysiology of visceral hypersensitivity in IBS patients [9,34]. Indeed, proinflammatory cytokines have algesic properties [47]. They can produce pain or hyperalgesia when they are exogenously administered [34], and cytokine antagonists reduce pain or hyperalgesia in animal models of inflammation [47]. For example, hypersensitivity to rectal distensions after intraperitoneal LPS application in rats was blocked by an IL-1 receptor antagonist [9]. Additional peripheral mechanisms connecting inflammation and pain sensitization may include increased numbers of mononuclear immune cells, T lymphocytes and mast cells activation [4,5,9]. In a rat model, LPS-induced visceral hypersensitivity could be blocked by a mast cell stabilizer indicating a pain sensitizing role of mast cells which were present in other sites than the gut [9]. Interestingly, experimental endotoxin administration increased intestinal permeability in humans [25], which in turn triggers systemic inflammation and may underlie increased rectal pain sensitivity in IBS patients [34,51].

To our knowledge, this is the first human experimental study complementing the increasing body of correlative findings to support the putative role of systemic immune activation in visceral hypersensitivity. We demonstrated that a transient systemic immune activation leads to decreased visceral sensory and pain thresholds and altered subjective pain ratings in healthy humans. Clearly, the results from this study are based on a small sample size, which bears the problem of limited statistical power and calls for future studies addressing the reproducibility of these findings. The mechanism or mechanisms mediating effects of endotoxin-induced inflammation on visceral pain sensitivity also remain an important subject of future research. The strength of this proof-of-principle study is the use of an established experimental model that allows assessment of the complex and interdependent effects of immunological, physiological, and behavioral changes after a systemic immune activation on pain thresholds and ratings in humans. Our results lend further support to the relevance of inflammatory processes in the pathophysiology of pain in IBS as well as IBD and underline the need for studies further elucidating the immune-to-brain communication pathways in gastrointestinal disorders.

Conflict of interest statement

The authors report no conflict of interest.

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References

[1] Adam B, Liebregts T, Gschossmann JM, Krippner C, Scholl F, Ruwe M, Holtmann G. Severity of mucosal inflammation as a predictor for alterations of visceral sensory function in a rat model. Pain 2006;123:179–86.
[2] Al-Khatib K, Lin HC. Immune activation and gut microbes in irritable bowel syndrome. Gut Liver 2009;3:14–9.
[3] Bahador M, Cross AS. From therapy to experimental model: a hundred years of endotoxin administration to human subjects. J Endotoxin Res 2007;13:251–79.
[4] Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinalidesi R. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 2004;126:693–702.
[5] Barbara G, Wang B, Stanghellini V, De Giorgio R, Cremon C, Di Nardo G, Trevisani M, Campi B, Geppetti P, Tonini M, Bunnett NW, Grundy D, Corinaldesi R. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. Gastroenterology 2007;132:26–37.
[6] Bercik P, Verdu EF, Collins SM. Is irritable bowel syndrome a low-grade inflammatory bowel disease? Gastroenterol Clin North Am 2005;34:235–45.
[7] Bielefeldt K, Davis B, Binion DG. Pain and inflammatory bowel disease. Inflamm Bowel Dis 2009;15:778–88.
[8] Caradonna L, Amati L, Magrone T, Pellegrino NM, Jirillo E, Caccavo D. Enteric bacteria, lipopolysaccharides and related cytokines in inflammatory bowel disease: biological and clinical significance. J Endotoxin Res 2000;6:205–14.
[9] Coelho AM, Fioramonti J, Bueno L. Systemic lipopolysaccharide influences rectal sensitivity in rats: role of mast cells, cytokines, and vagus nerve. Am J Physiol Gastrointest Liver Physiol 2000;279:G781–90.
[10] Collins SM, Piche T, Rampal P. The putative role of inflammation in the irritable bowel syndrome. Gut 2001;49:743–5.
[11] Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46–56.
[12] DellaGioia N, Hannestad J. A critical review of human endotoxin administration as an experimental paradigm of depression. Neurosci Biobehav Rev 2010;34:130–43.

[13] Dinan TG, Clarke G, Quigley EMM, Scott LV, Shanahan F, Cryan J, Cooney J, Keeling PWN. Enhanced cholinergic-mediated increase in the pro-inflammatory cytokine IL-6 in irritable bowel syndrome: role of muscarinic receptors. Am J Gastroenterol 2008;103:2570–6.
[14] Eisenberger NI, Berkman ET, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. Biol Psychiatry 2010;68:748–54.
[15] Eisenberger NI, Inagaki TK, Mashal NM, Irvin MR. Inflammation and social experience: an inflammatory challenge induces feelings of social disconnection in addition to depressed mood. Brain Behav Immun 2010;24:558–63.
[16] Elsenbruch S. Abdominal pain in irritable bowel syndrome: a review of putative psychological, neural and neuro-immune mechanisms. Brain Behav Immun 2011;25:386–94.
[17] Elsenbruch S, Rosenberger C, Bingel U, Forsting M, Schedlowski M, Gizewski ER. Patients with irritable bowel syndrome have altered emotional modulation of neural responses to visceral stimuli. Gastroenterology 2010;139:1310–9.
[18] Elsenbruch S, Rosenberger C, Enck P, Forsting M, Schedlowski M, Gizewski ER. Affective disturbances modulate the neural processing of visceral pain stimuli in irritable bowel syndrome: an fMRI study. Gut 2010;59:489–95.
[19] Engler H, Doenlen R, Engler A, Riether A, Prager G, Niemi MB, Pacheco-López G, Krügel U, Schedlowski M. Acute amygdaloid response to systemic inflammation. Brain Behav Immun 2011;25:1384–92.
[20] Gardiner KR, Anderson NH, Rowlands BJ, Barbul A. Colitis and colonic mucosal barrier dysfunction. Gut 1995;37:530–5.
[21] Gardiner KR, Halliday MI, Barclay GR, Milne L, Brown D, Stephens S, Maxwell RJ, Rowlands BJ. Significance of systemic endotoxemia in inflammatory bowel-disease. Gut 1995;36:897–901.
[22] Grigoleit JS, Oberbeck JR, Lichte P, Kobbe P, Wolf OT, Montag T, del Rey A, Gizewski ER, Engler H, Schedlowski M. Lipopolysaccharide-induced experimental immune activation does not impair memory functions in humans. Neurobiol Learn Mem 2010;94:561–7.
[23] Gwee K. Post-infectious irritable bowel syndrome, an inflammation–immunological model with relevance for other IBS and functional dyspepsia. J Neurogastroenterol Motil 2010;16:30–4.
[24] Hautzinger M, Bailer M, Worall H, Keller F. Beck Depressions Inventar (BDI). Bern: Hans Huber; 1995.
[25] Hietbrink F, Besselink MGH, Renooij W, de Smet MBM, Draisma A, van der Hoeven H, Pickkers P. Systemic inflammation increases intestinal permeability during experimental human endotoxemia. Shock 2009;32:374–8.
[26] Ito H. Treatment of Crohn's disease with anti-IL-6 receptor antibody. J Gastroenterol 2005;40:32–4.
[27] Keohane J, O'Mahony C, O'Mahony L, O'Mahony S, Quigley EM, Shanahan F. Irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease: a real association or reflection of occult inflammation? Am J Gastroenterol 2010;105:1789–94.
[28] Knowles CH, Aziz Q. Basic and clinical aspects of gastrointestinal pain. Pain 2009;141:191–209.
[29] Kullmann JS, Grigoleit JS, Schedlowski M. Effects of an acute inflammation on memory performance, mood and brain activity. Z Med Psychol 2011;20:108–17.
[30] Liebregts T, Adam B, Bredack C, Roth A, Heinzel S, Lester S, Downie-Doyle S, Smith E, Drew P, Talley NJ, Holtmann G. Immune activation in patients with irritable bowel syndrome. Gastroenterology 2007;132:913–20.
[31] Maier SF, Watkins LR. Immune-to-central nervous system communication and its role in modulating pain and cognition: implications for cancer and cancer treatment. Brain Behav Immun 2003;17:S125–31.
[32] Mayer EA. Gut feelings: the emerging biology of gut–brain communication. Nat Rev Neurosci 2011;12:453–66.
[33] Murray C, Sanderson DJ, Barkus C, Deacon RM, Rawlins JN, Bannerman DM, Cunningham C. Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. Neurobiol Aging, in press. doi:10.1016/j.neurobiolaging.2010.04.002.
[34] O'Malley D, Quigleya EMM, Dinana TG, Cryana JF. Do interactions between stress and immune responses lead to symptom exacerbations in irritable bowel syndrome? Brain Behav Immun 2011;25:1333–41.
[35] Pastor Rojo O, Lopez San Roman A, Albeniz Arbizu E, de la Hera Martinez A, Ripoll Sevillano E, Albillos Martinez A. Serum lipopolysaccharide-binding protein in endotoxemic patients with inflammatory bowel disease. Inflamm Bowel Dis 2007;13:269–77.
[36] Pollmacher T, Mullington J, Korth C, Schreiber W, Hermann D, Orth A, Galanos C, Holsboer F. Diurnal variations in the human rest response to endotoxin. J Infect Dis 1996;174:1040–5.
[37] Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, Pollmacher T. Cytokine-associated emotional and cognitive disturbances in humans. Arch Gen Psychiatry 2001;58:445–52.
[38] Rogler G, Andus T. Cytokines in inflammatory bowel disease. World J Surg 1998;22:382–9.
[39] Rosenberger C, Elsenbruch S, Scholle A, De Greiff A, Schedlowski M, Forsting M, Gizewski ER. Effects of psychological stress on the cerebral processing of visceral stimuli in healthy women. Neurogastroenterol Motil 2009;21:740–5.
[40] Schirbel A, Reichert A, Roll S, Baumgart DC, Buening C, Wittig B, Wiedenmann B, Dignass A, Sturm A. Impact of pain on health-related quality of life in patients with inflammatory bowel disease. World J Gastroenterol 2010;16:3168–77.
[41] Schulz P, Schlotz W, Becker P. Trierer Inventar zum chronischen Stress (TICS). Gottingen: Hogrefe; 2004.
[42] Sparkman NL, Buchanan JB, Heyen JR, Chen J, Beverly JL, Johnson RW. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. J Neurosci 2006;26:10709–16.
[43] Spielberger CD, Gorsuch RL, Lushene RE. Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychology Press; 1970.
[44] Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology 2009;136:1979–88.
[45] Spiller RC. Overlap between irritable bowel syndrome and inflammatory bowel disease. Dig Dis 2009;27:48–54.
[46] Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute *Campylobacter enteritis* and in post-dysenteric irritable bowel syndrome. Gut 2000;47:804–11.
[47] Ueceyler N, Schaefers M, Sommer C. Mode of action of cytokines on nociceptive neurons. Exp Brain Res 2009;196:67–78.
[48] Watkins LR, Maier SF. Immune regulation of central nervous system functions: from sickness responses to pathological pain. J Int Med 2005;257:139–55.
[49] Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 2011;25:181–213.
[50] Zhang JH, Huang YG. The immune system: a new look at pain. Chin Med J 2006;119:930–8.
[51] Zhou Q, Zhang B, Verne GN. Intestinal membrane permeability and hypersensitivity in the irritable bowel syndrome. Pain 2009;146:41–6.