

Alterations on peripheral blood B cell subsets induced by allergic rhinitis

Ana Henriques · Rodrigo Nunes · Graça Loureiro ·
António Martinho · Maria Pais · António Segorbe-Luís ·
Hélder Trindade · Celso Pereira · Artur Paiva

Received: 4 November 2014 / Accepted: 10 February 2015
© Springer Basel 2015

Abstract

Objectives and design Here we evaluated whether allergic rhinitis to *Dermatophagoides pteronyssinus* induces alterations on circulating B cell subsets.

Materials and methods Circulating B cell subsets and isotype expression on antigen-experienced B cells from allergic patients under conventional pharmacological treatment (NO-SIT, $n = 15$) and under subcutaneous immunotherapy (SCIT, $n = 33$), and non-allergic subjects (NC, $n = 25$) were analyzed by flow cytometry.

Results In allergic patients, we found a significant decrease in IgM⁺ and IgG⁺ memory B cells and an increase

in IgA⁺ memory B cells. Additionally, the numbers of circulating IgA⁺ plasmablasts in allergic patients were also increased, while those cells expressing IgM were reduced. **Conclusions** Allergic patients have a disturbed B cell subsets distribution which seems to underlie rhinitis pathogenesis and remain unchanged after SCIT.

Keywords Subcutaneous-specific immunotherapy · B cell subsets · Allergic rhinitis · *Dermatophagoides pteronyssinus*

Introduction

Several factors may specifically contribute to the pathophysiology of allergic rhinitis (AR), including the local synthesis of immunoglobulin (Ig) E in nasal mucosa, the up-regulation of Th2 cytokines or the alteration in the number of regulatory T (Treg) cells [1]. In fact, repeated exposure to aeroallergens cause B cell activation and maturation into plasma cells producing specific IgE antibodies which bind to specific receptors on the surface of basophils and mast cells [2]. Interestingly, despite the influence of AR on immune cells and the impact of subcutaneous immunotherapy (SCIT) therapy in the specific antibody response within the nasal mucosa being relatively well known [3–5], the potential systemic effect of AR on peripheral blood (PB) B cell subsets remains largely unknown.

Therefore, in the present study we aimed to identify alterations in the different circulating B cell compartments in AR patients with monosensitization to *Dermatophagoides pteronyssinus* (Dpt) based on their Ig expression patterns. Moreover, we looked for a possible correlation between IgE⁺ B cells and serum IgE levels.

Responsible Editor: Mauro Teixeira.

A. Henriques and R. Nunes have equally contributed to this work and both should be considered as first author.

A. Henriques · R. Nunes · A. Martinho · M. Pais ·
H. Trindade · A. Paiva (✉)

Blood and Transplantation Center of Coimbra, Portuguese
Institute of Blood and Transplantation, Coimbra, Portugal
e-mail: Artur.Paiva@IPST.min-saude.pt

A. Henriques
Faculty of Medicine of the University of Coimbra, Coimbra,
Portugal

R. Nunes · A. Paiva
Escola Superior de Tecnologia da Saúde de Coimbra/Coimbra
Health School, Coimbra, Portugal

G. Loureiro · A. Segorbe-Luís · C. Pereira
Imunoallergy Department, University Hospital Center of
Coimbra, Coimbra, Portugal

Materials and methods

Patients

Overall, 48 AR patients were included in this study, all of them with at least 2 years of perennial symptoms, wheal higher than 3 mm related to histamine response and the values of serum-specific IgE to *D. pteronyssinus* (ImmunoCAP, Thermo Scientific) at least class 2 (CAP): a group of 15 respiratory allergic patients monosensitized to mites (age: 26 ± 7 years; sex: 5 males and 10 females) who were undergoing treatment implemented according to the recommended guidelines (oral systemic anti-histamines, nasal and bronchial corticotherapy and bronchodilators for patients with asthma) and who had not been submitted, in the past, to specific immunotherapy, and that displayed the same clinical profile and the exclusion criteria as the SCIT group (NO-SIT); and a group composed of 33 patients (age: 30 ± 11 years; sex: 19 males and 14 females) with AR monosensitized to mites under active maintenance SCIT treatment (duration of SCIT: 28 ± 13 months) with allergoid Dpt extract Depigoid (Leti, Madrid, Spain) with monthly maintenance dose of 0.5 ml volume (Der p 1 = $7.2 \mu\text{g/ml}$)(SCIT) and who had not been previously undergone to systemic treatment. When included in the study, all patients presented clinical efficacy to treatment and in all of them the induction phase was achieved after 6 doses of weekly injection, followed by 1 injection every 4 weeks as a maintenance dose for a minimum of 12 months. Of note, all the analyses were performed during a period of clinical stabilization in both AR groups.

Healthy individuals group

A control group (NC) was selected composed of 25 healthy individuals (age: 30 ± 11 years; sex: 13 males and 12 females).

Biological samples

Fresh PB was collected from patients and healthy controls into K3-EDTA (Becton–Dickinson Biosciences, San Jose, CA, USA). The blood sample was performed in peripheral vein, before the SCIT administration in case of the SCIT group.

Ethical standards

The study protocol was approved by the Ethical Committee from Coimbra 185 University Hospital. All participants

gave and signed an informed consent form, and the principles of Helsinki Declaration were respected.

Determination of total and specific IgE

Quantitative determination of serum t-IgE was performed using nephelometry method. Total IgE concentration (kU/l) was determined comparing the results with the calibrators provided. Serum s-IgE to *D. pteronyssinus* was determined using the ImmunoCAP fluorescence enzyme immunoassay technique (Phadia, Uppsala, Sweden). To evaluate the test results, the response from the patient samples was converted to concentrations using a calibration curve. The range of measurements was 0.35–100 KU_A/l.

Flow cytometric data acquisition and analysis

The distribution of three B cell subsets—transitional/naïve (CD19⁺/CD20⁺/CD27[−]/CD38^{+/−}), memory (CD19⁺/CD20⁺/CD27⁺/CD38[−]) B cells, and plasmablasts (CD19⁺/CD20[−]/CD27⁺⁺/CD38⁺⁺)—was evaluated using a FACSCanto flow cytometer (BD, San José, CA, USA), using a lyse and wash procedure, as previously described [6], and following the below combination of monoclonal antibodies: CD19-PC7 (clone J3-119), CD27-PC5 (clone 1A4LDG5), CD45-KO (clone J.33), IgA-PE (clone B3506B4) from Beckman–Coulter (Miami, USA); CD20-PB (clone 2H7) from eBiosciences (San Diego, CA, USA); CD38-APC-H7 (clone HB7), IgG-FITC (clone G18-145) and IgM-APC (clone G20-127) obtained from Becton–Dickinson Biosciences (BD; San Jose, CA; USA); and IgE PE (clone BE5) from EXBIO (Prague, Czech Republic). Data acquisition was performed using a two-step procedure. Information from the total cellularity was first acquired using 5×10^4 cells/tube and then $\geq 2 \times 10^5$ B cells were acquired after a live gate on CD19⁺ cells. Plasmablasts were defined by concomitant higher expression of CD27 and CD38 as well as by an increased FSC and lower expression of CD19. Data were analyzed with Infinicyt 1.4 software (Cytognos SL, Salamanca, Spain). Results illustrate the percentage of positive cells within each cell subset.

Statistical analysis

The statistical analysis (Mann–Whitney *U* test) was performed using SPSS software (version 16.0, SPSS, Chicago, IL). *P* values <0.05 were classified as statistically significant.

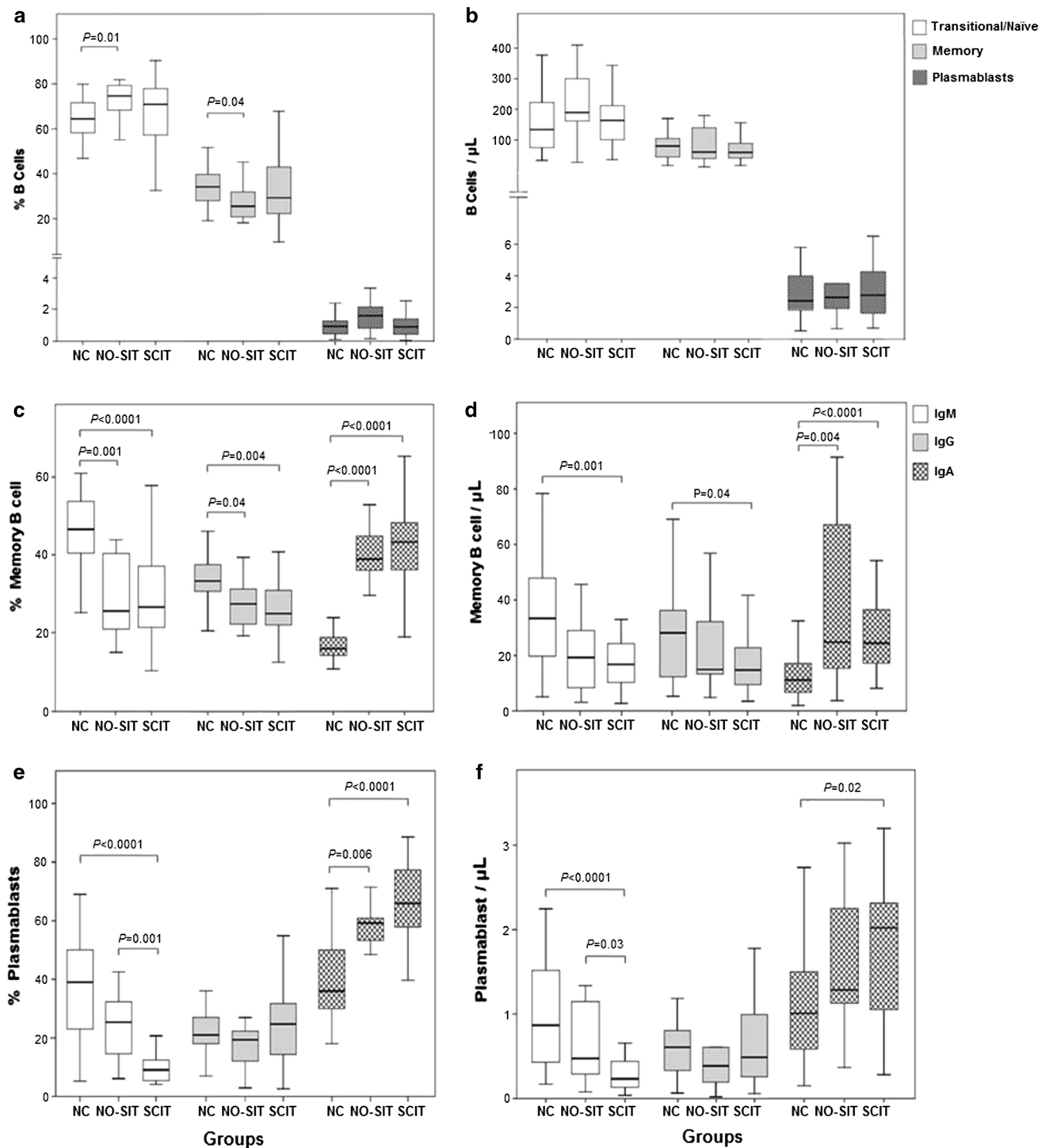


Fig. 1 Relative (a) and absolute numbers (b) of each B cell subset (transitional/naïve, memory and plasmablasts) and unique Ig-heavy chain expression on antigen-experienced B cells (c, d, e and f) in the studied groups: NC healthy individuals, NO-SIT allergic patients

never submitted to allergen-specific immunotherapy, SCIT patients submitted to allergen-specific subcutaneous immunotherapy. * $P < 0.05$, statistical significance

Results

Relative and absolute numbers of peripheral B cell compartments and their immunoglobulin expression in allergic patients and healthy individuals

No differences were observed in the relative and absolute numbers of peripheral B lymphocytes when comparing the different studied groups (Fig. 1a) whereas within B cell subsets (transitional/naïve, memory and plasmablasts), an increased proportion of transitional/naïve cells with a consequent decrease of memory cells was observed in NO-SIT patients, when compared with healthy individuals ($P < 0.05$) (Fig. 1b). Comparatively to healthy individuals, a significant increase of the frequency of $\text{IgA}^+\text{CD27}^+$ memory B cells followed by a decrease of those expressing IgM and IgG was found ($P < 0.05$), although without differences between the two patient groups (Fig. 1c, d). In addition, a marked increase in the relative and absolute numbers of IgA^+ plasmablasts followed by a decrease in those expressing IgM was found in AR patients, particularly in those under SCIT (Fig. 1e, f). Despite the majority of PB B cell subsets not expressing IgE, a decreased proportion of IgE^+ B cells was found in SCIT versus NO-SIT—0.13 (0.02–0.64) vs. 0.23 (0.13–3.13), respectively, $P = 0.02$. No IgE^+ plasmablasts were found either in allergic patients or in controls.

Serum total and specific IgE levels

Slightly lower total serum IgE (t-IgE) levels were found in NO-SIT patients when compared with the SCIT patients (295 ± 231 kU/l vs. 527 ± 796 kU/l, respectively), although not reaching statistical significance. Conversely, no differences were observed between the two AR groups regarding serum-specific IgE (s-IgE) levels (NO-SIT: 49 ± 29 kU_A/l; SCIT: 47 ± 37 kU_A/l). However, using the ratio of s-IgE over t-IgE a significant decrease in SCIT group was observed when compared with NO-SIT (0.13 ± 0.08 vs. 0.23 ± 0.1 , $P = 0.02$) although lacking significant correlation with IgE^+ memory B cells (data not shown).

Discussion

In the present study, we evaluated the PB distribution of B cell subsets in AR patients under SCIT or pharmacotherapy. An increased proportion of transitional/naïve B cells followed by the decreased proportion of memory B cells was found in AR NO-SIT patients when compared to NC. In contrast, no alterations on absolute numbers were observed. These results might be explained, at least in part,

considering the clinical stabilization status of both AR groups.

On the other hand, we focused our attention in isotype expression on antigen-experienced B cells from allergic patients under SCIT and under conventional pharmacological treatment and non-allergic subjects. Therefore, the analysis of Ig expression showed that, independent of the applied treatment, a marked higher proportion of memory B cells expressing IgA coupled with a decrease of those expressing IgM and IgG was found in allergic patients when compared to non-allergic subjects. Interestingly, in AR the frequency of IgA^+ plasmablasts was also increased and more evident in patients under Dpt-SCIT exclusively due to a lower proportion of IgM^+ plasmablasts. Overall, the increased switch to IgA by antigen-experienced B cells in AR patients may highlight the biological effect of IgA on mucosal defense, targeted for the first step of inhaled allergen exposure, and be able to minimize the clinical progression of allergic symptoms [7]. Therefore, the increased proportion of IgA is expected to be more pronounced in patients under SCIT and support or even synergize the advantageous effects of allergen-specific IgG [8].

Until now, circulating IgE^+ B cells are rare and difficult to quantify [9], even in allergic subjects. In agreement with others, the infrequent IgE^+ B cells detected suggest that the isotype switching to IgE^+ B cells and their proliferation and maturation to IgE-producing plasmablasts/plasma cells may take place in the nasal mucosa where dendritic cells, T cells and B cells participate in germinal center-like reactions [10, 11]. Despite their low numbers, we found a decreased proportion of IgE^+ B cells in SCIT compared to NO-SIT, which is in line with results published by Donohoe et al. [12]. Moreover, there was no correlation between IgE^+ B cell frequency and serum IgE measurements whereas a relationship between serum IgE levels and basophil expression of high-affinity IgE receptor ($\text{Fc}\epsilon\text{RI}$) was previously reported [13].

Taken together, we demonstrated that AR leads to significant alterations of the B cell compartment in PB of allergic patients as compared to non-allergic subjects. Whether these changes result from a direct effect of allergen in B cell subsets or are induced by therapy remains to be elucidated.

Conflict of interest None.

References

1. Broide DH. Allergic rhinitis: pathophysiology. *Allergy Asthma Proc.* 2010;9:370–4.
2. Holgate ST. Innate and adaptive immune responses in asthma. *Nat Med.* 2012;18:673–83.

3. Zhang H, Cardell LO, Bjorkander J, Benson M, Wang H. Comprehensive profiling of peripheral immune cells and subsets in patients with intermittent allergic rhinitis compared to healthy controls and after treatment with glucocorticoids. *Inflammation*. 2013;36:821–9.
4. Gelardi M, Peroni DG, Incorvaia C, Quaranta N, De Luca C, Barberi S, Dell'albani I, Landi M, Frati F, de Beaumont O. Seasonal changes in nasal cytology in mite-allergic patients. *J Inflamm Res*. 2014;7:39–44.
5. Cappella Antonio, Durham Stephen R. Allergen immunotherapy for allergic respiratory diseases. *Hum Vaccin Immunother*. 2012;8:1499–512.
6. Henriques A, Teixeira L, Inês L, Carneiro T, Gonçalves A, Martinho A, Pais ML, da Silva JA, Paiva A. NK cells dysfunction in systemic lupus erythematosus: relation to disease activity. *Clin Rheumatol*. 2013;32:805–13.
7. Holt PG, Strickland DH, Wikström ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. *Nat Rev Immunol*. 2008;8:142–52.
8. Daniel C, Repa A, Wild C, Pollak A, Pot B, Breiteneder H, Wiedermann U, Mercenier A. Modulation of allergic immune responses by mucosal application of recombinant lactic acid bacteria producing the major birch pollen allergen Bet v 1. *Allergy*. 2006;61:812–9.
9. Perez-Andres M, Paiva B, Nieto WG, Caraux A, Schmitz A, Almeida J, Vogt RF Jr, Marti GE, Rawstron AC, Van Zelm MC, Van Dongen JJ, Johnsen HE, Klein B, Orfao A. Primary Health Care Group of Salamanca for the study of MBL: human peripheral blood B-cell compartments: a crossroad in B-cell traffic. *Cytometry B Clin Cytom*. 2010;78(Suppl 1):S47–60.
10. Cameron L, Gounni AS, Frenkiel S, Lavigne F, Vercelli D, Hamid Q. S epsilon S mu and S epsilon S gamma switch circles in human nasal mucosa following ex vivo allergen challenge: evidence for direct as well as sequential class switch recombination. *J Immunol*. 2003;171:3816–22.
11. Takhar P, Smurthwaite L, Coker HA, Fear DJ, Banfield GK, Carr VA, Durham SR, Gould HJ. Allergen drives class switching to IgE in the nasal mucosa in allergic rhinitis. *J Immunol*. 2005;174:5024–32.
12. Donohoe PJ, Hedde RJ, Sykes PJ, Fusco M, Flego LR, Zola H. IgE1 cells in the peripheral blood of atopic, nonatopic, and bee venom-hypersensitive individuals exhibit the phenotype of highly differentiated B cells. *J Allergy Clin Immunol*. 1995;95:587–96.
13. Lopes A, Azenha P, Teodósio C, Inácio M, Silva I, Loureiro G, Martinho A, Luís AS, Trindade H, Pereira C, Paiva A. Impact of allergic rhinitis and specific subcutaneous immunotherapy on peripheral blood basophils of patients sensitized to *Dermatophagoides pteronyssinus*. *Allergy Asthma Clin Immunol*. 2013;9:40.