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Patients with Crohn's disease exhibit a dysregulated lymphocyte response to corticosteroids: Implications in stress-induced disease aggravation and failure of therapy

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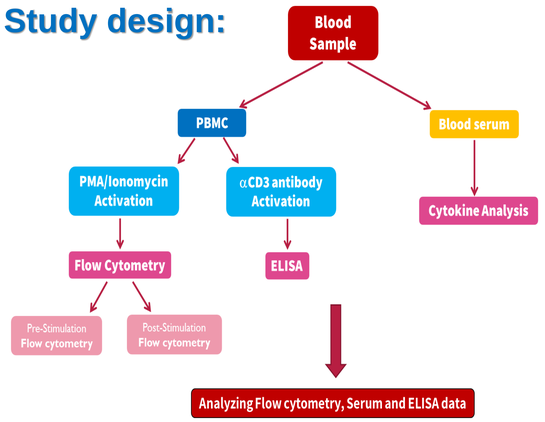
Background:

Crohn's disease (CD) engenders both psychological and physiological stresses. Our previous studies demonstrated that prolonged stress and chronic inflammation cause dysregulation of glucocorticoid (GC) signaling in different immune subsets, with consequent reduced sensitivity to GCs, so-called steroid resistance, and a shift towards a more aggressive immune response. We aim to identify and characterize patterns of steroid resistance in adult CD patients in comparison to healthy controls (HC).

Study design:



Methods:

- We analyzed leukocyte subset functionality and their responsiveness to GCs using peripheral blood mononuclear cells (PBMCs) isolated from CD patients (mean age 35 years, HBI in the range 5–15) and from age and sex matched healthy controls.
- PBMCs underwent stimulation with anti-CD3 without or with methylprednisolone (MP, 1–10,000 ng/ml) followed by measuring secreted levels of IL-2 (24 hours), TNFa (24 hours), IL-10 (48 hours), IFN-γ (48 hours), and IL-17 (72 hours) with ELISA.
- In addition, PBMCs were activated with PMA/ionomycin for 4 hours and analyzed for cytokine production with flow cytometry.

Conflict of Interest: All authors and physician collaborators declare that they have no competing interests to report.

Dose dependent steroid inhibition of cytokine production by PBMCs

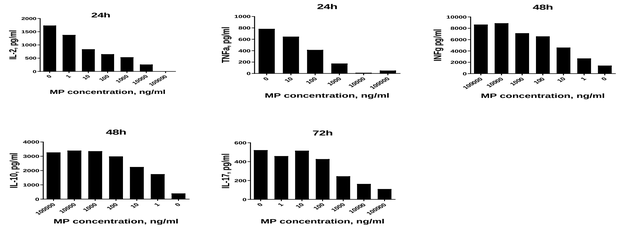


Figure 1: Steroid sensitivity assay was performed with aCD3-stimulated PBMCs. MP in increasing doses (1-100000 ng/ml) was added to the medium. Supernatants were collected 24, 48 and 72 hours following activation and cytokines were measured by ELISA. **MP caused a progressive reduction of PBMC cytokine production.**

Reduction of TNFa-producing cells in the CD8 T-cell population shown within CD patients and HC

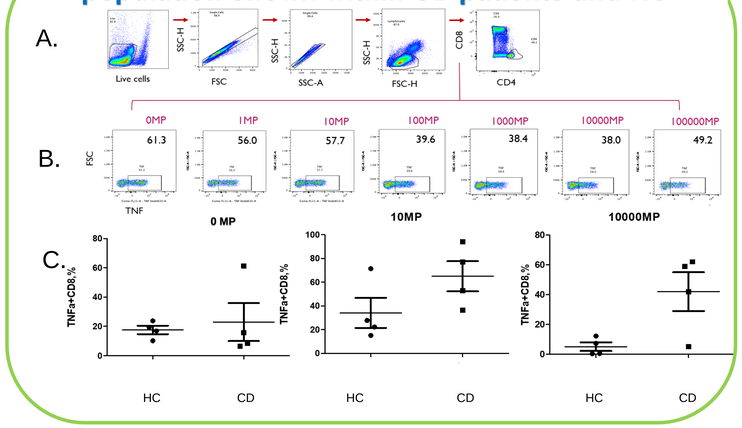


Figure 3: **MP in increasing doses caused a progressive reduction of TNFα producing cells in the CD8 population within healthy controls**.

A. Gating strategy for TNFα production within the CD8 T-cell population.
B. The effect was less pronounced in CD (n=4) than controls (n=4).
C. The difference between CD and HC became statistically significant at MP 10000 and 100000 ng/ml where some CD patients showed steroid resistance.

Results:

Reduced steroid sensitivity in CD patients vs. HC

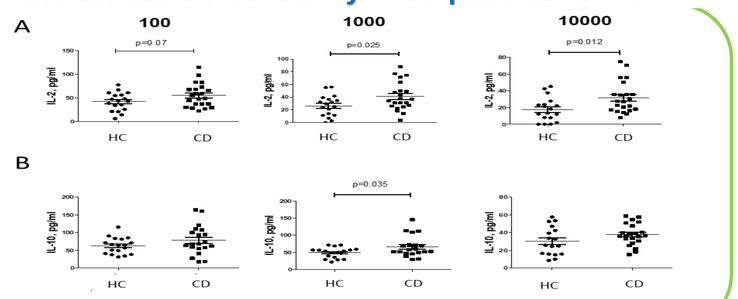


Figure 2: Cytokine production by aCD3-activated PBMCs in presence of different concentrations of MP was compared between CD patients (n=22) and Healthy controls (n=18). Cytokine production for non-inhibited cells was defined as 100% production and the levels in each steroid concentration were compared to it. The graphs represent relative production levels in presence of 100 (left), 1000 (middle) and 1000 (right) ng/ml of MP. **CD patients demonstrated reduced steroid sensitivity**. The difference became statistically significant for IL-2 and IL-10 production.

Cytokines levels in serums of CD patients and HC

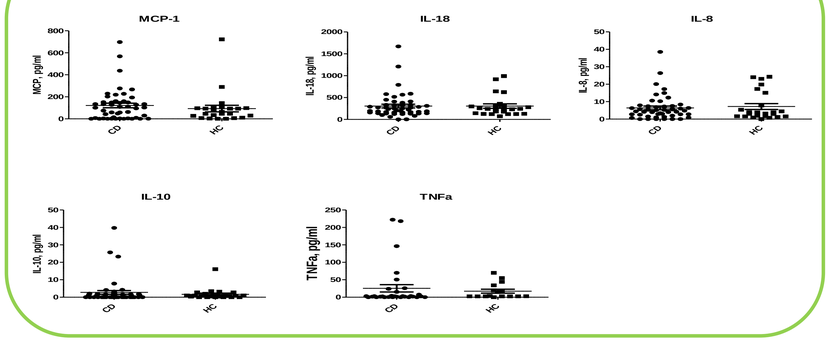


Figure 4: The levels of 13 key inflammatory cytokines were tested in people with CD (n=48) and healthy age- and sex-matched controls (n=24). Except for a few CD patients who had elevated levels of circulating cytokines**, no significant differences were observed between the groups.**

Conclusions

1. Immune cell subsets in certain people with CD exhibit a loss of steroid sensitivity.
2. This may be indicative of a dysregulated hypothalamus-pituitary-adrenal (HPA) axis, a phenomenon which may not only augur an inadequate response to corticosteroid therapy, but also may contribute to CD pathogenicity along with symptoms of anxiety and depression.