RESEARCH PAPER | Medical Science | Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Regulatory T Cells in Pediatric Lupus Nephritis

KEYWORDS

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ABSTRACT *CD4+ CD25+ regulatory T cells (Tregs) are critical in maintaining self-tolerance and preventing organ-specific autoimmunity. Their role in pediatric lupus nephritis (LN), an autoimmune disease characterized by inappropriate regulation of hyperactivated B and T cells, has not been clearly defined. Using flow cytometry to determine cell populations of CD4+ CD25+ T cells and CD8+CD122+ Tregs in pediatric LN patients and healthy subjects. The frequency of CD4+ CD25+ Tregs was significantly decreased in patients with active LN compared with patients with inactive LN and with controls (3.72% ± 1.43% versus 4.89% ± 1.37% and 9.4% ± 1.17%, respectively; P ≤0.01 and P < 0.001, respectively), The frequency of CD8+CD122+ was significantly decreased in active LN as compared to healthy control group (9.69%±3.9 versus 12.43%±3.87% and 29.81%±5.5; P:<0.01 and ≤0.001 respectively and there was insignificant difference in active LN as compared to inactive LN (9.69%±3.9 versus 12.43%±3.87% , P>0.05). We demonstrated reduced CD4+ CD25+ Treg levels indicating a role of CD4+ Tregs in the pathogenesis of SLE.*

Introduction

Systemic lupus erythematosus (SLE; also referred to as "lupus") is a chronic autoimmune, multisystem, and multifactorial disease characterized by the presence of autoantibodies to nucleic acid, proteins, and nucleoprotein complexes. The initial immunizing immunogen that drive the development of SLE are unknown, but characteristics of the immune response in SLE suggest that it is an antigen-driven condition.(1)

Systemic lupus erythematosus (SLE) is characterized by several functional abnormalities of the immune system, including impaired T cell responses and B cell hyper-reactivity. Regulatory T-cells, formerly called suppressor T-cells, stop the T-cells from becoming activated towards the end of an immune reaction. They also suppress dysfunctional T-cells that mistakenly attack body cells because they are identified as foreign invaders. Regulatory or suppressor T cell subsets that prevent harmful self-reactive immune responses originate in the thymus or can develop in the periphery. Regulatory $CD4^+$cells, $CD8^+$ cells, and NKT cells have been described elsewhere (1,2,3).

Regulatory T cells are classified into two groups: naturally occurring regulatory T cells and induced regulatory T cells. Naturally occurring tregs develop during normal T-cell maturation in the thymus and represent 1-2% of CD4+ T cells in the peripheral blood. CD4+ cells that constitutively express CD25, the α-chain of the IL-2R, comprise a unique lineage of thymus-derived regulatory T cells that have a potent contact-dependent mechanism of action (4, 5). These cells have been called "natural" regulatory T cells are characterized by their capacity to actively suppress T cell proliferation in vitro (6). In contrast to the mouse, the suppressive capacity of human Treg has been shown to be restricted to CD4+T cells with the highest expression levels of CD25, whereas CD4+ T cells with intermediate expression of CD25 might also contain activated T cells(7). Other T cells down-regulate immune responses by producing immunosuppressive cytokines.

The regulatory cells play important roles in immune response. Among these cells, CD4+CD25+ regulatory T (Treg) cells are a well-documented cell subpopulation. CD4+CD25+ Treg cells have got an effect in self-tolerance. Therefore, the difference of these cells was documented in the patients with autoimmune disease and graft-versus host disease [8-10].

Moreover, $CD4^+CD25^+$ Treg cell activity has been observed in lupus nephritis (LN). The importance of $CD4^+CD25^+$ regulatory T cells has been confirmed in many types of autoimmune diseases. However, other cells, especially those of CD8 T cell lineage, have also been suggested as regulatory T cells. Recently, $CD8^+CD122^+$ cells are described as another naturally occurring regulatory T cells in mice [8]. Experimental autoimmunity is exacerbated in CD122 depleted mice [9] and these cells suppress autoimmune processes. The status of $CD8^+CD122^+$ cells has not been known so far in lupus nephritis patients.

CD25 and CD122 are functionally closely related because these molecules are the α and β-chains of the IL-2 receptor, respectively (**13**). Therefore, we first evaluated the expression pattern of these molecules on different T-cell subpopulations and the outcome of antibody mediated depletion using anti-CD25 or anti-CD122 antibodies.

We aimed to assay the frequency of CD4+ CD25+ T cells in peripheral blood of pediatric patients with lupus nephritis (LN), and to investigate the expression differences of CD122+ on CD8+T cells between LN patients and healthy subjects.

PATIENTS AND METHODS

Patients and controls.

The study cohort included 50 patients diagnosed as having lupus nephritis (81% female and 19% male; mean age 16.4 ± 6.16 years according to the American College of Rheumatology (ACR) criteria, and 25 healthy control subjects (61% female and 39% male; mean age 9.9± 3.75 years) who had no history of autoimmune diseases, infectious diseases, malignancies, or immunosuppressive therapy. (The clinical characteristics of the patients and controls are available upon request from the author). The study was approved by the Mansoura pediatric hospital Mansoura University, and written informed consent was obtained from all participants.

Monoclonal antibodies (mAb) and flow cytometry.

The following mAb and reagents were used in this study: fluorescein isothiocyanate (FITC)–conjugated or peridinin chlorophyll A protein–conjugated anti-CD3 mAb, FITC-conjugated anti-CD25 mAb, FITC-conjugated anti-CD8 mAb, PE-conjugated anti-CD4 mAb, PE-conjugated and anti-CD122 mAb,

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

Isolation of peripheral blood: Peripheral venous blood samples were collected into tubes containing EDTA, and peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient centrifugation for 20 min at 1500 rpm without braking using Ficoll-Paque Plus solution. The bands of cell were pipette carefully into another centrifuge tube filled up with phosphate buffer saline (PBS) or hanks solution and mixed. After centrifugation for 10min at 1800 rpm, lymphocyte sediment were fixed with ice cold absolute alcohol 1ml and preserved in +4C° until analysis.

Statistical analysis: Data were explored, processed and analyzed using the statistical package for the social science, windows version 16, USA (SPSS PC+ version 16 software). Variable with normal distribution were expressed as mean ± SD. In these variables, the T test was applied for group differences.

Results

Table 1. Demographic and clinical characteristics of patients and control subjects

	Control	Inactive	Active
Case no.	18	17	20
Sex (male\Female)	7\11	4\13	3\17
Age years Range	9.91±3.75 5.5-17	13.41±3.66 7-17	14.7±3.48 4-19
AntidsDNA(IU/ml)	7.5±5.5	50.77±23.53	247.38±168.23
Creatinine(mg/dl)	0.44±0.12	0.59±0.4	0.96±1.1
CRP(mg/l)	5.92±2.68	19.34±2.63	44.2±17.08
ANA	9.5±3.9	76.19±12.87	78.19±10.37

Demographic and clinical characteristics of subjects

The control group was matched with the patient group for both age and sex. Within the study group, patients with active SLE disease had significantly anti-dsDNA levels (Table 1).

Percentages of Tregs in pediatric LN

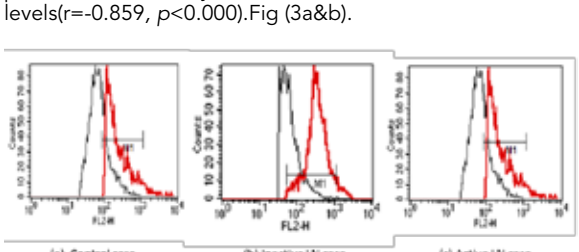
As regard to flow cytometry analysis data, the representative figures of comparison of $Cd8^+CD122^+$ Tcells and $CD4^+CD25^+$Tcells between control and patients groups as shown in fig.1&2.

The percentage of $CD122^+$ cells among $CD8^+$ lymphocytes levels in blood samples obtained from active LN patients was significantly decreased as compared to inactive LN and healthy control group (9.69%±3.9 versus 12.43%±3.87% and 29.81%±5.5 respectively). *P*:<0.01 and ≤0.001 respectively. Fig.(1)

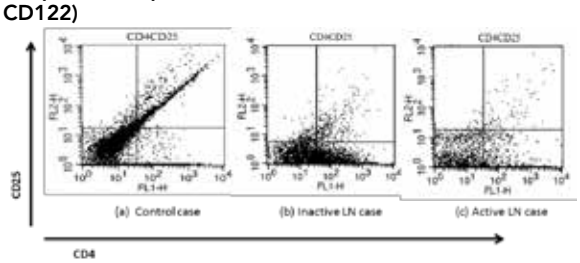
Patients with active LN had a significantly decreased percentage of $CD25^+$ lymphocytes in $CD4^+$Tcells compared with patients with inactive LN and healthy controls (3.72% ± 1.43% versus 4.89% ± 1.37% and 9.4% ± 1.17%, respectively; P ≤0.01 and P < 0.001, respectively).Fig.(2)

Correlation of disease activity with frequency of CD4+ CD25+ Tregs

The frequency of $CD4^+CD25^+$Tregs in LN patients was inversely correlated with serum anti-dsDNA levels (r=-0.699, *p*<0.000). Also, the frequency of $CD8^+CD122^+$Tregs in LN patients was inversely correlated with the serum anti-dsDNA levels(r=-0.859, *p*<0.000).Fig (3a&b).



Fig(1):Flow cytometry histograms for CD8+CD122+ show positive expression for both two marker according to M1(positive expression value) (black color CD8, red color CD122)



Fig(2): Comparison of flow cytometric analysis of double stain CD4 CD25T-cell populations between control and patient. The peripheral blood was stained with FITC-conjugated anti-CD4, PE-conjugated anti-CD25. Cells were gated on lymphocytes via their forward- and side-scatter properties.

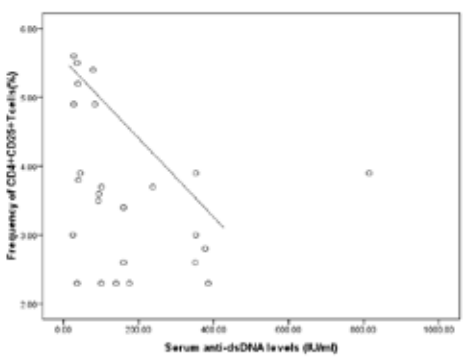


Fig.(3a)

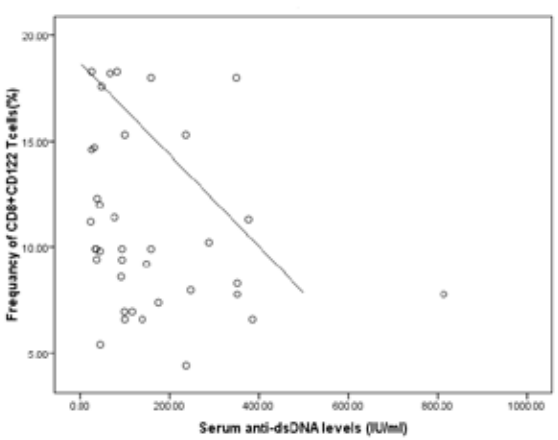


Fig.(3b)

Fig.3:(a)The frequency of $CD4^+$ $CD25^+$ Tregs was inversely correlated with serum anti-dsDNA levels.(b) The frequency of $CD8^+$ $CD122^+$ Tregs was inversely correlated with serum anti-dsDNA levels.

Discussion

SLE is an autoimmune disease characterized by loss of immune tolerance to self-antigens. Some workers suggested that there was a defective function of suppressor T cells in SLE disease; others indicated that active patients with SLE have low number of CD4+ Treg cell in their peripheral blood. (**Hayashi et al.,2009**)

It is well known that, Tregs have an important role in control-

Volume : 3 | Issue : 10 | Oct 2013 | ISSN - 2249-555X

ling autoimmune responses and inflammation. Any changes in Treg number or function or phenotype could lead to the development of autoimmune disease such as SLE. **(Horwitz 2008)**

$CD8^+CD122^+$ cells are newly identified regulatory cells in mice. These cells are naturally occurring regulatory cells. They play essential roles in the maintenance of immune homeostasis.(**Karagöz et al.,2009**) In our result, the expression of $CD122^+$ on $CD8^+$ was significantly reduced in pediatric LN patients as compared to healthy control. This finding may indicate that $CD8^+CD122^+$ may play a role in the pathogenesis of LN.

In our study, the $CD4^+$% from pediatric LN patients were significantly increased as compared to healthy control. On the other hand, the $CD25^+$ expression on $CD4^+$ was significantly reduced.

The obtained result was in agreement with **Liu *et al.*, (2004), and (2006) Horwitz (2008)**. The former reported a decreased percentage of $CD4^+CD25^+$ T cells. The later demonstrated that in human lupus Treg defects play a major role in the perpetuation of this disease.

Also **Lee *et al.*, (2006)** demonstrated that, the number of $CD4^+$ $CD25^+$ T cells in the peripheral blood of pediatric patients with active SLE is significantly decreased. Suggesting that the decreased number of $CD4^+$ $CD25^+$ T cells in the peripheral blood of pediatrics patients with SLE constitutes a defective Treg population and contributes to the pathogenesis of SLE.

While in **Alvarado-Sanchez *et al.*, (2006)** study, no significant differences in the levels of all regulatory cell subsets studied in SLE patients. On the other hand, the study of **Azab *et al.*, (2008**) demonstrated that the levels of $CD4^+CD25^+$ % T cells in SLE patients were higher than in the control group but it was statistically insignificant. This confliction with our results may be attributed to each the differences in patients age and conclusion.

Data regarding the functional activity and the numbers of $CD4^+CD25^+$ cell population in patients with SLE are conflicting (**Crispin et al. 2003; Liu et al. 2004; Miyara et al. 2005; Alvarado-Sanchez et al. 2006; Lee et al. 2006**). Conflicting results might be due to the differences in the administrated therapies. Patients with SLE, as usual, are on the treatments that include cytostatics and steroids.

Seo et al., (2002) showed that, activation of anti-dsDNA-producing auto-reactive B cells in mice requires help from $CD4^+$ helper T cells and the overcoming of the suppression of $CD4^+$ $CD25^+$ Tregs. The activated $CD4^+$ helper T cells presumably rescue self-reactive B cells from apoptosis and stimulate them to form autoantibodies.

Lee et al., (2006) demonstrated that the frequency of $CD4^+$ $CD25^+$ T cells in our patients with SLE was inversely related to both the serum level of anti-dsDNA and the disease activity. This finding was compatible with our finding. These results imply that a breakdown of tolerance as a result of impaired Treg function contributes to the excessive autoantibody formation seen in active SLE.

In conclusion: CD4+ CD25+ T cells in pediatric patients with active LN were significantly decreased, suggesting a role for a decreased Treg population in pediatric patients with LN. Also, diminished production of IL-2 may contribute to this defect. Based on these results, it becomes conceivable to design strategies to amplify the decreased function of Tregs in patients with active LN by specific therapeutic interventions with low-dose IL-2.

REFERENCE

. Maloy, K. J., and F. Powrie. 2001. Regulatory T cells in the control of immune pathology. Nat. Immunol. 2:816. | 2. Miller, A., O. Lider, A. B. Roberts, M. B. Sporn, Maloy and H. L. Weiner. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of transforming growth factor _ after antigen-specific triggering. Proc. Natl. Acad. Sci. USA 89:421. | 3. Sharif, S., G. A. Arreaza, P. Zucker, Q. S. Mi, J. Sondhi, O. V. Naidenko, M. Kronenberg, Y. Koezuka, T. L. Delovitch, J. M. Gombert, et al. 2001. Activation of natural killer T cells by _-galactosylceramide treatment prevents the onset and recurrence of autoimmune type 1 diabetes. Nat. Med. 7:1057. | | 4. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 1995. Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 155:1151. | 5. Suri-Payer E., Amar A. Z., Thornton A. M., and Shevach E. M. (1998): CD4_CD25_ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J. Immunol. 160:1212. | 6. Baecher-Allan, C., Brown J. A., Freeman G. J., and Hafler D. A. | (2001): CD4_CD25high regulatory cells in human peripheral blood. J. Immunol.; 167:1245–1253. | | 7. Shevach, E. M. (2000): Regulatory T cells in autoimmunity. | Annu. Rev. Immunol. 18:423. | 8. Valencia X., Yarboro C., Illei G., Lipsky P.E.(2007): Deficient CD4+CD25high T regulatory cell function in patients with active systemic lupus erythematosus. J.Immunol.; 178:2579-88. | 9. Hoffmann P., Ermann J., Edinger M., Fathman C.G., Strober S.(2002): Donor-type CD4(+)CD25(+) regulatory T cells suppress lethal acute graft-versus-host disease after allogeneic bone marrow transplantation. J.Exp.Med.;196:389-99. | 10. Taylor P.A., Lees C.J., Blazar B.R.(2002) The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. Blood;99:3493-99. | 11. McLachlan SM, Nagayama Y, Pichurin PN, Mizutori Y, Chen CR, Misharin A et al. (2007): The link between Graves' disease and Hashimoto's thyroiditis: a role for regulatory T cells. Endocrinology;148:5724-33. | | 12-Bonelli M, Savitskaya A, Steiner CW, Rath E, Smolen J, and Scheinecker C.(2009): Phenotypic and Functional Analysis of CD4+CD25+Foxp3+T Cells in Patients with Systemic Lupus Erythematosus. J Immunol; 1689-1695. | | 13-Triplett, Todd A., Mahr, MD, FACAAI, and Meet. (2012). "Defining a functionally distinct subset of human memory CD4+ T cells that are CD25POS and FOXP3NEG". journal of european immunology 42 (7). | | 14-Filaci G., Bacilieri S., Fravega M., Monetti M., Contini P., Ghio M., Setti M., Puppo F., and Indiveri F.(2001): Impairment of CD8+ T Suppressor Cell Function in Patients with Active Systemic Lupus Erythematosus. J Immunol 2001; 166:6452-6457 | 15-Moller, G. (1988). Do suppressor T cells exist? Scand. J. Immunol. 27:247. | 16-Gershon, R. K., and K. Kondo. (1971). Infectious immunological tolerance. Immunology21:903 | | 17-Crispin JC, Martinez A, Alcocer-Varela J.(2003): Quantification of regulatory T cells in patients with systemic lupus erythematosus. J Autoimmun; 21:273–6. | | 18-Liu MF, Wang CR, Fung LL, Wu CR.(2004) Decreased CD4+CD25+ T cells in peripheral blood of patients with systemic lupus erythematosus. Scand J Immunol; 59:198–202. | | 19-Viglietta, V., Baecher-Allan C., Weiner H. L., and Hafler D. A. (2004): Loss of functional suppression by CD4+CD25+regulatory T cells in patients with multiple sclerosis. J. Exp. Med. 199: 971–979. | | 20- Azab N.A., Bassyouni I.H., Emad Y., Abd El-Wahab G.A., Hamdy G., Mashahit M.A.(2008): CD4+CD25+ regulatory Tcells (TREG) in Systemic Lupus Erythematosus (SLE) patients: The possible influence of treatment with corticosteroids. Clinical Immunology 127, 151–157. | 21- Alvarado-Sánchez B, Hernández-Castro B, Portales-Pérez D, Baranda L, Layseca-Espinosa E, Abud-Mendoza C, Cubillas-Tejeda A, and González-Amaro R (2006). "Regulatory T cells in patients with systemic lupus erythematosus". J Autoimmun 27(2): 110–118. | 22- Lee J. H., Wang L. C., LinY. T., Yang Y.H., Lin D.T., and Chiang B. L.(2006) Inverse correlation between CD4+ regulatory T-cell population and autoantibody levels in paediatric patients with systemic lupus erythematosus Immunology; 117, 280–286. | 23- Miyara, M., Amoura, Z., Parizot, C., Badoual, C., Dorgham, K., Trad, S., Nochy, D., Debre, P.,Piette, J.C. and Gorochov, G. (2005) Global natural regulatory T cell depletion in active systemic lupus erythematosus. J. Immunol. 175, 8392–8400. | 24- Horwitz D.A.,(2008): Regulatory T cells in systemic lupus erythematosus: past, present and future. Arthritis Research & Therapy 2008, 10:227-15. | 25- Seo SJ, Fields ML, Buckler JL, et al.(2002): The impact of T helper and T regulatory cells on the regulation of anti-double-stranded DNA B cells. Immunity; 16:535–46. | | 26-Hayashi T, Adachi C, Hasegawa K. (2009): Systemic treatment of anti-CD4CD25 T cell monoclonal antibody exacerbates sialoadenitis in submandibular glands during the early life in lupus-prone female NZB x NZWF mice. J Oral Pathol Med;38(2):234-40 | 27- Karagöz B., BİLGİ O., SAYAN Ö, ERİKÇİ A.A. , ÖZGÜN A.,YILMAZ B,SÜCÜLLÜ I., TÜRKEN O. and KANDEMİR E.(2009): CD122 Expression on CD8+ T cells in peripheral blood of advanced stage cancer patients. Anatol J Clin Investig:3(1):1-3 |