



Excessive Body Weight and Immunological Response in Children with Allergic Diseases

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Abstract

The prevalence of allergy and obesity is sharply on the rise in children. However, the nature of a mutual relation of the two conditions remains unclear. The aim of the study was to assess the impact of excessive body weight on the immune response in children with allergies. There were 56 children with allergies, aged 4–15 years, included into the study (41 with asthma and 15 with atopic dermatitis). Based on the body mass index, children were divided into two groups: normal weight (body mass index (BMI) <85th percentile) and excessive weight (BMI ≥ 85th percentile). The immunological parameters were evaluated by flow cytometry. We found that children with excessive body weight had a significantly lower percentage of CD4⁺ lymphocytes and a higher percentage of natural killer T cells (NKT) and CD16/56⁺

lymphocytes than those with normal weight. In the group with allergy, a significant positive association was noticed between BMI and the percentage of human leukocyte antigen (HLA)-DR-specific CD3. Further analysis was done after dividing the allergy group into the children with normal and excessive weight. There were an adverse association between BMI and the percentage of CD8⁺ lymphocytes in those with normal weight and a positive one between BMI and the percentage of CD4⁺ in those with excessive weight. We conclude that excessive body weight plays a major role in mediating the immunological response in children with allergy.

Keywords

Allergic diseases · Asthma · Atopic dermatitis · Body weight · Immunological response · Lymphocytes

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1 Introduction

The prevalence of allergy and obesity in children has sharply increased of late. A recent report by WHO (2016) estimates is that over 340 million children and adolescents aged 5–19 years have excessive body weight and obesity, which is an increase from 4% in 1975 to 18% in 2016. The

exact prevalence of overweight and obesity in the population of Polish children remains unknown due to variable assessment criteria and variable age groups included into the relevant studies. The Health Behavior in School-aged Children (HBSC) study conducted in 2013–2014 has reported that 2.4% of children in Poland, aged 11–15 years, are obese and 12.4% are overweight (Oblacińska 2015). Another investigation performed in the OLAF study has revealed the excessive weight in the population of Polish children, aged 6–19 years, is in a range of 14.1–17.9%, depending on the region (Grajda et al. 2011). The latest UNICEF (2013) report shows that weight gain in the Polish children progresses at the fastest pace among the European countries.

Another rising health problem in the Polish population are allergic diseases. The latest data from the ECAP study in three age groups (6–7 years old, 13–14 years old, and young adults 20–44 years old) show that allergic diseases affect about 40% of these populations. The most frequent diagnosed allergic ailments are rhinitis (24–30%), asthma (11%), and atopic dermatitis (9%) (Samoliński et al. 2014).

The available data concerning the association of obesity and allergy do not provide equivocal information. Obesity is associated with a chronic inflammation process of low intensity, known as metabolic inflammation in which adipokines, such as leptin, resistin, and visfatin, produced by fat cells are involved. Recent studies show that the formation of these factors leads to numerous metabolic irregularities and modulates the immunological response. Metabolic and immune systems are strictly related. Consumption of excessive amount of food by obese people is identified by pattern recognition receptors as a harmful, stress-inducing biological event. Fat tissue, consisting mainly of adipocytes, requires preadipocytes, macrophages, fibroblasts, and vascular inflammatory cells for its growth. The exact mechanism of inflammation progression is an area of limited understanding. It is suggested that a hypoxic milieu of adipocytes, oxidative stress, and impairment of peroxisome

proliferator-activated receptors could have a crucial meaning (Góralaska et al. 2015).

The role of overweight and obesity in asthma progression is proven (Granell et al. 2014; Egan et al. 2013; Flaherman and Rutherford 2006). Further, more than 26% of children suffering from asthma are obese (Lang et al. 2012). A coincidence between asthma and obesity also has to do with more severe exacerbations of asthma (Ahmadizar et al. 2016), requiring mechanical ventilation and longer hospitalization (Okubo et al. 2016) and with a worse response to inhaled corticosteroids (Forno et al. 2011). However, although asthma may associate with allergic rhinitis (Bousquet et al. 2012), there is no evidence that rhinitis coexists with obesity (Han et al. 2016). Data regarding the association between atopic dermatitis and obesity are inconsistent either (Sybilski et al. 2015; Zhang and Silverberg 2015). Therefore, this study seeks to define the influence of excessive body weight on the immune system elements in children with allergies. We addressed the issue by evaluating the phenotype of peripheral blood lymphocytes such as CD3⁺, CD4⁺, CD8⁺, CD19⁺, CD16/56, natural killer T cells (NKT), human leukocyte antigen (HLA)-DR-specific CD3, and natural regulatory T lymphocytes (CD4⁺CD25^{high}CD127[−]Foxp3⁺) and the content of the pro-inflammatory interleukin (IL)-1, IL-2, IL-6, IL-17A, IL-22, tumor necrosis factor alpha (TNFα), and interferon gamma (IFNγ) and the anti-inflammatory cytokines (IL-4, IL-10, and transforming growth factor beta (TGFβ)).

2 Methods

2.1 Study Participants

The study group consisted of 56 children with allergies aged 4–15 years hospitalized at the Department of Pediatrics, Pediatric Nephrology and Allergology of the Military Institute of Medicine in Warsaw, Poland, since August 2014 till September 2016. In this group there were

Table 1 Criteria for participation in the study

Inclusion criteria	Exclusion criteria
Positive results of skin prick tests and/or increased concentration of total IgE	Age < 4 years and > 18 years
	Acute respiratory tract infection
Age > 4 years and < 18 years	Coexisting autoimmune system diseases
Consent to participate in the study	Allergen-specific immunotherapy
	Exacerbation of allergic disease

41 children with asthma and 15 children with atopic dermatitis. Atopic background was confirmed by the elevated content of total serum IgE and by positive results of skin prick tests with common alimentary and inhaled allergens. The patients were in a stable period of the disease, with no symptoms of acute respiratory tract infection. The inclusion and exclusion criteria are presented in Table 1.

We tallied the following data: disease duration, severity of symptoms, previous diagnostic procedures, and treatment. All children included into the study had a physical examination with the measurement of body height and weight. The children were divided into two groups based on the body mass index (BMI; kg/m^2), according to the WHO recommendation:

- Group I: normal body weight (BMI < 85 percentile)
- Group II: excessive body weight (BMI $\geq$ 85 percentile)

Body height was measured with the accuracy to 0.5 cm in the upright standing position without shoes and weight with use of a legalized and standardized scale with the accuracy to 100 g for children without shoes and wearing light clothes. Based on measurements of body height and weight, the body mass index (BMI) was calculated according to applicable formula.

Calculated BMI value was applied on the centile grids. Following the WHO recommendations, overweight was defined as BMI at or above the 85th percentile and below the 95th percentile and obesity as BMI at or above the 95th percentile. In further analysis, overweight and obesity are referred to as excessive body weight.

2.2 Analysis of Immunological Indices

2.2.1 Phenotype of Peripheral Blood Lymphocytes

Immunophenotyping of CD3⁺/19⁺, CD4⁺/CD8⁺, CD19⁺, CD16/56, NKT, and HLA-DR-specific CD3 lymphocytes was performed in whole blood specimens, collected into ethylenediamine-tetraacetic acid (EDTA)-primed tubes. A 100 μl of blood was taken for analyses. The samples were incubated with 20 μl of primary antibodies against the lymphocytic antigens for 20 min at room temperature, using an IMK Plus kit (BD Biosciences, Warsaw, Poland). Next, lysis of erythrocytes was done, using FACS Lysing solution (BD Biosciences, Warsaw, Poland), and the cells were washed with phosphate buffer solution (PBS) twice and fixed in 1% solution of paraformaldehyde in PBS. Cells were analyzed in a FACS Calibur Flow Cytometer (BD Immunocytometry Systems, San Jose, CA), equipped with CellQuest Pro software (BD Biosciences, Warsaw, Poland). The results of lymphocyte phenotypes were presented as the mean percentages $\pm$ SD.

2.2.2 Natural T Regulatory (Treg) Lymphocytes

To characterize nTreg cells, whole blood samples (100 μl) were stained with the primary antibodies against CD4-PerCP, CD25-APC, and CD127-FITC (BD Biosciences, Warsaw, Poland) or with an appropriate isotype control with the addition of CD4-PerCP antibody. After a 20-min incubation, lysis of erythrocytes was performed; the cells were washed with PBS. Then, cells were fixed and permeabilized in a specific buffer and

incubated for 40 min at 2–8 °C. Then, cells were stained with FoxP3 PE or isotype IgG1 kappa PE antibody for 45 min in the dark at room temperature. Next, cells were washed twice with PBS, fixed in 300 µl of 1% paraformaldehyde in PBS, and examined by flow cytometry. Ten thousand counts of CD4 PerCP-positive cells was the upper limit of cytometric acquisition. The percentage of nTreg cells (FoxP3⁺, CD25^{high}, and CD127^{low}) was counted in CD4-positive cells. The results were presented as the mean percentages $\pm$ SD.

2.2.3 Cytokines

After “on clot” blood collection and serum isolation (centrifugation at 2,000 $\times$ g, 20 min, 4 °C), samples were stored in aliquots at –80 °C, until the analysis was conducted. The content of selected cytokines was determined in the serum using the Human Th1/Th2/Th17 Cytokine Kit (cat. no. 560484, BD Biosciences, Warsaw, Poland), according to the manufacturer’s recommendations. Briefly, 50 µl of bead pellet, 50 µl of undiluted serum, and 50 µl of a detection reagent were added to the cytometric tubes. Dilutions of cytokines tested were prepared accordingly (standard curves) (Table 2). Samples were incubated for 3 h at room temperature. Then, they were centrifuged twice (500 $\times$ g, 5 min), and the bead pellet was resuspended in 300 µl of wash buffer. The samples were evaluated with flow cytometry (FACS Calibur Flow Cytometer, BD Immunocytometry Systems, San Jose, CA).

The cytokines investigated were divided into two groups:

- Pro-inflammatory: IL-1, IL-2, IL-6, IL-17A, IL-22, TNF α , and IFN γ
- Anti-inflammatory: IL-4, IL-10, and TGF β

The results were presented as mean $\pm$ SD concentrations (pg/ml).

2.3 Statistical Analysis

Data distribution was evaluated with the Kolmogorov-Smirnov and Lilliefors tests. Statistical differences between normally distributed data were tested with Student’s *t*-test. Otherwise, the Mann-Whitney U test was used. Correlation analysis was performed using Spearman’s factor for variables without normal distribution or Pearson’s factor for variables with normal distribution. A *p*-value <0.05 defined statistically significant differences. The analyses were made using a commercial STATISTICA v12 package (StatSoft, Tulsa, OK).

3 Results

Excessive body weight was present in 16 (29%) out of the 56 children participating in the study, out of whom 8 were obese and 8 were overweight. Detailed characteristics of the two groups

Table 2 Cytokine concentration and standard dilution

Tube label	Concentration (pg/ml)	Cytokine standard dilution
1	0 (negative control)	No standard dilution
2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8
8	1250	1:4
9	2500	1:2
10	5000	Top standard

are presented in Table 3. There were no significant differences in the absolute or percentage values of eosinophils and in total IgE antibodies between both groups (Table 4).

Children with excessive weight had a significantly lower percentage of CD4⁺ lymphocytes (30.6 ± 6.1 vs. 36.7 ± 7.9 , $p = 0.01$) and higher percentages of NKT and CD16/56⁺ lymphocytes (3.9 ± 1.9 vs. 2.8 ± 1.9 ; 15.0 ± 5.7 vs. 11.6 ± 5.7 , $p = 0.05$, respectively) than those with normal weight. There were no significant differences between the other immunological indices assessed (Table 5).

We used the BMI percentile value as an objective measure of nutritional status to evaluate the association between obesity and immunological indices, irrespective of children's age. In the entire group of children with atopy, there was a significant association between BMI and the percentage of CD3 anti-HLA-DR lymphocytes. There also were an inverse association between BMI and CD8 percentage in children with normal weight and positive association between BMI and CD4 percentage in children with excessive weight (Table 6; Figs. 1, 2, and 3). There was no significant association between BMI percentile and atopy indicators, such as total IgE or eosinophils, either in the entire group or subgroups with normal and excessive weight.

Table 3 Characteristics of children with normal (Group I) and excessive body weight (Group II)

	Group I ($n = 40$)	Group II ($n = 16$)
Age (year; mean $\pm$ SD)	9 ± 4	9 ± 3
Gender (M/F)	23/17	11/5
BMI (kg/m^2 ; median, q_{25} – q_{75})	16 (15–18)	23 (20–25)
Height (cm; mean $\pm$ SD)	134 ± 23	144 ± 19
Weight (kg; median, q_{25} – q_{75})	25 (21–47)	48 (20–25)

4 Discussion

A more severe course of allergic diseases in children with excessive weight than that in children with normal weight has been well established (Peters et al. 2018). Children with asthma and obesity have a worse asthma control and worse quality of life (van Gent et al. 2017; Borrell et al. 2013). Excess adipose tissue has also an impact on lung function (Schatz et al. 2015). However, the influence of obesity on the immunological system in children with allergies is still poorly known.

The majority of studies that address the relationship between obesity and immune system have been conducted in non-allergic children. In contrast, in the present study, we assessed the influence of excessive weight on the immunological system's responses in children with atopy. We noticed a higher percentage of eosinophils in children with normal weight compared to overweight or obese ones. However, the difference did not reach a statistical significance. The study conducted by Wu et al. (2011) has shown that adipose tissue eosinophilia mitigates chronic inflammation and positively influences glucose metabolism. Those authors also noticed an inverse association between the amount of adipose tissue and the number of eosinophils. Hence, eosinophils may play a crucial role in shaping the adipose tissue function. The mechanisms that underlie the adipose tissue homeostasis are yet an area of limited understanding. Bolus et al. (2018) have examined whether normalization of the eosinophil count would result in a body weight reduction and improvement in carbohydrate metabolism. The study was conducted in mice, fed with a high content of fat in the chow, followed by parenteral administration of recombinant interleukin 5 (rIL-5) to normalize the

Table 4 Atopy indicators in children with normal body weight (Group I) and excessive body weight (Group II)

	Group I ($n = 40$)	Group II ($n = 16$)	p
Eosinophils ($\times 10^3/\mu\text{l}$; mean $\pm$ SD)	0.62 ± 0.42	0.42 ± 0.34	0.09
Eosinophils (%; mean $\pm$ SD)	7.68 ± 4.68	5.40 ± 4.48	0.10
Total IgE (IU/ml; median, q_{25} – q_{75})	293 (204–780)	488 (291–1153)	0.28

Table 5 Immunological indices in children with normal (Group I) and excessive (Group II) body weight

	Group I (<i>n</i> = 40)	Group II (<i>n</i> = 16)	<i>p</i>
Peripheral T lymphocytes (%; mean $\pm$ SD)			
CD3	65.6 $\pm$ 6.9	62.6 $\pm$ 7.5	ns
CD19	16.5 $\pm$ 4.4	17.1 $\pm$ 5.9	ns
CD4	36.7 $\pm$ 7.9	30.6 $\pm$ 6.1	0.01
CD8	27.6 $\pm$ 5.1	30.6 $\pm$ 5.9	ns
CD16/56	11.6 $\pm$ 5.7	15.0 $\pm$ 5.7	0.05
NKT	2.8 $\pm$ 1.9	3.9 $\pm$ 1.9	0.05
CD3 anti-HLA-DR	5.8 $\pm$ 3.1	7.0 $\pm$ 3.5	ns
Natural regulatory T lymphocytes (%; median, q ₂₅ -q ₇₅)			
Tregs	0.7 (0.4–0.9)	0.5 (0.4–0.9)	ns
Cytokines – pro-inflammatory (pg/ml; median, q ₂₅ -q ₇₅)			
IL-1	0.0 (0.0–0.1)	0.0 (0.0–0.8)	ns
IL-2	1.7 (0.0–2.8)	2.3 (1.2–2.9)	ns
IL-6	2.1 (1.6–3.2)	2.4 (2.1–3.6)	ns
IL-17A	5.9 (2.6–11.0)	3.3 (0.8–7.1)	ns
IL-22	8.5 (2.6–43.0)	8.4 (0.0–17.1)	ns
TNF α	1.6 (1.2–1.8)	1.5 (1.3–1.9)	ns
IFN γ	0.0 (0.0–1.4)	0.0 (0.0–1.3)	ns
Cytokines – anti-inflammatory (pg/ml)			
IL-4 (median, q ₂₅ -q ₇₅)	1.3 (0.0–1.6)	0.5 (0.0–1.5)	ns
IL-10 (median, q ₂₅ -q ₇₅)	1.9 (1.7–2.5)	2.3 (1.9–3.1)	ns
TGF β (mean $\pm$ SD)	437.2 $\pm$ 170.1	457.9 $\pm$ 140.7	ns

NKT, natural killer T cells; Tregs, regulatory T cells; IL, interleukin; TNF α , tumor necrosis factor alpha; IFN γ , interferon gamma; TGF β , transforming growth factor beta

Table 6 Associations between body weight, represented by the percentile value of body mass index (BMI), with the phenotypes of peripheral lymphocytes and cytokines in children with normal (Group I) and excessive weight (Group II)

	All children	Group I	Group II
BMI versus	<i>r</i>	<i>r</i>	<i>r</i>
CD3	−0.17	−0.10	0.43
CD19	0.21	0.26	−0.09
CD4	−0.14	0.15	0.45*
CD8	−0.01	−0.34*	−0.17
CD16/56	0.04	−0.19	−0.45
NKT	0.11	−0.09	−0.29
CD3 anti-HLA-DR	0.30*	0.17	0.19
Tregs	0.15	0.28	−0.03
IL-2	0.05	0.04	−0.47
IL-4	−0.14	0.00	0.19
IL-6	0.25	0.26	−0.35
TNF	0.13	0.17	−0.24
IL-10	0.13	0.22	0.02
IFN γ	−0.04	−0.06	−0.01
IL-17A	−0.05	0.12	−0.39
IL-1	0.02	−0.09	−0.22
IL-22	−0.08	0.08	−0.20
TGF β	−0.12	−0.26	0.39

r linear regression coefficient; **p* < 0.05; acronyms of lymphocytes and cytokines same as in Table 5

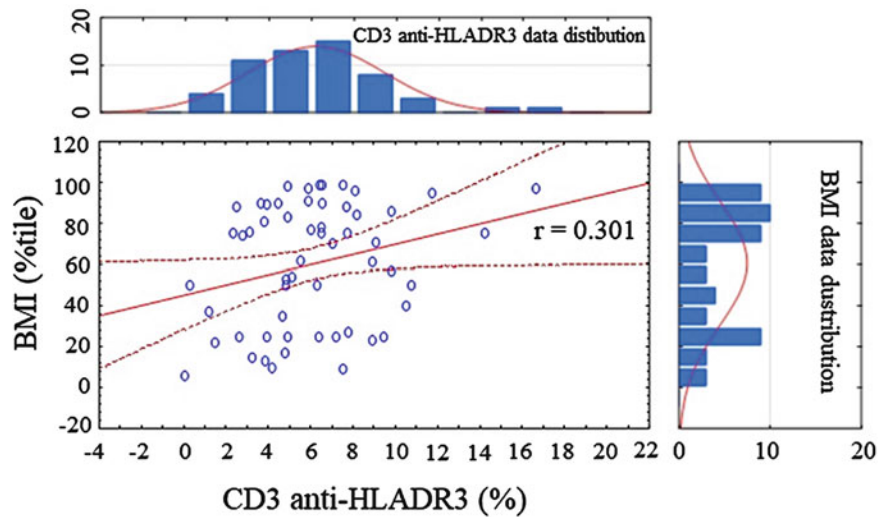


Fig. 1 Association between the percentile of body mass index (BMI) and CD3 anti-HLADR3 lymphocyte count in the entire study group of children. Solid line, linear regression line; dashed lines, 95% confidence intervals; side panels, distribution of data presented on the vertical and horizontal axes

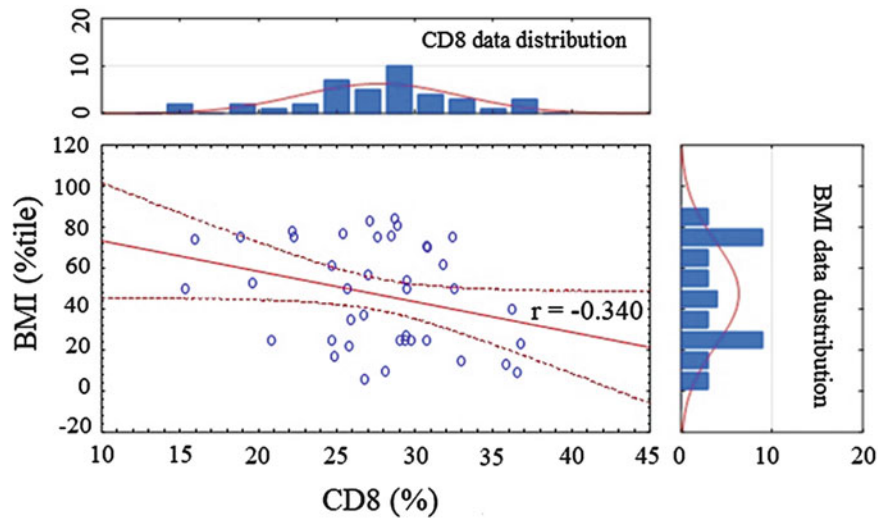


Fig. 2 Association between the percentile of body mass index (BMI) and CD8 lymphocyte count in children with normal body weight. Solid line, linear regression line; dashed lines, 95% confidence intervals; side panels, distribution of data presented on the vertical and horizontal axes

eosinophil count. There was no significant difference in the metabolic tests and no body weight reduction noticed after 8 weeks despite a three-fold increase in the eosinophil count.

Excessive body weight is associated with the inflammatory processes in which immune cells also are involved. Th2 lymphocytes, natural

regulatory T lymphocytes (Tregs), and eosinophils are present in the adipose tissue of slim persons, whereas there is an inflow of macrophages, which are conducive to inflammation in obese persons (Górska et al. 2015; Exley et al. 2014). Available data suggest that the phenotype of macrophages depends on the amount of

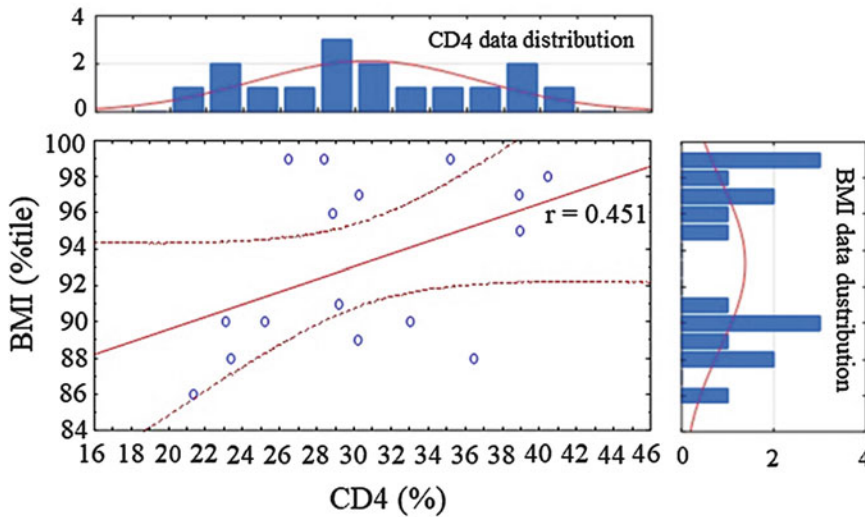


Fig. 3 Association between the percentile of body mass index (BMI) and CD4 lymphocyte count in children with excessive body weight. Solid line, linear regression line;

dashed lines, 95% confidence intervals; side panels, distribution of data presented on the vertical and horizontal axes

the adipose tissue. In slim persons, mostly M2 macrophages are present, while in obesity there are M1 macrophages which, activated by Th1 cells, stimulate release of the pro-inflammatory $\text{TNF}\alpha$. In contrast, M2 macrophages are stimulated by Th2 lymphocytes and inhibit the inflammatory propensity (Sharma et al. 2018; Lumeng et al. 2007). Yet, it is unknown whether the inflammatory process linked to obesity is limited to the adipose tissue or it is associated with a systemic reaction. The present study showed a higher percentage of macrophages in the population of peripheral lymphocytes in obese children than that in children with normal weight. Thus, it is possible that the inflammation of obesity leads to a systemic reaction. Similar observations have been made by Periyalil et al. (2018) who show that obesity associates with systemic inflammation and with a more severe respiratory track inflammation, irrespective of the coexisting asthma. However, visceral adipose tissue contains more macrophages in obese patients with asthma than without asthma. The number of macrophages also is associated with disease severity.

There are many other cells involved in the inflammatory process coexisting with obesity.

Recent studies reveal that the presence of CD1d on adipocyte surface activates NKT cells (Satoh and Iwabuchi 2016). A higher percentage of NKT cells is present in children with excessive weight compared to those with normal weight. NKT cells are a subpopulation of T lymphocytes. They influence many stages of the immune response through cytokine secretion (Crosby and Kronenberg 2018). Experimental studies show that these cells play a role in insulin resistance (Lynch et al. 2012; Ohmura et al. 2010). The role of NKT in the development of obesity is an area of limited understanding, but there is an adverse association between NKT cells and body weight (Sag et al. 2014). Owing to increased production of cytokines by NKT cells, typical for Th2 lymphocytes such as IL-4 and IL-10, M2 macrophages become activated and mitigate inflammation associated with obesity. In contrast, some other studies show that obesity development slows down when NKT cells are not present (Wu et al. 2012). Recently, Satoh and Iwabuchi (2018) have reviewed the influence of NKT cells on the development of obesity and point that their modulating influence on adipocytes depends on lipids in the diet and in particular on the intestinal microbiome.

Other studies point to the role of Treg lymphocytes in insulin resistance and obesity (Matarese et al. 2010). A reduction in Tregs results in increases in insulin and pro-inflammatory cytokines. Conversely, increased number of Tregs induces IL-10 and improves insulin action in the mouse (Feuerer et al. 2009). There is an apparent reduction in Tregs in obese persons (Agabiti-Rosei et al. 2018; Yun et al. 2010). In line with that, we noticed in the present study a decreased percentage of Tregs in children with excessive weight compared to those with normal weight, although the difference failed to reach statistical significance. This lack of significance in Treg changes might be explicable by not taking into account the influence of physical activity on the metabolism and immune responses: the element we failed to control in the study. A prospective study of Momesso dos Santos et al. (2015) has shown that regular moderate physical exercise results in inhibition of inflammation in obese children through modulating the immunological response.

This study showed that an increase in BMI is associated with the percentage of activated CD3 anti-HLA-DR. The HLA-DR molecule is a marker of T-lymphocyte activation and appears, on average, 3–5 days after binding with the antigen (Holling et al. 2004). The assessment of T-lymphocyte activation based on the presence of HLA-DR may indirectly reflect B-lymphocyte stimulation and antibody synthesis. Therefore, the findings suggest the impact of excessive body weight on T lymphocytes as with increasing BMI also increases the percentage of activated CD3⁺ lymphocytes, which points to the stimulation of the immune system. We also noticed that children with excessive weight have a lower percentage of CD4⁺ lymphocytes, but their number increases with increasing BMI. This finding is in line with that of Schindler et al.'s (2017) who have found an association between obesity and the occurrence of increased level of CD4⁺: the cells that are a major source of IL-17A. That study shows the association between BMI and Th17 lymphocytes based on the investigation of 65 children of whom 15 were obese.

We conclude that excessive body weight importantly and adversely modulates the immunological response in children with allergy. The macrophages and NKT are the immune cells that play a pivotal role in the modulation of immunological responsiveness.

Acknowledgments Funded by grant no. 323 of the Military Institute of Medicine in Warsaw, Poland.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Military Institute of Medicine (permit 123/14).

Informed Consent Written informed consent was obtained from all legal guardians of individual participants included in the study.

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