

Frequencies of circulating CD4+CD25+CD127low cells in atopics are altered by bronchial allergen challenge

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ABSTRACT

Background Recent studies in rodents revealed that regulatory T cells (T reg cells) with CD4+CD25+ phenotype can exert suppressive effects on experimentally-induced allergic airway inflammation and airway hyper-reactivity. It is unclear however, whether modulations of bronchoconstriction responses in human subjects might be related to T reg cells. We report here for the first time the changes in frequency of circulating lymphocytes with putative T reg cell phenotype (CD4+CD25+CD127low) in relation to bronchoconstriction phenotype following an intrabronchial allergen challenge.

Material and methods Thirty-one house dust mite sensitive patients were challenged with *Dermatophagoides pteronyssinus* extract (Dp). Eleven isolated early responders (IER) were compared with nine dual (early and late) responders (DR) and to 11 non-responders (NR). Frequencies of peripheral blood CD4+CD25+CD127low lymphocytes were assessed in all groups of patients by using three-parameter flow cytometry before, and six and 24 h, after allergen inhalation.

Results At baseline, frequencies of CD4+CD25+CD127low lymphocytes were not statistically different among NR, IER and DR. When all individuals were analyzed together, a statistically significant decrease in frequency of CD4+CD25+CD127low lymphocytes was observed 6 h after the bronchial challenge. Interestingly, such a pattern was found consistently only in NR, while IER and DR displayed varying responses resulting in a trend similar to that of NR. Twenty-four hours after the bronchial challenge, frequencies of CD4+CD25+CD127low lymphocytes in all groups tended to return to baseline values.

Conclusions Our data indicate that bronchial allergen inhalation in sensitive patients (predominantly in those who did not develop significant bronchoconstriction) is associated with a decrease of proportion of peripheral lymphocytes with regulatory T cell phenotype.

Keywords Airway hyper-reactivity, IL-2R, IL-7R, T reg cells.

Eur J Clin Invest 2008; 38 (3): 201–204

Introduction

CD4 + regulatory T cells (T reg cells) play a vital role in controlling allergic responses and their function is disturbed in the active stage of allergic inflammation [1–3]. Recent experimental studies in rodents revealed the protective role of CD4+CD25+ regulatory T cells against the development of allergic airway disease [4–6]. Nonetheless, the potential role of T reg cells in modulating allergic asthmatic reactions in humans remains poorly understood. Recently, the use of a combination of monoclonal antibodies (mAbs) against CD4, CD25 (the α chain of the IL-2 receptor), and CD127 (the α chain of the IL-7 receptor) was proposed for flow cytometry-based delineation of a well-separated CD4+CD25+CD127low population of putative T reg cells [7–9].

Those cells, as compared with other cell subsets, were the most highly suppressive in functional assays, poorly responsive to TCR signalling and had the highest levels of Foxp3. Here we use this novel flow-cytometric approach and report the first demonstration of time-course quantitative analysis of lymphocytes with T reg cell phenotype in relation to different bronchoconstriction patterns following intrabronchial allergen challenge.

Materials and methods

Thirty-one individuals aged 21–44 years with a history of dyspnoea and cough after house dust exposure and with skin prick-test reactivity to *Dermatophagoides pteronyssinus* (Dp) and

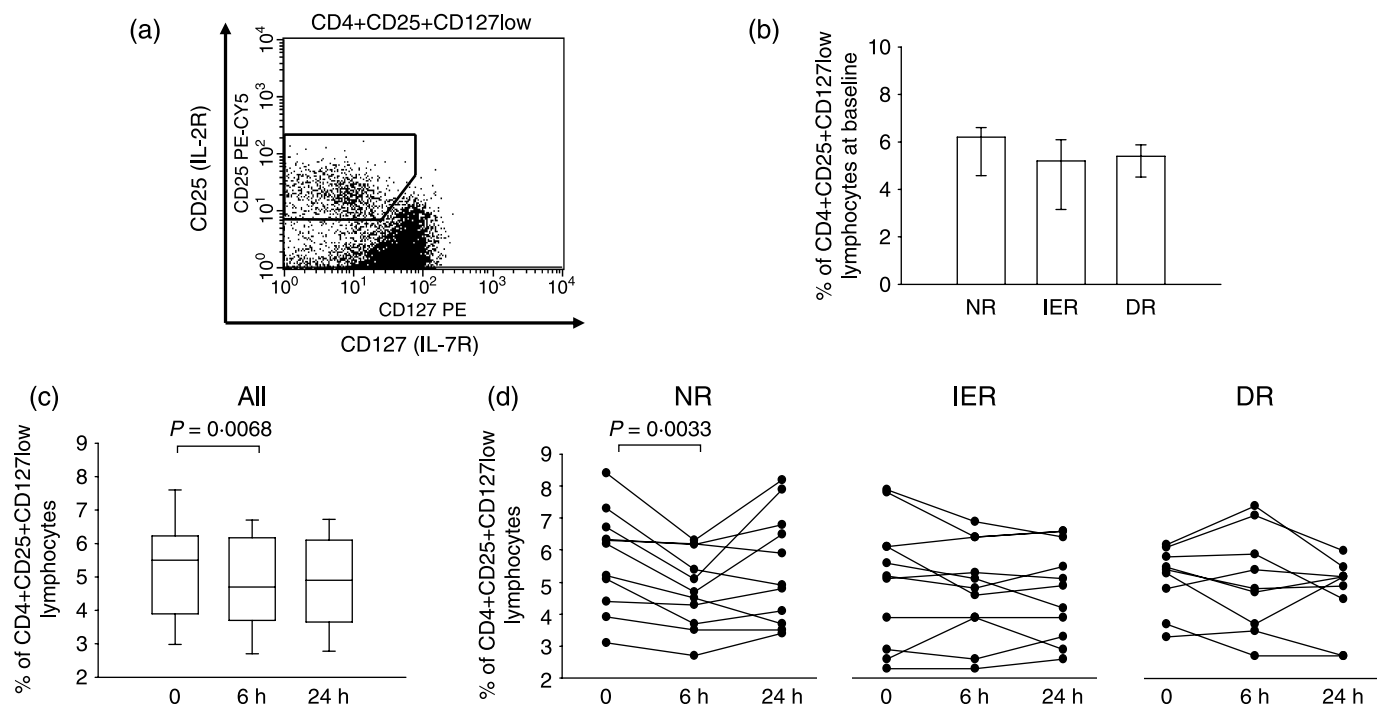


Figure 1 (a) Flow cytometric dot plot characteristic for studied individuals. Plots are gated for CD4+ T lymphocytes and CD25+CD127low lymphocytes are boxed. (b) Frequency of CD4+CD25+CD127low lymphocytes in NR ($n = 11$), IER ($n = 11$) and DR ($n = 9$) at baseline (before allergen challenge). Data are presented as vertical bars representing the median values. Length of whiskers represents 25th and 75th percentiles. Kruskal–Wallis ANOVA test was used to analyze statistically significant differences among groups. A P -value of < 0.05 was considered to indicate statistical significance. (c) Time course analysis of CD4+CD25+CD127low lymphocyte frequencies in all house dust mite-sensitive subjects ($n = 31$) before and 6 and 24 h after bronchial allergen challenge. Data are depicted as box plots. Medians are noted by inside bars and the boxes span the 25th and 75th percentiles while whiskers represent the 5th and 95th percentiles. Statistical analysis was performed with the use of non-parametric Wilcoxon test. Statistically significant results were identified by a P -value of < 0.05 . (d) Detailed time course analysis of frequencies of CD4+CD25+CD127low lymphocytes in NR ($n = 11$), IER ($n = 11$) and DR ($n = 9$) before and 6 and 24 h after bronchial allergen challenge. Statistical analysis was performed with the use of non-parametric Wilcoxon test. Statistically significant results were identified by a P -value of < 0.05 .

Dermatophagoides farinae were recruited for the study and hospitalized in our department for 48h observation. All study participants were non-smokers and were never treated for asthma. All subjects were challenged intrabronchially with Dp extract according to a protocol described elsewhere [10]. Increasing doses of allergen (0.8, 4, 20, 100, 500 and 2500 SBE) were administered using De Vilbis #646 nebulizer attached to a Rosenthal – French dosimeter. Forced expiratory manoeuvres were performed 15 min after inhalation of each dose of the allergen extract. Allergen inhalations were continued until either at least 20% reduction in forced expiratory volume in 1 s (FEV1) or a cumulative dose 5000 SBE was reached. Subsequently, FEV1 was measured every 15 min during the first hour after challenge, every 60 min during the next 11 h and again after 24 h. Depending on the development or not of early asthmatic response (at least 20% fall of FEV1 within 30 min) or dual asthmatic response (early phase followed by late phase that starts after 3–5 h and is maximal after 6–12 h), subjects

were grouped as isolated early responders (IER), dual responders (DR) or non-responders (NR). There were 11 IER, nine DR and 11 NR. EDTA blood was drawn before, and six and 24 h after, the allergen challenge. The study protocol was approved by local Ethics Committee. Fresh whole blood samples were incubated for 30 min at room temperature with 20 μ L of mAbs: anti-CD4 FITC (BD PharMingen, Erembodegen, Belgium), anti-CD25 PE-Cy5 (IL-2R, BD PharMingen, Erembodegen, Belgium), and anti-CD127 PE (IL-7R, Immunotech, Marseille, France). The cells were washed twice with phosphate-buffered saline, and fixed with 2% paraformaldehyde. Flow cytometry analysis was performed with the use of a FACSCalibur cytometer (BD Immunocytometry Systems, San Jose, USA) as described elsewhere [11,12]. All samples were run in a blinded fashion. First, lymphocytes were gated by a combination of forward and side scatter. The gating of CD4+CD25+CD127low T lymphocytes was performed as described elsewhere [9] (Fig. 1a).

Results

The baseline levels of CD4+CD25+CD127low lymphocytes were not statistically different among NR, IER and DR [6.2% (IQR, 2.28%) vs. 5.2% (IQR, 3.2%) vs. 5.4% (IQR, 1%), respectively, (Fig. 1b). Combined analysis of data from all individuals revealed a statistically significant decrease in the frequency of CD4+CD25+CD127low lymphocytes 6 h after the bronchial challenge from 5.4% (IQR, 2.3%) to 4.8% (IQR, 2.46%), $P = 0.0068$ (Fig. 1c). Interestingly, the same analysis performed for different groups of responders revealed that only NR demonstrated the consistent pattern of a significant decrease in the frequency of CD4+CD25+CD127low lymphocytes from 6.2% (IQR, 2.28%) to 4.7% (IQR, 2.46%), $P = 0.0033$, (Fig. 1d). In contrast, in IER and DR, we observed varying responses among subjects and, despite a trend similar to that of NR, the changes in frequencies of CD4+CD25+CD127low lymphocytes did not reach statistical significance: IER: from 5.2% (IQR, 3.2%) to 4.8% (IQR, 2.5%), $P = 0.15$, DR: from 5.4% (IQR, 1%) to 4.8% (IQR, 2.2%), $P = 0.85$. Twenty-four hours after the bronchial challenge, frequencies of CD4+CD25+CD127low lymphocytes in all groups tended to return to baseline values: NR: from 4.7%, (IQR, 2.46%) to 4.9% (IQR, 3.1%), $P = 0.1$; IER: from 4.8% (IQR, 2.5%) to 4.9% (IQR, 3.1%), $P = 0.95$; DR: from 4.8% (IQR, 2.2%) to 5.2% (IQR, 0.7%), $P = 0.32$.

Discussion

In this study, for the first time, the frequencies of lymphocytes with T reg cell phenotype were quantified in the setting of an allergen challenge in sensitive human subjects. The most intriguing result of our report is that allergen inhalation, predominantly in subjects not developing an asthmatic reaction, was associated with a decrease in the frequency of circulating CD4+CD25+CD127low T lymphocytes. Importantly, we observed exactly the same pattern of putative T reg cell changes with the use of other, subtly different approaches for phenotypic delineation of T reg cells (i.e. gating proposed by Hartigan O'Connor *et al.* [7] and Liu *et al.* [8] or gating for CD4+CD25high T cells only – data not shown). Thus we confirm that a bronchial allergen challenge in atopic subjects causes significant alteration of numbers of peripheral lymphocytes with T reg cell phenotype. It is unclear now whether the portion of T reg cells may migrate to airways following a bronchial challenge with allergen and whether T reg cells themselves could contribute to suppression of bronchoconstriction. This hypothesis comes from experimental studies performed in mice that revealed a protective role of either adoptively transferred or non-depleted autologous T reg cells against the development of airway hyper-reactivity [4–6]. Thus, further studies will be required to better explore the potential capacity of T reg cells to suppress allergen-induced bronchoconstriction in human subjects.

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Received 4 September 2007; accepted 6 December 2007

Acknowledgements

The authors wish to thank Steven Snodgrass (NCI, NIH, Bethesda, MD) for editorial assistance and Teresa Michno for laboratory assistance. The work was supported by grants from the Medical University of Bialystok (Nos 3-35521P and 3-06729P).

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