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Is there a possible correlation between antibodies against lipopolysaccharide, intestinal and blood-brain barrier proteins in IBD subjects?

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Dear Editor,

It has been established that individuals with inflammatory bowel disease (IBD) have greater risk for increased intestinal permeability [1,2]. This condition facilitates the translocation of enteric bacterial lipopolysaccharide (LPS) into the bloodstream. LPS by itself is inflammatory, but it also triggers the flux of tumor necrosis factor-α, which has been shown to play a role in multiple autoimmunities [3]. This mechanism explains the increase of comorbidities, from arthritis to psoriasis [4–7], seen in IBD disorders, Crohn's disease (CD) and ulcerative colitis (UC).

Neurological comorbidities in IBD are seen less frequently [8]; however, the critical nature of neurological dysfunctions warrants investigation into a possible link between the translocation of LPS, as seen in IBD, to a breakdown of the blood-brain barrier (BBB). A breach of the BBB often precedes the onset of neuroautoimmunity [9]. We examined the co-occurrence of LPS antibody with barrier proteins such as occludin, zonulin, S100B and human aquaporin-4 (AQP4).

It has been shown that LPS levels are increased in patients with IBD [10,11]. However, it has also been shown that LPS and similar molecules have an extremely short half-life (from 2 to 4 min), and that levels of such molecules can fluctuate greatly from day to day or even hour to hour because of the body's immune response to these molecules [12,13]. The measurement of antibodies to these molecules has been demonstrated to be more stable and reliable [13]. Therefore, we used ELISA methodology to test sera from 188 subjects with IBD who were positive for anti-*Saccharomyces cerevisiae* antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA), and compared them with sera from 196 healthy controls using commercial kits from Inova. Then, all subjects were assessed for serum immunoglobulin IgG, IgA and IgM reactivities to LPS, as well as barrier tissue proteins occludin + zonulin, (AQP4), and S100B using in-house ELISA.

At 2 SD above the mean, 72% of patients with IBD showed elevations in LPS IgG antibodies, 41% showed elevations in IgA antibodies, and 33% in IgM antibodies.

In the LPS IgG positive group, the IgG immune reactivity was 48% for occludin + zonulin, 42% for AQP4, and 34% for S100B. There was nearly a 2-fold increased risk of having antibodies to barrier proteins occludin + zonulin, S100B and AQP4, if LPS IgG antibodies were elevated. Specifically, the IgG risk ratio was 1.5 (1.2–2.0) for occludin + zonulin (p-value 0.001); 1.7 (1.3–2.2) for S100B (p-value < .0001); and 1.2 (0.9–1.6) for AQP4 (p-value 0.1).

In the IgA positive group, the IgA immune reactivity was 40% for occludin + zonulin, 52% for AQP4, and 57% for S100B. There was a 2–4-fold increased risk of having antibodies to barrier proteins if LPS IgA antibodies were elevated. Specifically, the IgA risk ratio was 2.2 (1.6–2.9) for occludin + zonulin (p-value < .0001); 3.8 (3.0–4.9) for S100B (p-value < .0001); and 3.2 (2.5–4.1) for AQP4 (p-value < .0001).

In the IgM positive group, the IgM immune reactivity was 55% for occludin + zonulin, 58% for AQP4, and 50% for S100B. There was a 2-fold increased risk of having antibodies to barrier proteins if LPS IgM antibodies were elevated. Specifically, the IgM risk ratio was 2.7 (2.1–3.6) for occludin + zonulin (p-value < .0001); 2.5 (1.9–3.3) for S100B (p-value < .0001); and 2.3 (1.8–3.0) for AQP4 (p-value < .0001). It is important to note that IgM reactivity to LPS had the highest percent of reactivity to all three self-proteins (39%) simultaneously.

Furthermore, statistical analysis was performed to study the relationships of LPS, occludin + zonulin, S100B and AQP4 using Pearson's coefficients. Linear trends were evaluated for each immunoglobulin using a scatter matrix. The p-value was adjusted for multiple comparisons using a Bonferroni adjustment. Risk analysis was conducted using chi-square analysis to determine the risk immunological reactivity to barrier proteins with individuals that exhibited elevated LPS antibodies.

Our results showed significant correlations between LPS antibodies and antibodies to barrier proteins. Moderate correlation coefficients were noted for IgA and IgM antibodies ($r = 0.5–0.6$), and small to moderate correlations were noted for IgG ($r = 0.2–0.4$). These relationships between LPS and barrier proteins are summarized in Table 1.

Our results also showed significant correlations among the barrier proteins with each other. Indeed our results showed a higher correlation coefficient with the IgA (0.6–0.7) and IgM (0.7–0.8) results. The IgG correlation coefficients were also statistically significant (0.4–0.5). These relationships between LPS and barrier proteins are summarized in Table 2.

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Table 1
Correlations analysis between LPS IgG, IgA, IgM and barrier proteins IgG, IgA and IgM.

Correlation of Lipopolysaccharides IgG with Barrier Proteins		
Barrier Proteins	Coefficient	P-value
S100B IgG	0.4	< 0.0001
AQP4 IgG	0.2	0.0004
Occludin-Zonulin IgG	0.4	< 0.0001

Correlation of Lipopolysaccharides IgA with Barrier Proteins		
Barrier Proteins	Coefficient	P-value
S100B IgA	0.6	< 0.0001
AQP4 IgA	0.6	< 0.0001
Occludin-Zonulin IgA	0.5	< 0.0001

Correlation of Lipopolysaccharides IgM with Barrier Proteins		
Barrier Proteins	Coefficient	P-value
S100B IgM	0.6	< 0.0001
AQP4 IgM	0.6	< 0.0001
Occludin-Zonulin IgM	0.6	< 0.0001

Table 2
Correlation analysis between barrier proteins.

Barrier Protein Antibody Correlations		
Correlations	Coefficient	P-value
S100B IgA and AQP4 IgA	0.7	< 0.0001
S100B IgA and Occludin-Zonulin IgA	0.7	< 0.0001
AQP4 IgA and Occludin-Zonulin IgA	0.6	< 0.0001
S100B IgG and AQP4 IgG	0.5	< 0.0001
S100B IgG and Occludin-Zonulin IgG	0.4	< 0.0001
AQP4 IgG and Occludin-Zonulin IgG	0.5	< 0.0001
S100B IgM and AQP4 IgM	0.8	< 0.0001
S100B IgM and Occludin-Zonulin IgM	0.7	< 0.0001
AQP4 IgM and Occludin-Zonulin IgM	0.7	< 0.0001

Our findings showed that, compared to healthy controls, IgG, IgA and IgM LPS antibodies are elevated in IBD patients. Although IgG had the highest percentage of elevations both in IBD patients and controls, the IgA and IgM reactions point to the specificity of LPS in IBD patients. In inflammatory gastrointestinal disorders IgA and IgM are the preferred biomarkers. IgA is reflective of mucosal immunity, and due to its polymeric quality can be transported from the gut to the blood stream, thus, making IgA more specific to the gut. The IgM antibody class is widely used to identify acute or inflammatory stage immune responses. IgA and IgM showed moderate correlation between LPS and barrier proteins. IgA and IgM also showed higher correlation between the barrier proteins and each other. These correlations show that IgA and IgM reactivity to LPS puts the IBD patient at greater risk for gut and BBB damage and therefore neuroautoimmunity.

The significant elevation of LPS antibodies in IBD patients versus healthy controls confirms that many patients with IBD have systemic inflammation from LPS [14,15]. Indeed, patients with IBD tend toward chronic and major comorbidities [9,16,17], which can significantly impact quality of life and increase healthcare costs [16,17]. Although neurological autoimmune disorders in IBD are rare [18], incidences are on the rise [19], while co-occurrence of psychological disorders with IBD are more common [20,21].

We conclude that patients with IBD should be screened for LPS antibody and its correlation with barrier protein antibodies in an effort to detect or prevent possible BBB damage and neurological comorbidities. There are limitations to our data analysis because we did not specifically separate ASCA- or ANCA-positive individuals; we also were not able to consider clinical symptomatologies because we utilized purchased commercial samples in our testing. Additional studies should then be performed on CD versus UC patients based on ASCA or ANCA positivity as well as clinical symptomatologies that are associated with individual disorders to identify any increased risk for neuroautoimmunity within the subgroups of IBD patients.

References

[1] Michielan A, D'Incà R. Intestinal permeability in inflammatory bowel disease: pathogenesis, clinical evaluation, and therapy of leaky gut. Mediators Inflamm 2015;2015:628157https://doi.org/10.1155/2015/628157. [PubMed: 26582965].

[2] Fukui H. Increased intestinal permeability and decreased barrier function: does it really influence the risk of inflammation? Inflamm Intest Dis 2016;1:135–45. https://doi.org/10.1159/000447252.

[3] Emi Aikawa N, de Carvalho JF, Artur Almeida Silva C, Bonfá E. Immunogenicity of anti-TNF-alpha agents in autoimmune diseases. Clin Rev Allergy Immunol 2010;38(2–3):82–9. https://doi.org/10.1007/s12016-009-8140-3. [PubMed: 19565360].

[4] Lin T-Y, Chen Y-G, Lin C-L, Huang W-S, Kao C-H. Inflammatory bowel disease increases the risk of peripheral arterial disease. Medicine (Baltimore) 2015;94(52):e2381. https://doi.org/10.1097/MD.0000000000002381. [PubMed: 26717386].

[5] Binus AM, Han J, Qamar AA, Mody EA, Holt EW, Qureshi AA. Associated comorbidities in psoriasis and inflammatory bowel disease. J Eur Acad Dermatol Venereol 2012;26(5):644–50. https://doi.org/10.1111/j.1468-3083.2011.04153.x. [PubMed: 21689167].

[6] Vlachos C, Gaitanis G, Katsanos K, Christodoulou D, Tsianos E, Bassukas I. Psoriasis and inflammatory bowel disease: links and risks. Psoriasis: Targets and Therapy 2016;6:73–92. https://doi.org/10.2147/PTT.S85194.

[7] Román AL, Muñoz F. Comorbidity in inflammatory bowel disease. rld J Gastroenterol 2011;17(22):2723–33. https://doi.org/10.3748/wjg.v17.i22.2723. [PubMed: 21734780].

[8] Morís G. Inflammatory bowel disease: an increased risk factor for neurologic complications. World J Gastroenterol 2014;20(5):1228–37. https://doi.org/10.3748/wjg.v20.i5.1228. [PubMed: 24574797].

[9] Liebner S, Dijkhuizen RM, Reiss Y, Plate KH, Agalliu D, Constantin G. Functional morphology of the blood-brain barrier in health and disease. Acta Neuropathol 2018;135(3):311–36. https://doi.org/10.1007/s00401-018-1815-1. [PubMed: 29411111].

[10] Magro DO, Kotze PG, Martinez CAR, Camargo MG, Guadagnini D, Calixto AR, et al. Changes in serum levels of lipopolysaccharides and CD26 in patients with Crohn's disease. Intest Res 2017;15(3):352–7. https://doi.org/10.5217/ir.2017.15.3.352. [PubMed: 28670232].

[11] Villarán RF, Espinosa-Oliva AM, Sarmiento M, De Pablos RM, Argüelles S, Delgado-Cortés MJ, et al. Ulcerative colitis exacerbates lipopolysaccharide-induced damage to the nigral dopaminergic system: potential risk factor in Parkinson's disease. J Neurochem 2010;114(6):1687–700. https://doi.org/10.1111/j.1471-4159.2010.06879.x. [PubMed: 20584104].

[12] Yao Z, Mates JM, Cheplowitz AM, Hammer LP, Maiseyeu A, Phillips GS, et al. Blood-borne lipopolysaccharide is rapidly eliminated by liver sinusoidal endothelial cells via high-density lipoprotein. J Immunol 2016;197:2390–9. https://doi.org/10.4049/jimmunol.1600702.

[13] Vojdani A, Vojdani E, Kharrazian D. Fluctuation of zonulin levels in blood vs stability of antibodies. World J Gastroenterol 2017;23(32):5669–79. https://doi.org/10.3748/wjg.v23.i31.5669.

[14] Guo S, Nighot M, Al-Sadi R, Alhmoud T, Nighot P, Ma TY. Lipopolysaccharide regulation of intestinal tight junction permeability is mediated by TLR4 signal transduction pathway activation of FAK and MyD88. J Immunol 2015;195:4999–5010. https://doi.org/10.4049/jimmunol.1402598. [PubMed: 26466961].

[15] Pasternak BA, D'Mello S, Jurickova II, Han X, Willson T, Flick L, et al. Lipopolysaccharide exposure is linked to activation of the acute phase response and growth failure in pediatric Crohn's disease and murine colitis. Inflamm Bowel Dis 2010;16(5):856–69. https://doi.org/10.1002/ibd.21132. [PubMed: 19924809].

[16] Bähler C, Schoepfer AM, Vavricka SR, Brüngger B, Reich O. Chronic comorbidities associated with inflammatory bowel disease: prevalence and impact on healthcare costs in Switzerland. Eur J Gastroenterol Hepatol 2017;29(8):916–25. https://doi.org/10.1097/MEG.0000000000000891. [PubMed: 28471826].

[17] Bernstein CN, Nabalamba A. Hospitalization-based major comorbidity of inflammatory bowel disease in Canada. Can J Gastroenterol 2007;21(8):507–11. [PubMed: 17703250].

[18] Lossos A, River Y, Eliakim A, Steiner I. Neurologic aspects of inflammatory bowel disease. Neurology 1995;45:416–21. [PubMed: 7898687].

[19] Gondim Fde A, Oliveira GR, Teles BC, Souza MH, Braga LL, Messias EL. A case-control study of the prevalence of neurological diseases in inflammatory bowel disease (IBD). Arq Neuropsiquiatr 2015;73(2):119–24. https://doi.org/10.1590/0004-282X20140223. [PubMed: 25742581].
[20] Gracie DJ, Ford AC. Irritable bowel syndrome-type symptoms are associated with psychological comorbidity, reduced quality of life, and health care use in patients with inflammatory bowel disease. Gastroenterology 2017;153(1):324–5. https://doi.org/10.1053/j.gastro.2017.05.037. [PubMed: 28572008].
[21] Varatharaj A, Galea I. The blood-brain barrier in systemic inflammation. Brain Behav Immun 2017;60:1–12. https://doi.org/10.1016/j.bbi.2016.03.010.

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