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COMPARISON OF CHANGES IN THE PERCENTAGES OF CD8⁺CD28⁻TCRαβ⁺ T CELL SUBPOPULATIONS IN ALLERGIC ASTHMA SUBJECTS vs CONTROLS BEFORE AND AFTER ANTI-CD3/ANTI-CD28/IL-2 STIMULATION *IN VITRO*

M. EUSEBIO¹, L. KRASZULA¹, M. KUPCZYK², P. KUNA² and M. PIETRUCZUK¹

¹Department of Laboratory Diagnostics, II Chair of Internal Medicine, Medical University of Lodz, Poland; ²Department of Internal Medicine, II Chair of Internal Medicine, Medical University of Lodz, Poland

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Asthma is a chronic inflammatory disease characterized by the migration of activated T cells into the bronchial mucosa. TGF-β and IL-10 have proved to regulate airway hyper-responsiveness and leukocytes recruitment to the airways of ovalbumin (OVA) sensitized mice. We examined relative changes in CD8⁺ T cell subpopulations between fifty allergic asthma subjects and twenty five age-matched healthy adults before and after anti-CD3/CD28 and IL-2 stimulation in the presence of IL-10 or TGF-β, focusing on CD62L and FoxP3 expressing TCRαβ⁺ cells. Severe asthma group had a significantly higher percentage of CD8⁺CD28⁻ and CD8⁺CD28⁻TCRαβ⁺CD62L^{high}FoxP3^{bright} T cells than other groups after enrichment. Compared to the baseline, co-stimulation with either IL-10 or TGF-β increased the percentage of CD8⁺CD28⁻ but decrease the percentage of CD8⁺CD28⁺ T cells within anti-CD3/anti-CD28/IL-2 activated CD8⁺ T cells in all groups. Co-stimulation with anti-CD3/anti-CD28/IL-2 in presence of either IL-10 or TGF-β decreased the frequencies of CD8⁺CD28⁻TCRαβ⁺CD62L^{high}FoxP3^{bright} T cells in severe asthma subgroup but increased this parameter in other groups. We suggest that altered high level expression of CD62L and FoxP3 on CD8⁺CD28⁻TCRαβ⁺ T cell is relevant to allergic asthma. These data have implications for further characterization of CD8⁺CD28⁻TCRαβ⁺ T cell subsets, with special emphasis on their implication in healthy or allergic immune response.

Allergic asthma is a chronic inflammatory disorder of the lung (1) initiated and maintained by the migration of allergen-specific T cells from the blood into the lung parenchyma. In recent years, several subsets of CD8⁺ T cells have been defined based on their differential expression of the T-cell costimulatory marker CD28 and the transcription factor FoxP3. Studies from several research groups including ours demonstrated the contribution of CD8⁺CD28⁻ T cells to the pathogenesis of asthma (2). Moreover, CD8⁺Foxp3⁺ T cells have been

shown to inhibit allergen-induced Th₂ immune response (3). CD8⁺ T cells can also be distinguished based on their surface expression of the lymph node homing molecules L-selectin (CD62L) into CD62L^{high} “central” (T_{CM}) and CD62L^{low} “effector” (T_{EM}) memory subsets (4). Human leukocytes express CD62L, the human homologue of the murine lymphocyte homing receptor, mel-14. This molecule mediates various aspects of immune system including cell communication, adhesion, and migration from the circulation into the lung. Altered

Key words: IL-10, TGF-β, FoxP3, anti-CD3/CD28, CD8⁺TCRαβ⁺ T cells, allergic asthma

Mailing address: Makandjou-Ola Eusebio, MD, PhD
Department of Laboratory Diagnostics,
II Chair of Internal Medicine,
Medical University of Lodz, Kopcinski 22 str.,
90-153 Lodz, Poland
Tel.: +48 42 677 69 83 Fax: +48 42 678 28 33
e-mail: eusemak@yahoo.com / mak.eusebio@umed.lodz.pl

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expression of this molecule correlates with impaired recruitment of leukocytes into the inflamed lung (5). Human CD62L⁺ T cells are proved to influence the production of IgG and IgA-producing cells by B cells (6). These findings lend strong support to the idea that CD62L plays a primordial role in allergic mediated pulmonary inflammation. Previous studies have shown that thymic derived conventional T cells, which co express T cell receptor (TCR) $\alpha\beta$ and either CD4 or CD8 molecules migrate to the periphery, whereas their unconventional counterparts which are characterized by the expression of either TCR $\alpha\beta$ or TCR $\gamma\delta$ with or without CD4 or CD8 populate non-lymphoid organs (7). Convincing reports indicate that CD8⁺CD28⁺TCR $\alpha\beta$ ⁺ T cells represent the most commonly encountered circulating T cells subset in the peripheral blood of adult subjects (8). However, the distribution of circulatory CD8⁺CD28⁺TCR $\alpha\beta$ ⁺ T cells expressing CD62L and FoxP3 has not been well examined. Immunomodulatory drugs modulate allergic immune responses through inhibition of the interleukin (IL)-2-dependent proliferation of human peripheral blood mononuclear T cell clones. Interestingly, co stimulation of T cells with the combination of anti-CD3/CD28 and IL-2 resulted in an increased expansion rate compared with cultures stimulated with IL-2 alone (9). Furthermore, in view of previous findings that TGF- β and IL-10 regulate antigen-induced airway hyper-responsiveness and the recruitment of leukocytes to the airways of ovalbumin (OVA) sensitized mice (10), we hypothesized that these cytokines may influence activated T cells adhesion to the lung vascular endothelium. To that end, we analyzed the distribution of different CD8⁺ T cell subsets before and after short term culture with anti-CD3/anti-CD28/IL-2 in the presence of IL-10 or TGF- β . We showed differences between allergic asthma and healthy subjects in regards to the relative percentages of enriched or anti-CD3/CD28 and IL-2 activated CD8⁺CD28⁺ T cell subpopulations. We present evidence that IL-10 and TGF- β regulate the expression of TCR $\alpha\beta$, CD28, CD62L and FoxP3 on untouched or anti-CD3/anti-CD28/IL-2 treated CD8⁺ T cells from healthy and allergic asthma subjects. These observations have implications for further characterization of the CD8⁺CD28⁺TCR $\alpha\beta$ ⁺ T cell subsets and with special emphasis on their role in allergic asthma.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the Medical University of Lodz, Poland. All participants were non-smokers and provided written informed consent in accordance with the Declaration of Helsinki before sample collection and assessment.

A total of twenty five severe allergic asthmatics (SA), twenty five mild to moderate asthmatics (MA), and twenty five healthy controls (NC) were studied. The participants underwent medical examination; spirometry and skin prick tests. The diagnosis of asthma was based on history of periodic wheezing, reversible airway obstruction as described in GINA guidelines. The severity distribution was based on the European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA) classification. Patients were clinically stable and had no recent history of an upper respiratory tract infection.

Blood collection

Anti-coagulated peripheral blood was drawn within one hour prior to skin prick test (SPT) to avoid the probable influence of this procedure on our results. The samples were kept on ice to prevent adhesion molecules receptor modulation.

Peripheral blood mononuclear cells (PBMC) isolation

Peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation (Histopaque 1077, density 1.073 kg/L). PBMCs purity was at least 97% as assessed by hematologic analyzer.

CD8⁺ T cell enrichment by magnetic-activated cell sorting (MACS)

CD8⁺ T cells were purified from PBMCs by negative selection using a Naive CD8 T Cell Enrichment Set (BD Biosciences, California). The cells were initially incubated with CD8⁺ T Cell Enrichment Cocktail (5 μ l per 1×10^6 cells) for 15 min at 4°C in the dark. After washing with a 10X excess volume of 1X BD IMagTM buffer, cells were centrifuged at 300 $\times$ g for 10 min, and the supernatant was decanted. The cells were incubated with BD IMagTM Streptavidin particles for 30 min at 4°C. The labeled cells were enriched using a BD IMagnetTM. The purity of CD8⁺ T cells was always higher than 97% as determined by flow cytometry.

CD8⁺ T cells culture conditions

For *in vitro* stimulation, freshly isolated CD8⁺ T cells were washed twice with RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) and suspended (1.0×10^6 cells/100 μ l) in the same medium supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), amino

acid supplements (2 mM glutamine), and antibiotics (100 U/mL penicillin; 100 mg/mL streptomycin) (Sigma). Cells were stimulated with anti-CD3 (10 μ g/mL), anti-CD28 (1 μ g/mL), and recombinant IL-2 (20 U/mL) in the presence of 10 ng/mL of IL-10 or TGF- β . Cultures were incubated for 3 days at 37°C under 5% CO₂ and 95% air atmosphere.

Immunofluorescence

Freshly isolated and cultured CD8⁺ T cells were stained according to standard procedures. Cells in suspension (1 $\times$ 10⁶ cells/100 μ L) were incubated with 10 μ L of the following conjugated monoclonal antibodies against CD3 [fluorescein isothiocyanate (FITC)] (clone UCHT1, IgG₁, κ), CD8 [American Cyanamid (Am Cyan)] (Clone SK1, IgG₁, κ), CD28 [peridinin chlorophyll protein-Cy5 (PerCP-Cy5)] (Clone CD28.2, IgG₁, κ), CD62L [phycoerythrin cyanine-5 (PE-CyTM 5)] (Clone DREG-56, IgG₁, κ), and TCR α/β (Pacific blue) (Clone T10B9.1A-31, IgG₁, κ) for 30 min in the dark. Cells were re-suspended in wash buffer and then incubated with 2 ml of 1x Human FoxP3 Buffer A for 10 min in the dark. Thereafter, cells were washed twice with 2 ml of BD Pharmingen Stain Buffer (FBS) and centrifuged. The aliquots of labeled CD8⁺ T cells suspension was washed with 2ml of BD Pharmingen Stain Buffer (FBS) and incubated with anti-anti-Human FoxP3 (allophycocyanin (APC)) (clone 259D/C7, IgG₁, κ) for 30 min in the dark. All steps were performed at 4°C. The labeled cells were washed with cold phosphate-buffered saline (PBS), and subsequently resuspended in 500 μ L of staining buffer prior to analysis.

Flow cytometry analysis

Samples were run on a bench top FACS CANTO II cytometer (BD Biosciences, USA) according to the manufacturer's instructions. Data were analyzed with FACS Diva software (Becton Dickinson). Gating of CD8, CD28, TCR α/β , CD62L surface markers and intracellular FoxP3 (Fig. 2) were determined using Fluorescence Minus One (FMO). This technique consists of selecting controls containing all markers except the one of interest. A total of 10,000 cells were detected at each time for each sample. Lymphocytes were gated by forward and side scatter characteristics (Fig. 1). Gates were standardized within individual samples to ensure a fully comparative data. Results are presented as median percentages of viable CD8⁺ T cell within the specific gated lymphocyte compartment.

Statistical analysis

All statistical analyses were performed using an unpaired, two-tailed Mann-Whitney test and Stat Soft's software (z o.o., Krakow, Poland). $P < 0.05$ was considered statistically significant.

RESULTS

Comparison of patient demographic data

Fifty patients with allergic asthma, including 25 males and 25 females, were enrolled in this project. Twenty-five healthy volunteers served as controls.

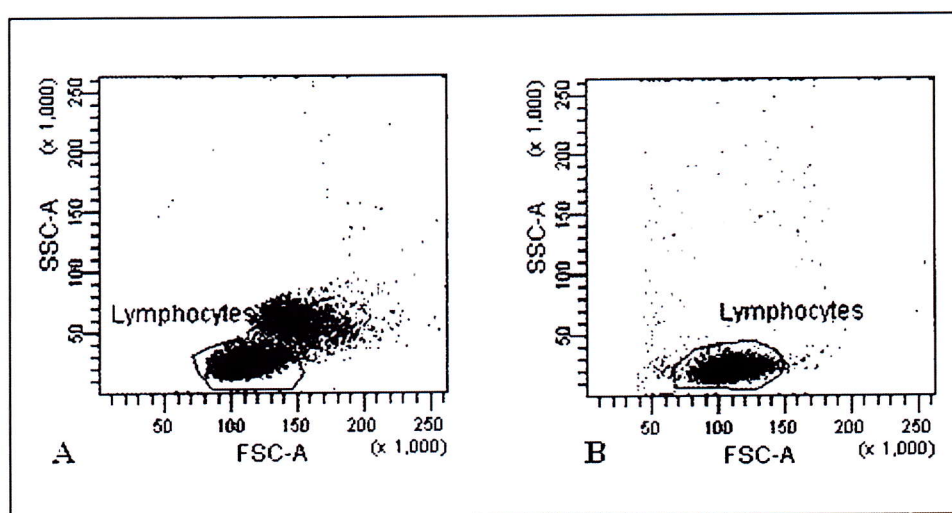


Fig. 1. Representative dot plots for lymphocytes. Cells were isolated from peripheral blood prior to staining. Lymphocytes were gated based on forward scatter/side scatter. Dot plots representing the lymphocyte gate after isolation (A) and magnetic bead purification (B) are shown.

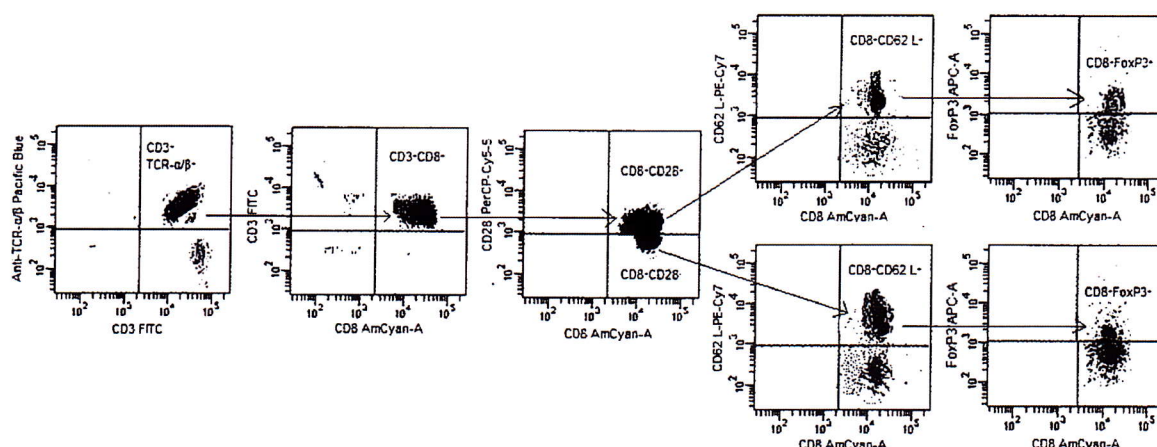


Fig. 2. Flow cytometric analysis of CD8⁺ T cells co expression CD28, CD62L, TCRαβ, and FoxP3 molecules. Untreated and treated CD8⁺ T cells from each group were separately stained with anti-TCRαβ, anti-CD3, anti-CD8, anti-CD28 and anti-CD62L and anti-FoxP3 monoclonal antibodies. CD3⁺CD8⁺T population were evaluated for the expression of selected markers by gating: CD3⁺ lymphocytes from TCRαβ⁺ cells population; CD8⁺ lymphocytes from CD3⁺ cells; CD28⁺ lymphocytes from CD8⁺ cells; CD62L⁺ lymphocytes from CD28⁺ or CD28⁻ cells; FoxP3⁺ lymphocytes from CD62L⁺ cells. Quadrants show staining of populations gated according to fluorescence-minus-one (FMO) control technique.

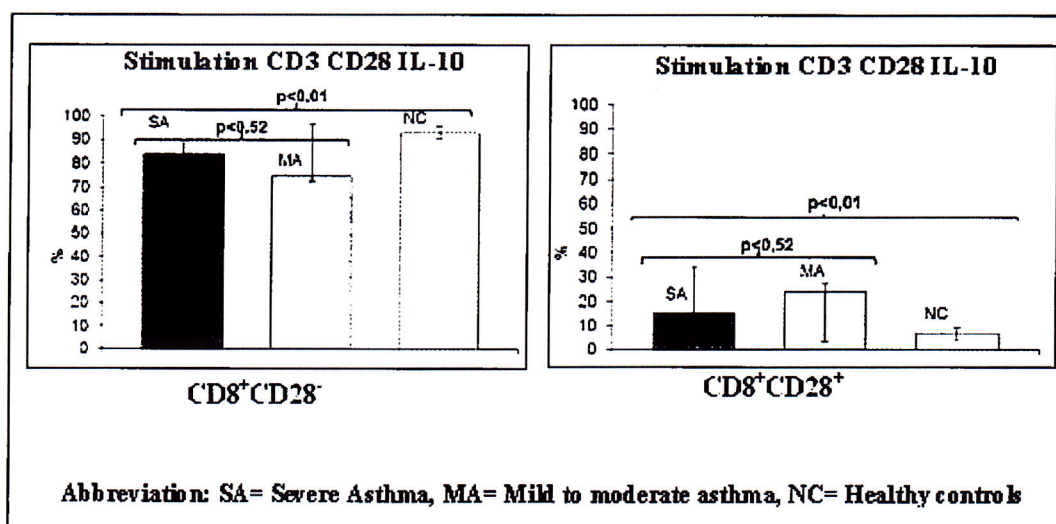


Fig. 3. Comparative analyses of the percentages of enriched CD8⁺CD28⁻ and CD8⁺CD28⁺ T cells using the Wilcoxon Mann-Whitney test. Untouched CD8⁺ T cells were magnetically purified by negative selection from peripheral blood mononuclear cells (PBMC) obtained from buffy coats fraction of peripheral blood according manufacturers' instructions. After staining with antibodies, the surface expression of CD3, CD8, and CD28 was assessed by flow cytometry. Shown are the relative percentages of CD8⁺CD28⁻ and CD8⁺CD28⁺ T cells. The bar graph shows the median $\pm$ Interquartile (IQ) of 4 independent experiments. P values of 0.05 or less were considered statistically significant.

The severe asthma group comprised 12 men and 13 women whereas the mild to moderate asthma group included 13 men and 12 women. The control group comprised 13 men and 12 women. The mean year of: severe asthma subjects was 48 ± 14 , mild to moderate asthma subjects 42 ± 13 , and controls 48.5 ± 14.5

patients.

Comparison of patients' clinical information

Allergic asthma subjects had common asthma symptoms and were skin prick test-positive (>3 mm wheal) for at least 1 of 9 common aeroallergens

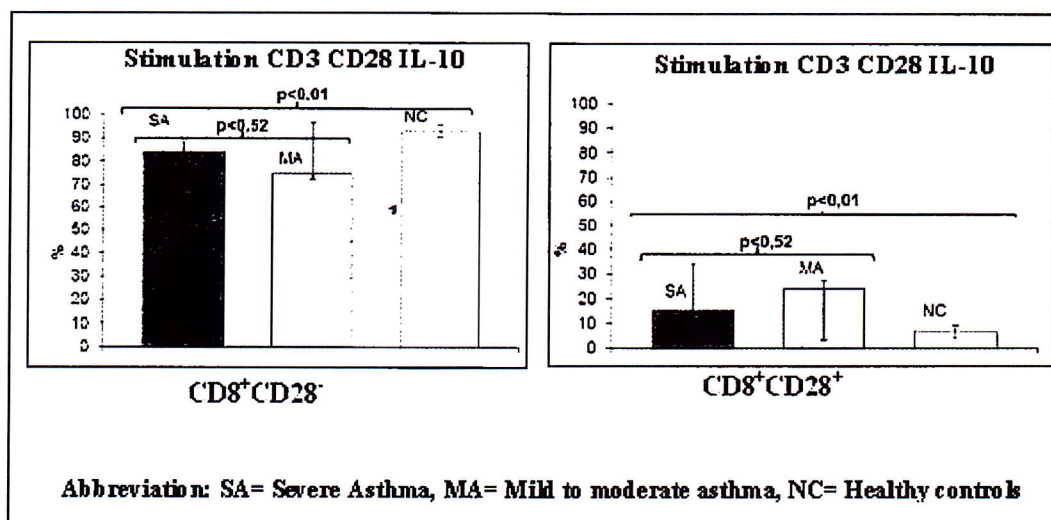


Fig. 4. Comparison of the percentages of in vitro activated CD8⁺CD28⁻ and CD8⁺CD28⁺ T cells cultures using the Mann-Whitney test. Anti-CD3/anti-CD28/IL-2-activated CD8⁺ T cells were cultured in the presence of IL-10. After 3 days in culture, the surface expression of CD3, CD8, and CD28 was assessed by flow cytometry. Shown are the relative frequencies of CD8⁺CD28⁻ and CD8⁺CD28⁺ T cells. The bar graph indicates the median $\pm$ Interquartile (IQ). P values of 0.05 or less were considered statistically significant.

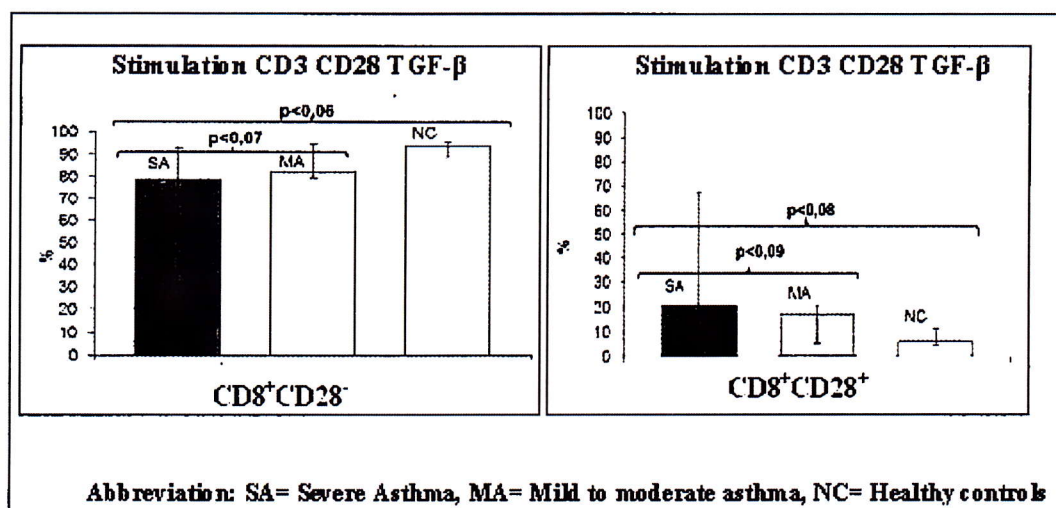


Fig. 5. Comparison of the percentages of in vitro activated CD8⁺CD28⁻ and CD8⁺CD28⁺ T cells cultures using the Mann-Whitney test. Anti-CD3/anti-CD28/IL-2-activated CD8⁺ T cells were cultured in the presence of TGF-β. After 3 days in culture, the surface expression of CD3, CD8, and CD28 was assessed by flow cytometry. Shown are the relative percentages of CD8⁺CD28⁻ and CD8⁺CD28⁺ T cells. The bar graph indicates the median $\pm$ Interquartile (IQ). P values of 0.05 or less were considered statistically significant.

extracts. Airway reversibility was defined as an increase of at least 12% or 200 ml in forced expiratory volume in 1s (FEV₁) 30 min after inhalation of two puffs of salbutamol (2x100 μg). Airflow obstruction was determined whenever FEV₁ and FVC were beneath 80% of the predicted values and FEV₁/FVC

ratio was below 75% of the predicted values. Severe asthma subjects had longer duration of disease 16 $\pm$ 8.7 than those with mild to moderate asthma 10 $\pm$ 6.3. The percentage predicted FEV₁ of severe asthma subjects was 67 $\pm$ 15.98, mild to moderate asthma 87.71 $\pm$ 16.12, and controls 96.6 $\pm$ 4. Patients

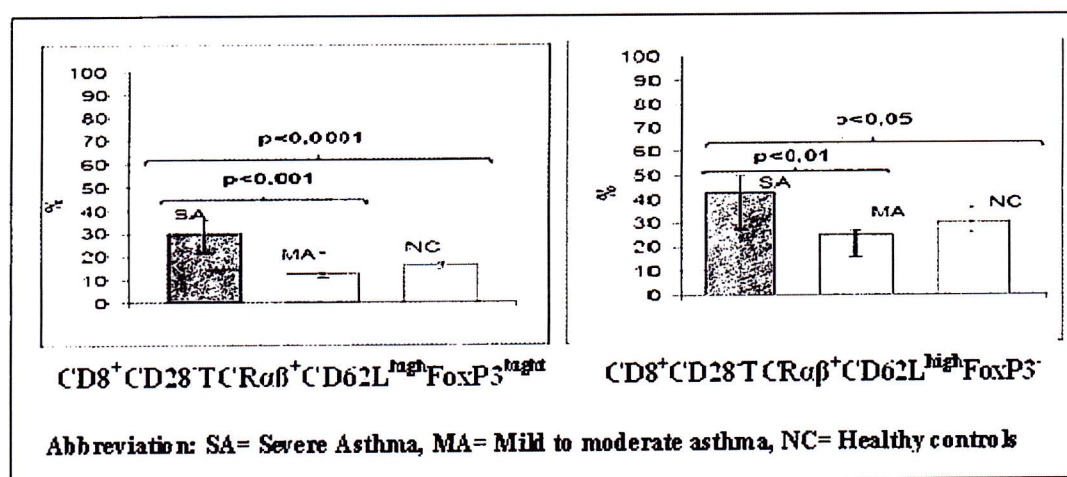


Fig. 6. Phenotype of enriched $CD8^+CD28^-TCR\alpha\beta^+CD62L^{high}FoxP3^{bright}$ T cells and $CD8^+CD28^-TCR\alpha\beta^+CD62L^{high}FoxP3^-$ T cells. Untouched $CD8^+$ T cells were purified by negative selection from PBMC obtained from peripheral blood according manufacturers' instructions. After staining with antibodies against specific antibodies, the surface expression of TCR $\alpha\beta$, CD3, CD8, CD28, CD62L, and the intracellular transcription factor FoxP3 by enriched $CD8^+$ T cells was assessed by flow cytometry. Shown are the relative percentages of $CD8^+CD28^-TCR\alpha\beta^+CD62L^{high}FoxP3^{bright}$ T cells as well as $CD8^+CD28^-TCR\alpha\beta^+CD62L^{high}FoxP3^-$ T cells. The bar graph shows the median $\pm$ Interquartile (IQ). P values of 0.05 or less were considered statistically significant.

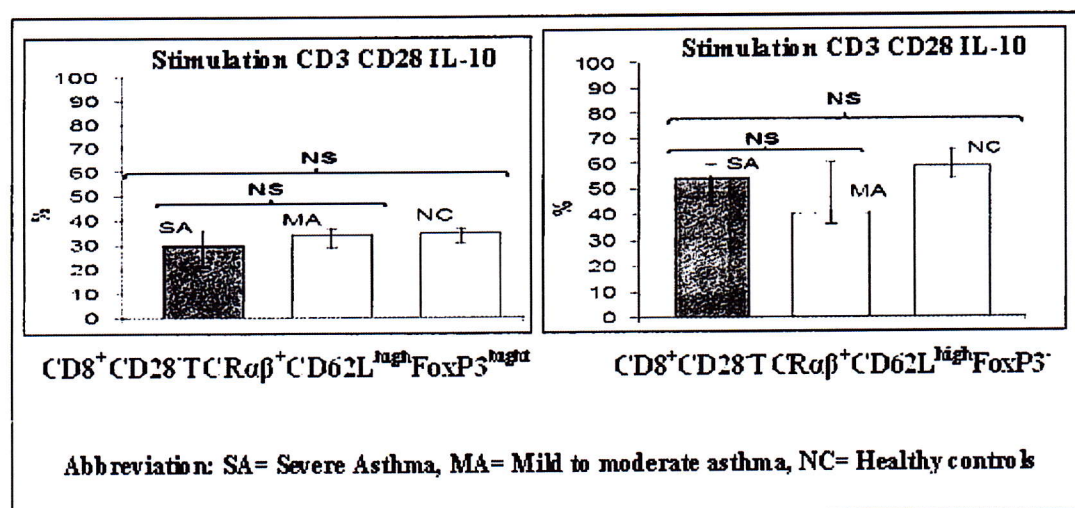


Fig. 7. Comparison of the effects of anti-CD3/anti-CD28/IL-2 and IL-10 co-stimulation on the proportion of $CD8^+CD28^-CD62L^{high}FoxP3^{bright}$ and $CD8^+CD28^-CD62L^{high}FoxP3^-$ T cells using the Mann-Whitney test. After 3 days in culture, the surface expression of TCR $\alpha\beta$, CD3, CD8, CD28, CD62L, and the intracellular transcription factor FoxP3 by $CD8^+$ T cells was assessed by flow cytometry. The $CD8^+CD28^-TCR\alpha\beta^+CD62L^{high}FoxP3^{bright}$ T cells and $CD8^+CD28^-TCR\alpha\beta^+CD62L^{high}FoxP3^-$ T cells frequencies are shown. The bar graph shows the median $\pm$ Interquartile (IQ). P values of 0.05 or less were considered statistically significant.

with severe asthma have taken either high dose of inhaled glucocorticosteroids (ICS) (800-1600 μ g), long-acting β -agonists (LABA) for at least a year. Patients with mild to moderate asthma received daily inhaled steroids (200-800 μ g) in combination with symptomatic therapy. All healthy controls had negative-skin prick test to all tested allergens, normal

chest X-Ray and baseline FEV₁.

Severe asthma group had a significantly higher percentage of $CD8^+CD28^-$ but lower percentage of $CD8^+CD28^+$ T cells in peripheral blood than others

Patients with severe allergic asthma (73.2 $\pm$ 65.6%) had significantly higher relative percentage of

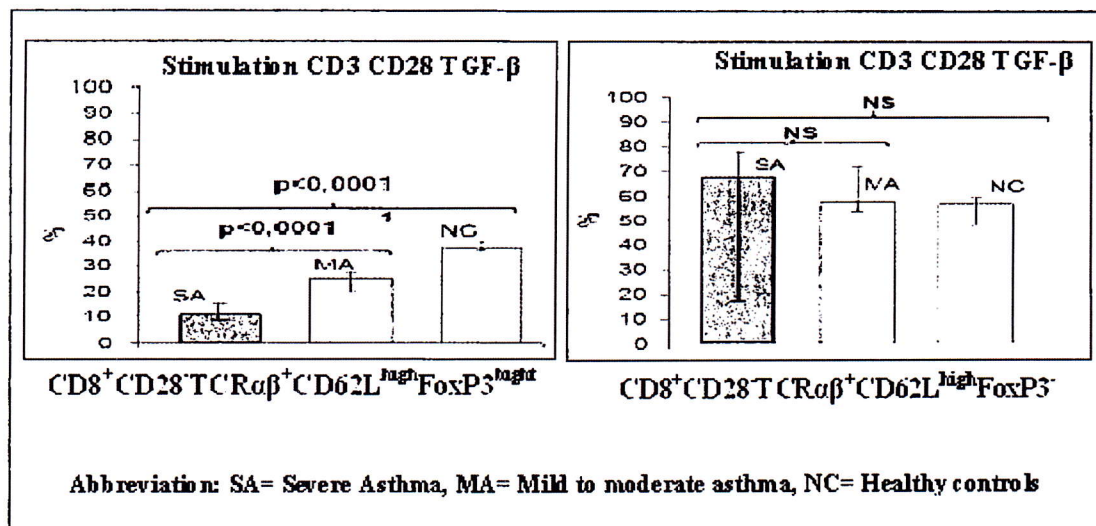


Fig. 8. Comparison of the effects of anti-CD3/anti-CD28/IL-2 and TGF-β co-stimulation on the proportion of CD8⁺CD28⁻CD62L^{high}FoxP3^{bright} and CD8⁺CD28⁻CD62L^{high}FoxP3⁻ T cells using the Mann-Whitney test. After 3 days in culture, the surface expression of TCRαβ, CD3, CD8, CD28, CD62L, and the intracellular transcription factor FoxP3 by CD8⁺ T cells was assessed by flow cytometry. The CD8⁺CD28⁻TCRαβ⁺CD62L^{high}FoxP3^{bright} T cells and CD8⁺CD28⁻TCRαβ⁺CD62L^{high}FoxP3⁻ T cells frequencies are shown. The bar graph shows the median ± Interquartile (IQ). P values of 0.05 or less were considered statistically significant.

CD8⁺CD28⁻ T cells within the isolated CD8⁺ T cells than those with mild to moderate allergic asthma (37.5±37.35%) (p<0.001) and controls (46.3±47.6%) (p<0.05). In contrast, the percentage of circulating CD8⁺CD28⁺ T cells within the freshly isolated CD8⁺ T cells was significantly lower in the severe asthma group (26.8±34.35%) than in the mild to moderate phenotype (61.7±62.75) (p<0.01) and controls (54.2±52.4) groups (p<0.02) (Fig. 3).

Co-stimulation with either IL-10 or TGF-β caused an increase in the percentage of CD8⁺CD28⁻ but a decrease in percentage of CD8⁺CD28⁺ T cells within anti-CD3/CD28 and IL-2 activated CD8⁺ T cells from all groups

Findings from immunotherapy trials suggest that anti-CD28 (αCD28) can enhance the stimulatory effects of αCD3 on T cells due to its capacity to mimic the B7.1 or B7.2 ligand (11). IL-2, which is an autocrine cytokine play a crucial role in the activation, growth, proliferation of antigen-specific T-cell clones. It has been demonstrated that anti-CD3/CD28 and IL-2 regulate T-cell proliferation in different ways (12). Some reports suggest that the stimulatory effects of αCD3/CD28 on CD8⁺ T cells are IL-2 independent (13) whereas other studies

have proven otherwise. Because IL-10 and TGF-β direct T-cell differentiation, we asked whether they play a role in the pathogenesis of AA, and whether they have some effects on TCRαβ, CD28, CD62L and FoxP3 expression on αCD3/αCD28/IL-2 treated CD8⁺ T cells. In relation to the baseline, co-stimulation with αCD3/αCD28/IL-2 and IL-10 resulted in a 1.14 (73.2±65.6% to 84.1±77%) fold upgrade in the percentage of CD8⁺CD28⁻ T cells in the SA subgroup compared with 1.99-fold (37.5±37.35% to 74.6±84.65%) in the MA subgroup and 2-fold (46.3±47.6% to (92.9±93.3%) increase in the controls group (Fig. 4). In contrast, co-stimulation with αCD3/αCD28/IL-2 and TGF-β resulted in a 1.77-fold (26.8±34.35% to 15.1±22.9%) decrease in the relative percentage of CD8⁺CD28⁺ T cells in the SA subgroup compared with 2.56-fold (61.7±62.75 to 24.1±15.35%) in the MA subgroup and 7.74 fold (54.2±52.4) to (7±6.65 %) in the NC group (Fig. 5).

Severe allergic asthma group had increased CD8⁺CD28⁻TCRαβ⁺CD62L^{high}FoxP3^{bright} T-cell frequencies than other groups after enrichment

Continuing OVA aerosol challenge has proven to increase the number of TCRαβ⁺ cells (14). Here, we identify and characterize CD8⁺CD28⁻

TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cells in peripheral blood of healthy and allergic asthma subjects before stimulation (baseline). The severe asthma subgroup had significantly higher relative proportion of CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cells (30.3 ± 28.85) than subjects with the mild to moderate asthma (12.8 ± 16.55) ($p < 0.001$) and controls (16.2 ± 16.45) ($p < 0.0001$), $p < 0.05$ (Fig. 6).

Compared to the baseline, α CD3/ α CD28/IL-2 and IL-10 or TGF- β stimulation decreased the frequencies of CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cells in severe asthma subgroup

Compared to the baseline, stimulation with α CD3/ α CD28/IL-2 and IL-10 slightly decreased the percentage of CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cells in severe asthma group (from 30.3 ± 28.85 to 29.8 ± 28.6) (Fig. 7). Similarly, co-stimulation with α CD3/ α CD28/IL-2 and TGF- β caused 2.75-fold (from 30.3 ± 28.85 to 11.0 ± 11.75) decrease in this parameter in this subgroup (Fig. 8).

Compared to the baseline, co-stimulation with α CD3/ α CD28/IL-2 and IL-10 or TGF- β increased the frequencies of CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cells in mild to moderate asthma and controls groups

Co-stimulation with α CD3/ α CD28/IL-2 and IL-10 resulted in 2.66-fold increase (12.8 ± 16.55 to 34.1 ± 32.55) in CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cell frequency in the mild to moderate asthma group and 2.12-fold increase (from 16.2 ± 16.45 to 34.5 ± 33.65) in controls group (Fig. 7). Similarly, co-stimulation with α CD3/ α CD28/IL-2 and TGF- β led to 1.90-fold (from 12.8 ± 16.55 to 24.4 ± 23.5) increase in this parameter in the mild to moderate asthma and 2.29-fold (from 16.2 ± 16.45 to 37.2 ± 37.85) increase in controls groups (Fig. 8).

DISCUSSION

Previous studies examining human T cells have reported that altered numbers of intraepithelial CD8⁺TCR $\alpha\beta^+$ T cells correlate with perpetuated inflammation (15). Moreover, an altered distribution of CD8⁺TCR $\alpha\beta^+$ T cells has been observed in

bronchial alveolar lavage (BAL) of allergic animal models (16). A growing body of evidence suggests an association between the expression levels of L-selectin, airway hyper responsiveness, and chronic allergic asthma (17). Increase percentages of circulating Foxp3⁺CD62L⁺ T cells are seen in graft-versus-host disease (GvHD) (18). Initial results from our research group established a link between frequencies of human circulatory CD8⁺CD25⁺ T cells expressing high level of FoxP3 and the development of allergic asthma (19). In light of these evidences, the present study shows an altered expression of CD8⁺CD28⁺TCR $\alpha\beta^+$ T cells subset expressing high level of CD62L and FoxP3 in allergic asthma subjects, indicating a role for this subset in the course and clinical outcome of allergic airway inflammation. This observation led us hypothesize that the migration of circulating FoxP3 expressing CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high} T cells to the airways is impaired in allergic asthma subjects as evidenced by their altered expression of adhesion molecule CD62L. Our observation is consistent with previous studies (20) demonstrating an increase expression of FoxP3⁺ T cells to offset the aggravated atopy and airway inflammation. However, it detracts from other reports which found no significant differences between the levels of L-selectin expressing-T cells from patients with asthma and healthy subjects (21). Previous studies have established a correlation between the proportion of FoxP3 and CD62L (22) expressing T cells and the levels of serum Immunoglobulin E (IgE). Due to the fact that the levels of serum IgE determine clinical expression and severity of allergic asthma (23), one would expect a higher expression of CD62L in allergic asthma subjects compared to controls. Surprisingly, the percentage of CD8⁺CD28⁺TCR $\alpha\beta^+$ CD62L^{high}FoxP3^{bright} T cells was increased in severe asthma subgroup whereas this parameter was decreased in mild to moderate asthma subgroup. There are several possible explanations for the discrepancies between the severe and mild to moderate asthma in relation to controls. T helper 2 (Th₂) cells and related cytokines such as IL-4 and IL-5 have long been presumed to play an exclusive role in asthma and allergy. In this perspective, Th₁ cells were thought to down modulate the effects of Th₂ cells, thus their protective role in asthma. Recently,

the pro-inflammatory properties of interferon- γ (IFN- γ), a cytokine secreted by Th₁ has begun to emerge. IFN- γ and IL-4 are proven to cross regulate each other. The former is thought to suppress Th₂ cells whereas the latter appears to inhibit Th₁ subset. It is becoming clear that IFN- γ secreting-Th₁ cell can predispose individuals to developing severe asthma. Interestingly, IFN- γ has been shown to increase CD62L expression on circulating leukocytes (24). Therefore, differences in cytokines profiles between severe and mild to moderate asthma subjects offer a possible explanation for our findings. Some reports indicate that IL-4 mediates airway inflammation by inhibiting IFN- γ (25), whereas others provide reliable evidences that IFN- γ inhibits IL-4 effects and up-regulate Foxp3 and CD62L (26). Thus, it could conceivably be hypothesized that, in severe allergic asthma subjects, IFN- γ suppresses IL-4, the dominant Th₂ cytokine to enhance Foxp3 and CD62L expression. Furthermore, Q576R polymorphism in the gene encoding the IL-4 receptor has been identified in patients with severe asthma (27). The difference between the allergic asthma groups may be partially explained by the IL-4 receptor polymorphism in severe asthma subjects. It has also been demonstrated that glucocorticoids have some effects on intracellular L-selectin mRNA (28) and FOXP3 expressions. We consider difference in glucocorticosteroids dose intake between asthma subgroups as another probable explanation for some discrepancies between expected theoretical and experimental data. Overall, considering the importance of homing adhesion molecule CD62L in T cell migration in the lungs, we speculate that any alteration of its expression can impede the infiltration of CD8⁺CD28⁺TCR $\alpha\beta$ CD62L^{high}FoxP3^{bright} T cells into the airways, perpetuating inflammation. It has been documented that costimulation of T cells with α CD3/CD28 and IL-2 greatly increases the proportion of FoxP3⁺ T cells (29). Our findings lend support to this idea by showing an increase frequency of CD8⁺CD28⁺TCR $\alpha\beta$ CD62L^{high}FoxP3^{bright} T cells in mild to moderate asthma and controls groups. Surprisingly, culture under similar condition led to a decrease in the relative percentage of this subset in severe allergic asthma subgroup. It has been shown that TCR-stimulated T cells from allergic asthma subjects are resistant to certain inhibitory effects of

TGF- β and IL-10 as compared with healthy controls (30). We hypothesized that the differential response of CD8⁺CD28⁺TCR $\alpha\beta$ CD62L^{high}FoxP3^{bright} T cells from allergic asthma subjects to the effects of TGF- β and IL-10 may provide another explanations for our findings. Albeit suggestive, our hypotheses need to be confirmed by larger and unbiased studies. In summary, we established a link between altered expression of high level of CD62L and FoxP3 on CD8⁺CD28⁺TCR $\alpha\beta$ T cell and allergic asthma. These data have important implications for further characterization of CD8⁺CD28⁺TCR $\alpha\beta$ T cells subsets and with special attention on their role in healthy or allergic immune response.

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