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In schizophrenia, increased plasma IgM/IgA responses to gut commensal bacteria are associated with negative symptoms, neurocognitive impairments and the deficit phenotype.

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Abstract

Increased gut permeability (leaky gut) with increased translocation of Gram-negative bacteria plays a role in the gut-brain axis through effects on systemic immune-inflammatory processes. Deficit schizophrenia is characterized by an immune-inflammatory response combined with a deficit in natural IgM antibodies to oxidative specific epitopes (OSEs), which are a first line defense against bacterial infections.

This study measured plasma IgA/IgM responses to 5 Gram-negative bacteria in association with IgM responses to malondialdehyde (MDA) and azelaic acid in 80 schizophrenia patients (40 with the deficit syndrome and 40 without) and in 38 healthy controls.

Deficit schizophrenia was characterized by significantly increased IgA responses to *Hafnei alvei*, *Pseudomonas aeruginosa*, *Morganella morganii* and *Klebsiella pneumoniae* as compared with non-deficit schizophrenia. The presence of deficit schizophrenia was highly predicted by increased IgA responses to *Pseudomonas putida* and IgM responses to all 5 Gram-negative bacteria and lowered natural IgM to MDA and azelaic acid with a bootstrap area under the ROC curve of 0.960 (2000 random curves). A large proportion of the variance (41.5%) in the PANSS negative score was explained by the regression on IgA responses to *K. pneumoniae* and IgM responses to the 5 enterobacteria coupled with lowered IgM antibodies to azelaic acid. There were significant associations between IgA levels to Gram-negative bacteria and Mini Mental State Examination, Boston naming test, Verbal Fluency and Word List Memory test scores.

These findings provide further evidence that deficit schizophrenia is a distinct phenotype of schizophrenia, which is characterized by an increased impact of Gram-negative commensal bacteria coupled with a deficit in natural IgM, pointing to aberrations in B1 cells. It is concluded that increased bacterial translocation and deficits in the compensatory immune-regulatory system

(CIRS) may drive negative symptoms and neurocognitive impairments, which are hallmarks of deficit schizophrenia.

Key words: immune, inflammation, natural IgM, B1 cells, oxidative stress, TRYCATs, schizophrenia, psychosis, psychiatry

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Introduction

There is now evidence that first episode psychosis (FEP) and acute psychotic relapses as well as chronic schizophrenia, treatment resistant and stable-phase schizophrenia are characterized by activation of the immune-inflammatory response system (IRS) (Smith and Maes, 1995; Anderson and Maes, 2013; Roomrunagwong et al., 2018b; Miller et al., 2011). The findings indicate an acute phase response, with increased levels of positive acute phase proteins, increased complement factors, and activation of M1 macrophagic, T helper (Th)-1 and Th-17 immune cell phenotypes (Maes et al., 1997b; Roomruangwong et al., 2018b).

These schizophrenia phenotypes are not only accompanied by an activated IRS, but also by a concomitant activation of the compensatory immune-regulatory responses system (CIRS) including activated Th-2 and T regulatory (Treg) immune phenotypes and increased levels of soluble cytokine receptor levels that have immune regulatory effects, such as soluble interleukin-2 receptor (sIL-2R), soluble tumor necrosis factor (sTNF-R)1 and sTNF-R2, and sIL-1R antagonist (sIL-1RA) levels (Roomruangwong et al., 2018b). As such, the CIRS exerts many immune-regulatory and anti-inflammatory activities, which tend to downregulate the IRS (Roomruangwong et al., 2018b). This is important since immune products of the IRS (IL-6, IL-1, TNF-α) as well as the CIRS (IL-4, IL-13, CCL-11 or eotaxin) may exert cytotoxic and neurotoxic effects thereby driving neuroprogression (Davis et al., 2014; 2016; Noto et al., 2018; Roomruangwong et al., 2018b; Maes and Carvalho, 2018).

Distinct schizophrenia-related phenotypes including FEP are characterized by a significantly elevated IRS / CIRS ratio, indicating a net immune-inflammatory response that does not appear to be sufficiently attenuated by an activated CIRS (Noto et al., 2018; Roomruangwong et al., 2018b). Moreover, schizophrenia is accompanied by deficits in the CIRS and hence more

prominent IRS responses after immune challenges (Noto et al., 2018; Roomruangwong et al., 2018b). For example, the levels of plasma Clara Cell secretory protein (CC16), an endogenous disulfide-bridged protein with protective and anti-inflammatory properties, are significantly decreased in schizophrenia patients (Maes et al., 1996; 1997a). Moreover, relatively lowered levels of plasma sTNF-R1, sTNF-R2, sIL-2R and sIL-1RA in FEP have been reported, which may worsen the clinical outcomes in FEP (Noto et al., 2018).

There is now accumulating evidence that deficit schizophrenia is a distinct nosological category, which is significantly discriminated from non-deficit schizophrenia by more severe neuro-immune aberrations, memory impairments, negative symptoms as well as psychotic, hostility, excitation and mannerism (PHEM) symptoms (Kanchanatawan et al., 2018a; 2018b). Increased IgA levels directed against tryptophan catabolites (TRYCATs) (indicating an overactivation of the TRYCAT pathway) are other biomarkers of deficit schizophrenia and negative and neurocognitive symptoms (Kanchanatawan et al., 2018a; 2018b). The cytotoxic and neurotoxic properties of increased TRYCATs such as picolinic acid (PA), xanthurenic acid (XA) and quinolinic acid (QA) are further augmented by increased levels of eotaxin or CLL-11, a Th-2-related product that may contribute to neurocognitive deficits and negative symptoms in schizophrenia (Sirivichayakul et al., 2018a; 2018b). However, a significant deficit in the CIRS, as indicated by lowered levels of natural IgM against TRYCATs and oxidative specific epitopes (OSEs), including malondialdehyde (MDA) and azelaic acid, appears to be the most prominent immune abnormality in deficit schizophrenia (Kanchanatawan et al., 2018a; Maes et al. 2018a). These IgM antibodies have specificity to self-antigens and OSEs and are present even without antigenic contact, although increased surface expression of these neoepitopes following lipid peroxidation and aldehyde formation may generate adaptive IgM responses towards the same

neoepitopes (Binder, 2012; Weismann and Binder, 2012; Diaz-Zaragoza et al., 2015; Thiagarajan et al., 2016; McMahon and Skaggs, 2016). Natural IgM, especially those directed to MDA, have strong immune-regulatory effects and in fact are an integral component of the innate first-line defense against microorganisms, including Gram-negative bacteria (Binder, 2012; Weismann and Binder, 2012; Diaz-Zaragoza et al., 2015). Therefore, we hypothesized that lowered natural IgM responses to MDA and azelaic acid in deficit schizophrenia could be accompanied by a greater impact of Gram-negative commensal bacteria affecting schizophrenia phenomenology including negative symptoms and neurocognitive impairments.

Hence, the present study was performed to examine whether a) deficit schizophrenia is accompanied by increased IgA/IgM levels to Gram-negative bacteria and whether these responses are positively associated with negative symptoms and neurocognitive deficits; and b) the deficits in IgM isotype antibodies to OSEs coupled with increased IgA/IgM responses to Gram-negative bacteria have cumulative effects on negative symptoms and neurocognitive impairments above and beyond the effects of each factor alone.

Methods

Participants

This study enrolled 38 healthy controls and 80 patients with schizophrenia. All patients were in a stable phase of schizophrenia without acute episodes for at least one year and they complied with the diagnostic criteria for schizophrenia according to DSM-IV-TR criteria. Using the Schedule for Deficit syndrome SDS (Kirkpatrick et al., 1989) schizophrenia patients were allocated to two groups, namely those with and those without deficit schizophrenia. All patients

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were outpatients at the Department of Psychiatry at the King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Healthy volunteers were recruited from the same catchment area.

Exclusion criteria for patients were: a) acute episodes of schizophrenia the year prior to inclusion; b) axis-1 DSM-IV-TR psychiatric disorders such as major depression, bipolar disorder, schizoaffective disorder, substance-use disorders and psycho-organic disorders; c) medical illness such as psoriasis, rheumatoid arthritis, COPD, diabetes (type 1 and 2) and inflammatory bowel disease; d) neurological disorders including stroke, Parkinson's disease, multiple sclerosis, and Alzheimer's disease; e) use of immunomodulatory drugs and antioxidant supplements and ω3-polyunsaturated fatty acids. Exclusion criteria for controls were: a) lifetime or current axis I diagnosis according to DSM-IV-TR criteria; and b) a positive family history of schizophrenia.

All controls and patients as well as the guardians of patients, namely parents or other close family members, provided written informed consent prior to participation in this study. The study was conducted according to International and Thai ethics and privacy laws. Approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (#298/57), which is in compliance with the International Guidelines for Human Research protection as required by the Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

Measurements

Clinical assessments

The authors used a semi-structured interview applied by the same senior psychiatrist, specialized in the treatment of schizophrenia (BK), in order to collect socio-demographic and

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clinical data. The Mini-International Neuropsychiatric Interview (M.I.N.I.) was used in a validated Thai translation (Kittirathanapaiboon and Khamwongpin, 2005) to make the diagnosis of schizophrenia, whilst the SDS was used to make the diagnosis of primary deficit schizophrenia. BK also scored the Positive and Negative Syndrome Scale (PANSS), with scores on the negative (PANSSneg) and positive (PANSSpos) subdomains (Kay et al., 1987). We computed (Kanchanatawan et al., 2018c) four z-unit weighted composite scores reflecting four different symptom dimension scores using items of the PANSS and the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), namely a) the psychotic dimension as sum of z score of PANSS P1 (delusion) (zP1) + zP3 (hallucinations) + zP6 (suspiciousness) + zBPRS11 (suspiciousness) + zBPRS12 (hallucinatory behavior) + BPRS15 (unusual thought content); b) the hostility dimension as: sum of zP7 (hostility) + zPANSS general14 (zG14, poor impulse control) + zBPRS10 (hostility) + zBPRS14 (uncooperativeness); c) the excitement dimension as: zP14 (excitement) + zP5 (grandiosity) + zBPRS8 (grandiosity) + zBPRS17 (excitement); and d) the mannerism dimension as: zG5 + zBPRS7 (both mannerism and posturing). The diagnosis of tobacco use disorder (TUD) was made using DSM-IV-TR criteria. Body mass index (BMI) was assessed the same day as the clinical interview and rating scale scoring as body weight (kg) / length (m^2).

The authors also measured different Consortium to Establish a Registry for Alzheimer's disease (CERAD)-Neuropsychological tests (CERAD, 1986). These were scored by a trained research assistant who was blinded to the clinical diagnosis. The CERAD tests were carried out the same day we also completed the semistructured interview and clinical scorings. The CERAD probes used in the current study were: a) the Mini-Mental State Examination (MMSE), which probes a more general neuropsychological defect including naming, orientation, memory,

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concentration and constructional praxis; b) the Boston naming test to probe naming; c) Verbal Fluency Test (VFT) to probe fluency and semantic memory; and d) Word List Memory (WLM) to probe learning ability and verbal episodic memory.

Assays

In patients and controls, fasting blood was sampled at 8.00 a.m. for the assay of IgA and IgM responses to five Gram-negative bacteria and IgM-mediated autoimmune responses directed against MDA and azelaic acid. A description of the measurements of IgM or IgA isotypes directed against the Gram-negative bacteria is provided elsewhere (Roomruanwong et al., 2017). "Antigens derived from five Gram-negative bacteria were assayed after sonication, namely *Hafnia alvei, Klebsiella pneumonia, Morganella morganii, Pseudomonas aeruginosa,* and *Pseudomonas putida.* Polystyrene 96-well plates (NUNC) were coated with 200 µl solution containing bacterial components at 4 µg/ml in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4°C for 16 h under agitation. Then, we added 200 µl blocking solution (PBS, Tween 20 0.05%, 5 g/l BSA) for 1 h and placed at 37°C. Following two washes with PBS, plates were filled up with 100 µl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 2.5 g/l BSA) and incubated at 37°C for 105 minutes. After three washes with PBS-0.05% Tween 20, plates were incubated at 37°C for 1 h with peroxidase-labeled anti-human IgM or IgA secondary antibodies diluted respectively at 1: 15,000 and 1: 10,000 in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). Afterwards, plates were washed three times with PBS-0.05%Tween 20, and incubated with the detection solution for 10 min in the dark. Chromogen detection solution (Tetramethylbenzedine) was used for the peroxidase assay at 16.6 ml per liter in 0.11 M sodium acetate trihydrate buffer (pH 5.5) containing 0.01% H_2O_2. The reaction was stopped with 25 µl 2-

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N HCl. After addition of stop solution (H_2SO_4 or HCl), the obtained, proportional absorbance in the tested sample (compared to established concentration of respective antibodies), was measured at 450 nm with one alpha of correction at 660 nm.

ELISA methods were used to measure IgM levels directed against conjugated azelaic acid and MDA (Maes et al., 2018). Azelaic acid and MDA were linked to fatty acid free-BSA according to previously described methods. “The detection of IgM autoantibodies to the conjugates was performed by an indirect ELISA tests (Maes et al., 2018). Briefly, polystyrene 96-well plates (NUNC) were coated with 200 µl solution containing the conjugates or BSA in 0.05 M carbonate buffer at pH 9.6. Well plates were incubated at 4°C for 16 h under agitation. Then, a 200 µl of blocking solution (PBS, 2.5 g/l BSA) was added for 1 h and placed at 37°C. Following three washes with PBS, plates were filled up with 100 µl of sera diluted at 1:1000 in the blocking buffer A (PBS, 0.05% Tween 20, 10% Glycerol, 2.5 g/l BSA, 1 g/l BSA-G) and incubated at 37°C for 2 h. After three washes with PBS-0.05% Tween 20, plates were incubated at 37°C for 1 h with peroxidase-labeled anti-human IgM secondary antibodies diluted respectively at 1: 15,000, in the blocking buffer (PBS, 0.05% Tween 20, 2.5 g/l BSA). They were then washed three times with PBS-0.05% Tween 20, and incubated with the detection solution for 10 min in the dark. Chromogen detection solution was used for the peroxidase assay at 8% in 0.1 M acetate and 0.01 M phosphate buffer (pH 5.0) containing 0.01% H_2O_2. The reaction was stopped with 25 µl 2-N HCl. ODs were measured at 492 nm using a multiscan spectrophotometer. All assays were carried out in duplicate. The intra-assay coefficients of variation (CV) were < 6%” (Maes et al., 2018).

Statistical analysis

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Analysis of variance (ANOVAs) was used to assess differences in continuous variables between groups, while analysis of contingency tables (X^2-test) was used to check associations between categorical variables. Multinomial logistic regression analysis was employed to assess associations between IgA/IgM levels to Gram-negative bacteria and diagnosis (deficit versus non-deficit schizophrenia versus normal controls). We employed binary regression analysis to assess the most significant predictors (IgM/IgA responses to Gram-negative bacteria) of deficit schizophrenia versus non-deficit schizophrenia with or without normal controls. Moreover, we computed Odds ratios (OR) and 95% confidence intervals. In the current study, we used multiple regression analysis to assess the best prediction of negative symptoms and neurocognitive test results (entered as dependent variables), using IgA/IgM responses to Gram-negative bacteria and IgM levels to MDA/azelaic acid as explanatory variables. In order to examine the effects of extraneous variables on the IgM/IgA responses to bacteria we employed multivariate general linear model (GLM) analysis with the IgM/IgA responses as dependent variables and age, sex, BMI, TUD, and the drug state of the patients as explanatory variables. Tests for between-subject effects were consequently employed to delineate the effects of significant independent variables on IgM/IgA responses. We used Receiver Operating Characteristics (ROC) analysis to compute the area under the ROC curve (including after 2000 bootstraps). The MDA and azelaic OD data were Ln transformed in order to normalize the data distribution of the IgM-responses and all OD data were processed in z transformations. We interpreted the bootstrapped (n=1000) results of regression analyses and report if there are differences between results with and without bootstrapping. Results of univariate GLM analysis with multiple comparisons were p-corrected for false discovery rate **(**FDR**) (**Benjamini and Hochberg, 1995**).** All results of regression analyses were also checked for collinearity using tolerance and VIF values. Since the IgM responses to

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Gram-negative bacteria were highly correlated (yielding significant collinearity in regression analysis) we used a z-unit weighted composite score reflecting total IgM responses to the 5 bacteria (zsumIgM), which was computed as the z transformation of the sum of all five z transformed IgM OD bacterial data. We also computed a zsumzIgA composite score (based on the IgA responses to the 5 bacteria) and divided the study group according to two subgroups based on the median-split method. Statistical analyses were performed using IBM SPSS windows version 22. Tests were 2-tailed and a p-value of 0.05 was used for statistical significance.

In order to examine the causal links among IgM/IgA responses to Gram-negative bacteria, IgM antibodies to MDA and azelaic acid, cognitive tests and symptoms we employed Partial Least Squares (PLS) analysis (SmartPLS; Ringle et al., 2014). PLS is a structural equation modeling technique that uses path modeling performed on latent vectors (LV) extracted from indicator variables (Ringle et al., 2014). A LV extracted from the six items of the SDS was the final output variable (reflecting SDS score or negative symptoms) and the direct explanatory variables were a LV extracted from three CERAD tests, namely True Recall, WLM and VFT (reflecting "memory impairments"), an LV extracted from IgA responses to Gram-negative bacteria, zsumzIgA (one indicator variable) and a LV extracted from both OSEs (reflecting natural IgM). Moreover, the model examines whether the effects of bacteria and IgM directed against OSEs are mediated by the memory LV (see Figure 2). As such, we use a multistep path mediated model (Cepeda-Carrion et al., 2018), namely from IgM/IgA to Gram-negative bacteria and IgM to OSEs → cognitive impairments (possible mediator) → negative symptoms. The quality of the model was assessed using SRMR < 0.08 as an overall model fit criterion. New constructs were accepted only when they showed a good reliability and discriminant validity, including Cronbach's alpha > 0.7, composite reliability > 0.7, and average variance extracted (AVE) > 0.500. Moreover, indicators

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of the latent constructs should have factor loadings > 0.5 and p values < 0.001 (Ringle et al., 2014). We used consistent PLS bootstrapping (2000 bootstraps) to compute path coefficients with exact p-value, total effects, total indirect and specific indirect effects.

Results.

1. Socio-demographic data

Table 1 displays the demographic, clinical and biomarker data in two subgroups divided according to the zsumzIgA composite score using the median split method (median=-0.0772019), yielding two study groups, a first with lower and a second with higher zsumIgA values, reflecting a lower versus higher bacterial load. The results of these multiple comparisons were not p-corrected for false discovery rate (and not adjusted for relevant confounding variables) because these results together with the results of intercorrelation matrices were employed to delineate the explanatory variables to be entered in the ultimate regression and PLS analyses. Table 1 shows no significant differences in age, gender, marital status, education, TUD, BMI, number of psychotic episodes, PANSS positive subscale score, psychosis, hostility, excitement, mannerism, IgM levels to MDA and azelaic acid and MMSE among these two study groups. Subjects in the high zsumzIgA composite score showed significantly higher SDS and PANSS negative subscale scores, lower BNT, VFT, WLM and True Recall values.

Associations between IgA/IgM responses to enterobacteria and (deficit) schizophrenia

Table 2 shows the associations between the IgA responses to Gram-negative bacteria and diagnosis into three classes, namely deficit versus non-deficit schizophrenia versus controls. Diagnostic categories were the dependent variables and biomarkers the explanatory variables.

Entry of the separate IgA and IgM values showed that the IgA values directed to *H. alvei, P. aeruginosa, M. morganii* and *K. pneumonia* were significantly associated with the deficit phenotype (versus the non-deficit phenotype) and also separated the deficit phenotype from controls (*H. Alvei* and *K. Pneumoniae*). There were no significant associations between the diagnostic groups and either IgA responses to *P. putida* and IgM responses to the 5 gram-negative bacteria. After considering the effects of IgM responses to MDA and azelaic acid in those multinomial regression analyses we found that IgM responses to MDA ($\chi2=32.90$, df=2, $p<0.001$), and azelaic acid ($\chi2=11.00$, df=2, $p=0.004$), IgA responses to *P. putida* ($\chi2=14.52$, df=2, $p=0.001$) and zsumIgM ($\chi2=22.79$, df=2, $p<0.001$) were significantly associated with diagnostic classes. Deficit schizophrenia was significantly separated from non-deficit schizophrenia and controls by higher IgA responses to *P. putida* and zsumIgM and lower IgM antibodies to MDA and azelaic acid.

Subsequently, we have also examined the discrimination of deficit schizophrenia from all other subjects or from non-deficit schizophrenia using binary logistic regression analysis. **Table 3** shows the results of these two regression analysis with diagnosis as dependent variable and biomarkers (together with age, sex and BMI) as explanatory variables. The first analysis shows that deficit schizophrenia (versus all other participants) was significantly associated with higher zsumIgM levels and IgA levels to *P. putida* and lower IgM levels to MDA and azelaic acid. 83.1% of all cases were correctly classified with a sensitivity of 70.0% (to detect deficit schizophrenia), a specificity of 89.7% and an area under the ROC curve of 0.927 (±0.024). **Figure 1** shows the measurements of the IgA/IgM responses to Gram-negative bacteria and IgM responses to MDA and azelaic acid in patients with deficit schizophrenia versus all other subjects (all in z transformations of the OD values). Natural IgM to MDA ($F=35.42$, df=1/116, $p<0.001$; partial eta

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squared=0.234) and azelaic acid (F=23.77, df=1/116, p<0.001; partial eta squared=0.170) were significantly lower in deficit schizophrenia as compared with all other subjects.

The second regression analysis shows that deficit schizophrenia versus non-deficit schizophrenia was significantly associated with higher zsumIgM levels and IgA levels to *H. alvei* and lower IgM levels directed to MDA and azelaic acid. 91.3% of all cases were correctly classified with a sensitivity of 92.5% (to detect deficit schizophrenia), a specificity of 90.0% and an area under the ROC curve of 0.963 (±0.020). The bootstrapped AUC ROC estimation (n=2000 random curves) was 0.960 with 95% confidence intervals of 0.915-0.992. Age, sex and BMI were not significant in this regression analysis.

Impact of extraneous variables

To delineate possible effects of age, sex, BMI, education and medications on the IgA/IgM responses to Gram-negative bacteria, multivariate GLM analysis was performed with the biomarkers (IgA and IgM levels to the 5 Gram-negative bacteria) and diagnosis (in three groups) as dependent variables. There were significant effects of sex ((F=2.02, df=10/98, p=0.039) and age (F=3.87, df=10/98, p<0.001), but not BMI (F=0.37, df=10/98, p=0.957) on the IgA/IgM responses. After p-correction for FDR, the IgA responses to *P. aeruginosa* (p=0.020), *M. morganii* (p=0.028) and *P. putida* (p=0.010) and IgM responses to *H. alvei* (p=0.033), *P. aeruginosa* (p=0.02), *M. morganii* (p=0.02), *P. putida* (p=0.02) and *K. Pneumoniae* (p=0.033) were higher in females than in males. After p-correction, there were significant and positive correlations between age and IgA responses to all 5 bacteria except *P. putida* (all p<0.011); and negative correlations between age and IgM responses to all 5 bacteria (all <0.010). In any case, all regression analyses used in this study were adjusted for possible effects of age and sex but those variables did not affect the

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association between biomarkers and diagnosis. There were no significant effects of education (F=1.75, df=10/97, p=0.080) and TUD (F=0.32, df=10/97, p=0.976) on the biomarkers. Multivariate GLM analysis showed no significant effects of the drug state of the patients on the biomarkers, namely risperidone (n=33, F=0.54, df=10/88, p=0.860), clozapine (n=10, F=1.58, df=10/88, p=0.127), haloperidol (n=8, F=0.99, df=10/88, p=0.457), perphenazine (n=20, F=0.60, df=10/88, p=0.810), antidepressants (n=26, F=0.54, df=10/88, p=0.859), mood stabilizers (n=12, F=1.07, df=10/88, p=0.398) and anxiolytics/hypnotics (n=27, F=1.02, df=10/88, p=0.437).

Associations between IgA/IgM responses to bacteria and schizophrenia phenomenology

In order to examine the associations between schizophrenia symptomatology and biomarkers we have entered the total SDS score and its 6 item scores, the PANSS positive and negative subscale scores, and 4 PHEM composite scores as dependent variables and IgA responses to 5 Gram-negative bacteria, zsumIgM and IgM antibody levels against MDA and azelaic acid as explanatory variables in multiple regression analyses. **Table 4** shows that 48.2% of the variance in the total SDS score was explained by the regression on IgM azelaic acid and education (inversely) and IgA to *K. pneumoniae* and zsumIgM (both positively). Also the 6 items of the SDS scale were significantly predicted (29.0-40.3% of the variance) by IgM to OSEs combined with zsumIgM and IgA responses to one of the 5 bacteria (most often *K. pneumonia*) and education. The PANSS positive subscale was not associated with any of the biomarkers, whereas 41.5% of the variance in the PANSS negative subscore was explained by the regression on IgM azelaic acid (inversely) and IgA to *K. pneumoniae* and zsumIgM (both positively). 16.8% of the variance in psychotic symptoms was explained by increased IgA levels to *K. pneumoniae* and education. 26.6% of the variance in the excitation composite score was explained by IgM to azelaic acid and

education (inversely) and IgA to *K. pneumoniae* (positively). There were no significant associations between hostility or mannerism and any of the biomarkers. Age and sex were not significant in the analyses shown in Table 4.

Associations among IgA/IgM responses to Gram-negative bacteria and neurocognitive tests

In order to assess the associations between IgA/IgM responses to Gram-negative bacteria and OSEs and neurocognitive tests we employed multiple regression analyses with the neurocognitive CERAD tests as dependent variables and the biomarkers, age, sex and education as independent variables. We found that 21.1% of the variance in the BNT test results was explained by education (inversely) and IgA responses to *K. pneumoniae*. 40.6% of the variance in the MMSE was explained by the regression on education, IgM against MDA (both negatively) and IgA to *P. putida* (the bootstrapped p values were significant for all 3 variables, including IgA against P. putida, namely p=0.043). 11.7% of the variance in the VFT was explained by IgA levels to *K. pneumoniae*. 33.7% of the variance in WLM test was explained by the regression on education, IgM levels against MDA (inversely) and IgA levels to *K. pneumoniae*. Table 5 shows also that there are positive associations between IgM against MDA or azelaic acid and zsumIgM composite score.

Results of PLS analysis

Figure 2 shows the outcome of the multistep PLS path analysis with path coefficients and exact p –values of the model presented in the methods section. We found that 73.4% of the variance in the LV extracted from the 6 SDS items was explained by memory LV, natural IgM LV, zsumIgM and IgA Gram-negative LV. 29.3% of the variance in the memory LV was explained by

the regression on natural IgM LV, zsumIgM and IgA Gram-negative LV. There were significant total and specific indirect effects of IgA Gram-negative bacteria LV (t=+2.44, p=0.015) and IgM against OSEs (t=2.15, p=0.032). There were total effects of IgA to Gram- LV on memory (t=+3.25, p=0.001) and SDS LV (t=+4.24, p<0.001) and of zsumIgM on SDS LV (t=3.50, p=0.001) and of natural IgM to OSEs on memory (t=+2.68,p=0.008) and SDS LV (t=-5.94, p<0.001).

Discussion

The first major finding of this study is that deficit schizophrenia is accompanied by a) significantly increased IgA responses to four Gram-negative bacteria, namely *H. alvei, P. aeruginosa, M. morganii and K. pneumoniae*; and b) and increased IgA responses to *P. putida* coupled with increased IgM responses to all 5 Gram-negative bacteria and lowered IgM responses to MDA and azelaic acid. These five bacteria, including *K. pneumonia*e, belong to the normal intestinal flora (Wiest, 2005; Todar, 2018), although the latter also belongs to the lung microbiome (O'Dwyer et al., 2016). Increased serum IgA levels directed against sonicated samples of Gram-negative bacteria (thus including LPSs) reflect LPS exposure (Pasternak et al., 2010) and, therefore, constitute an indirect assay of increased bacterial translocation into the blood or mesenteric lymph nodes (MLNs) with or without systemic spreading of the bacteria. Translocated bacteria into the MLNs may prime and activate immune cells in the MLNs thereby inducing an immune response (Kwa et al., 2006). Therefore, our results indicate that in deficit, but non non-deficit, schizophrenia, Gram-negative bacteria may have translocated from the gut (and/or lung) thereby increasing bacterial load and inducing a systemic immune response.

Previously, we have shown that major depression, bipolar disorder and chronic fatigue syndrome are accompanied by increased bacterial translocation (Maes et al., 2007; 20018; 2012;

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2013a; Maes et al., in preparation). In patients with major depression significant associations were found between IgA against Gram-negative bacteria and IgG responses to oxidized low-density lipoprotein cholesterol, serum lysozyme levels and IgM responses to MDA and azelaic acid, indicating that bacterial translocation is associated with peripheral immune-inflammatory and autoimmune processes (Maes et al., 2013a). Moreover, in CFS it was reported that increased IgA to Gram-negative bacteria are significantly associated with increased serum levels of IL-1, TNF-α, neopterin, elastase and autoantibodies directed to serotonin (Maes et al., 2012; 2013b), indicating that increased bacterial translocation may cause peripheral activation of M1 macrophagic, Th-1, neutrophil and autoimmune pathways.

Apart from LPSs, bacteria contain many immunogenic proteins and pathogen-associated molecular patterns (PAMPs), which may induce immune-inflammatory responses (Hoppe et al., 2012). Nevertheless, best documented are the effects of LPSs triggering the Toll-Like Receptor 4 (TLR4) complex, a receptor of the innate immune system. Following TLR4 activation, an intracellular cascade is induced leading to activated cell signalling networks, including nuclear factor (NF)-κB and MAPK, which in turn may induce the production of pro-inflammatory cytokines and inducible nitric oxide synthase (iNOS) (Lucas and Maes, 2013; Chan et al., 2001). Moreover, LPS may stimulate the production of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, with consequent activation of superoxide and hydrogen peroxides, and cyclooxygenase-2 and lysozyme by mononuclear phagocytes (Lin et al., 2011; Check et al., 2010; Peng et al., 2005; Pai and Sodhi, 1991; Lewis et al., 1990). Moreover, the increased IgA levels against Gram-negative bacteria measured here are significantly associated with TRYCAT pathway activation, which may be explained by effects of LPSs or its downstream products, including pro-inflammatory cytokines, inducing the TRYCAT pathway (Maes et al., submitted). LPSs have

profound neurotoxic effects leading to neurodegenerative processes which are mediated by microglial activation (Arai et al., 2004). Gut microbiota may increase the permeability of the BBB (Braniste et al., 2014), while LPS can access the brain via different routes, including circumventricular organs and area postrema as well as via peripheral nerves signal transduction (Zakaria et al., 2017). Moreover, Gram-negative bacteria encountering environmental stress may produce outer membrane vesicles (OMVs), which contain antigens, PAMPs and virulence factors (Muraca et al., 2015; Anand and Chaudhuri, 2016; Ellis and Kuehn, 2010; Ellis et al., 2010). OMVs may increase the permeability of the blood-brain barrier and be delivered to the brain (Muraca et al., 2015). Interestingly, some OMVs are pathogenic and have pro-inflammatory effects, whilst other OMVs may have immune-regulatory functions, for example by inducing immune tolerance (Kaparakis-Liaskos and Ferrero, 2015; Shen et al., 2012). As such, increased bacterial translocation could contribute to the immune-inflammatory pathophysiology of mental disorders, including deficit schizophrenia.

The second major finding of this study is that increased IgA and IgM responses to Gram-negative bacteria are significantly associated with negative, but not positive, symptoms. Recently, we have shown that neuro-immune biomarkers to a large extent determine the variance in negative symptoms as measured with the SDS (and its 6 items) and the PANSS negative subscale, including increased levels of eotaxin (or CCL-11, a Th-2-related product) and IL-10 (a Treg cytokine) and increased production of neurotoxic TRYCATs (Kanchanatawan et al., 2018a; Sirivichayakul et al., 2018a; 2018b). Thus one possible explanation is that bacterial translocation, as established in the present study, may play a role by inducing different neuro-immune pathways that lead to increased neurotoxic effects and thus neuroprogression. Our negative findings on possible associations between Gram-negative bacteria and positive symptoms should be explained by the lack of

specificity of the latter (Kanchanatawan et al., 2018c). Indeed, we have shown that "positive symptomatology" is not a viable concept as these symptoms should be "dissected" into more relevant symptoms dimensions, including psychosis (delusions, hallucinations), hostility, excitation and mannerism. As such, the current study detected significant associations between IgA/IgM responses to Gram-negative bacteria and psychosis and excitation. Again, psychosis and excitation are significantly associated with increased IL-10 levels (reflecting immune activation) and increased neurotoxic TRYCATs, which may be induced by increased bacterial translocation (Kanchanatawan et al., 2018c; Sirivichayakul et al., 2018b). Future research should address the question whether the effects of bacterial translocation on negative symptoms, psychosis and excitation are mediated by changes in the immune system and TRYCAT patterning.

The third major finding of this study is that IgA and IgM responses to Gram-negative bacteria explain part of the variance in neurocognitive deficits, including a more general impairment in neurocognitive functioning (MMSE), naming ability (BNT), and semantic (VFT) and episodic memory (WLM). Previously, it was shown that not only activation of immune-inflammatory pathways and oxidative stress, but also gut microbiota are involved in the cognitive decline seen in patients with diabetic encephalopathy (Xu et al., 2017). Gut microbiota increase the permeability of tight-junctions of the BBB, which in turn allow induction of cognitive impairments (Braniste et al., 2014). In humans, administration of low-dose LPS affects long-term memory performance for emotional stimuli, but not accuracy in working memory (Grigoleit et al., 2011). In rodent models, acute, subchronic and chronic administration of LPSs significantly induces diverse cognitive dysfunctions in learning and (working and spatial) memory, which are associated with increased expression of M1 macrophagic cytokines, IL-1β, amyloid-β, induced-apoptosis in the brain and / or attenuated hippocampal neurogenesis (Hauss-Wegrzyniak et al., 1998; Zhu et

al., 2014; Skelly et al., 2017; Valero et al., 2014). Based on these findings some authors have proposed LPS-induced memory impairments in rats as a model of Alzheimer's disease (Zakaria et al., 2017) and LPS exposure in the prenatal period in the rodent as a model of schizophrenia (Meyer, 2014). Interestingly, based on a new cognitive model of schizophrenia (Sirivichayakul et al., 2018a), the current study found that the effects of IgA (but not IgM) responses to Gram-negative bacteria on negative symptoms are in part mediated by memory deficits, indicating that more direct pathways not related to memory impairments are involved. As such, increased bacterial translocation in deficit schizophrenia may aggravate neurocognitive impairments and negative symptoms associated with immune activation, increased eotaxin levels and neurotoxic TRYCAT levels.

The fourth major finding of this study is that Gram-negative bacteria coupled with lower IgM to MDA and azelaic acid are, together, strong predictors of deficit schizophrenia and negative symptoms above and beyond the effects of each factor alone. Natural IgM to MDA and azelaic acid are natural antibodies, which are produced by B1 cells and play a key role in protection and remediation of infections by neutralizing invading pathogens (Rothstein et al., 2013). These natural antibodies are constitutively secreted by B1 cells even without antigen presentation and as such constitute a "pre-existing shield" protecting against infections "in the lag time for adaptive antibody production" (Rothstein et al., 2013; Aziz et al., 2015). Thus, recovery from different types of infections depends heavily upon these poly- and autoreactive B1 natural autoantibodies (Rothstein et al., 2013). Besides their key role in antimicrobial defenses these natural IgM autoantibodies also have homeostatic properties by clearing apoptotic cells and potentially inflammatory (Aziz et al., 2015) and oxidative (Roomruanwong et al., 2018a) molecules. This may explain that mice lacking natural IgM are more prone to infectious, immune-inflammatory and

oxidative stress-related disorders, including infections with *Streptococcus pneumoniae* and influenza virus, arteriosclerosis and autoimmune disorders (Aziz et al. 2015). As such, lower natural IgM to OSEs in deficit schizophrenia are accompanied by a greater impact of Gram-negative bacteria on negative symptoms, psychosis, excitation and neurocognitive impairments. Nevertheless, it should be stressed that our study does not indicate any involvement of Gram-negative bacteria in the pathophysiology of schizophrenia per se: the effects of Gram-negative bacteria only appear in people with low natural IgM and therefore their effects are confined to the deficit phenotype.

The main limitation of the study is that we used a case-control design which does not allow to establish firm causal inferences. Secondly, it would have been even more informative if we had examined IgA/IgM responses to other Gram-negative gut commensal bacteria. The findings pertaining the ROC analysis deserve replication in an independent cohort.

Figure 2 shows a summary of the findings in deficit schizophrenia. Trigger factors including infections may activate the IRS and the CIRS (Roomruangwong et al., 2018b). Nevertheless, when there are pre-existing deficits in the CIRS, including lowered levels of Clara Cell protein (CC16) and soluble interleukin-2 receptor (sIL-2R) and soluble tumor necrosis factor receptor (TNFR1/R2), the immune-regulatory CIRS functions may be insufficient thereby increasing risk towards detrimental effects of an activated IRS (Roomruangwong et al., 2018b). The latter coupled with ensuing oxidative processes may affect long interspersed element-1 (LINE-1) partial methylation patterns, which in turn may cause more profound aberrations in neuro-oxidative and neuro-immune pathways (Kalayasiri et al., 2018). Many IRS products have cytotoxic and neurotoxic effects, which may cause neuroprogression and, consequently, schizophrenia phenomenology (Roomruangwong et al., 2018b). These products include M1macrophagic-related

cytokines such as IL-1, TNF-α and IL-6; Th-1 products such as interferon-γ, IL-12 and picolinic acid (PA), xanthurenic acid (XA) and 3-OH-kynurenine (3HK); and Th-2 products such as IL-4, IL-5, IL-13 and CCL-11 (eotaxin) (Kanchanatawan et al., 2018a; Sirivichayakul et al., 2018a; 2018b; Roomruanwong et al., 2018b). However, pre-existing deficits in B1 cell functions including lower natural IgM (Maes et al., 2018), are accompanied by increased neurotoxic effects of Gram-negative bacteria, quinolinic acid (QA) and nitrosylated and nitrated proteins (Kanchanatawan et al., 2018a; Maes et al., 2018). All those factors together may contribute to negative symptoms, psychosis, excitation and neurocognitive deficits thereby shaping the deficit phenotype.

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Conflict of interest

The authors have no conflict of interest with any commercial or other association in connection with the submitted article.

Author’s contributions

All the contributing authors have participated in the manuscript. MM and BK designed the study. BK recruited patients and completed diagnostic interviews and rating scale measurements. MM

carried out the statistical analyses. All authors (BK, MM, SS and AC) contributed to interpretation of the data and writing of the manuscript. All authors approved the final version of the manuscript.

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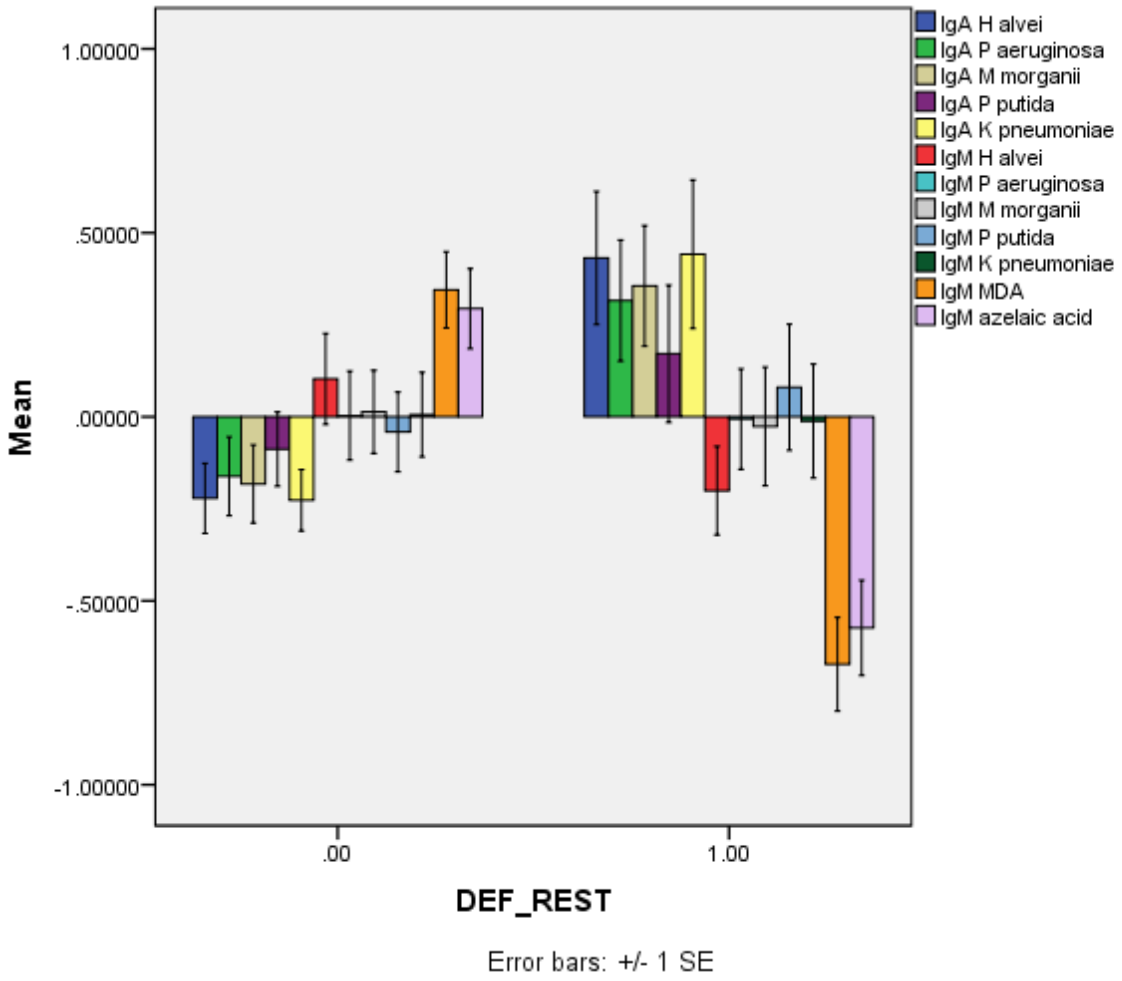

Figure 1. Measurements of IgA and IgM levels (in z transformations) to different Gram-negative bacteria and natural IgM to malondialdehyde (MDA) and azelaic acid in patients with deficit schizophrenia (1) versus patients with non-deficit schizophrenia and controls (0).

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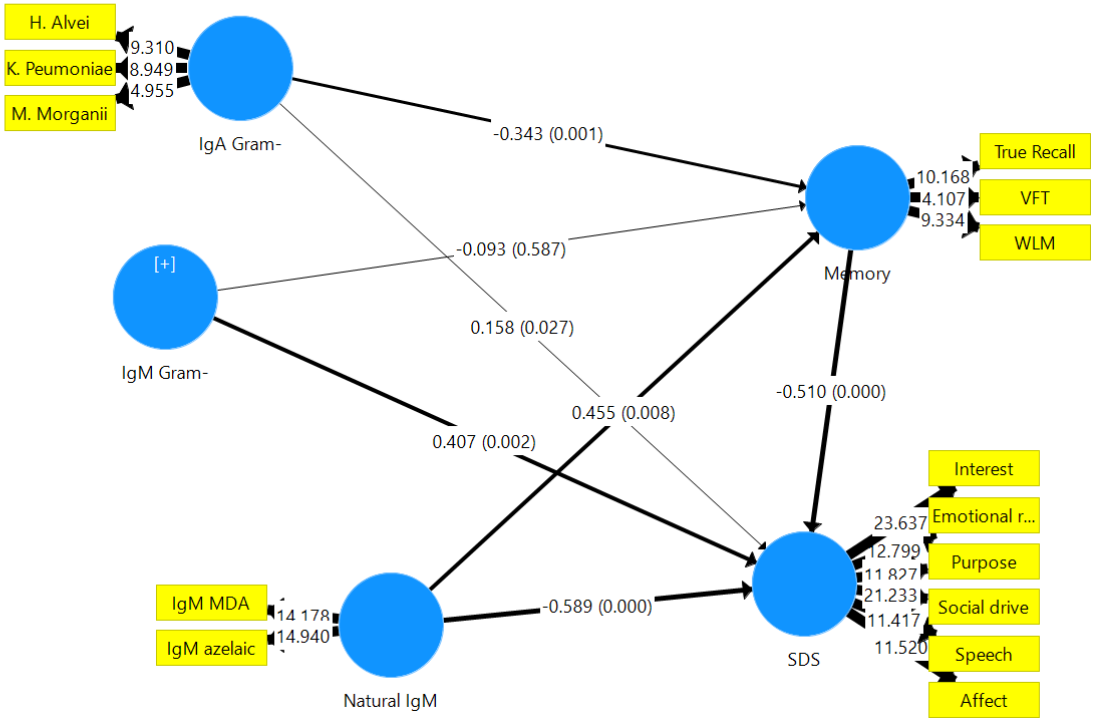

Figure 2. Results of PLS path modelling with a latent vector (LV) extracted from the 6 SDS symptoms as outcome variable and as explanatory variables: a) a LV extracted from verbal fluency test (VFT), word list memory (WLM) and true recall (named "memory"); b) a LV extracted from IgA to Gram-negative bacteria (named: "IgA Gram-"), c) IgM to sum of 5 Gram- bacteria (named "IgM Gram-"), and d) a LV extracted from IgM to malondialdehyde (MDA) and azelaic acid (named "Natural IgM). Shown are p coefficients (with exact p values) for the inner model, and t-values for the outer model.

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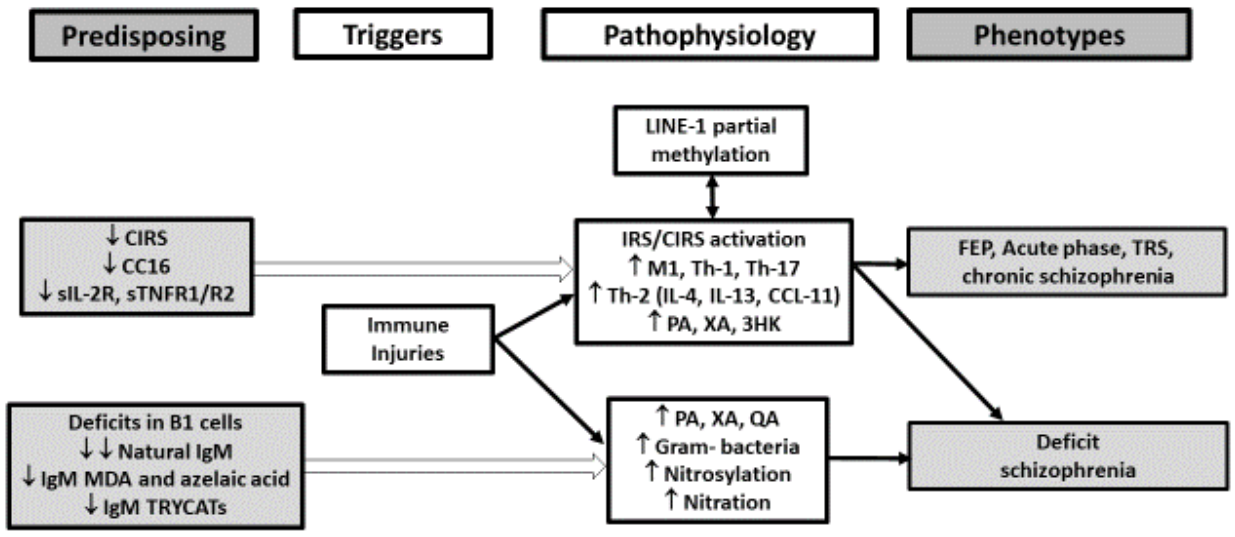

THE IRS-CIRS THEORY OF DEFICIT SCHIZOPHRENIA

Figure 3. The IRS (immune-inflammatory response system) and CIRS (compensatory immune-regulatory system) theory of deficit schizophrenia. See Conclusions to the paper for abbreviations.

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Table 1. Socio-demographic, clinical and biomarker data in subjects with higher versus lower IgA responses to Gram-negative bacteria.

Variables	zsumIgA < -0.0772019 (n=59)	zsumIgA ≥ -0.0772019 (n=59)	F/X^2/Ψ	df	p
Age (years)	38.0 (11.7)	41.9 (11.6)	3.20	1/116	0.076
Gender (M/F)	29/30	37/22	2.20	1	0.138
Education (years)	13.5 (3.9)	12.3 (5.0)	2.03	1/116	0.157
Single / married / separated	41/13/4	41/11/6	0.57	2	0.753
TUD (N/Y)	55/4	56/3	Ψ=-0.036	-	0.697
BMI (kg/m^2)	24.6 (5.2)	23.9 (4.5)	0.58	1/111	0.447
Number of psychotic episodes	2.3 (2.8)	1.9 (2.6)	0.53	1/111	0.470
SDS total score	3.4 (5.1)	5.7 (6.2)	4.70	1/114	0.032
PANSS negative	13.2 (9.1)	17.4 (11.0)	5.06	1/115	0.026
PANSS positive	11.7 (7.5)	12.3 (6.6)	0.27	1/115	0.604
Psychosis	-0.129 (0.953)	0.160 (1.039)	2.45	1/115	0.120
Hostility	-0.015 (1.011)	0.036 (1.005)	0.08	1/115	0.780
Excitement	-0.127 (0.937)	0.154 (1.053)	2.36	1/116	0.128
Mannerism	-0.094 (0.978)	0.121 (1.026)	1.35	1/115	0.248
HC / Nondeficit / deficit SCZ	20/26/13	18/14/27	8.61	2	0.014
IgM MDA (z scores)	0.005 (1.028)	-0.005 (0.980)	0.00	1/116	0.953
IgM azelaic acid (z scores)	-0.070 (1.045)	0.070 (0.957)	0.57	1/116	0.451
BNT	14.0 (1.2)	12.9 (1.7)	16.83	1/115	<0.001
VFT	22.4 (6.9)	19.5 (7.8)	4.35	1/116	0.039

MMSE	27.1 (2.9)	26.1 (3.9)	2.55	1/116	0.113
WLM	19.6 (5.5)	17.2 (5.4)	5.61	1/116	0.019
True Recall	7.2 (2.2)	6.4 (2.2)	4.13	1/116	0.045

All results are shown as mean (±SD).

$F/X^2/\Psi$: results of analyses of variance (F) or analyses of contingency analyses (X^2) or Ψ coefficient;

TUD: tobacco use disorder; BMI: body mass index;

SDS: total score on the Schedule for Deficit Syndrome; PANSS: total score on the Positive and Negative Syndrome Scale; BPRS: Brief Psychiatric Rating Scale

Psychotic dimension: computed as z score PANSS P1 (delusion) (zP1) + zP3 (hallucinations) + zP6 (suspiciousness) + zBPRS11 (suspiciousness) + zBPRS12 (hallucinatory behavior) + BPRS15 (unusual thought content); Hostility dimension: computed as zP7 (hostility) + zPANSS general14 (zG14, poor impulse control) + zBPRS10 (hostility) + zBPRS14 (uncooperativeness); Excitement-grandiosity dimension: computed as zP14 (excitement) + zP5 (grandiosity) + zBPRS8 (grandiosity) + zBPRS17 (excitement); Mannerism-posturing dimension: computed as zG5 + zBPRS7 (both mannerism and posturing);

HC: healthy controls / non-deficit schizophrenia / deficit schizophrenia;

IgM MDA and azelaic acid: IgM antibody titers against malondialdehyde and azelaic acid;

BNT: Boston naming test; VFT: verbal fluency test; MMSE: Mini mental State Examination; WLM: Word List Memory.

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Table 2. Results of multinomial logistic regression analysis with diagnosis (into three groups) as dependent variable and IgA/IgM responses to Gram-negative bacteria as explanatory variables. Groups are: healthy controls (HC) and patients with (Def) and without (Non-Def) deficit schizophrenia.

Independent Variables	Nagelkerke X^2, df, p	Dichotomies	Wald	df	p	OR	95% CI intervals
IgA *H. Alvei*	0.132 X^2=14.75, df=2, p=0.001	Non-Def / HC Def / HC Def / Non-Def	2.52 4.25 11.27	1 1 1	0.112 0.039 0.001	0.63 1.77 2.81	0.36 – 1.12 1.03 – 3.04 1.54 – 5.14
IgA *P. aeruginosa*	0.064 X^2=6.86, df=2, p=0.032	Non-Def / HC Def / HC Def / Non-Def	0.59 3.05 6.05	1 1 1	0.443 0.081 0.014	0.84 1.52 1.82	0.53 – 1.32 0.95 – 2.45 1.13 – 2.94
IgA *M. morganii*	0.093 X^2=10.24, df=2, p=0.006	Non-Def / HC Def / HC Def / Non-Def	2.33 2.62 8.84	1 1 1	0.127 0.105 0.003	0.68 1.47 2.17	0.41 – 1.12 0.92 – 2.35 1.30 – 3.62
IgA *K. pneumoniae*	0.142 X^2=15.88, df=2, p<0.001	Non-Def / HC Def / HC Def / Non-Def	3.40 3.96 11.83	1 1 1	0.065 0.047 0.001	0.56 1.67 2.97	0.30 – 1.04 1.01 – 2.75 1.60 – 5.52
zsumIgM	0.560 X^2=81.21, df=8, p<0.001	Non-Def / HC Def / HC Def / Non-Def	0.02 16.57 15.46	1 1 1	0.900 <0.001 <0.001	1.04 10.54 10.12	0.55 – 1.96 3.39 – 32.75 3.19 – 32.08
IgA *P. putida*		Non-Def / HC Def / HC	5.06 4.95	1 1	0.024 0.026	0.52 2.57	0.29 – 0.92 1.12 – 5.89

		Def / Non-Def	12.64	1	<0.001	4.94	2.05 – 11.91
IgM MDA		Non-Def / HC	1.07	1	0.300	1.46	0.71 – 2.98
		Def / HC	9.24	1	0.002	0.12	0.03 – 0.47
		Def / Non-Def	12.12	1	<0.001	0.08	0.02 – 0.34
IgM azelaic acid		Non-Def / HC	0.00	1	0.995	0.99	0.46 – 2.15
		Def / HC	8.38	1	0.004	0.12	0.03 – 0.50
		Def / Non-Def	7.98	1	0.005	0.12	0.03 – 0.52

OR: Odd's ratio, 95%CI: 95% confidence intervals with upper and lower limits

zsumIgM: composite score computed as sum of all z transformations of the IgM responses to the 5 Gram-negative bacteria assayed in our study; IgM MDA and azelaic acid IgM antibody titers against malondialdehyde and azelaic acid;

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Table 3. Results of binary logistic regression analyses with deficit schizophrenia (Def) as dependent variable and the IgA/IgM responses directed to Gram-negative bacteria and IgM titers to malondialdehyde (MDA) and azelaic acid as explanatory variables.

Dependent variables	**Nagelkerke Model X^2**	**Significant explanatory variables**	**B (SE)**	**W**	**df**	**p**	**OR**	**95% CI**
#1. Def/rest	0.651	IgA *P. putida*	1.22 (0.41)	8.91	1	0.003	3.38	1.52 – 7.52
	75.00, df=4, <0.001	zsumIgM	2.34 (0.56)	17.38	1	<0.001	10.36	3.45 – 31.11
		IgM MDA	-2.28 (0.64)	11.20	1	0.001	0.10	0.03 – 0.39
		IgM azelaic acid	-2.12 (0.72)	8.81	1	0.003	0.12	0.03 – 0.49
#2. Def/No-Def	0.785	zsumIgM	2.99 (0.82)	13.36	1	<0.001	19.88	4.00 – 98.75
	71.03, df=4, <0.001	IgM MDA	-3.09 (1.06)	8.52	1	0.004	0.05	0.01 – 0.36
		IgA *H. alvei*	2.00 (0.67)	8.91	1	0.003	7.39	1.99 – 27.50
		IgM azelaic acid	-2.00 (0.87)	5.28	1	0.022	0.14	0.03 – 0.75

Def/rest: the logistic regression analysis are performed with deficit schizophrenia (Def) as dependent variable and no-def or rest (controls + no-def) as reference group;

zsumIgM: composite score computed as sum of all z transformations of the IgM responses to the 5 Gram-negative bacteria assayed in our study;

OR: Odds ratio, 95% confidence intervals (CI).

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Table 4. Results of stepwise multiple regression analyses with severity of schizophrenia symptoms as dependent variables and IgA/IgM responses to Gram negative bacteria and IgM isotype titers to malondialdehyde (MDA) and azelaic acid as explanatory variables.

Dependent Variables	Explanatory variables	BE (SE)	t	p	R2	Model F	df	p
SDS	IgM azelaic acid IgA *K. pneumoniae* zsumIgM Education	-4.23 (0.70) 1.52 (0.48) 2.34 (0.71) -0.31 (0.12)	-6.05 3.16 3.31 -2.48	<0.001 0.002 0.001 0.015	0.482	17.00	4/73	<0.001
Restricted affect	IgM azelaic acid zsumIgM Education IgA *P. aeruginosa*	-0.80 (0.15) 0.47 (0.15) -0.06 (0.03) 0.22 (0.11)	-5.34 3.12 -2.18 2.05	<0.001 0.003 0.032 0.044	0.351	9.87	4/73	<0.001
Diminished emotional range	IgM azelaic acid Education zsumIgM	-0.89 (0.16) 0.36 (0.16) 0.33 (0.16)	-4.88 -3.12 3.04	<0.001 0.003 0.003	0.329	12.09	3/74	<0.001
Poverty of speech	IgM MDA IgA *M. morganii* Education	-0.44 (0.09) 0.32 (0.10) -0.05 (0.02)	-4.81 3.31 -2.32	<0.001 0.001 0.023	0.359	13.80	3/74	<0.001
Curbing of interest	IgA *K. pneumoniae* IgM azelaic acid zsumIgM	0.37 (0.10) -0.71 (0.14) 0.43 (0.14)	3.86 -4.98 2.97	<0.001 <0.001 0.004	0.388	15.65	3/74	<0.001
Diminished sense of purpose	IgA *K. pneumoniae* IgM azelaic zsumIgM	0.37 (0.11) -0.60 (0.16) 0.34 (0.16)	3.43 -3.70 2.13	0.001 <0.001 0.037	0.290	10.06	3/74	<0.001

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Diminished social drive	IgM azelaic zsumIgM IgA *K. pneumoniae*	-0.92 (0.16) 0.57 (0.16) 0.38 (0.11)	-5.60 3.49 3.41	<0.001 0.001 0.001	0.403	16.68	3/74	<0.001
PANSS positive	-							
PANSS negative	IgA *K. pneumoniae* IgM azelaic acid zsumIgM	2.78 (0.91) -6.70 (1.30) 4.29 (1.23)	-5.18 3.48 -2.22	<0.001 0.001 0.030	0.415	13.10	4/74	<0.001
Psychotic symptoms	IgA *K. pneumoniae* Education	0.27 (0.10) -0.05 (0.03)	2.68 -2.10	0.009 0.039	0.168	7.67	2/76	0.001
Hostility	-							
Excitation	IgA *K. pneumoniae* IgM azelaic acid Education	0.28 (0.09) -0.27 (0.10) -0.06 (0.02)	3.05 -2.66 -2.56	0.003 0.010 0.012	0.266	9.20	3/76	<0.001
Mannerism	-							

*All dependent variables were entered as z-scores (the IgM data to MDA and azelaic acid were first Ln transformed)

zsumIgM: composite score computed as sum of all z transformations of the IgM responses to the 5 Gram-negative bacteria assayed in our study;

SDS: total score on the Schedule for Deficit Syndrome; PANSS: total score on the Positive and Negative Syndrome Scale;
BPRS: Brief Psychiatric Rating Scale. Psychotic dimension: computed as z score PANSS P1 (delusion) (zP1) + zP3 (hallucinations) + zP6 (suspiciousness) + zBPRS11 (suspiciousness) + zBPRS12 (hallucinatory behavior) + BPRS15 (unusual thought content); Hostility dimension: computed as zP7 (hostility) + zPANSS general14 (zG14, poor impulse control) + zBPRS10 (hostility) + zBPRS14 (uncooperativeness); Excitement-grandiosity dimension: computed as zP14 (excitement) + zP5 (grandiosity) + zBPRS8 (grandiosity) + zBPRS17 (excitement); Mannerism-posturing dimension: computed as zG5 + zBPRS7 (both mannerism and posturing);

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Table 5. Results of multiple regression analyses with neurocognitive tests as dependent variables and IgA/IgM responses Gram-negative bacteria as primary explanatory variables.

Dependent Variables	Explanatory variables*	BE (SE)**	t	p	Model R^2	Model F	Model df	Model p
BNT	Education IgA *K. pneumoniae*	0.10 (0.02) -0.22 (0.10)	3.99 +2.22	<0.001 0.029	0.211	10.17	2/76	<0.001
MMSE	Education IgM MDA IgA *P. putida*	0.51 (0.08) 0.72 (0.32) -0.59 (0.32)	6.60 2.28 -1.84	<0.001 0.026 0.069#	0.406	17.33	3/76	<0.001
VFT	IgA *K. pneumoniae*	-2.86 (0.08)	-3.21	0.002	0.117	10.33	1/78	0.002
WLM	Education IgM MDA IgA *K. pneumoniae*	0.09 (0.02) 0.24 (0.08) -0.20 (0.08)	4.30 2.85 -2.42	<0.001 0.006 0.018	0.337	12.89	3/76	<0.001
IgM MDA	zsumIgM Age	0.55 (0.09)	5.93	<0.001	0.323	29.22	1/78	<0.001
IgM azelaic acid	zsumIgM	0.64 (0.08)	8.13	<0.001	0.459	66.07	1/78	<0.001

* Entered are also age and sex

**All dependent variables were entered as z-scores (the IgM data were first Ln transformed)

MDA: malondialdehyde; zsumIgM: composite score computed as sum of all z transformations of the IgM responses to the 5 Gram-negative bacteria assayed in our study;

#Significant after bootstrapping (n=1000), see Results section