Peripheral Dendritic Cells in Asthma

Á Cseh,[1] A Bohács,[2] B Szalay,[1] G Losonczy,[2] T Tulassay,[3] B Vásárhelyi,[1,4] L Tamási[2]

[1]*Research Group for Pediatrics and Nephrology, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary*

[2]*Department of Pulmonology, Semmelweis University, Budapest, Hungary*

[3]*First Department of Pediatrics, Semmelweis University, Budapest, Hungary*

[4]*Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary*

Key words: Asthma. Lymphocyte. Dendritic cell. Regulatory T cell.

Palabras clave: Asma. Linfocito. Célula dendrítica. Célula T reguladora.

Dendritic cells (DCs) affect helper T cell (T_H) cell commitment [1]. The two major DC subsets are myeloid DCs (mDCs), which shift the T-cell response in the direction of T_H2 cells [2], and plasmacytoid DCs (pDCs), which induce regulatory T cells (Tregs) and which are major inhibitors of adaptive immunity [3]. We studied the association between prevalence of DC subsets and specific target cells (Tregs, T_H1 $CD4^+$, and T_H2 $CD4^+$ cells) and asthma symptoms.

We enrolled 28 patients (15 men, 13 women) with persistent asthma of differing severity (values expressed as median [interquartile range]). The Asthma Control Test (ACT) [4] score was 20 (16-22), median age was 32 (29-39) years, and the body mass index was 23.37 (21.04-26.92). Patients were taking inhaled corticosteroids (beclomethasone or equivalent) at 800 (500-1000) µg/d and long-acting ß-agonists at 2 (0-4) puffs/d. Forced vital capacity was 93% (80%-104%) of predicted, forced expiratory volume in 1 second was 82% (72%-103%) of predicted, peak expiratory flow was 74% (67%-93%) of predicted, airway resistance was 0.31 (0.20-0.39) kPa/s/L, and peripheral oxygen saturation was 98% (96%-99%). The exclusion criteria were acute exacerbation of asthma, severe comorbidity, medication other than inhaled corticosteroids or long-acting ß-agonists, infection during the previous 6 weeks, and smoking.

Blood samples were taken after informed consent was obtained. Peripheral blood mononuclear cells (PBMCs) were isolated and stained with surface markers (CD4, CD11c, CD123, CCR4, CXCR3, HLA-DR, Lin1, CD3, CD14, CD16, CD19, CD20, and CD56) (BD Biosciences Pharmingen, San Diego, California, USA) and FoxP3 (eBioscience, San Diego, California, USA) [5]. Fifty-four healthy controls were also enrolled. The Mann-Whitney test, Spearman test, and multiple regressions were applied using Statistica 8 (Statsoft, Tulsa, Oklahoma, USA).

Total DC prevalence (Lin1–HLA-DR^+/PBMCs) was comparable in both asthma patients and controls (1.19 [0.77-1.54] vs 1.16 [0.60-2.60]). The prevalence values for mDC ($CD11c^+CD123^-$/DCs) and pDC ($CD123^+CD11c^-$/DCs) were also comparable (59.64 [52.07-65.19] vs 63.65 [56.02-72.31] and 31.36 [18.26-36.22] vs 25.57 [22.25-32.34], respectively). The mDC/pDC ratio was lower in asthma patients than in controls (1.95 [1.48-2.92] vs 2.53 [1.93-3.51], *P*=.038). The prevalence of T_H1 and T_H2 cells ($CD4^+CXCR3^+CCR4^-$/$CD4^+$ and $CD4^+CCR4^+CXCR3^-$/$CD4^+$, respectively) was also comparable (25.56 [19.74-31.97] vs 35.64 [29.46-45.64], and 3.01 [1.50-4.40] vs 2.10 [1.79-3.58], respectively), while the T_H1/T_H2 ratio was lower in patients with asthma (7.85 [4.40-10.12] vs 9.34 [6.39-11.47], *P*=.039). Treg ($CD4^+FoxP3^+$/$CD4^+$) prevalence was also similar in both groups (2.70 [1.45-4.40] vs 3.02 [2.26-5.80]).

The ACT score correlated inversely with the prevalence of mDC and positively with the prevalence of pDC (r=–0.607, *P*=.013 and r=0.650, *P*=.007, respectively) (Figure). The ACT score correlated with the prevalence of T_H1 and inversely with the prevalence of T_H2 (r=0.491, *P*=.017; r=–0.417, *P*=.048). The correlations remained significant after adjustment for age, body mass index, and gender, but ceased after adjustment for the antiasthmatic drugs used.

Recent experiments demonstrated that the elimination of mDCs abrogates T_H2 response [6]. pDCs are regarded as modulators of the immune response by inducing Treg cells [7]. Our results were consistent with those of other authors, namely, we also found that pDCs predominated (ie, low mDC/pDC ratio), although we could not confirm previous findings on high pDC prevalence [8]. The lack of higher pDC prevalence in our study may be due to specific patient characteristics, as we enrolled patients with poorer asthma control (ACT≤19) and, therefore, lower pDC prevalence (Figure).

Asthma control is determined by drug use. In addition, recent studies demonstrated that inhaled corticosteroids and long-acting ß-agonists influence DC subsets [9,10]. In our study, there was no direct impact of antiasthmatic drugs on peripheral DC prevalence. However, these drugs may still influence cell prevalence values, as the correlation between asthma control and cell prevalence ceased to be observed after adjustment for drug use [8-10].

Our data suggest that DC phenotype is strongly associated with general well-being, as reflected by the total ACT score, namely, lower mDC and higher pDC values are associated with better asthma control. This finding reinforces the potential role of DCs as biomarkers for the assessment of asthma control.

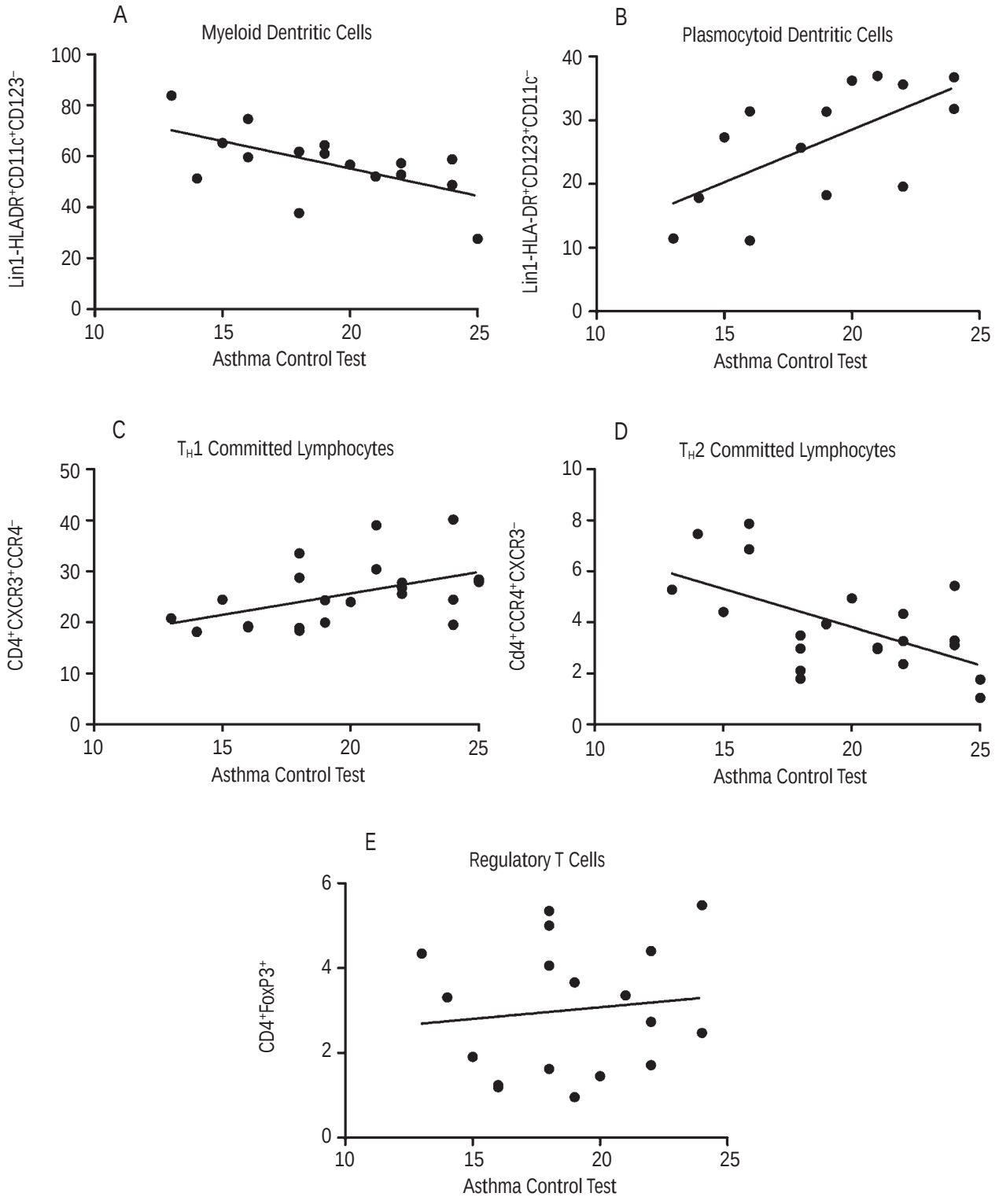

Figure. Correlations between total asthma control test scores and the prevalence of (A) myeloid dendritic cells (r=–0.607, P=.013), (B) plasmacytoid dendritic cells (r=0.650, P=.007), (C) T_H1 committed lymphocytes (r=0.491, P=.017), (D) T_H2 committed lymphocytes (r=–0.417, P=.048), and (E) regulatory T cells (nonsignificant).

Acknowledgments

This work was supported by grants TÁMOP-4.2.2-08/1/KMR-2008-0004 and OTKA K-68758.

References

1. Lambrecht BN, Hammad H. Biology of lung dendritic cells at the origin of asthma. Immunity. 2009;31:412-24.
2. Kool M, Lambrecht BN. Dendritic cells in asthma and COPD: opportunities for drug development. Curr Opin Immunol. 2007;19:701-10.
3. de Heer HJ, Hammad H, Kool M, Lambrecht BN. Dendritic cell subsets and immune regulation in the lung. Semin Immunol. 2005;17:295-303.
4. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, Murray JJ, Pendergraft TB. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol. 2004;113:59-65.
5. Švec P, Vásárhelyi B, Pászthy B, Körner A, Kovács L, Tulassay T, Treszl A. Do regulatory T cells contribute to Th1 skewness in obesity? Exp Clin Endocrinol Diabetes. 2007;115:439-43.
6. van Rijt LS, Jung S, Kleinjan A, Vos N, Willart M, Duez C, Hoogsteden HC, Lambrecht BN. In vivo depletion of lung $CD11c^+$ dendritic cells during allergen challenge abrogates the characteristic features of asthma. J Exp Med. 2005;201:981-91.
7. Ito T, Yang M, Wang YH, Lande R, Gregorio J, Perng OA, Qin XF, Liu YJ, Gilliet M. Plasmacytoid dendritic cells prime IL-10-producing T regulatory cells by inducible costimulator ligand. J Exp Med. 2007;204:105-15.
8. Matsuda H, Suda T, Hashizume H, Yokomura K, Asada K, Suzuki K, Chida K, Nakamura H. Alteration of balance between myeloid dendritic cells and plasmacytoid dendritic cells in peripheral blood of patients with asthma. Am J Respir Crit Care Med. 2002;166:1050-4.
9. Yokomura K, Suda T, Matsuda H, Hashizume H, Asada K, Suzuki K, Chida K. Suplatast tosilate alters DC1/DC2 balance in peripheral blood in bronchial asthma. J Asthma. 2005;42:567-70.
10. Saeki S, Matsuse H, Kondo Y, Machida I, Kawano T, Tomari S, Obase Y, Fukushima C, Kohno S. Effects of antiasthmatic agents on the functions of peripheral blood monocyte-derived dendritic cells from atopic patients. J Allergy Clin Immunol. 2004;114:538-44.

Manuscript received January 22, 2010; accepted for publication May 18, 2010.

Áron Cseh MD
First Department of Pediatrics
Bókay u. 53.
1083 Budapest, Hungary
E-mail: cseharon@gmail.com

Comparison of Basophil Activation Test Results in Blood Preserved in Acid Citrate Dextrose and EDTA

N Sousa,[1] R Martínez-Aranguren,[2] M Fernández-Benitez,[2] F Ribeiro,[1] ML Sanz[2]
[1]*Immunoallergology Department, Coimbra University Hospitals, Portugal*
[2]*Department of Allergology and Clinical Immunology, Clinica Universidad de Navarra, Pamplona, Spain*

Key words: ACD. Aeroallergens. Basophil activation test. Blood storage. EDTA.

Palabras clave: ACD. Aeroalérgenos. Test de activación de basófilos. Preservación de sangre. EDTA.

The basophil activation test (BAT) complements skin tests and specific immunoglobulin (Ig) E determination in analyzing immediate-type reactions to allergens such as aeroallergens [1], hymenoptera venom [2], latex [3], foodstuffs, and drugs [4]. This technique enables the number of provocation tests, which are often dangerous, to be substantially lowered. However, no single universal protocol is available, and results vary considerably. Sturm et al [5] recently compared blood from venom-allergic patients that was collected in EDTA tubes with the same blood after 18 hours' storage. They reported a significant drop in the percentage of basophil activation leading to a very high rate of negative BAT results. The results of our analysis were not consistent with these observations.

The objective of this study, therefore, was to compare basophil activation between blood preserved in acid-citrate dextrose (ACD) tubes (BD Vacutainer Systems, Plymouth, UK) and blood preserved in K3EDTA (EDTA) tubes (BD Vacutainer Systems) at 0, 24, and 48 hours' storage at 4ºC in patients allergic to *Dermatophagoides pteronyssinus* or *Phleum pratense*. These allergens were chosen because BAT has been thoroughly validated for both and the positivity criteria are universally accepted [1].

Three patients with allergic rhinitis, asthma, or both, and proven allergy to *D pteronyssinus* or *P pratense* were randomly enrolled from our clinic. Allergy was confirmed by a suggestive clinical history with positive results for skin tests, specific IgE, or both. After informed consent detailing the purpose of this study was obtained, peripheral blood was collected using ACD and EDTA tubes. BAT with either preservative free of *D pteronyssinus* extract (final concentrations 1.411 and 0.353 mg/mL) or preservative free of *P pratense* extract (final concentrations 0.148 and 0.037 mg/mL) (Bial Aristegui, Bilbao, Spain) was performed on a blood sample from each patient (ACD and EDTA) immediately after collection and again at 24 and 48 hours. The blood was stored at 4ºC. BAT was performed on the buffy coat fraction using CD63 as the activation marker, as reported elsewhere [1]. Data were analyzed using a FACScan flow cytometer (Becton Dickinson, New Jersey, USA).

After data analysis, an individual threshold for basophil activation was defined for each patient. The threshold used

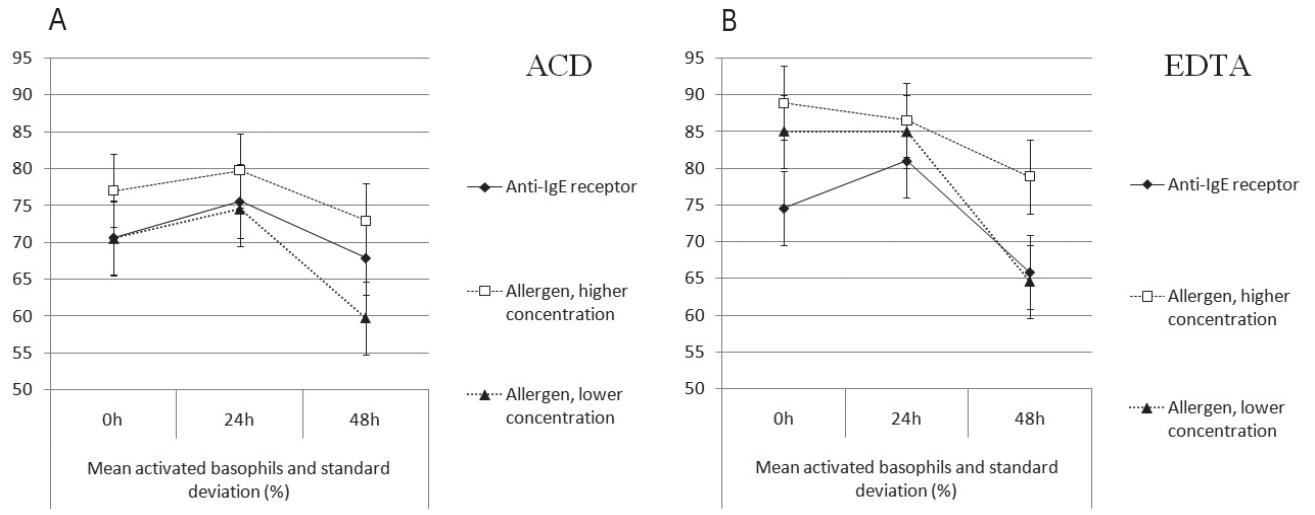

Figure. Percentage of activated basophils in response to monoclonal anti-IgE receptor and both allergen concentrations (*Dermatophagoides pteronyssinus* and *Phleum pratense*) after 0, 24, and 48 hours' storage at 4ºC. A, ACD tubes; B, EDTA tubes. ACD indicates acid-citrate dextrose.

to evaluate both the ACD and EDTA samples at the different timepoints was applied to ensure an accurate comparison. The percentage of activated basophils to monoclonal anti-IgE receptor and both concentrations of allergens used are presented in the Figure.

After stimulation with monoclonal anti-IgE receptor and both aeroallergens, we found that basophil activation was similar in fresh blood and blood stored for 24 hours. Even after 48 hours, basophil activation was still substantial. The differences between ACD and EDTA tubes were negligible. These results contradict those of Sturm et al [5], which revealed a substantial reduction in the number of activated basophils to anti-IgE and to hymenoptera venom in venom-allergic patients. Although our patients were allergic to aeroallergens and not to hymenoptera venom, basophil reactivity has been shown to be very high in both [1,2].

In conclusion, our study showed that the results of the BAT using fresh blood were similar to those for blood stored for 24 hours at 4ºC. There was slight drop in activation after 48 hours, although the results remained clearly positive. A similar study should be performed using drug allergens.

References

1. Sanz ML, Sanchez G, Gamboa PM, Vila L, Uasuf C, Chazot M, Dieguez I, Oehling A, De Weck AL. Ag-induced basophil activation:CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. Clin Exp Allergy. 2001;31:1007-13.
2. Eberlein-König B, Rakoski J, Behrendt H, Ring J. Use of CD63 expression as marker of in vitro basophil activation in identifying the culprit in insect venom allergy. J Invest Allergol Clin Immunol. 2004;14:10-16.
3. Ebo DG, Lechkar B, Schuerwegh AJ, Bridts C H, De Clerck LS, Stevens WJ. Validation of a two-color flow cytometric assay detecting in vitro basophil activation for the diagnosis of IgE-mediated natural rubber latex allergy. Allergy. 2002:57:706-12.
4. Sanz ML, Gamboa PM, Mayorga C. Basophil activation tests in the evaluation of immediate drug hypersensitivity. Curr Opin Allergy Clin Immunol. 2009;9:298-304.
5. Sturm GJ, Kranzelbinder B, Sturm EM, Heinemann A, Groselj-Strele A, Aberer W. The basophil activation test in the diagnosis of allergy: technical issues and critical factors. Allergy. 2009;64:1319-26.

Manuscript received March 2, 2010; accepted for publication May 24, 2010.

Nuno Alexandre Gaspar Sousa
S. Imunoalergologia, HUC
Praceta Mota Pinto, 3000
Coimbra, Portugal
E-mail: Nunogasparsousa@gmail.com

Basophil Activation Test in a Case of Systemic Hypersensitivity Reaction to Infliximab With Good Tolerance to Another Anti-TNF-α Agent (Adalimumab)

L Manso,[1] B Polo,[2] M Fernández-Nieto,[1,4] B Sastre,[3,4] V del Pozo,[3,4] J Sastre[1,4]

[1]*Servicio de Alergia, Fundación Jiménez Díaz, Madrid, Spain*

[2]*Servicio de Digestivo, Fundación Jiménez Díaz, Madrid, Spain*

[3]*Servicio de Inmunología, Fundación Jiménez Díaz, Madrid, Spain*

[4]*CIBER de Enfermedades Respiratorias (CIBERES)*

Key words: Systemic hypersensitivity reaction. Basophil activation test (BAT). Infliximab. Adalimumab. Drug allergy.

Palabras clave: Reacción de hipersensibilidad sistémica. Test de activación de basófilos (TAB). Infliximab. Adalimumab. Alergia a fármacos.

Monoclonal antibodies became available for clinical use in the early 1980s, after Köhler and Milstein [1] described the hybridoma technique, which made it possible to use biological agents to treat numerous conditions. Since then, several immune system mediators have been cloned and modified and are now used to treat inflammatory diseases. One of the most important groups is that of the tumor necrosis factor (TNF) α antagonists, namely, infliximab, adalimumab, etanercept, and certolizumab, the latter having recently been approved for human use by the United States Food and Drug Administration. Several adverse reactions to TNF-α antagonists have been reported. These are mostly local or minor systemic reactions [2], although the literature also provides descriptions of severe systemic reactions and anaphylaxis [3,4]. Recently, an immunological mechanism was associated with injection site reactions after administration of etanercept and adalimumab [5], and another group reported a large number of desensitizations [6].

We report the case of a 40-year-old man with fistulizing Crohn disease who developed general malaise, cutaneous flushing, palpitations, and urticaria on his arms upon administration of the fifth dose of the chimeric antibody infliximab. At the time, the patient was also undergoing treatment with azathioprine. Infliximab (225 mg diluted in 250 mL of 0.9% sodium chloride) was administered by continuous infusion (infusion rate of 2 mL/min). The patient was premedicated with oral dexchlorpheniramine and methylprednisolone. Symptoms started when the patient had received 100 mg of infliximab and disappeared when the infusion was stopped. A new dose of methylprednisolone was administered. The patient was discharged 2 hours later without symptoms.

One month after the reaction, the patient underwent an allergy workup at our department. Skin prick with mouse dander, hamster dander, and latex extracts elicited negative results. The results of prick-by-prick test with infliximab (0.01, 0.1, and 1 mg/mL) and intradermal skin testing (0.01 and 0.1 mg/mL) were negative. Therefore, a single-blind intravenous challenge was performed with infliximab. The concentration used was the same as in previous administrations, although this time at half the infusion rate (1 mL/min). When the patient had received 2.5 mg of infliximab (about 3 min after starting the infusion), he began to experience general malaise, pruritus, and cutaneous flushing. Intramuscular adrenaline (a single dose of 0.3 mL), methylprednisolone, and dexchlorpheniramine were administered. The patient was discharged 4 hours later with no symptoms. Following this positive result in the intravenous challenge, a basophil activation test (BAT) was performed 4 weeks after the last reaction to elucidate the immunological mechanism involved in the reaction. BAT (Basotest, ORPEGEN Pharma, Heidelberg, Germany) was performed with infliximab and adalimumab (in order to study tolerance to other TNF-α antagonists) using whole blood obtained from the patient and a control, as previously described [7]. The drug concentrations used were 0.001, 0.002, 0.05, and 0.1 mg/mL for infliximab and 0.5, 1, 25, and 50 mg/mL for adalimumab. Negative results were obtained for both agents at all concentrations. Administration of adalimumab was well tolerated by the patient.

Hypersensitivity reactions to monoclonal antibodies have been reported, although very few have an immunoglobulin E–mediated mechanism [8]. Such a mechanism had been postulated in allergic reactions to TNF-α antagonists, yet no clear evidence has come to light [9]. To our knowledge, this is the first report of BAT being used to analyze the reaction to a TNF-α antagonist.

In conclusion, we report the case of a patient who suffered a systemic hypersensitivity reaction after administration of the TNF-α antagonist infliximab. The patient tolerated administration of adalimumab, as previously described [10]. The results of BAT suggest that an IgE-mediated mechanism is not involved.

Acknowledgments

These results were presented as an oral communication in the Oral Communication Session "Various I" at the International Symposium on Drug Hypersensitivity (Logroño, Spain, 2009), organized by SEAIC.

Editorial assistance was kindly provided by Oliver Shaw.

References

1. Köhler G, Millstein C. Continuous cultures of fused cells secreting antibody of predefined specifity. Nature. 1975;256:495-7.
2. Vergara G, Silvestre JF, Betlloch I, Vela P, Albares MP, Pascual JC. Cutaneous drug eruption to infliximab: report of 4 cases with an interface dermatitis pattern. Arch Dermatol. 2002;138:1258-9.
3. Sugiura F, Kojima T, Oba M, Tsuchiya H, Ishiguro N. Anaphylactic reaction to infliximab in two rheumatoid arthritis patients who had previously received infliximab and resumed. Mod Rheumatol. 2005;15:201-3.

4. Chávez-López MA, Delgado-Villafaña J, Gallaga A, Huerta-Yáñez G. Severe anaphylactic reaction during the second infusion of infliximab in a patient with psoriatic arthritis. Allergol Immunopathol (Madr) 2005;33:291-2.
5. Benucci M, Manfredi M, Demoly P, Campi P. Injection site reactions to TNF-a blocking agents with positive skin tests. Allergy. 2008;63:138-9.
6. Brennan PJ, Bouza TR, Hsu FI, Sloane DE, Castells MC. Hypersensitivity reactions to mAbs: 105 desensitizations in 23 patients, from evaluation to treatment. J Allergy Clin Immunol. 2009;124(6):1259-66.
7. Manso L, Heili S, Fernández-Nieto M, Sastre B, Sastre J. Basophil activation in two cases of hydrochlorothiazide-induced noncardiogenic pulmonary edema. Allergy. 2010;65:135-6.
8. Baudouin V, Cusiak A, Hadad E, Schandene L, Goldman M, Loirat C, Abramowicz D. Anaphylactic shock caused by immunoglobulin E sensitization after retreatment with the chimeric anti-interlekin-2-receptor monoclonal antibody basiliximab. Transplantation. 2003;76:459-63.
9. Domm A. A patient's reaction to infliximab. Ann Allergy Asthma Immunol. 2003;90:298-301.
10. Stallmach A, Giese T, Schmidt C, Meuer SC, Zeuzem SS. Severe anaphylactic reaction to infliximab: successful treatment with adalimumab - report of a case. Eur J Gastroenterol Hepatol. 2004;16:627-30.

Manuscript received March 10, 2010; accepted for publication May 24, 2010.

Joaquín Sastre
Servicio de Alergología
Fundación Jiménez Díaz
Avda. Reyes Católicos, 2
28040 Madrid, Spain
E-mail: jsastre@fjd.es

Efficacy of the Slow Dose-up Method for Specific Oral Tolerance Induction in Children With Cow's Milk Allergy: Comparison With Reported Protocols

H Kaneko, T Teramoto, M Kondo, H Morita, H Ohnishi, K Orii, E Matsui, N Kondo
Department of Pediatrics, Graduate School of Medicine, Gifu University, Gifu, Japan

Key words: Cow's milk allergy. Specific oral tolerance induction. Slow dose-up method.

Palabras clave: Alergia a leche de vaca. Inducción específica de tolerancia oral. Método de dosificación lento.

The basic treatment for adverse reactions to food has traditionally been to avoid the offending food [1]. Specific oral tolerance induction (SOTI) achieved by oral exposure to increasing doses of the specific food allergen seems to be a promising therapeutic approach. No standard protocol for SOTI has been designed and there is wide variation among hospitals.

The study population comprised 10 patients (aged 4-14 y) referred to Gifu University Hospital (Gifu, Japan) with allergy to cow's milk. Diagnosis was based on serum CAP system fluorescent enzyme immunoassay (cow's milk–specific immunoglobulin [Ig] E, 0.92 IU/mL to >100 IU/mL; mean 18.2 IU/mL) and a positive allergic reaction to cow's milk during the 3 months before the beginning of the study (7 patients) or a positive allergic reaction to a double-blind placebo-controlled food challenge (3 patients) (Table).

The initial dose of our slow dose-up method was 1 daily drop of cow's milk (approximately 0.025 mL) in 20 mL of water. The dose was increased every 2 weeks. The challenge was performed at Gifu University Hospital under medical supervision. When symptoms appeared, the daily dose was not increased and the previous dose was repeated. The children were then carefully assessed. A reaction was considered to be positive in the presence of at least one of the following symptoms: urticaria/angioedema or erythema with pruritus; rhinitis and/or conjunctivitis; bronchial asthma; vomiting and/or diarrhea with abdominal pain; and general malaise or loss of consciousness.

Eight patients completed the protocol and were able to tolerate 100 mL of cow's milk. The 2 patients who did not complete the protocol experienced symptoms, one with urticaria following a 5-mL dose and the other with perioral exanthema following a 20-mL dose. In these patients, the daily dose of cow's milk was not increased and the last dose was repeated after 2 weeks. The same symptoms reappeared with each repeated dose. Therefore, the patients could not continue the protocol. Three patients experienced mild side effects, including perioral exanthema, which did not require medication or dose reduction.

The protocol of Staden et al [2] required 2 months to reach the maximum dose (250 mL). The adverse reactions observed were exacerbation of eczema in 13 of 25 patients and urticaria in 11. Seven of the 20 patients (35%) tolerated milk and were considered the control group. The protocol of Longo et al [3] consisted of a rush-phase SOTI and a slow increasing-phase SOTI. Five of 30 patients who underwent SOTI required epinephrine for the treatment of adverse reactions. Eleven of 30 patients who underwent SOTI (36%) achieved complete tolerance to cow's milk. In our slow dose-up method, the severity and frequency of adverse reactions were almost the same as those of the protocols mentioned above.

Protocols are divided into 2 groups on the basis of the time required to reach the maintenance doses. Rush immunotherapy is performed in the hospital [3-5] and consists of doubling the doses of cow's milk every 1 or 2 hours. It takes approximately 1 week to achieve the maintenance dose. The other is performed at home. The time to reach the maintenance doses varies from 65 to 200 days [2,6-9].

We previously observed transiently elevated lymphocyte stimulation [10] and interferon γ production with ß-lactoglobulin, and the CD4CD25 ratio increased after SOTI (data not shown). These immunological changes may be associated with milk-allergen–specific regulatory T cells.

SOTI is a promising method for the treatment of food allergy. Although we consider it to be safe and effective, it should be performed by trained staff and following a standard protocol.

Table. Patient Characteristics and Serum IgE Levels Before SOTI

Case	Sex	Age, y	Symptoms Presented With Cow's Milk Before SOTI	IgE (IU/mL)	Specific IgE (kU_A/mL)			
					Cow's Milk	Casein	ß-Lactoglobulin	Symptoms During SOTI
Patients who completed the protocols								
1	F	5	Cough, erythema with pruritus	86.7	1.6	2	<0.34	No symptoms during SOTI
2	M	6	Perioral exanthema	250	9.5	12	0.79	Perioral exanthema (5 mL of cow's milk)
3	M	7	Conjunctivitis, bronchial asthma	1500	42	67	1.8	No symptoms during SOTI
4	M	7	Cough, urticaria, perioral exanthema	1400	>100	>100	6.7	Perioral exanthema (2 mL of cow's milk)
5	F	8	Erythema with pruritus	120	0.92	1.2	<0.34	No symptoms during SOTI
6	M	8	Erythema with pruritus	600	1.6	1.1	0.68	No symptoms during SOTI
7	F	9	Cough, urticaria, perioral exanthema	710	4.9	8	<0.34	Perioral exanthema (20 mL of cow's milk)
8	M	10	Cough, erythema with pruritus	88	4	5.3	<0.34	No symptoms during SOTI
Patients who did not complete the protocols								
1	M	4	Cough, erythema with pruritus	120	11	9	7.2	Urticaria (5 mL of cow's milk, repeatedly induced)
2	M	14	Erythema with pruritus	520	6.5	3.4	0.98	Perioral exanthema (20 mL of cow's milk, repeatedly induced)

Abbreviations: Ig, immunoglobulin; SOTI, specific oral tolerance induction.

Acknowledgments

This study was partially supported by a grant from the Ministry of Health, Labor and Welfare of Japan.

References

1. Sicherer SH Sampson HA. Food allergy. J Allergy Clin Immunol. 2006;117:S470-5.
2. Staden U, Rolinck-Werninghaus C, Brewe F, Wahn U, Niggemann B, Beyer K. Specific oral tolerance induction in food allergy in children: efficacy and clinical patterns of reaction. Allergy. 2007;62:1261-9.
3. Longo G, Barbi E, Berti I, Meneghetti R, Pittalis A, Ronfani L, Ventura A. Specific oral tolerance induction in children with very severe cow's milk-induced reactions. J Allergy Clin Immunol. 2008;121:343-7.
4. Bauer A, Ekanayake Mudiyanselage S, Wigger-Alberti W, Elsner P. Oral rush desensitization to milk. Allergy. 1999;54:894-5.
5. Staden U, Blumchen K, Blankenstein N, Dannenberg N, Ulbricht H, Dobberstein K, Ziegert M, Niggemann B, Wahn U, Beyer K. Rush oral immunotherapy in children with persistent cow's milk allergy. J Allergy Clin Immunol. 2008;12:418-9.
6. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG. A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. Allergy. 2004;59:980-7.
7. Patriarca G, Nucera E, Pollastrini E, Roncallo C, De Pasquale T, Lombardo C, Pedone C, Gasbarrini G, Buonomo A, Schiavino D. Oral specific desensitization in food-allergy children. Dig Dis Sci. 2007;52:1662-72.
8. Zapatero L, Alonso E, Fuentes V, Nartinez MI. Oral desensitization in children with cow's milk allergy. J Investig Allergol Clin Immunol. 2008;18:389-96.
9. Caminiti L, Passalacqua G, Barberi S, Vita D, Barberio G, De Luca, R Panjo GB. A new protocol for specific oral tolerance induction in children with IgE-mediated cow's milk allergy. Allergy Asthma Proc. 2009;30:1-6.
10. Kondo N, Shinbara M, Inoue R, Fukao T, Kaneko H, Teramoto T, Tashita H. Inhibition of interferon-gamma production from lymphocytes stimulated with food antigens by a beta2-agonist, procaterol, in patients with food-sensitive atopic dermatitis. J Invest Allergol Clin Immunol. 1997;7:225-8.

Manuscript received March 6, 2010; accepted for publication June 28, 2010.

Hideo Kaneko, MD, PhD
Derpartment of Pediatrics, Graduate School of Medicine, Gifu University
Yanagido, Gifu, 501-1194, Gifu
Japan
E-mail: hideo@gifu-u.ac.jp

Metrorrhagia as an Uncommon Symptom of Anaphylaxis

MA Gonzalo-Garijo,[1] I Pérez-Rangel,[1] MI Alvarado-Izquierdo,[2] R Pérez-Calderón,[1] S Sánchez-Vega,[1] MA Zambonino[1]
[1]*Department of Allergology and Clinical Immunology, Infanta Cristina University Hospital, Badajoz, Spain*
[2]*Allergology Unit, Coria Hospital, Coria, Spain*

Key words: Anaphylaxis. Hypersensitivity. Metrorrhagia. Vaginal bleeding.

Palabras clave: Anafilaxia. Hipersensibilidad. Metrorragia. Hemorragia vaginal.

Anaphylaxis is a severe allergic reaction that usually presents with mucocutaneous, gastrointestinal, respiratory, cardiovascular, or neurologic symptoms and signs. The diagnosis is based primarily on clinical criteria. Involvement of the skin is reported in 80% to 90% of episodes, the respiratory tract in up to 70%, the gastrointestinal tract in up to 45%, the cardiovascular system in up to 45%, and the central nervous system in up to 15% [1,2]. The objective of this work was to assess the presence of metrorrhagia during anaphylaxis, as vaginal bleeding is an unusual clinical manifestation of this condition.

We diagnosed 65 cases of anaphylaxis (0.95% of all patients attending the clinic for the first time) over a 10-year period (2000-2009). Thirty-six cases occurred in

Table. Clinical Characteristics and Causative Agents

Patient Number	Age, y	Symptoms	Onset[a]	Agent	Treatment	Resolution of Anaphylaxis
1	52	Dizziness, hypotension, rhinitis, wheezing, generalized erythema, urticaria, nausea, diarrhea, abdominal pain, metrorrhagia	12 h	Trimethoprim-sulfamethoxazole	Corticosteroids and antihistamines (oral and IV)	4 d
2	37	Headache, dizziness, hypotension, pharyngeal itching, generalized rash, lower abdominal pain, nausea, vomiting, diarrhea, metrorrhagia	4 h	Amoxicillin-clavulanic acid	Corticosteroids and antihistamines (IV), adrenaline (SC), saline infusion	6 h
3	39	Dizziness, generalized itching and erythema, urticaria, dyspnea, wheezing, abdominal cramping, vomiting, diarrhea, metrorrhagia	6 h	Amoxicillin-clavulanic acid	Corticosteroids and antihistamines (IM)	4 h
4	35	Dizziness, warmth, cutaneous itching, abdominal cramping, metrorrhagia	1 h	Hymenoptera venom	Unknown (IM)	2 h
5	38	Dizziness, palmar and plantar itching followed by generalized itching with erythema and urticaria, conjunctival hyperemia, hydrorrhea, dyspnea, lower abdominal pain, metrorrhagia	1 h	Pollen immunotherapy	Corticosteroids and antihistamines (IM), adrenaline (SC)	3-4 h
6	38	Dizziness, hypotension, itching (palmar, plantar, otic, and pharyngeal), lower abdominal pain, metrorrhagia	1 h	Amoxicillin	Corticosteroids and antihistamines (IM), adrenaline (SC)	2 h
7	42	Dizziness, hypotension, palmar and plantar itching, urticaria, dyspnea, dysphagia, diarrhea, lower abdominal pain, metrorrhagia	6 h	Unknown	Corticosteroids and antihistamines (IM), oxygen, inhaled bronchodilators	8 h

Abbreviations: IM, intramuscular; IV, intravenous; SC, subcutaneous
[a] Time between onset of anaphylaxis and onset of metrorrhagia

women (55.4%) and 29 in men (44.6%). Only 7 women (19%) experienced vaginal bleeding in association with the anaphylactic episode. The clinical characteristics and causative agents of these 7 cases are shown in the Table. Mean age was 40 years. Onset of anaphylaxis was always acute, ranging from 15 to 30 minutes after the trigger, and metrorrhagia appeared from 1 to 12 hours (mean, 4.4 h) after onset. All the patients complained of abdominal pain and underwent additional examinations (abdominal X-ray, ultrasound scan, and—in some cases—gynecological evaluation).

Anaphylaxis has been defined as a serious allergic reaction that is rapid in onset and can be fatal [1]. Prevalence is estimated at 0.05% to 2%, and the rate of occurrence appears to be increasing, mainly in young people [2]. The pathogenesis of anaphylaxis involves immunological mechanisms (immunoglobulin [Ig] E–mediated or non–IgE-mediated) and nonimmunological mechanisms (eg, physical factors, alcohol, opioids). Idiopathic anaphylaxis, currently a diagnosis of exclusion, makes it possible to identify unrecognized triggers. Regardless of the trigger and mechanism, activation of mast cells and basophils results in the rapid release of immediate mediators (preformed and lipid) and delayed mediators (cytokine secretion). Severity and mortality are affected by age, concomitant diseases (eg, asthma, cardiovascular disorders, or mastocytosis), and concurrent medication [1,2]. The most common cause of anaphylaxis in childhood is food allergy, whereas drugs and hymenoptera venom are more common in adults [3]. In any case, anaphylaxis is unpredictable, underrecognized by patients, and underdiagnosed by health care professionals [1].

According to previous reports [4-6], metrorrhagia is a rare symptom of anaphylaxis and is not usually included in the list of possible symptoms of this condition. However, the most recent expert consensus adds "uterine contractions in postpuberal female patients" to the possible manifestations of anaphylaxis [2]. The pathogenic mechanism of this symptom has not been established, but the effects of mediators on smooth muscle secondary to histamine and other agents might explain this finding. In our series, only 7 patients (19%) experienced this symptom, and the most common trigger was drugs (mainly antibiotics). Alcoceba et al [4] described a series with 5 patients (12% of all women with anaphylaxis) in which the most common trigger of anaphylaxis was hymenoptera venom (4 patients). There has been 1 report of metrorrhagia secondary to cold allergy [5] and another following immunotherapy [6]. In our series, metrorrhagia was not associated with anaphylaxis when the patients were treated in the emergency room. Awareness of metrorrhagia in the context of anaphylaxis could help avoid unnecessary complementary examinations.

References

1. Simons FE. Anaphylaxis: Recent advances in assessment and treatment. J Allergy Clin Immunol. 2009;124:625-36;quiz 637-8.
2. Simons FE. Anaphylaxis. J Allergy Clin Immunol. 2010;152:S161-81.
3. Tang ML, Osborne N, Allen K. Epidemiology of anaphylaxis. Curr Opin Allergy Clin Immunol. 2009;9:351-6.
4. Alcoceba E, Marquès L, Lara S. Metrorrhagia in anaphylactic reactions. Allergy. 2009;64(Suppl. 90):214.
5. Frank DE. Metrorrhagia due to allergy to cold. Ann Allergy. 1947;5:574.
6. Toubi E, Kessel A, Golan TD. Vaginal bleeding – a rare complication of immunotherapy. Allergy. 1997;52:782-3.

Manuscript received June 17, 2010; accepted for publication June 29, 2010.

M Ángeles Gonzalo-Garijo
Hospital Universitario Infanta Cristina
Sección de Alergología
Carretera de Portugal, s/n
06080 Badajoz
Spain
E-mail: magonzalog@telefonica.net