Distribution of T-Lymphocyte Subsets and their Cytokines in 2 – 5 Year Old Indian Children with Autism Spectrum Disorder

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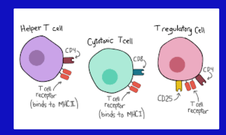
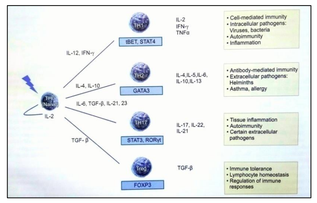


Background

- Autism Spectrum Disorder (ASD) is a common neuro-developmental disorder of children, with a global prevalence of 1.7%, and 0.8-1.1% in India.
- The underlying etiopathogenesis is still unknown.
- Immune dysregulation has been proposed as children with ASD have more infections, allergies, asthma, and eczema than the general population.
- Animal models of pregnant mice and monkeys showed socio-behavioural changes in the offspring (like ASD) after exposure to certain antigens.
- Antenatal triggers may cause ASD in genetically predisposed individuals.
- Studies of individuals with ASD found aberrant T lymphocyte subsets, dendritic cells & cytokines.
- T lymphocytes can be T helper cells (Th 1 & 2) that stimulate immunity, CD25 T regulatory (T reg) cells that suppress immunity, and Th 17 cells that are pro-inflammatory.
- None of the studies have been done in young children.



Objectives

- To measure T-lymphocyte subsets (Th1, Th2, Th17 and T Reg cells) & cytokines (IFN γ, IL 4, IL 17 and TGF β) in 2 – 5 year old Indian children with ASD

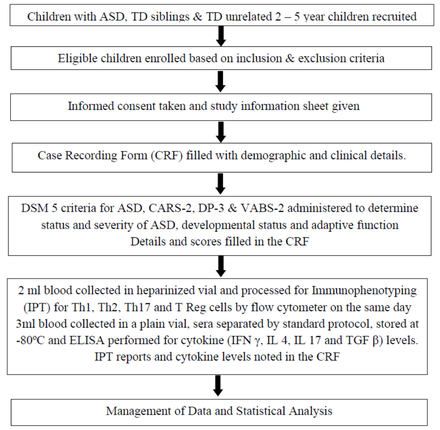
Methodology

- **Study design**: Cross sectional observational study at LHMC, New Delhi (Nov' 2018 – Mar' 2020)
- **Inclusion criteria**: Children between 2-5 years of age diagnosed with ASD (DSM-5 criteria for ASD)
- Typically developing (TD) unrelated 2 – 5 year old's
- TD 2-5 year siblings of children with ASD (No ASD, normal development and adaptive function)
- **Exclusion criteria**: Anyone with acute febrile illness within 2 weeks, on steroids >7 days or vaccinated within 12 weeks, or known immunodeficiency.
- **Sample Size:** 80 ASD based on 4 p (1-p)/ d^2), where prevalence (p) of CD4 (+) CD25 (+) T Reg cells in ASD is 73.3% and margin of error (d) 10%.
- Any number of TD siblings found in study period
- 30 TD unrelated children for baseline T lymphocyte subset and cytokine levels (since no Indian data)
- **Psychometric Evaluation:** DSM 5 criteria for ASD & Childhood Autism rating Scale 2nd edition, Developmental Profile 3rd edition and Vineland Adaptive Behavior Scale 2nd edition
- **Immunological assessment**

i) Flow cytometer (BC 500) for Immunophenotyping (IPT) of T lymphocyte subsets (CD45, CD4, CD8, CD25, CD127, IL 17A and IFN γ)

ii) Standard ELISA kits for cytokine assessment (IFN γ, IL 4, IL 17 and TGF β)

Flow of the study protocol



Results

- **Demographic Profile**
- 80 ASD (54 M, 26F), 10 TD siblings (5M, 5F), & 20 TD (7M, 13 F), unrelated 2–5-year children
- **Psychometric Profile**
- ASD: 87.5% severe, and 2.5% mild to moderate
- **Immunological Profile**
- Dysregulated levels of CD4+25+127- T cells (T reg) and CD4+8+ T cells (cytotoxic) were found in 72 (90%) and 68 (72.5%) cases of ASD
- Children with ASD and siblings had significantly lower % of CD4+ CD25+ 127- T cells (T reg) than TD; 1.28 ± 1.45, 1.10 ± 0.66 and 3.77 ± 2.78.
- Percentage of CD8+ 1L4 T cells (Th2) in cases with ASD (12.74 ± 14.49) were significantly more than TD children (4.18 ± 3.69)

Results…

- Levels of IL 17α was higher in children with ASD (15.279 ± 14.78 pg/ml) compared to siblings (8.309 ± 5.98 pg/ml) and TD children (10.755 ± 8 49 pg/ml), but not statistically significant.
- The level of IFN-γ was significant more in ASD (19.571 ± 10.292%) and siblings (15.216 ± 2.563%), than TD, but normal.
- The level of TGF-β was more in ASD (210.62 ± 208.16 pg/ml) than siblings (126.18 ± 88.96 pg/ml) and TD 186.80 ± 138.74 pg/ml) though not statistically insignificant

Conclusions

- Involvement of T-helper 2 cells & T cytotoxic cells suggests the neuroimmune axis is affected in ASD.
- The significant decrease in T-Reg cells in children with ASD and their siblings indicates a role of the maternal immune system that affects both.
- This supports the hypothesis of an unknown trigger causing ASD due to immune dysregulation in genetically predisposed individuals.
- Whether these T reg cells are naïve or activated, and can be targeted in immunomodulation by restoring the Th 2/ Th 1 pathway imbalance may be targets for further research
- The role of T regs and related cytokines as biomarkers is also worth exploring

All authors have declared no conflict of interest and no financial disclosures

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