Author’s response to reviews

Title: A step beyond the hygiene hypothesis – immune-mediated classes determined in a population based study

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Author’s response to reviews:
General comments

We are very grateful for the careful reading and supportive remarks of the reviewers. Their suggestions helped us to improve the text.

Reviewer #1:

This is an interesting paper setting out to (i) to develop an integrated approach to immune system programming by classifying comorbidity patterns of childhood infections and atopic diseases together with adverse childhood events using the latent class analysis (LCA) as the statistical model, and (ii) to characterize the immune-mediated classes established based on white blood cell (WBC) counts and inflammatory markers as well as their association patterns with other groups of diseases and sociodemographic variables. The database came from CoLaus|PsyCoLaus, a large epidemiological study in Switzerland, in which 6734 35-75-year-old subjects took part in the baseline examination in 2003-2006 and 5064 in the second survey in 2009-2012. The authors report that sharing a specific immune-mediated class different from the neutral class [resilient, atopic, mixed or adverse childhood experiences (ACE class)] modifies the risk for most chronic inflammatory diseases and neurodevelopmental / mental disorders. In particular, the mixed class emerged as a new and unanticipated phenomenon. The LCA analysis was based on infectious childhood diseases, atopic diseases and childhood adversities and came up with five classes.

Comments:mpact

1. What is the evidence for the statement on page 25 that immune-mediated classes consolidate during the first years of life? What is the impact later immunological insults?

This is indeed one of the most intriguing questions that result from this analysis. We discussed this question on page 22 and 23 (section "Time of onset of childhood infections and timing issues"). Since the frequencies and the age of onset of childhood infections are known, they serve for a preliminary orientation. They allow to conclude whether a shift towards a specific class other than the neutral class is already present or not. Decreased frequencies of childhood infections such as chickenpox, measles or mumps (CMM) are most clearly prominent in the
resilience class and indicate that a protective mechanism is already active. In the mixed class, we found increased frequencies for some infections (scarlet, pertussis, rubella) again indicating that a specific immune vulnerability is already established. The shifts seem to be less progressed in the ACE and the atopic class, since they are only partly reflected in the CMM infections. As further discussed, it is not possible to conclude from our data whether class changes and different sequences of class membership occur. Indirect evidence suggests that the differentiation of immune-mediated classes might end by adolescence.

3. All inflammatory markers analyzed are proinflammatory measures, and no anti-inflammatory markers. e.g. IL-10 have been analyzed. The authors had no possibility to influence the selection of immunological markers in the current setting, but in the Discussion section it would be appropriate to discuss the selection of immunological markers and its potential impact on the outcome of the LCA.

Thank you for this hint. We added the following remark in the second paragraph of the Discussion section:

"In addition, the range of inflammatory markers that served to characterize the classes was limited. The selection focused on markers that were known and were intensively discussed at the beginning of the study in the early 2000 years. In the meantime, the range of inflammatory markers of interest has impressively grown and includes both anti-inflammatory markers (e.g. IL-10) and pro-inflammatory markers (e.g. IL-8, IL-23). It is left to subsequent studies to examine their interplay in the different classes."

Reviewer #3:

The report submitted by Ajdacic-Gross et al. presents a novel comprehensive approach to better characterize the complex network of immune programming in childhood in the context of infectious, atopic and mood disorders based on a latent class analysis (LCA) in males and females, separately. Data were obtained from CoLaus/PsyColaus a population based study conducted in Switzerland. LCA revealed five major classes corresponding to a resilient, neutral, atopic, infectious/atopic and an ACE (adverse childhood experiences) type.
General comment.

LCA emerged within the last years as a potent statistical method to characterize clinically relevant pattern in large study populations. LCA thereby serves as tool to better understand the so far latent network between basic physiological pathways and clinical signs, somatic as well as psychiatric manifestations. The report is thereby timely and ambitioned. Based on the postulates of the hygiene hypothesis, this report aims to contribute to the ongoing discussion of common roots in somatic and psychiatric disorders mediated by the immune system and stimulated by exogenous exposures such as ACE and infections. It is not surprising that the LCA confirms the currently mostly accepted opinion of a relationship between early immune programming and later disease development. Moreover, the immune mediated classes identified and comprehensively discussed in this report fitting well to the principles of the hygiene hypothesis and subline relationship between chronic inflammatory processes and mood disorders and, vice versa, resilience in association with balanced immune response. In sum, as stated by the authors, the LCA serves as a pattern recognition tool based on the reduction of dimension and thereby might simplify reality. Nevertheless, this report contributes new evidence to the field of early immune programming and disease development.

Special comments: 1. The report is fluently written, the introduction allocates the basic hypothesis and the aim of the study vividly. In the method section the authors concisely describe both questionnaires and statistical operations used in this study. The main part of the results are presented adequately.

Namely, the last paragraph should not be presented in bullets and in more detail as this part is the clinically the most relevant aspect.

We reformulated this paragraph and added further details. However, we tried to avoid anticipating the discussion or more specific analyses. Therefore, the focus is on basic issues such as sexual dimorphism.

The figures should be edited and improved in quality.

In the figures 2 and 3, we changed the thick blue dashed line into a plain thin blue line. Moreover, we moved the hay fever item behind the asthma items so that the curve of the atopic
class is easier to interpret. We also corrected the label text in the figures 6 and 7 (missing "p=" in some instances) and enlarged it by 1 point.

The discussion section highlights the specific attributes of the five classes precisely and well substantiated by literature references. I highly appreciate and support the rationale between the positive association ulcer, H. pylori -infection status and the "Old friends" hypothesis and the decreased susceptibility for mental disorders in the resilient class.

2. The authors should give some brief information about the CoLaus-study, e.g. the survey is based on instruments in French language. Is the study also conducted in German, Italian and/or Rhaeto-Romanic parts?

The study was conducted with the residents of Lausanne, a city in the French part of Switzerland (see the first paragraph of the section on the CoLaus|PsyCoLaus cohort, page 8). The use of languages is more homogeneous in Lausanne than, for example, in Geneva or in German speaking urban parts of Switzerland. We added a remark on the language of the survey:

"The survey was conducted in French language."

In order to better document the differentiation between the CoLaus and PsyCoLaus parts (and subsamples) we more clearly addressed the specific study parts in this paragraph and the sequence of the sample allocation.

3. Were somatic disorders surveyed by explicitly questioning for doctor's diagnosis or just self-reported?

This was described in the section describing the DIGS (third paragraph on page 9). The questions explicitly addressed whether the subject was diagnosed with a specific condition. However, this cannot be taken for granted in all conditions (e.g., childhood infections).

A specific case is migraine that was assessed with the DIHS. We introduced additional text on page 9 to provide this specific information:
"The lifetime prevalence of migraine was assessed according to the criteria of the International Classification of Headache Disorders (ICHD-II), using the validated French version of the Diagnostic Interview for Headache Syndromes (DIHS)."

4. The authors report to assess hsCrP. Why not CrP? As nearly every subject displays low levels of CRP without any signs of inflammation the assessment of high-sensitive CrP is questionable in the clinical and epidemiological context.

The hsCRP was preferred since it is a high sensitive assay which allows to detect changes of CRP levels in the very low range. This is important for a general population study where hsCRP has been associated with cardiovascular disease and other chronic disorders. CRP assay is not as sensitive in the low range as hsCRP and is suitable for clinical situations where the focus is on systemic inflammation.

5. WBC strongly depends on some life-style factors that might act as confounders such as smoking. Were models based on WBC adjusted for common confounders?

Thank you for this suggestion. Since the analyses focus on pattern recognition (see methodological remarks on page 13), we adjusted the analyses neither for repeated testing nor for confounders. The latter is left for subsequent analyses that examine specific associations in more detail, then using regression models or SEM.