Lower regulatory T cell and higher P-gp expression predisposes relapse in Nephrotic Syndrome

Narayan Prasad[1], Akhilesh Jaiswal[1], Vikas Agarwal[2] Brijesh Yadav[1], Mohit Rai[2]

Department of Nephrology[1] and Clinical Immunology[2]

Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, INDIA

E-mail: narayan.nephro@gmail.com, narayan@sgpgi.ac.in

introduction

- Idiopathic Nephrotic syndrome(NS) is one of the most common glomerular disease in children and characterized by loss of permselectivity of glomerular filtration barrier, and massive proteinuria.

  Giuseppe D'Amico et al Kidney International 2003
- Nephrotic children usually respond to glucocorticoids (GC) during the first episode of the disease. However, approximately 50–60% of them experience frequently relapsing or steroid-dependent course.

  Hogg RJ et al PARADE, *Pediatrics 2000*
- Indirect evidences suggest that NS is a consequence of dysfunction of CD4+ T cell function

  Shalhoub RJ, lancet 1974
- Steroid non responsiveness may be because of disturbances in T cell subset population and/ or, because of one of the factors responsible for the acquired steroid resistance that modulate the disease response to pharmacological interventions, such as P-gp expression.

  Higgins CF. Annu Rev Cell Biol 1992
- The expression of P-gp on different immune cells suggest that P-gp may influence cell mediated immune response.

  Klimecki et al Blood 1994
- Worse response to steroid or dependency in NS may be due to over expression of P-gp. Increased P-gp activity may be because of MDR-1 gene polymorphism.

  Wasilewaska et al. Pediatr Nephrol 2006
- Patients of NS carrying homozygous mutants of single nucleotide polymorphism (SNP) G2677T/A are prone to develop steroid resistance.

  Prasad N et al NDT 2011
- P-gp acts as an efflux pump and removes its substrates from inside the cell to outside.
- P-gp appears to be a double edged sword which protects the cells against the accumulation of xenobiotics and toxins in the cytoplasm
- Simultaneously by limiting intra lymphocytic concentration, P-gp is capable of limiting the ability of steroids to bind to their receptors resulting in poor corticosteroid response

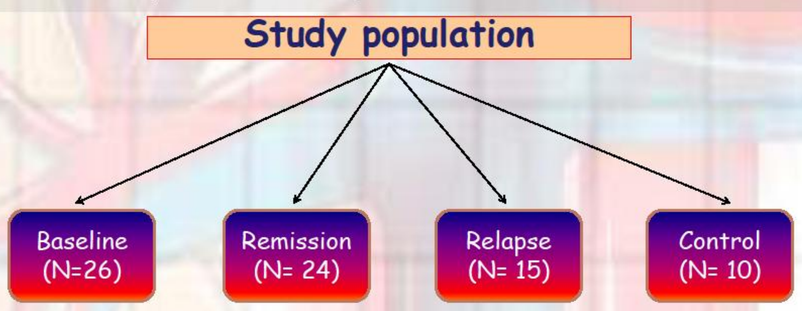
  Arthritis Rheum 2005; 52:1676-1683

objectives

- To identify the frequency of $CD4^+CD25^+FoxP3^+$ T regulatory cells, $CD4^+IFN\text{-}\gamma^+$ Th1 and $CD4^+IL\text{-}4^+$ Th2 cells with their respective regulatory cytokines (Il-10 and TGF-β) and effector cytokines (IFN-γ and IL-4) at baseline before steroid therapy, during remission and at relapse of NS.
- To analyze the expression of P-gp on peripheral blood lymphocytes at baseline, during remission and at relapse of NS .
- To analyze the correlation between P-gp expression and regulatory and effector cytokines in NS patients.

materials and methods

Patients and controls : Total 26 INS patients (age=8±4 years, male=21), 24 patients achieved remission following steroid therapy and15 relapsed on follow up (mean 8.86± 3.9 months) and 10 healthy control children.



All patients were recruited as per criteria of ISKDC

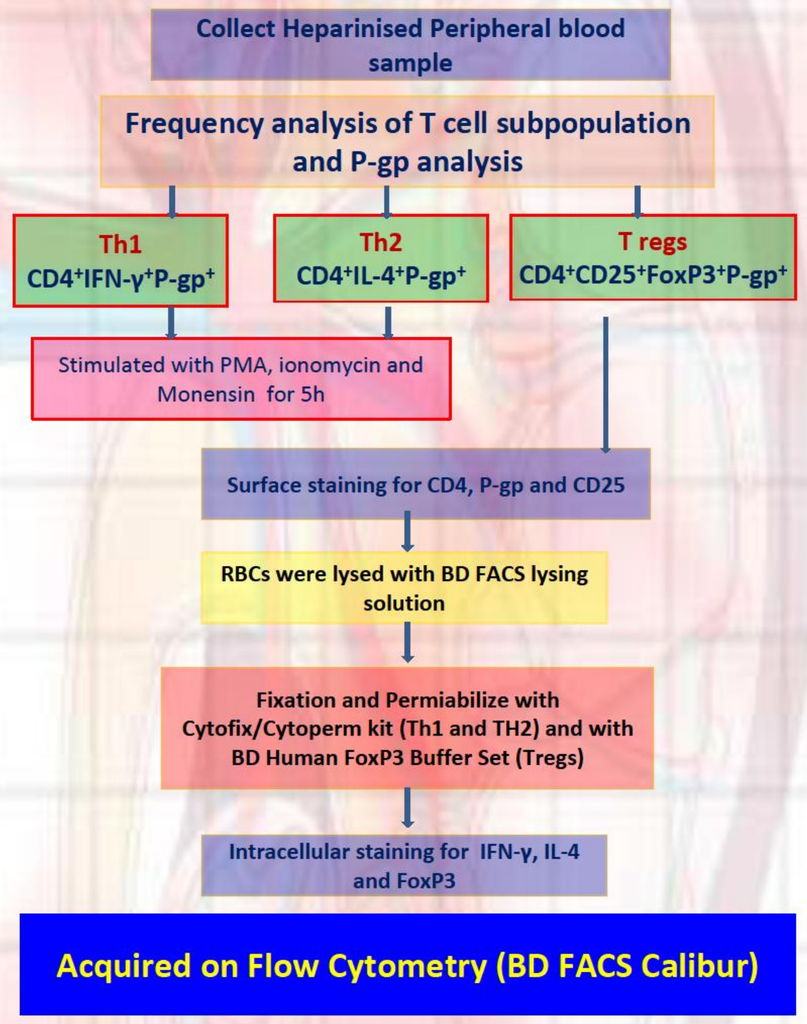
In *vitro* culture of PBMCs and Cytokine Estimation

PBMC isolation by Ficol gradient method

1 x 10^6 cells/ml in media with PMA and ionomycin at 37°C, 5% CO2, 100% humidity culture for 24h

Supernatant elisa for IL-10, IFN-γ, IL-4 and TGF-β

materials and methods



Acquired on Flow Cytometry (BD FACS Calibur)

results

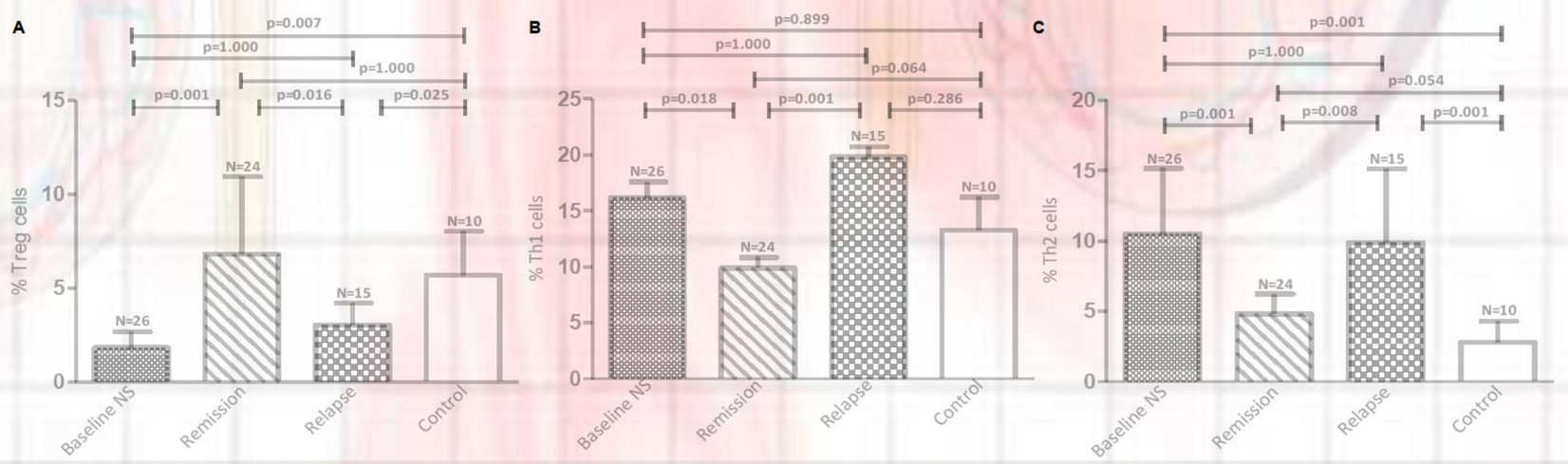


Fig 1 | Results are expressed as the percentage of CD4+CD25+FoxP3+Treg, CD4+IFN-γ+Th1 and CD4+IL-4+Th2 cells in CD4+ lymphocyte in blood. Significant increase in Treg cells at remission as compared to the baseline and healthy controls and again decreased after relapse (**1A)**. Th1 and Th2 cells were significantly decreased in remission as compared to baseline values and healthy controls and again increased at relapse of NS (**1A, 1B)**.

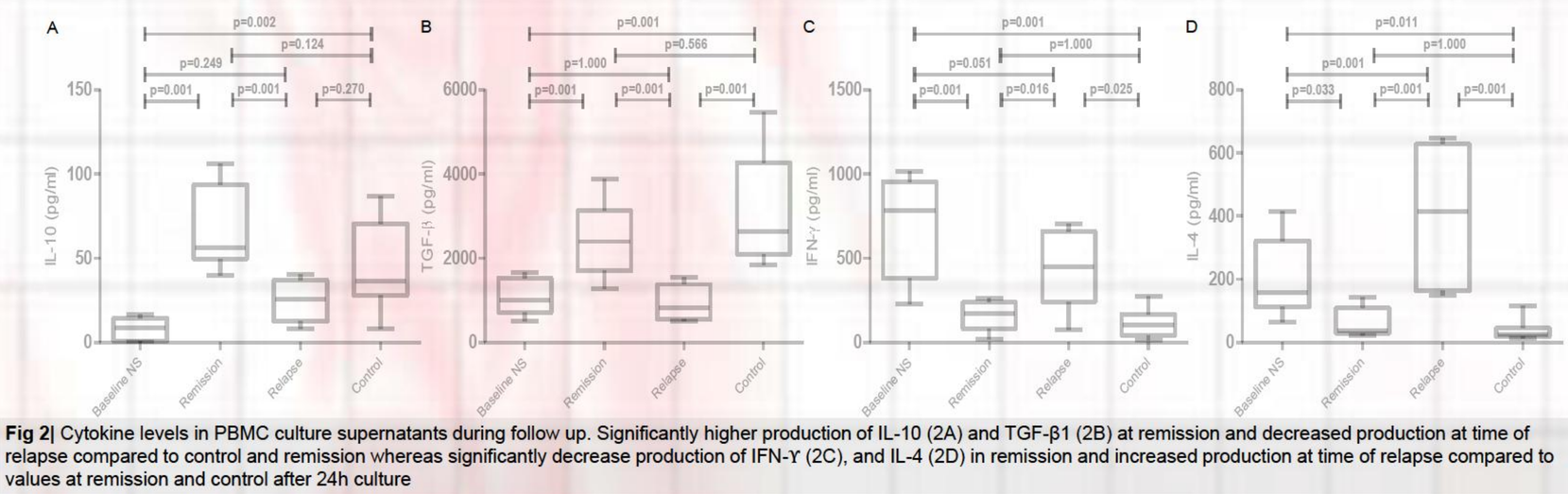


Fig 2| Cytokine levels in PBMC culture supernatants during follow up. Significantly higher production of IL-10 (2A) and TGF-β1 (2B) at remission and decreased production at time of relapse compared to control and remission whereas significantly decrease production of IFN-γ (2C), and IL-4 (2D) in remission and increased production at time of relapse compared to values at remission and control after 24h culture

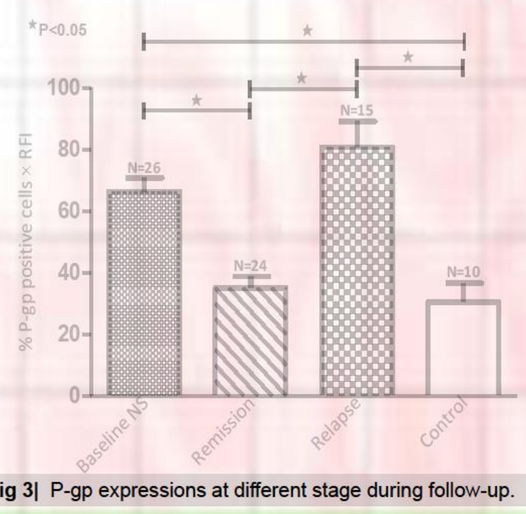


Fig 3| P-gp expressions at different stage during follow-up.

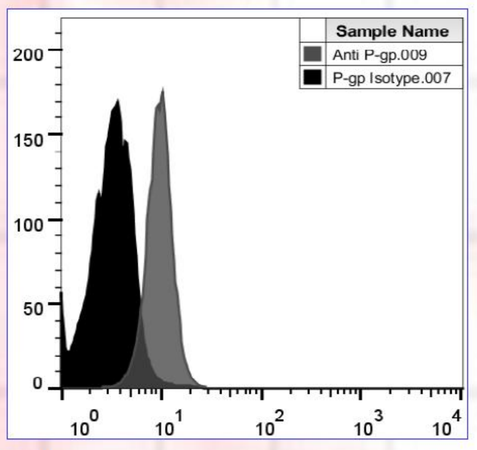


Fig 4| Relative fluorescence intensity (RFI) in one of the representative sample from a patient with resistant NS.

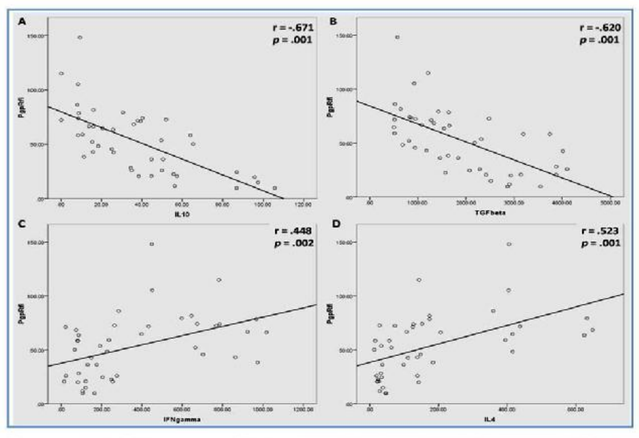


Fig 5| Pearson's Correlation of P-gp expression with different cytokines profiles

conclusions

- ✓ The regulatory T cells and their cytokines was observed to be higher during the remission compared to the values at baseline and during relapse while Th1 and Th2 cells and their cytokine was higher at baseline and during relapse of NS.
- ✓ P-gp expression on lymphocytes was significantly higher at baseline, decreased during remission and again increased at relapse of NS.
- ✓ P-gp expression increases with increase of Teff cells and their cytokines and decreases with decline of Tregs and their cytokines.
- ✓ Monitoring of P-gp expression may be helpful tool in monitoring and treatment of relapsing INS.

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K) Clinical Nephrology, primary and secondary glomerulonephritis.

Narayan Prasad

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