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IL-10 Receptor Expression on Lymphocytes and Monocytes in Children with Food Allergy

To the editor,

The article by Crealy et al “Prevalence of IgE mediated food allergy in children with inflammatory bowel disease” [1] provides further evidence of the common pathways that disrupt the gut homeostasis leading to perpetuation of inflammatory activity in the bowel manifesting as food allergy, inflammatory bowel disease (IBD) or both. Numerous studies in adults and children have investigated the mechanisms that abrogate oral tolerance and the active suppression of immune responses to antigens and microbes.

Emerging evidence highlights the critical role of interleukin 10 (IL-10) as a key mediator of different cell populations and intestinal immune responses. More specifically, the IL-10/IL-10R receptor (IL-10R) signaling pathway has been shown to modulate both innate and adaptive immune responses in the gastrointestinal (GI) tract of humans and animal models [2]. IL-10R is a heterodimeric cell surface complex consisting of IL-10Ra and IL-10Rb chains. The extracellular ligand binding domain of IL-10Ra forms specific high affinity interactions with IL-10, whereas IL-10Rb is characterized by low affinity and is a shared receptor with other class 2 cytokines [3].

In normal states, the IL-10/ IL-10R complex initiates a cascade of reactions leading to the phosphorylation of signal transducer and activator of the transcription 3 (STAT3) and

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resulting in the suppression of inflammatory cytokines (interleukin 6, tumor necrosis factor α , interleukin 1 β) [4], [5]. On the contrary, IL-10 deficient mice present with spontaneous enterocolitis [6] and infantile onset IBD has been associated with loss of function variants in both subunits of IL-10R [7]–[9].

We would like to present our preliminary data of the expression of IL-10R on peripheral blood lymphocytes and monocytes in children with IgE- mediated food allergy compared with age- and sex - matched healthy controls. The diagnosis of IgE- mediated food allergy was established based on the published guidelines in children who developed specific signs and symptoms on the exposure to the food antigen(s) along with antigen-specific IgE titer ≥ 0.35 kU/L (ImmunoCAP100 system phadia, Uppsala, Sweden) and/or positive skin prick test(s) to the offending food antigen(s). The healthy controls had negative personal or family history of atopic diseases (FA, asthma, hay fever, atopic dermatitis), negative specific IgE antibodies against the most common food antigens (milk, soya, egg, wheat, fish) and IgE level within the normal range. Children with chronic or immune mediated disorders or recent febrile illness (within 3 months) were excluded from the both groups.

We investigated the expression of IL-10R on the surface of T and B lymphocytes and on the surface of monocytes in total 44 patients, 23 with IgE- mediated food allergy and 21 healthy controls. We chose to determine the expression of IL-10Ra because it specifically binds IL-10. The demographic characteristics of the participants are summarized in table 1.

IL-10R1 (IL-10Ra) expression on the surface of CD4+ helper T cells, CD19+ B cells and CD14+ monocytes was determined by flow cytometry with the use of monoclonal antibodies with the following specificities and fluorochrome conjugations: CD3-PC5 (UCHT1), CD4-FITC (13B8.2), CD14-FITC (RM052), CD19-PC7 (J3-119), CD45-ECD (J33) were purchased from Beckman Coulter (Minnesota, Miami, USA); CD210a- PE (IL-10Ra) and the appropriate isotype- matched control antibody- PE were purchased from BD PharMingen, San Diego, CA, USA. Fresh whole blood samples were incubated for 15 minutes at room temperature with monoclonal antibodies protected from light. The red blood cells were lysed with the EXCELLYSE solution (EXBIO, Praha Czech Republic) diluted 1/10 and incubated at room temperature in the dark for another 10 minutes. Samples were centrifuged at 300g for 5 minutes and the supernatant was discarded. The pellet was

suspended in PBS saline to adjust cell suspension to 1×10^6 cells/500 μ L. The acquisition of 50,000 events was performed within 30 minutes on a FC500 Beckman Coulter flow cytometry analyzer. Lymphocytes were identified by their forward (FSC) and side scatter (SSC) characteristics, whereas helper T cells and B cells were identified by the expression of the specific markers anti-CD3/anti-CD4 and anti-CD19 respectively. Monocytes were identified by their forward (FSC) and side scatter (SSC) characteristics and the anti-CD14 antibody. The data are presented as the mean fluorescence intensity (MFI) within each cell subset as shown in Figure 1. Throughout the study we compared patients versus healthy controls and MFI values of unstained (isotype control) and stained cells for each cell subset.

Our results indicate that the highest expression of IL-10R was observed on monocytes in both food allergy patients and healthy controls. There was no difference in the expression of IL-10R on monocytes between children with food allergy and healthy controls neither on the surface of CD19 B lymphocytes (table 1). On the contrary, the expression of IL-10R on CD4⁺ Th lymphocytes was significantly higher in patients with food allergy compared to controls ($p < 0.0001$, table 1, Figure 2). The expression of IL-10R on CD4⁺ T lymphocytes wasn't correlated with serum IL-10 levels in 16/23 patients with food allergy that they had their serum cytokine determined.

Our preliminary results could indicate that the significantly higher expression of IL-10R on CD4⁺ T lymphocytes in food allergy patients compared with healthy controls reflects a compensatory mechanism of the organism to overcome the lower levels of IL-10 that have been reported in patients with active food allergy [10], [11]. Our data did not confirm a negative correlation between the expression of IL-10R and the levels of serum IL-10 and this can be attributed to the small sample size and also the fact that the patients with food allergy had similar low levels of serum IL-10 (range 2.87- 4.35 pg/ml). The higher expression of IL-10R on CD4⁺ T lymphocytes, but not on B lymphocytes or monocytes, in food allergy patients could also suggest a disruption of the IL-10/IL-10R signaling pathway in food allergy in which the cellular immune responses are mediated by T lymphocytes. Further studies on the expression, as well as the function of IL-10R on both unstimulated and stimulated immune cells including CD4⁺ T cell subsets (i.e. Tregs, Th17) are required to shed more light into the complex pathways that disturb the gut homeostasis and to elucidate how IL-10 signaling modulates the inflammatory activity in the GI tract.

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	Food allergy Group	Controls	p
	n=23	n=21	
Age [years; mean±SD (range)]	4.9 ± 2.46 (1-11)	5.2 ± 3 (1-10)	0.734
Male/female sex	12/11	11/10	1.0
Expression of IL10-receptro (IL10R1, MFI*)			
on CD4+	1.24± 0.18	0.69±0.2	<0.0001
on CD19+	1.02 ± 0.2	1.08±0.4	0.729
on monocytes	3.58±0.5	3.7±0.46	0.376

MFI: Mean Fluorescence Intensity

Table 1. Demographic data and expression of IL10-receptor in patients with food allergy and controls

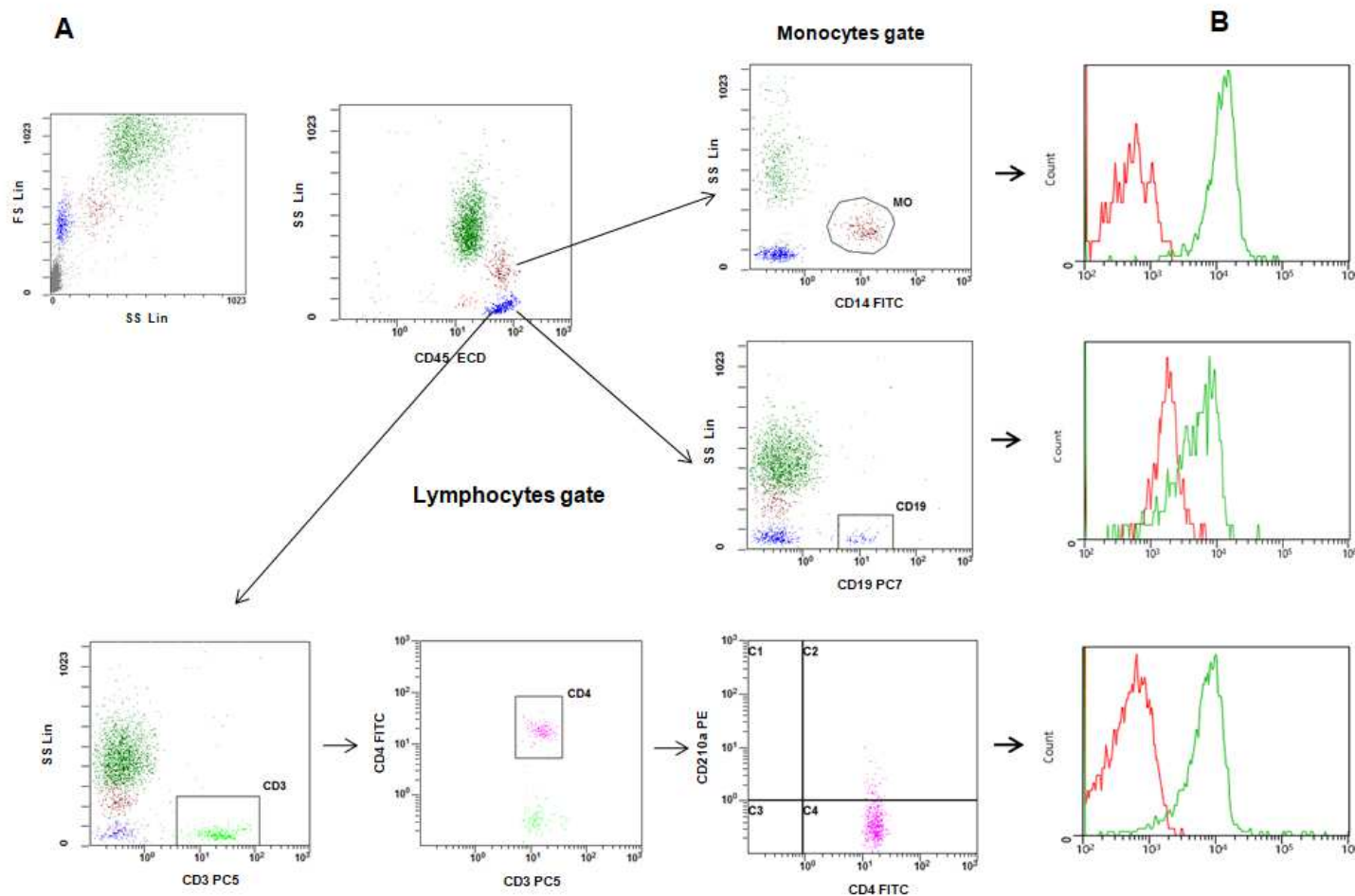


Fig. 1. Gating strategy and IL-10R expression in PBMC lymphocyte subsets and monocytes.

(A) The acquisition and analysis gates were restricted to the lymphocytes (CD19+ B cells, CD3+CD4+ T cells) and monocytes as determined by the forward scatter (FSC) and side scatter (SSC) characteristics of these cells combined with the CD45 expression. A CD3 versus SS display was used to identify T-cells, CD19 versus SS for B-cells and CD14 versus SS for monocytes.

(B) IL-10R expression (MFI) in CD3+CD4+ T cells, CD19+ B cells and monocytes. Red line histograms represents isotype control (unstained cells) and green line histograms represents cells stained with CD210a (IL-10R1).

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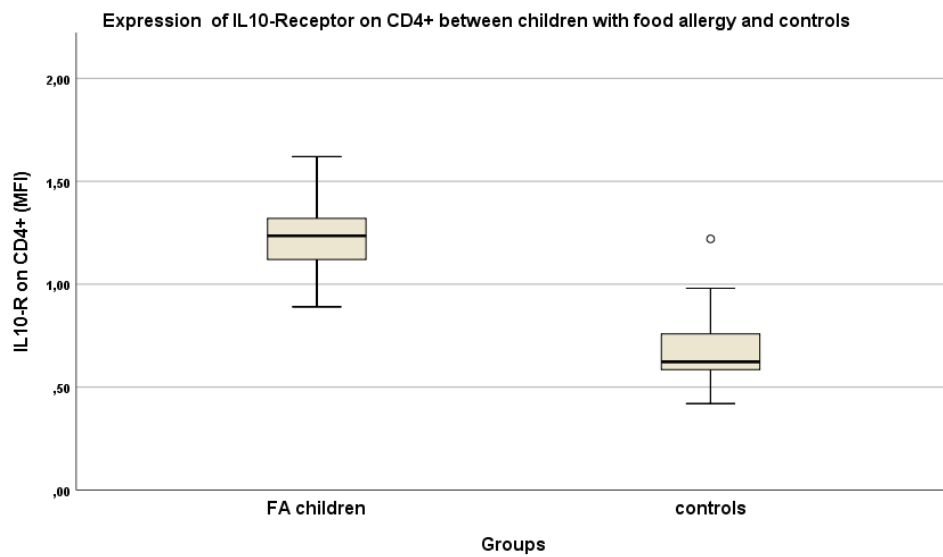


Figure 2. Expression of IL-10 R on CD4+ T lymphocytes in children with food allergy and controls