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Natural Regulatory T Cells in Some Parasitic Diseases

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Abstract

Parasitic infection in human alimentary tract causes a significant change in immune system through its continuous antigens secretion. The aim of this study was to estimate the change in natural regulatory T cell population in peripheral blood of patients infected with different types of alimentary tract parasites. Regulatory T cells (CD4+CD25+Foxp3+) were detected in eighty patients infected with intestinal parasites and forty healthy volunteers using flow cytometry technique. Statistical analysis showed a significant increase in regulatory T cell percentage in infected patients compared to healthy group (P<0.001). Patients infested with Giardia showed significantly higher CD4+CD25+Foxp3+ cell percentages than those infested with other parasites (P<0.001). Also, mixed infestation showed significantly higher CD4+CD25+Foxp3+ cell percentages than single infestation. In conclusion, natural regulatory T cell frequencies (CD4+CD25+Foxp3+) increase significantly in patients with parasitic diseases compared to healthy controls. The higher levels were associated with mixed infection compared to single infection, and in older than younger patients.

Keywords: Regulatory T cell; CD4+CD25+FOXp3+; Parasitic diseases; Giardia

Introduction

There is a continual exposure to large number of food-borne pathogens within alimentary tract, which represent a major challenge to the immune system to differentiate between harmful and innocuous antigens; which can be achieved by suppressor T cells and regulatory T (Treg) cells [1-3]. Regulatory T cells refer to subset of immune cells which suppress immune reactivity via suppressive cytokines and signal [4]. Treg cells can be divided into two types: natural Treg (nTreg) and inducible Treg (iTreg) cells [5]. Natural regulatory T cells develop as a result of contact with self-antigens in the thymus and go to periphery to enforce self-tolerance before pathogen exposure. Natural regulatory Tcells include CD4, CD25+, and FOXp3+[6]. Inducible regulatory T cells develop in the periphery from conventional CD4+ T cells after exposure to signals as regulatory cytokines, immunosuppressive drugs, or antigen presenting cells conditioned by microbial products [7]. Natural Treg cells participate in immune response to many, if not, all infectious agents. Usually they serve to restrain exuberant immune reactivity which in many chronic infections benefits the host by limiting tissue damage [8]. However, the nTreg response may handcap the efficiency of protective immunity [9-11]. Parasites are still, even in the 21st Century, remarkably prevalent across the world [12]. Parasites have evolved in many different ways to avoid detection by their host and to manipulate their host's immune system in order to gain an advantage and survive [13]. The induction and/or manipulation of Tregs by parasites may be one such method to aid their survival [14].

The aim of this study was to estimate the change in natural regulatory T cell population in patients infected with different types of alimentary tract parasites.

Material and Methods

Seventy children attending Mansoura children hospital, and 50 adults were enrolled for this study; sixty eight were males and fifty two were females. Mean age for children 8.71 years (range: 5-12 years), while mean age for adult 40.6 years (range: 33-47 years). Fifty children and 30 adults were infected with intestinal parasites and 20 children and 20 adults were the healthy control of matched age and sex after seven negative stool samples on seven alternative days. Peripheral heparinized blood samples were obtained from both parasitized and healthy control.

Exclusion criteria: malnutrition, any form of immune deficiency, inflammatory diseases, diabetes mellitus, coexistent malaria or leishmania, previous antiparasitic treatment in three months before.

Coprological study: direct smear, Formol-Ether concentration method [15] and acid fast stain were used for *Coccidea* [16], Gomori's trichrome stain [17], Weber's trichrome stain for *Microsporidia* [18] and agar plate culture for S*trongyloides stercoralis* [19].

Flow Cytometric Analysis (FCA): The following anti-human monoclonal antibodies were used: phycoerythrin (PE)–anti-CD4, fluorescein isothiocyanate (FITC)–labeled-anti-CD25, phycoerythrin-cyanine 5 (RPE-CY5)–labeled anti-Foxp3, (eBioscience, San Diego, USA) and their appropriate isotype controls (Dakocytoformation, Denmark). Fixation and permeabilization of cells was done using Intraprep kits reagent (immunotech, Beckman Coulter, Marseille, France). Phorbol myristate acetate (PMA) and ionomycin were purchased from Sigma, USA.

Peripheral blood mononuclear cells (PBMCs) were isolated, after centrifugation of heparinized blood over Ficoll-Hypaque at 500 × g for 15 min, from healthy control subjects and patients. The PBMCs were cultured in RPMI 1640 medium that contained 100 U/mL penicillin, 100 U/mL streptomycin and 10% fetal bovine serum. Cell density was adjusted to 2×10^6cells/mL. Cells were stimulated by adding 50 ng/mL PMA, 1 µg/mL ionomycin and 10 µg/mL BFA to the medium for 5 h at 37°C under 5% CO_2. Stimulated cells were centrifuged at 1200 g for 7 min at 10°C, washed, and then surface stained by incubating cells ($1\text{-}2\times10^6$/mL) for 30 min at room temperature in the dark with fluorescence labeled anti-CD3 and anti-CD4. For intracellular staining, cells were subsequently treated with cell fixation and permeabilization reagents following the manufacturer's instructions, and then incubated with a fluorescence labeled anti-Foxp3 for 30 min at 4°C. Cells were re-suspended in PBS and analyzed using the EPICS XL flow cytometer. Appropriate isotypic controls were used to determine specific binding for each fluorescent channel. T-regulatory cells were defined as the percentage of Foxp3+cells in CD4+CD25+ T cells.

Statistical Analysis: The statistical analysis of data was done by using excel program and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 16. Kolmogorov-Smirnov test was done to test the normality of data distribution. Qualitative data were presented as frequency and percentage. Quantitative data were presented as median, range, mean and standard deviation. For comparison between two groups; student t-test and Mann-Whitney test (for non-parametric data) were used. Multivariate logistic regression analysis was done using data age, and parasitic infestation status. Odds ratios (ORs) were calculated and are given within 95% confidence intervals (CI). Relationships between different results were examined using Spearman's rank correlation test. A value of $p<0.05$ was considered significant in all statistical tests at confidence interval 95%.

Ethical statement: Written consent was obtained from all adults and parents of children prior to enrollment in this study.

Results

There is no significant differences between parasitized and non-parasitized subjects regarding age and sex. Parasitized subjects showed significantly higher CD4+CD25+Foxp+% when compared to non-parasitized ($p<0.001$). Also, the comparison of CD4+CD25+Foxp+ in the studied group according to type of parasites versus control shows highly significant CD4+CD25+Foxp+% in patients infected with each type of parasite than those with no parasites ($p<0.001$). Subjects infested with Giardia show significantly higher CD4+CD25+Foxp+ % than those infested with other parasites ($p<0.001$). On the other hand, subjects infested with Schistosoma mansoni show significantly lower CD4+CD25+Foxp+% than those infested with other parasites ($p<0.001$). On the other hand, subjects infested with mixed infestation show significantly higher CD4+CD25+Foxp+% than those infested with single infestation ($p=0.028$).

Degree of parasitic infestation was stratified into mild, moderate and heavy. CD4+CD25+Foxp3+ proportion show significant differences between all categories ($p=0.009$) and mild versus heavy ($p=0.003$). No significant differences were found between mild versus moderate and moderate versus heavy ($p=0.144$, 0.245 respectively).

Multivariate analysis was done using age (Adult versus children) and parasitic infestation status in relation to CD4+CD25+Foxp+% (above median versus below median) as a dependent factor. It shows that parasitic infestation status is risk factor for higher CD4+CD25+Foxp+% ($p<0.001$, OR=3.024, 95% CI=1.082- 6.236). In addition, adults are susceptible for higher CD4+CD25+Foxp+% ($p<0.001$, OR=3.333, 95% CI=2.561-6.549).

Discussion

Parasitic challenges to the host are met by a wealth of humoral and cellular responses which may ends by tissue damage. So, to avoid this tissue damage, induction of T-cell hyporesponsiveness and bystander suppression is mandatory [20]. Immune modulation by parasitic infection is believed to be mediated by natural and inducible Treg cells. Treg cells mediate their actions via induction of cell cycle arrest, apoptosis and inhibition of pro-inflammatory cytokines [21]. This may be beneficial to host in chronic infections as preventing immune mediated pathology. Too early functioning of Tregs may ends in uncontrolled parasite growth and severe disease [22].

In this study, CD4+CD25+Foxp+% show highly significant results in parasitized patients than control. Also, parasitic infestation status is risk factor for higher CD4+CD25+ Foxp+% ($p<0.001$, OR=3.024, 95% CI=1.0.82- 6.236). Tregs induced by parasites cripples host immunity and suppress antiparasitic effector cells [14]. The expanded profile of Tregs in parasitic infections was reported by Maizels and Smith [23], which may be attributed to activation of pre-existing (natural or thymic) Tregs or the de novo induction of Tregs from naive peripheral Th0 precursors. Parasitic nematode infections have been shown to induce regulatory cell expansion in both mice and humans [24].

Also Montes et al. [25], reported increased proportions of CD4+CD25+Foxp3+ Tregs in patients with Strongyloides stercoralis and human T lymphocyte virus -1 coinfection in comparison to normal controls which leads to decreased antigen driven production of IL-5 and lower eosinophil counts. Those results suggest a role for these cells in blunting antigen-driven protective responses. *E.histolytica* triggers development of Treg populations during chronic phases of disease that repress the development of responder T cells [21].

In the present study, patient infested with Giardia show significantly higher CD4+CD25+Foxp+% than those infested with other parasites ($p<0.001$). The relatively higher CD4+CD25+Foxp+% in Giardia lamblia in relation to other parasites can be attributed to the fact that G. lamblia causes little or no inflammation in humans [26]. Giardia actively down regulates the inflammatory response [27], which may be mediated via T regulatory cells. As evidenced by IL-8 was not induced, in contrast to what is typically seen in intestinal infections that cause inflammation. Its low level during Giardia infection partly explains the low levels of inflammation. Also, by analyzing the gene expression of several different cytokines in human intestinal epithelial cell lines 5 h after G. lamblia infection in vitro did not reveal any cytokines that were induced at a high level [28,29].

On the other hand, patient infested with Schistosomiasis show significantly lower CD4+CD25+Foxp+% than those infested with other parasites ($p<0.001$). This lower CD4+CD25+Foxp+% observed in schistosomal infection in comparable to other parasites can be attributed to earlier stage of infection or previous treatment. Watanabe et al. [30], stated that not all Schistosoma mansoni-infected individuals develop high percentages of circulating Tregs. The effective treatment decreases the proportion of Tregs and their phenotypes, possibly because of the removal of constant exposure to antigens from intravascular, egg-producing adult worms. Also, Singh et al. [31], found that schistosomal granulomatous livers at 8 and 16 weeks after infection showed 10 and 30 fold increases in Foxp3 expression compared with normal liver. Also, the percentage of Treg cells in granuloma rose from 12% at 8 weeks to 88% at 16 weeks after infection.

Also, Mixed infestation showed significantly higher CD4+CD25+Foxp+% than single infestation ($p=0.028$) and heavy infestation showed significantly higher CD4+CD25+Foxp+% than mild infestation ($p=0.003$). This also reported by Minigo et al. [32], Todryk et al. [33], who stated that higher Tregs numbers are associated with increased parasite load and development of human infection caused by P. falciparum.

In the present study, being an adult is a risk factor for higher CD4+CD25+Foxp+% ($p<0.001$, OR=3.333, 95% CI=2.561-6.549). The numbers of CD4 + natural Tregs increase with age [8,34-36] which leads to declining antimicrobial immune responses.

In conclusion, natural regulatory cells CD4+CD25+Foxp+% increase significantly in late parasitic diseases compared to healthy control, also they significantly increase with mixed infection compared with single infection, and significantly increase with age.

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