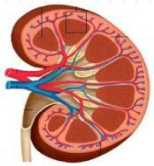

CD4+CD25+Treg cells may be useful for treatment of IgA nephropathy

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Agenda

- Defined of IgA nephropathy(IgAN)
- The relationship between the tonsils and IgAN
- CD4+CD25+Treg cells and their roles
- Hypothesis and designed
- Results and Discussions

Defined of IgAN

IgAN is characterized by the accumulation of IgA deposits, predominantly in the glomerular mesangium, and represents the most common form of glomerulonephritis.

Tonsils were closely related tolgAN.

- Urinary findings were deteriorated after tonsil stimulation in patients with IgAN.
- Tonsillectomy can improve the urinary findings,keep stable renal function, improve mesangial proliferation, decrease IgA deposits and have a favorable effect on long-term renal survival in some IgAN patients

CD4+CD25+Treg cells and their roles

- CD4+CD25+Treg cells are of critical importance to the maintenance of tolerance by inhibiting the activation and proliferation of autoreactive T cells.
- Depletion of the minor CD4+CD25+ Treg cells results in the development of organ-specific autoimmunity.
- Autoimmune diseases can be prevented by reconstitution of the animals with CD4+CD25+Treg cells.
- CD4+CD25+Treg cells are regulators in almost all of the animal models of human organ-specific diseases, transplant rejection and allergic diseases.

Hypothesis

- Some patients with recurrent chronic tonsillitis have not suffered from renal disease, implying that it is possible to find a balance between immunity and tolerance.
- Some patients, however, suffered from IgAN along with recurrent chronic tonsillitis. It could be hypothesized that a numericaland/or functional deficit of CD4+CD25+Treg cells in the tonsils of IgAN patients might trigger the development of the diseases

Desiged

- The lymphocytes were isolated from tonsils of all subjects (including 37 IgAN cases and 37 controls without renal diseases)
- The CD4+CD25+ cells were measured by flow cytometry.
- Expression of J chain mRNA was analyzed by in situ hybridization (ISH) and the dimeric IgA-producing cells were identified by immunofluorescence and fluorescent ISH)

Desiged

- Tonsillar CD4+CD25+ Treg cells were isolated by magnetic beads.
- A total of 2 × 10 6 CD4+CD25+ Treg cells were transferred into rats that were previously orally immunized over a period of 14 weeks and subsequently received an injection of BSA into the tail vein on 3 consecutive days.
- Urine protein and erythrocytes were measured.
- Glomerular injury was assessed by histopathology.
- Plasminogen activator inhibitor type 1 (PAI-1), interleukin (IL)-6 and transforming growth factor (TGF)-β1 in mesangial cells of rats were examined by RT-PCR.

Results

- The number of CD4+CD25+ Treg cells was significantly lower in cases than in controls
- The number of dimeric IgA-producing cells was significantly higher in cases than controls
- The CD4+CD25+ cells number was negatively correlated with that of dimeric IgA-producing cells.

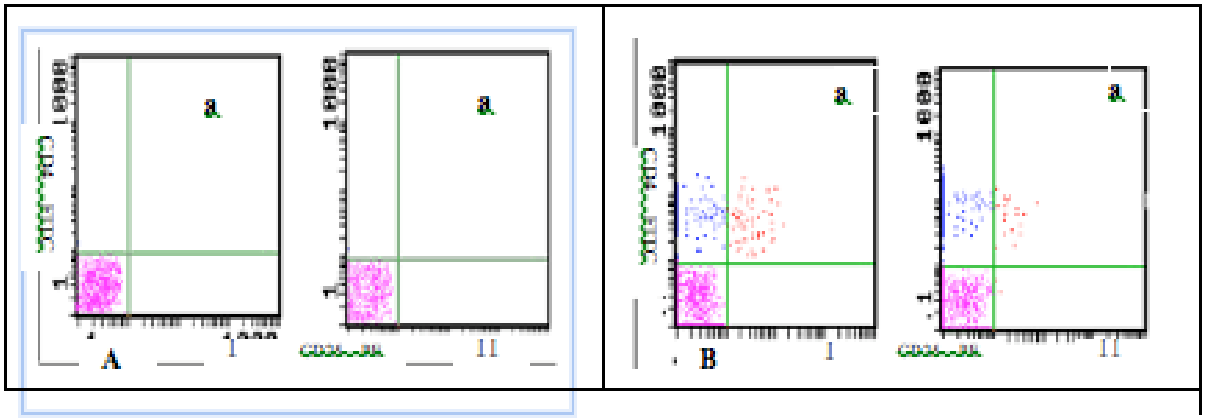
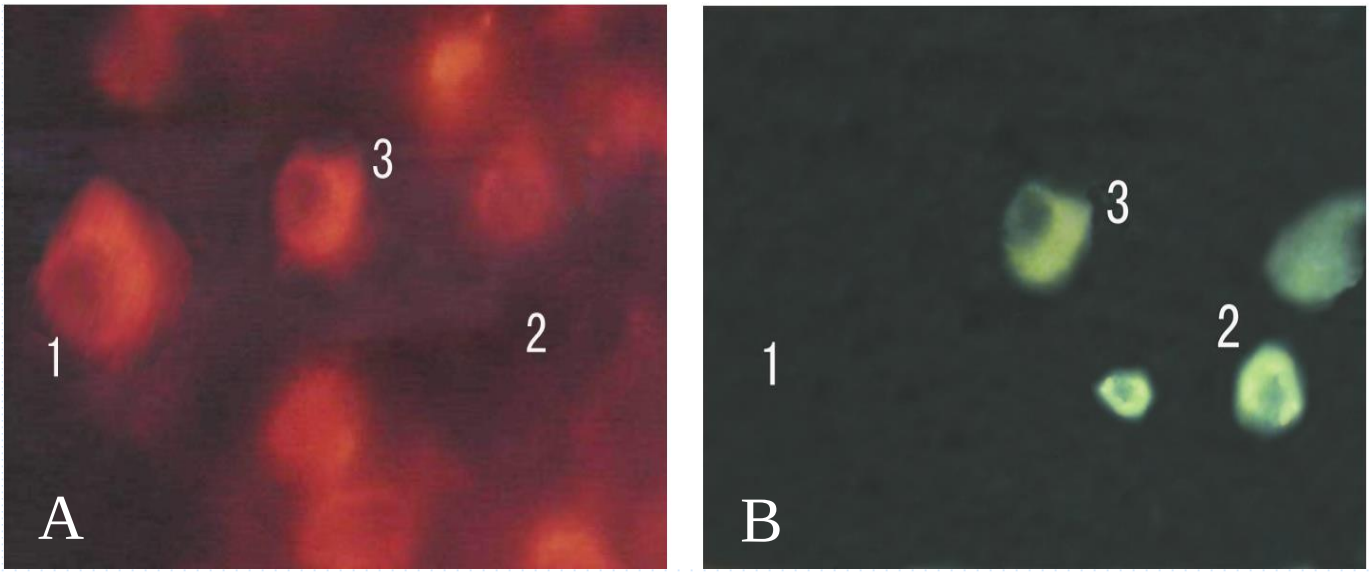
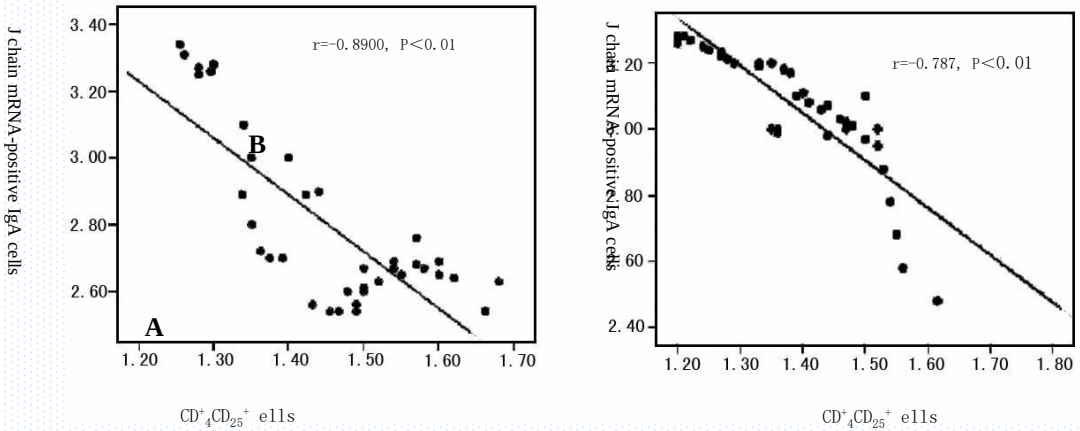


Fig.1 The analysis of CD4+CD25+ total T cell in freshly isolated tonsillar lymphocytes which were gated on lymphocytes flow cytometric method .CD4+CD25+ T regulatory cells were counted using indicated gates. α(CD4+CD25+ T regulatory cells). I: Control patient and II:IgAN patient. A: The negatively analysis of CD4+CD25+ in tonsillar lymphocytes(without monoclonal antibodies anti-human CD4,anti-human CD25) B: The analysis of CD4+CD25+ in tonsillar lymphocytes of IgAN and control patient. The percentage of $CD4^+CD25^+$ cells population were significantly decreased in IgAN patients compared to the patients without renal disease



The analysis of J chain mRNA-positive IgA cells in freshly isolated tonsillar lymphocytes by Combined immunofluorescence and fluorescent ISH
(A). J chain mRNA-positive cells visualized under UV (578nm)
(B). IgA plasma cells visualized under UV (490nm).
Cell 1, IgA-negative(B),J chain mRNA-positive(A);
cell2, IgA-positive (B),J chain mRNA-negative(A);
cell 3, lgA-postive(B),J chain mRNA-positive(A)
The percentage of J chain mRNA-positive cells IgA population were significantly increased in IgAN patients compared to the patients without renal disease.



Tonsilar lymphocytes showed correlations between CD4+CD25+ Treg and J chain mRNA-positive IgA cells.

A:Lymphocytes from patients with IgAN showed negative correlations between CD4+CD25+ Treg and J chain mRNA-positive IgA cells;

B: Lymphocytes from patients without renal disease showed negative correlations between CD4+CD25+ Treg and J chain mRNA-positive IgA cells .

Results

- IgA deposition in the mesangial region and the glomerular planar area and the number of cells from IgAN patients were significantly higher than in rats that received CD4+CD25+ Treg cells from the control group, although they were dramatically lower compared with rats treated without CD4+CD25+ Treg cells.
- levels of PAI-1, IL-6 and TGF-β1 expression in renal mesangial cells of rats that received CD4+CD25+ Treg cells from IgAN patients were significantly higher than in rats that received CD4+CD25+ Treg cells from the control group, although they were dramatically lower compared with rats treated without CD4+CD25+ Treg cells.

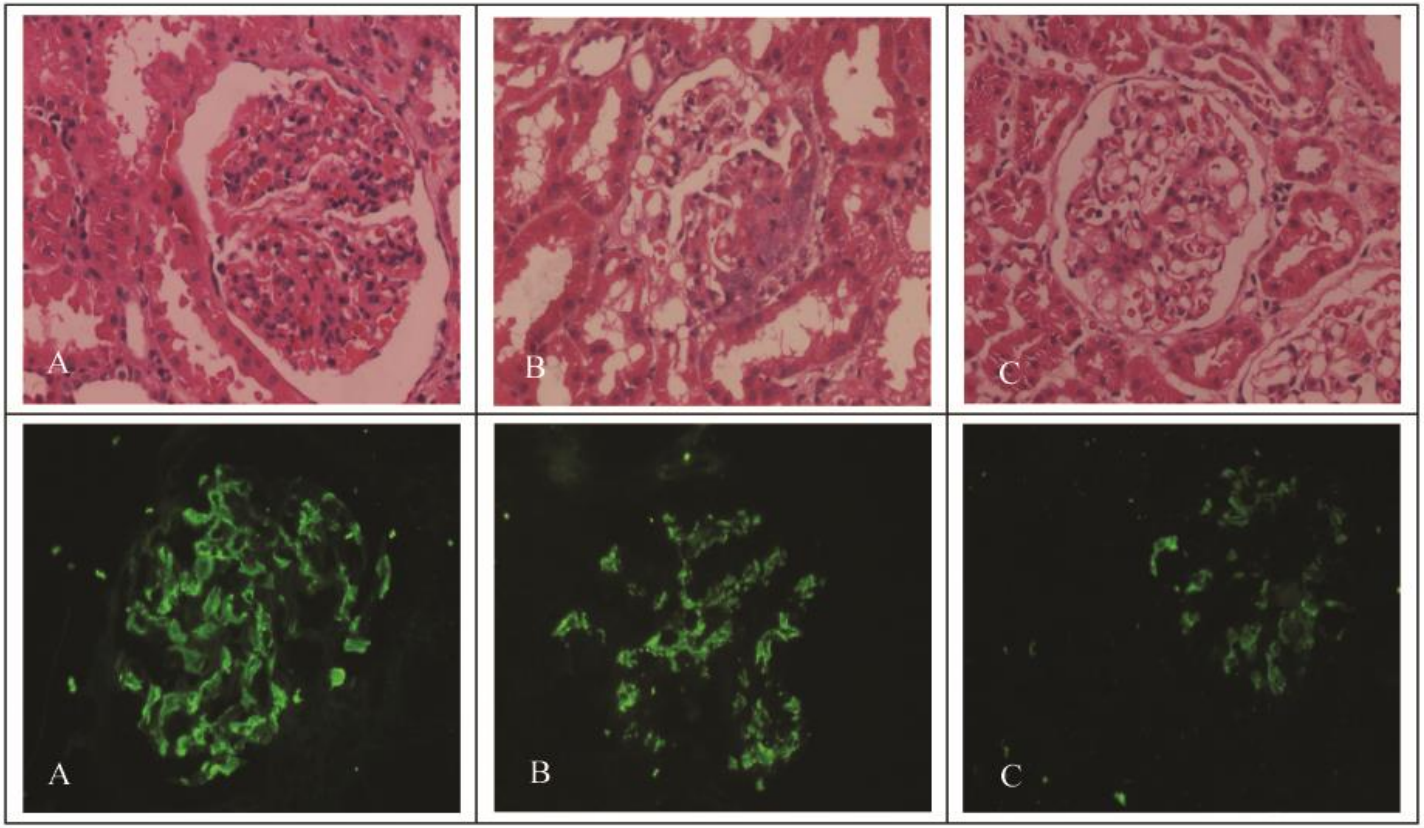


Fig.1 Histopathological findings in rats(40x)

The glomerular planar area and the average number of cells in the glomeuli in IgAN groups rats compared to rats treated with the control groups. The former were significantly increased compared to control groups, although significantly decreased compared with in rats treated without CD_4+CD_{25}+ Treg cells. The intensity of IgA deposition in the mesangial region of rats treated with IgAN groups was significantly higher than in control groups, although significantly decreased compared with in rats treated without CD_4+CD_{25}+ Treg cells.

A. rats treated without CD_4+CD_{25}+ Treg cells

B. CD_4+CD_{25}+ Treg cells - IgAN -treated rats

C. CD_4+CD_{25}+ Treg cells - control -treated rats

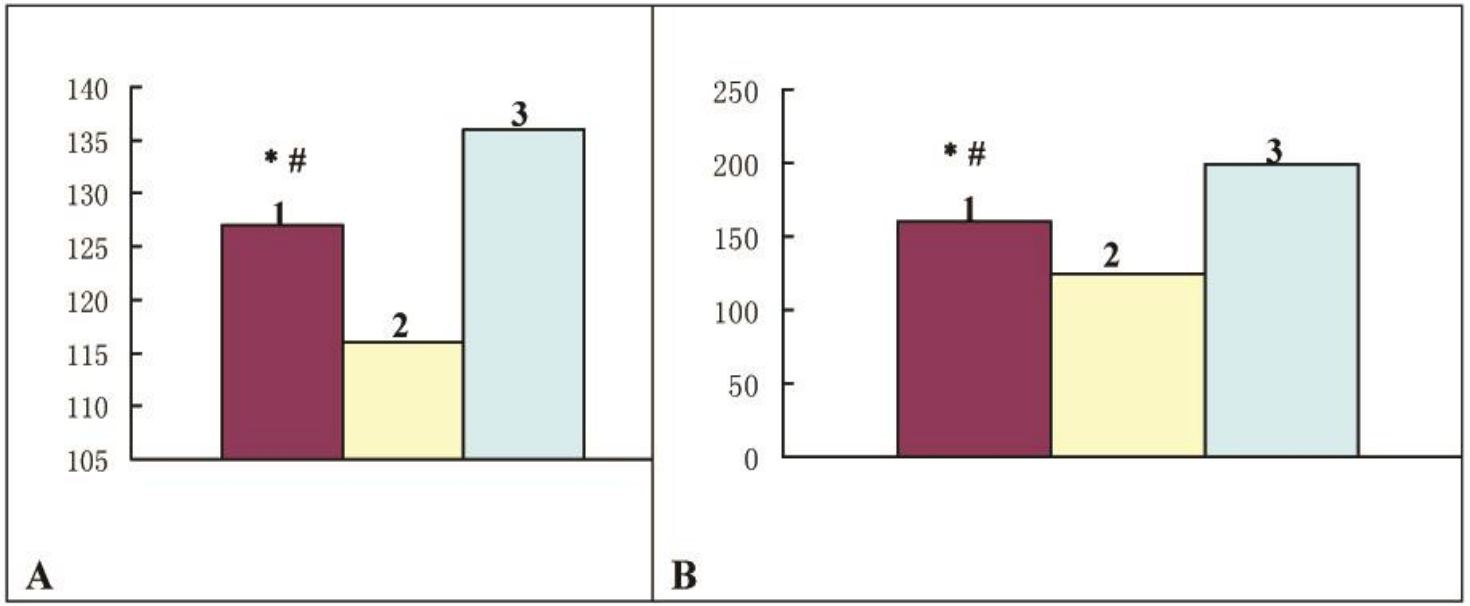


Fig.2 Glomerular average planar area and the average number of cells in the glomeuli

A. Glomerular average planar area

B. Glomerular average number of cells

1. CD4+CD25+ Treg cells - IgAN -treated rats
2. CD4+CD25+ Treg cells - control -treated rats
3. rats treated without CD4+CD25+ Treg cells

Glomerular planar area and the average number of cells in the glomeruli in IgAN groups were significantly increased compared to control groups (all * $P < 0.05$), although significantly decreased compared with in rats treated without $CD_4^+CD_{25}^+$ Treg cells(all $^{\#}P < 0.05$)

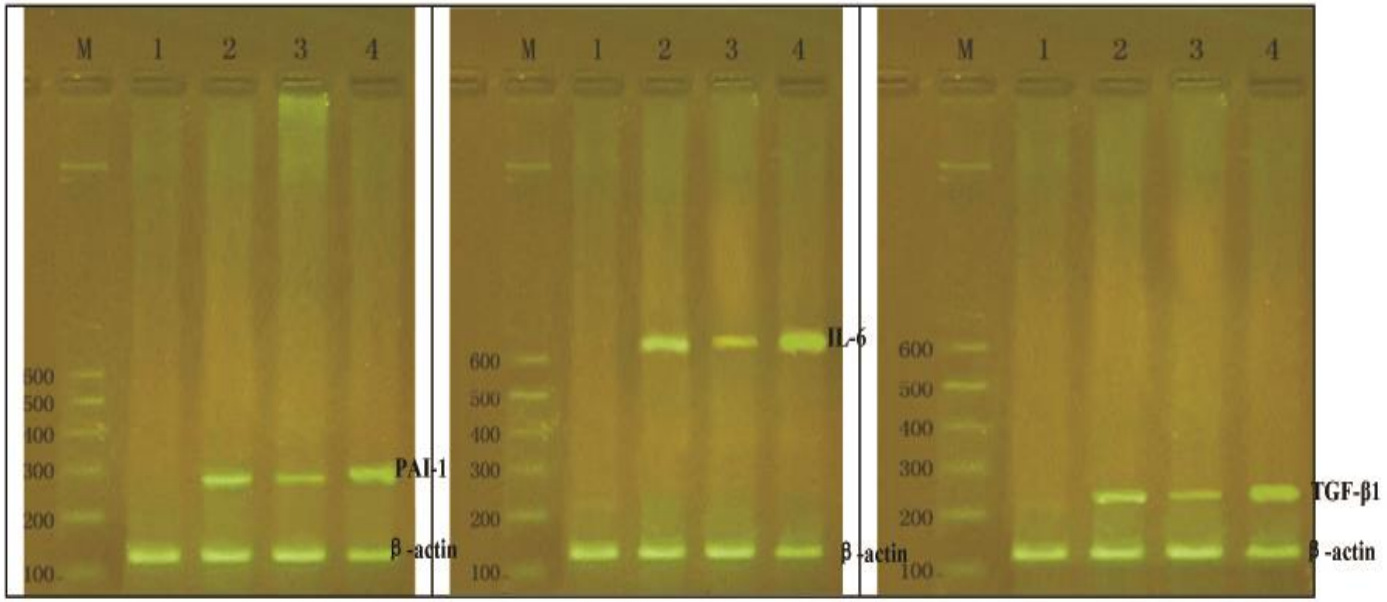


Fig. 3 RT-PCR of PAI-1 mRNA ,IL-6 mRNA, TGF-β1 mRNA in cultured rat renal mesangial cells

Expression of of PAI-1 mRNA, Il-6 mRNA ,and TGF- 1 mRNA in renal mesangial cells from rats received CD_4+CD_{25}+ Treg cells from IgAN groups were considerably increased compared to in control groups, although significantly decreased compared with in rats treated without CD_4+CD_{25}+ Treg cells.

1. Negative control (omission of primer)
2. CD_4+CD_{25}+ Treg cells - IgAN -treated rats
3. CD_4+CD_{25}+ Treg cells - control -treated rats
4. rats treated without CD_4+CD_{25}+ Treg cells

Discussion

- CD4+CD25+ cell numbers in the tonsillar tissues of IgAN patients were significantly lower than those in the control group.
- Our experiments have shown that dimeric IgA-producing cells were significantly increased in IgAN patients compared with the control group.
- The CD4+CD25+ cells number was negatively correlated with that of dimeric IgA-producing cells.

Discussion

- IgA deposition in the mesangial region and the glomerular planar area and the number of cells from IgAN patients were significantly higher than in rats that received CD4+CD25+ Treg cells from the control group, although they were dramatically lower compared with rats treated without CD4+CD25+ Treg cells.
- levels of PAI-1, IL-6 and TGF-β1 expression in renal mesangial cells of rats that received CD4+CD25+ Treg cells from IgAN patients were significantly higher than in rats that received CD4+CD25+ Treg cells from the control group, although they were dramatically lower compared with rats treated without CD4+CD25+ Treg cells.

Discussion

- There is a numerical and/or functional $CD4^+CD25^+$Treg cells deficit in IgAN patients.
- Under the conditions of a decrease of $CD4^+CD25^+$Treg cells number and/or functional deficit, lymphocytes from IgAN patients have significantly stronger responses to antigens which lead to higher levels of cytokine.
- Excessive cytokine production might enhance the switching of antibody production from one isotype to another e.g. from IgM to IgA .
- Therefore large amounts of IgA are present in the serum of IgAN patients.
- IgA are deposited in glomerular mesangium via the circulatory system where they activate complement or disturb the balance between blood coagulation and plasminogen; release inflammatory factors; and injure renal tissue.
- Altering the $CD4^+CD25^+$ Treg cells number and/or enhancing $CD4^+CD25^+$ Treg cells responses might be useful in the prevention and treatment of IgAN.

Discussion

- In summary, IgAN is common glomerular disorders that can potentially progress to end-stage renal disease in some patients.
- $CD4^+CD25^+$ Treg cells are of critical importance to the maintenance of tolerance.
- $CD4^+CD25^+$Treg cells are regulators of almost all of the animal models of human organ-specific diseases, transplant rejection, and allergic diseases.
- A numerical and/or functional $CD4^+CD25^+$ Treg cells deficit in IgAN might induce the development of the diseases.
- Altering the number of $CD4^+CD25^+$Treg cells and/or enhancing $CD4^+CD25^+$ Treg cells responses might be useful in the prevention and treatment of IgAN .

Thank for your attention!