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Is rectoperineal fistula in anorectal malformations an ectopic anal canal with an internal anal sphincter?

Czy przetoka odbytniczo-kroczowa w wadach odbytu i odbytnicy jest ektopowym kanałem odbytu ze zwieraczem wewnętrznym odbytu?

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Abstract

Authors tried to determine the role of an internal anal sphincter (IAS) in patients with rectoperineal fistula undergoing PSARP, with or without IAS-saving procedure.

Materials and methods: 20 girls and 6 boys with rectoperineal fistula undergoing PSARP between 1993-2008 were included in the study. An IAS-saving operation was performed in 19 children (73.1%), 17 girls and 2 boys, while 7 children underwent regular PSARP with resection of narrowed distal portion of fistula.

Results: Functional postoperative result according to the „10" score was good (8-10 pts.) in all 26 children. There was no significant difference in RAP (16.3 *vs.* 16.9 *vs.* 17.2 cmH_2O) and ACL (1.7 *vs*. 1.7 *vs*. 1.9 cm) values between children with and without preserved IAS and the reference group. Positive RAIR was observed in 13 of 19 children with preserved IAS, and in 2 of 7 patients after resection of IAS, but this incidence did not differ statistically ($p = 0.095$). The major functional disorder in the examined children was constipation, observed in 9 children after sphincter-saving procedure and in 2 patients after regular PSARP, but the difference was not significant ($p = 0.658$).

Conclusions: An IAS is important, but not a decisive factor in fecal continence and IAS-saving procedures are associated with high incidence of constipation. Authors suggest that IAS can be spared, wherever it was possible, *i.e.* the fistula is wide (at least 8 mm) and it will not impair function of the neoanus.

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Key words: anorectal malformations, rectoperineal fistula, internal anal sphincter, anorectal manometry, posterior sagittal approach.

Streszczenie

Autorzy podjęli próbę oceny roli zwieracza wewnętrznego odbytu (ZWO) u pacjentów z przetoką odbytniczo-kroczową po operacji wady odbytu metodą PSARP, z wycięciem lub oszczędzeniem ZWO.

Materiały i metody: Badaniami objęto grupę 20 dziewczynek i 6 chłopców z przetoką odbytniczo-kroczową operowanych metodą PSARP w latach 1993-2008. Operację oszczędzającą ZWO przeprowadzono u 19 dzieci (73,1%), 17 dziewczynek i 2 chłopców, natomiast u 7 pacjentów wykonano operację PSARP z wycięciem zwężonego, dystalnego odcinka przetoki.

Wyniki: Pooperacyjny wynik czynnościowy wg skali „10" był dobry (8-10 pkt.) u wszystkich 26 dzieci. Nie stwierdzono istotnej różnicy w wartościach RAP (16,3 *vs*. 16,9 *vs.* 17,2 cmH_2O) i ACL (1,7 *vs.* 1,7 *vs.* 1,9 cm) u dzieci z i bez ZWO oraz grupą referencyjną. RAIR wywołano u 13 spośród 19 dzieci z zachowanym ZWO oraz u 2 spośród 7 pacjentów bez ZWO, bez różnicy statystycznej ($p = 0,095$). Najpoważniejszym zaburzeniem czynnościowym u dzieci były zaparcia obserwowane u 9 pacjentów z zachowanym ZWO i u 2 dzieci po klasycznej operacji PSARP, bez różnicy statystycznej ($p = 0,658$).

Wnioski: ZWO jest ważnym, ale nie decydującym czynnikiem w mechanizmie trzymania stolca, a operacjom oszczędzającym ZWO towarzyszy wysoki odsetek zaparć w okresie pooperacyjnym. Autorzy sugerują oszczędzenie ZWO, jeżeli jest to możliwe, tzn. ujście przetoki jest szerokie (co najmniej 8 mm) i nie upośledza to czynności nowego odbytu.

Słowa kluczowe: wady odbytu i odbytnicy, przetoka odbytniczo-kroczowa, zwieracz wewnętrzny odbytu, manometria odbytnicza, dostęp strzałkowy tylny.

Introduction

Before de Vries and Peña introduced the Posteriori Sagittal Anorectoplasty (PSARP) for surgical correction of anorectal malformations (ARM) in 1982 [1], the major concern of pediatric surgeons had been how to create a new anus in an anatomical position and place the rectum within the muscle structures without causing damage to them. The appearance of the neoanus and the postoperative functional results have improved markedly with a new procedure as compared with other operations [2, 3]. However, in corrective operations for ARMs little attention has been paid to the preservation of the terminal rectum and the rectourogenital or rectoperineal fistula [4]. Only a few authors have advocated the importance of the mentioned structures and have suggested the preservation of the internal anal sphincter (IAS) in the reconstruction of the anal canal in children with ARMs [5-7].

In this paper the authors report the histological and manometric results in patients with the rectoperineal fistula undergoing PSARP, with or without an internal sphincter-saving procedure.

Material and methods

From 1993 to 2008, 94 children (50 girls and 44 boys) with ARMs were treated by the author (J.N.) in Pediatric Surgery Department of Medical University of Lodz, Poland. Posterior sagittal anorectoplasty (PSARP) [1] was performed in 91 children [2]. Twenty six patients, 20 girls and 6 boys, with the rectoperineal fistula were included in the present study. An internal sphincter-saving operation was performed in 19 children (73.1%), 17 girls and 2 boys, without colostomy creation. Seven children (26.9%) had colostomy done initially and underwent regular PSARP with the resection of a narrowed distal portion of the fistula (Table 1.). Tissue specimens were sectioned longitudinally for a histological examination.

Table 1. Surgical management of children with rectoperineal fistula.

Initial management	PSARP		PSARP with IAS preservation		Total
	F	M	F	M	
Colostomy	3	4	-	-	7
Without colostomy	-	-	17	2	19
Total	3	4	17	2	26
	7		19		
Constipation	2		9		11

All the children underwent a clinical and manometric evaluation 2 to 10 years (mean 3.3 yrs) after the surgical correction of ARM. The clinical assessment was based on the quantitative „10" score, modified by the author (J.N.) Kelly's score [8], in which the result was classified as: 10-8 points – good (normal bowel function), 7-4 points – satisfactory (with no or slight social limitations), 0-3 points – poor (total incontinence).

The manometric technique has been described elsewhere [2, 9]. The criteria for the functional internal sphincter were the presence of high resting anal pressure (RAP) and the positive rectoanal inhibitory reflex (RAIR). The anal canal length (ACL), which resembles the length of the zone of both sphincters adherence to the rectal wall, was measured additionally.

The obtained results were subjected to a statistical analysis using Chi square test and t-test (Statistica 9.1 PL package)

Results

The functional result according to the „10" score was good (8-10 pts.) in all 26 children. There was no significant difference in RAP and ACL values between children with and without preserved IAS and the results were comparable with the values obtained in the reference group. Positive RAIR was

observed in 13 of 19 children with preserved IAS, and occasionally (2 of 7 patients) after the resection of IAS, but this incidence did not differ statistically ($p = 0.095$) (Table 2.).

Table 2. Results of anorectal manometry in children after correction of rectoperineal fistula. RAP - resting anal pressure, RAIR - rectoanal inhibitory reflex, ACL – anal canal length.

Procedure	No. of patients	Mean RAP (cmH_2O)	Mean ACL (cm)	Positive RAIR
PSARP	7	16.3	1.7	2 (28.6%)
PSARP with IAS preservation	19	16.9	1.7	13 (68.4%)
Total	26	16.8	1.7	15 (57.7%)
Reference group	40	17.2	1.9	40

The major functional disorder in the examined children was constipation, observed in 11 (42.3%) out of 26 children after the surgical correction of the rectoperineal fistula. The incidence of constipation was higher after the sphincter-saving procedure (9 children) comparing with regular PSARP (2 patients), but the difference was not significant ($p = 0.658$) (Table 1.). The physical examination did not reveal an anal stricture in any of these patients. In all the cases constipation was treated with diet and occasional enemas; in two children Debridat (Trimebutine) and Coordinax were effective.

Discussion

The internal anal sphincter (IAS) has been described as the thickening of the circular smooth muscle layer in the most distal part of the rectal wall [4, 10]. IAS is believed to contribute about 85% in magnitude to the resting anal pressure (RAP) and the rectoanal inhibitory reflex (RAIR) [11] and to be closely related to continence after the repair of ARM [12, 13]. Histological and

manometric studies have shown that there is a thickened muscle ring as well as the transitional epithelium present at the most distal end of rectourogenital or rectoperineal fistula [12, 14]. Rintala et al. suggest that these are structures of the normal anal canal and, therefore, rectourogenital or rectoperineal communication is, in fact, an ectopic anal canal [7, 15].

The specimen obtained from the children with ARM at the time of surgery enabled studies on the physiology of smooth muscles in the imperforated bowel and fistulas to the urogenital tract [14]. The examined fragments of the muscles showed *in vitro* reactions characteristic for IAS; the inhibitory reaction after electrostimulation, relaxation after pharmacological α-adrenergic and β-adrenergic stimulation and contraction or relaxation after cholinergic stimulation. Although these results suggest that the distal fragment of the rectal smooth muscles layer in children with ARM functionally corresponds to IAS in healthy individuals, the clinical implications of this observation are not fully clear [14]. Because the majority of rectourogenital fistulas are very narrow and thin-walled, from the perspective of the operative technique it may be difficult to identify and preserve IAS during the operation of ARM with urogenital fistulas, where the rectum and urethra (or vagina) share a common wall, which in its distal part is created only by two layers of mucosa [1, 3]. Even if a surgeon succeeds to separate and preserve the whole distal segment of the fistula, there will be a smooth muscle missing in the part of the bowel wall separated from the urethra or vagina there is no smooth muscle [14]. In some patients with high ARM during the corrective operation the most distal part of the bowel is removed, and a more proximal segment of the bowel is used for anoplasty – in consequence these patients have no IAS. Some of them present various degrees of incontinence, while others show good bowel control and are continent [6, 13, 16]. Lin et al. reported no significant difference in the incidence of positive RAIR and in the RAP values between the patients who had and who did not have the sphincter-saving procedure. They also propagated RAIR in children with no IAS after the resection of the distal rectum during Rehbein's procedure [17].

Yoo et al. confirmed in animal models that the resection of the 2 and 4 cm distal segment of the bowel with the internal sphincter impaired neither continence nor significantly decreased RAP in operated dogs [18]. The results of several studies on children after the surgical correction of ARM by PSARP show that the preservation of the distal fragment of the rectal wall smooth muscle layer may improve the postoperative functional results. Children with IAS preserved after the radical surgery and with positive RAIR show statistically better continence than those in whom this structure had been removed [5, 7, 13, 14, 19]. On the other hand, patients after the surgical correction of ARM develop disorders of the bowel function regardless of the presence of the internal sphincter. Decreased RAP and continence disorders were reported in patients with preserved IAS and normal RAIR [20, 21]. Constipation was observed as a major functional complication in children with preserved IAS as a result of inhibited peristalsis in the rectum [22]. The rectoperineal fistula is a low ARM with well developed sacrum and muscles [1, 2, 4]. The opening of the fistula is wide and efficient for defecation in majority of cases, although it can be stenotic, making passing meconium and stools difficult or impossible in number of patients. These children need colostomy creation as early as possible to prevent the chronic distention of the rectum and irreversible damage to the bowel wall [2, 23]. Postoperative results are good in these ARM, all the patients are continent with good appearance of the neoanus [1-4]. We compared postoperative results in the children with the rectoperineal fistula who underwent a sphincter-saving procedure or regular PSARP. There was no statistical difference in RAP values between both groups, and RAIR was evoked in number of the children of both groups. However, we observed constipation in 11 children after the correction of rectoperineal fistula; in 9 after the sphincter-saving procedure and in two after regular PSARP. Seven children had colostomy created initially due to the stenotic opening of the fistula and underwent regular PSARP with the resection of the distal narrowed segment of the rectum.

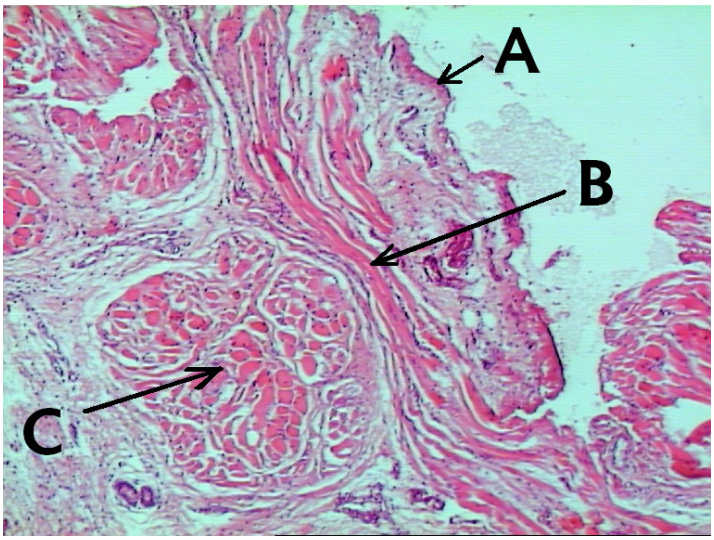

Fig. 1. Micrograph of transverse section of the rectoperineal fistula in girl.
A – an anal-type mucosa, B – circular layer of smooth muscles (IAS),
C – longitudinal layer of smooth muscles.

Tissue specimens were taken from these patients and sectioned longitudinally for a histological examination. An anal-type mucosa and circular layer of smooth muscles was found in all the specimens (Fig. 1.). Nineteen children who underwent the sphincter-saving procedure had no colostomy done because of the wide opening of the fistula – at least 8mm checked by Hegar's dilators. Nine out of 11 patients with constipation recruited from children with preserved IAS and with no colostomy. It is possible that both elements played a role in constipation, but of children without IAS only one presented constipation. Constipation after the repair of ARM has mainly been associated with low anomalies [16, 20], although a sphincter-saving procedure can cause constipation also in children operated due to high and intermediate ARMs [22]. The prognosis of constipation in patients after IAS-saving procedure is unclear, however in all patients in the present series, the symptoms of constipation were ameliorated with medical treatment.

Conclusions

Our results suggest that the IAS is an important, but not decisive factor in fecal continence, because children without IAS were clinically continent. Manometrical results support this thesis as well, because there was no decrease of the resting anal pressure in patients without IAS. The author (J.N.) observed incontinence in children with undeveloped or injured surgically voluntary muscles. We suggest that IAS can be spared, wherever possible, *i.e.* the fistula is wide (at least 8 mm) and it will not impair the function of the neoanus.

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Current view about basophils and their significance in the allergic immune response

Aktualna wiedza o bazofilach i ich roli w reakcjach alergicznych

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Abstract

Basophils are one of major effector cells participating in the immune response despite the fact that they constitute a small population of peripheral blood leukocytes. They are activated by cross-linking of an antigen with IgE through FcεRI and then they release a wide range of mediators, implicated in the pathogenesis of allergic diseases. Basophils are essential cells in IgG-mediated systemic anaphylaxis and currently are considered as antigen-presenting cells. This review summarizes recent studies and reports on basophils, their origin and role in the immune response, and their participation in diseases. Widening of our knowledge about basophils can help us to understand better the mechanisms of the complex processes in which those cells are involved.

Key words: cytokines, Th1/Th2 response, late-phase reaction (LPR), releasability, cell differentiation.

Streszczenie

Bazofile to jedne z najistotniejszych komórek uczestniczących w odpowiedzi immunologicznej, mimo iż stanowią niewielką populację we krwi obwodowej. Ulegają aktywacji w krzyżowej reakcji wiązania antygenu przez przeciwciało IgE do receptora FcεRI. Uwalniają szerokie spektrum mediatorów, co przekłada się na implikacje w patogenezie chorób alergicznych. Odgrywają także znaczącą rolę w uogólnionych reakcjach anafilaktycznych z udziałem przeciwciał IgG, a także jako komórki prezentujące antygen. Ta praca jest podsumowaniem aktualnej wiedzy o bazofilach, ich pochodzeniu i roli w reakcjach alergicznych oraz znaczeniu w jednostkach chorobowych. Poszerzanie wiedzy z tego zakresu jest niezbędne do lepszego zrozumieniu mechanizmów skomplikowanych procesów, w których bazofile uczestniczą.

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Słowa kluczowe: cytokiny, odpowiedź Th1/Th2, zjawisko „*basophil releasability*", reakcja późnej fazy (LPR), różnicowanie komórek.

Abbreviations:

MCs	– mast cells
FcεRI	– the high affinity receptor for immunoglobulin E
CD	– clusters of differentiation
CFU-Eo/Baso	– colony-forming unit-eosinophil/basophil
IL	– interleukin
GM-CSF	– granulocyte-macrophage colony stimulating factor
CFU-Baso/Mega	– colony-forming unit-basophil/megakaryocyte precursor
IRF-2	– interferon regulatory factor 2
Th2	– T helper cells with profile 2
Ig	– immunoglobulin
ITIM	– immunoreceptor tyrosin-based inhibition motive
LIR7	– leukocyte immunoglobulin-like receptor 7
APC	– antigen-presenting cells
MHC class II	– major histocompatibility complex class II
LPR	– the late-phase reaction
NGF	– nerve growth factor
HRF	– histamine-releasing factor
LTC4	– leukotriene C4
ERK	– extracellular signal-regulated kinase
sIgA	– secretory IgA
MCP-1	– monocyte chemoattractant protein-1
RANTES	– Regulated on Activation Normal T Cells Expressed and Secreted
CCR	–specific receptors for CC chemokines
PAF	– platelet activating factor
TSLP	– thymic stromal lymphopoietin
GzmB	– granzyme B
VLA-4	– adhesive molecules on vascular endothelial cells
sLT	– sulfidoleukotrienes
PAR-2	– protease-activated receptors
IgE-CAI	– immunoglobulin E-chronic allergic inflammation
CU	– chronic idiopathic urticaria

Introduction

Basophils are granulocytic leucocytes, one of major effector cells participating in the immune response. They constitute a small population of peripheral blood leukocytes below 1% [1]. Basophils are derived from unstimulated multipotential stem cells. They complete their differentiation in the bone marrow before entering the bloodstream, whence they are recruited into tissues, at the sites of inflammatory or immune responses [2]. Basophils have a short lifespan of several days and they do not proliferate once they mature [2-3].

Basophilic granulocytes were described for the first time in 1879 by Paul Ehrlich. Nevertheless, they were overlooked as minor and possibly redundant blood mast cells (MCs) and analyzed as a surrogate of the tissue mast cells [4-5]. Due to the lack of understanding of cellular and molecular biology of human basophils and their immunological functions, studies on human basophils have long been hampered. Moreover, there were no adequate methods for cell isolation. Studies on basophil functions were also diminished because of the low sensitivity of the assays used to measure mediator release, or the absence of appropriate animal models exhibiting basophil abnormalities. In the early 1990s improved methods for basophil isolation and purification and techniques for assessing the response and activation mechanisms of basophils became more sensitive and specific, even for that limited number of cells. In spite of the considerable progress, the role of basophils *in vivo* in allergy and other diseases is still poorly known [6-8]. Recent studies and reports on basophils, their role in immune response and participation in diseases can help us to understand better the mechanisms of these complex processes and provide targeted effective treatment for patients.

Specific markers for basophil detection

After many years of difficult investigations, a few basophil-specific monoclonal antibodies have been found. They are able to recognize intracellular or surface structures that do not react with other leukocytes or mast cells, such as Bsp-1, 2D7, BB1, 97A6 (fig. 1) [4, 6, 9-10].

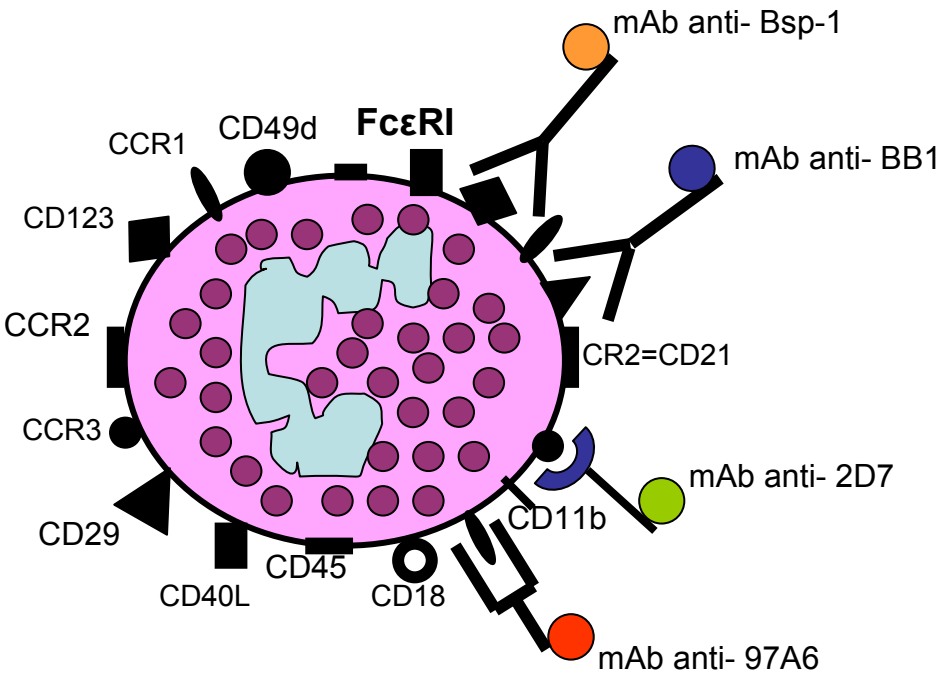

Fig. 1. Constitutive receptors and basophil-specific monoclonal antibodies useful in basophil identification.

Those unique markers for human basophils are needed to determine precisely the role of these cells in diseases. The Bsp-1, 45kDa surface protein was detected by Bodger as a selective marker for basophils [11]. Antibody 97A6 is directed against cell surface antigen, expressed during basophil activation [12]. The 2D7 and BB1 are selective intracellular markers in basophils and are useful for immunohistochemistry [9, 13-14]. 2D7 recognized by a mouse IgG1κ monoclonal antibody binds to a protein of 72-76kDa produced at an early stage in basophil development from bone marrow progenitors [15]. The 2D7 ligand may facilitate the assessment of basophil involvement in diseases such as anaphylaxis, asthma, and atopic dermatitis [15-16]. Basophils occur at the site of inflammation along with mast cells. It is important to distinguish both types of the cells because they respond differently to pharmacologic agents; and this differentiation may influence the treatment [15]. BB1 antibody recognizes a large macromolecular complex of approximately 5000kDa, called basogranulin, secreted along with histamine upon cell stimulation [17]. Immunostaining with this monoclonal antibody has revealed that basophils are rare in nasal biopsies from asthmatic subjects with seasonal allergic rhinitis out of the season, and the intranasal administration of allergen can induce a rapid influx of basophils into nasal mucosa of rhinitic subjects [18].

Ways of basophil activation

Basophils express constitutive receptors (useful in basophil identification *in vitro*) for IgE, IgG, cytokines, chemokines, complement components and other soluble stimuli that include lipids, histamine and small molecular weight peptides (fig. 1). The main receptor of basophils is the high affinity receptor for immunoglobulin E (FcεRI) on their surface. They are activated by cross-linking of FcεRI-bound IgE with bi- or multi-valent antigen [1], and then release a spectrum of mediators that have been implicated in the pathogenesis of allergic diseases, including asthma. They degranulate preformed mediators, such as histamine, tryptase, and also induce the de novo synthesis and secretion

of arachidonic acid metabolites and cytokines i.e. IL-4, IL-13 [19-20]. The level of IgE regulates FcεRI expression on human basophils. The long incubation of basophils without IgE *in vitro* resulted in the slow dissociation of this immunoglobulin in the environment and the decrease in FcεRI expression. Basophil cultures with IgE caused up-regulation of FcεRI expression [19]. The effect of IgE level was confirmed by utilizing humanized anti-IgE monoclonal antibodies (rhuMAB-E25) virtually eliminating circulating IgE. A receptor through which IgE induces this up-regulation is not known yet, but research in this area may give hope for novel therapeutic approaches to the management of allergic disease. It is known that mutations resulting in amino acid substitution in the human β-chain of FcεRI may be linked to atopic diseases [21-23].

The origin of basophils

Up today the basophil lineage remains a matter of debate [4]. It is still confusing whether basophils possess a lineage-restricted progenitor or whether they share a common ancestor with mast cells, eosinophils, or even megakaryocytes [2]. The knowledge of the pathway leading to basophil differentiation in humans could be very useful for a better comprehension of early phases in the pathogenesis of allergic diseases [8]. There are few studies and hypotheses concerning basophil ancestry (Table 1).

Table 1. Different hypothesis concerning basophils ancestry.

References	**Ancestor of basophils**
Li L [27]	common ancestor with mast cells
Florian S [24]	distinct lineage but the same hematopoietic progenitor CD34(+)
Denburg JA [28]	common ancestor with eosinophils
Dy M [29]	common ancestor with megakaryocytes

Florian et al. using a large series of monoclonal antibodies detected clearly distinct clusters of differentiation (CD) expression on basophils and mast cells, thus providing further evidence that these two types of cells represent distinct lineages [24], although both types derived from CD34+ hematopoietic progenitor cells [2, 25]. It is known that they differ from mast cells in their ultrastructure, expression of surface antigens, and response to growth factors [25-26].

On the other hand, Le L et al. [27] reported the presence of metachromatic cells with features of both basophils (blood location, segmented nuclei, Bsp-1 expression) and mast cells (expression of c-kit, tryptase, chymase, carboxypeptidase A) in the peripheral blood of patients with asthma, allergy, and an allergic drug reaction. These basophilic cells with mast cell-like proteases localized in the peripheral blood may have important biological implications in the regulation of homeostasis and can contribute to the development of allergic diseases [27]. The detection of basophilic marker Bsp-1 among the human mast cell line HMC-1 provides further support for the existence of a hybrid mast cell/basophil progenitor [4]. However, the concept of the existence of bipotential precursor, CFU-Eo/Baso (colony-forming unit-eosinophil/basophil), is not excluded. It is suggested that basophil and eosinophil lineages are often associated with the same colony and such association is also observed *in vivo* in humans. The injection of interleukin 3 (IL-3) or granulocyte-macrophage colony stimulating factor (GM-CSF) induces basophils and eosinophils, as well as an increased number of precursors of both lineages in atopic patients [28].

Moreover, recent studies have presented the novel statement about common basophil/megakaryocyte precursor (CFU-Baso/Mega) existence [4, 29]. Thus, all these reports confirm that basophil ancestor is still unclear.

Contribution to the immune responseTh2 - polarization

Mast cell and basophil functions overlap or complement one another during IgE-associated acquired immune response, and these roles include both effector and immunoregulatory activities [1, 10]. Basophils are important effector cells in allergy and in the host response to parasitic infections, such as helminths and ectoparasitic ticks. These infections induce strong IgE response [30]. The cells are directed by chemotactic factors at the sites of inflammation where they release proinflammatory mediators and toxic granule products [31]. Current data indicate that basophils and not dendritic cells are indispensable as antigen-presenting cells (APC) in allergen-induced activation of Th2 response. They present the antigen via major histocompatibility complex class II (MHC class II) and costimulatory molecules such as CD40, CD86 and CD54 [35, 41, 51]. They also generate large quantities of cytokines such as IL-4, IL-5, IL-13 promoting the activation of T helper cells with profile 2 (Th2-profile) and, at the same time, inhibiting Th1 differentiation [32-37]. All their properties have provided a new insight into the possible role of basophils in the pathogenesis of allergic disorders such as asthma, atopic dermatitis, and immunity to pathogens. The above-mentioned cytokines are key regulators of the Th2 response and their specific immunoregulatory functions can enhance IgE production. For example, IL-4 derived from basophils affects the differentiation of naive CD4+ T cells into Th2 cells *in vitro* and *in vivo* [5, 7, 36, 38-41]. Wedemeyer et al. suggested there a potential positive feedback mechanism [1]. Thus, a high level of IgE enhancing FcεRI surface expression on basophils influences the increase in FcεRI-dependent release of IL-4 and/or IL-13. Finally, this results in a higher level of IgE [1]. It could be one of the reasons why basophils are considered contributors to IgE-mediated anaphylaxis [42].

Moreover, Hida et al. [7] provided *in vitro* and *in vivo* evidence for the role of basophils in the initiation of Th2 response and the regulation of the Th1/Th2 balance. This report presented a novel mechanism for the suppression of excess Th2 polarization, mediated by interferon regulatory factor 2 (IRF-2).

IRF-2 deficiency causing the accelerated expansion of basophils led to the elevation of IL-4 production. It was confirmed when the depletion of basophils reduced the levels of IL-4. The spontaneous Th2 polarization *in vivo* in IRF-2-deficient mouse model was indeed greatly reduced concomitantly with the reduction in basophil numbers. Thus, the negative regulation of basophil numbers by IRF-2 would be critical for the host defense against infection [7].

Furthermore, it has been recently emphasized that basophils are essential cells in IgG-mediated systemic anaphylaxis. Such evidence suggests their role as initiators rather than effectors of allergic inflammation [43-46]. Apart from the receptor for IgE, basophils express two types of immunoglobulin G (IgG) receptors, FcγRIIA and FcγRIIB. The activation of FcγRIIA with IgG-antigen complexes leads to mediator release, whereas FcγRIIB transduces inhibitory signals because of the presence of inhibition motives ITIM (immunoreceptor tyrosin-based inhibition motive) in the intracellular sequence [4, 47]. Sloane et al. [48] identified leukocyte immunoglobulin-like receptor 7 (LIR7), common with Fc receptor γ chain, cross-linking with IgE eliciting release of basophilic mediators: histamine, LTC4, and IL-4. These findings indicate that LIR7 is the novel signaling receptor on human basophils which,as unique,activates cells in an IgE-independent way. Therefore, it can explain severe reactions to a stimulus in nonatopic patients with Th2 airway inflammation [48]. The recent studies reported by Bleharski et al. show that LIR7 is overexpressed in skin lesions of lepromatous leprosy, in which the Th2 response is dominant [49]. LIR7 in the colligation with LIR3 (involving ITIMs) or with FcεRI substantially inhibited release of basophilic mediators. The balance between activating and inhibitory signals delivered by several families of receptors is critical for the functions of leukocytes in the immune responses [48]. Several stimuli, unspecific for an allergen, can activate basophils. This way of activation confirms a significant role of basophils in the development of anaphylaxis and allergic inflammation even in the absence of IgE (IgE-independent mechanism) [4, 39, 50].

Late-phase response

When the immediate hypersensitivity reaction partially diminishes, the late-phase reaction (LPR) can be observed 6-9 hours after the exposure to the allergen [1, 52]. Recent investigations suggest that LPR has a substantial meaning in the pathogenesis of chronic allergic diseases rather than the early phase. There are several lines of evidence that basophils can contribute to LPR. Basophils infiltrate tissues many hours after the immediate reaction to the administered antigen and cause mediator release in the LPR. Secreted immunoregulatory cytokines potentially regulate the immune response of the other cells participating in allergic inflammation and resulting in an amplification of the overall reaction. Persistent chronic allergic inflammation can affect remodeling of the tissues and these structural changes are often associated with functional alterations [53]. Basophils are present in the cellular infiltrate in the bronchial tissue of atopic asthmatic patients [31] and their number increases after the allergen challenge. They are key effectors in the cutaneous LPR to allergens [16, 54] and dominate some T cell-mediated delayed-type hypersensitivity responses. In humans, basophils are recruited in LPRs at the sites of inflammation in the skin, nose and lower airways [1, 54].

Mast cells and, probably, basophils represent the sources of mediators and cytokines that can have anti-inflammatory potential. Some of these cells also express cell surface receptors that can mediate down-regulation of FcεRI-dependent signaling [19, 22]. Thus, basophils may potentially participate in the attenuation, as well as in the initiation or perpetuation of acquired immune responses [1].

Essential cytokines for basophil priming

Basophilic release of mediators (releasability) in patients with allergic diseases is also controlled by many biochemical processes connected with cytokines and their stimulating effects on inflammatory cells [55], (Table 2).

Cytokines such as IL-3, but also IL-5, GM-CSF, nerve growth factor (NGF), histamine-releasing factor (HRF) present at sites of allergic inflammation are capable of priming the release of mediators such as histamine and leukotriene C4 (LTC4) from basophils and can contribute to the process of their differentiation [6, 9].

Table 2. Mediators affecting on basophils.

Mediator	**Effect on basophils**
IL-3	- priming mediators release (i.e. IL-4, IL-13, histamine) [7, 23, 76]
	- priming cell proliferation and maturation [7]
	- making basophils more sensitive to chemokine activity [60]
	- priming adhesion to endothelial ligands [60]
	- priming chemotaxis [60]
	- priming participation of basophils in Th2 differentiation [25]
	- growth factor activity [4, 39]
	- intensification of specific allergen-induced activation [69]
	- promoting of basophilia [2]
IL-5	- priming cell proliferation and maturation [7, 59]
	- priming mediators release [9]
GM-CSF	- priming mediators release [6, 9]
MCP-1	- stimulation of mediators release
	- chemotactic activity [60]
HRF	- priming mediators release [6, 9]
NGF	- priming cell activation
	- priming mediators release [6, 9]
RANTES	- chemotactic activity [60]

Interleukin 3 signals diverged into, at least, 2 distinct pathways leading to either proliferation or cytokine production in basophils [7]. IL-3 might be produced constantly *in vivo* at low levels by T cells and has the longest duration of action. It can also induce phenotypic alteration of mature basophils [56-57]. IL-3 after binding its receptor (CD123) and subsequent receptor dimerization, activates JAK2 kinase, most likely due to its transphosphorylation. JAK2 is relevant in transmitting signals for cellular functions and it results in the activation of extracellular signal-regulated kinase (ERK), which primes LTC4 release. Furthermore, the recent studies show that basophils may synthesize IL-3, which suggests the ability to regulate their own priming *in vivo* [58].

The NGF also activates ERK pathway but it interacts via the other receptor called Trk. The finding that basophils express the Trk receptor is remarkable and may indicate the participation of basophils as crucial cells in inflammatory immune reactions even in the deficiency of eosinophils [56].

Secretory IgA (sIgA) also stimulates basophils to release both histamine and LTC4, but only if the cells have first been primed by the pretreatment with IL-3, IL-5, or GM-CSF. Because sIgA is the most abundant immunoglobulin isotype secreted in the mucosa, it is suggested that it may contribute to basophil activation during immune responses at mucosal sites [59].

It is worth mentioning that the monocyte chemoattractant protein-1 (MCP-1), chemotactic agent for basophils, similarly as HRF, stimulates these cells to histamine release and it is claimed as a substantial factor in the maintenance of inflammation in LPR [60]. For basophil influx, other significant chemokines such as MCP-2, MCP-3, MCP-4, and RANTES are involved but they are more feeble promoters in the mediator release than MCP-1. Basophils respond to those CC chemokines with specific receptors (CCRs) and they predominantly express CCR3, detected in the tissues, nose, and lungs during the allergic late-phase reaction. Basophil signaling pathway via sequential and cooperatively acting chemokines might be a mechanism explaining *in vivo* differences in their participation in the immune response [4, 61].

Mediators released by basophils

Basophils are a potent source of mediators, preformed as histamine, tryptase, and synthesized de novo such as arachidonic acid metabolites, platelet activating factor (PAF) [62], thymic stromal lymphopoietin (TSLP) [41] and cytokines (fig. 2). Altered basophil releasability is suspected to play a crucial role in asthmatic and allergic disease [38, 63], in the initiation, development, and maintenance of the inflammation [57]. The mediators evoke multiple local effects, including enhanced local vascular permeability, increased cutaneous influx, arteriolar dilation and the increased loss of intravascular fluid from post capillary venules; and other effects such as itching, swelling, redness, rhinitis, as well as the hay fever and allergic conjunctivitis.

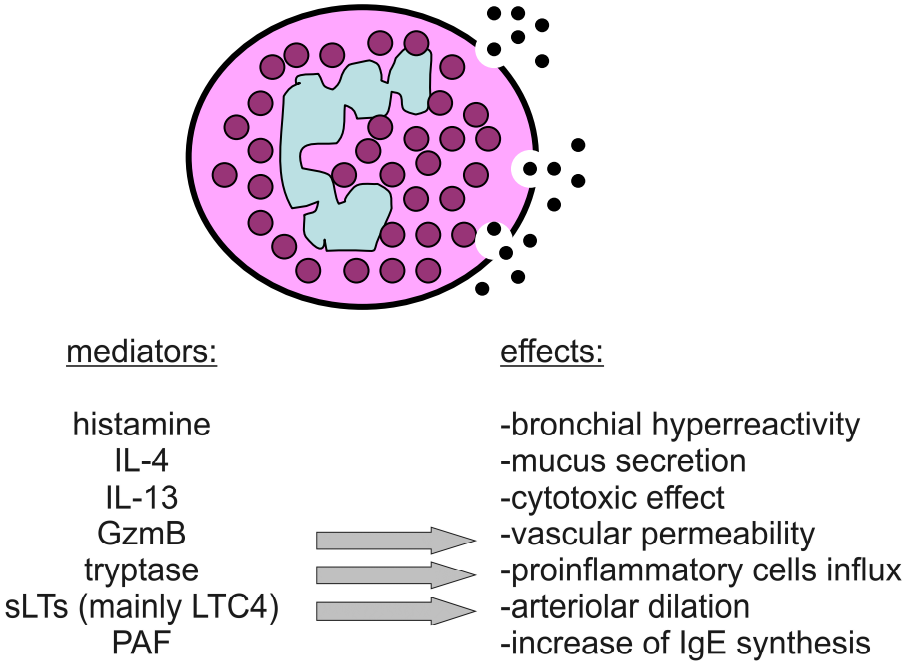

Fig. 2. Effect of mediators released by basophils.

Apart from the local elicitation of allergic reactions (*i.e.* asthma), systemic anaphylaxis represents an acute hypersensitivity response that typically involves multiple organ systems and can lead to death [1].

Histamine as the main mediator released during basophil activation, described for the first time in the 1960s [64], is an indicator of clinical severity in allergic diseases (IgE-dependent) [65]. Enhanced spontaneous histamine release was observed in basophils isolated from patients with food allergy, atopic asthma after inhaled allergen challenge and atopic dermatitis [6, 66]. Nevertheless, the recent studies suggest that in IgG-mediated reactions PAF is the main mediator released by basophils instead of histamine [44] and it increases vascular permeability with higher potency than histamine [62, 67].

IL-13 is regarded as another basophilic key cytokine in the pathogenesis of asthma. The level of IL-13 after allergen provocation indicates the severity of the local LPR [68]. The presence of IL-13 correlates with granzyme B (GzmB) which may be considered as a potential mediator of asthma. Several studies show that the level of GzmB reflects the severity of LPR similarly as the level of IL-13 [69].

Substantial amounts of IL-4, together with IL-13, are detected after allergen activation. Both cytokines contribute to enhancing IgE production or driving Th2 differentiation [1-2, 40]. They can also influence the expression of VLA-4, adhesive molecules on vascular endothelial cells, which indicates the role of activated basophils in the adherence and selective migration of eosinophils into the inflammatory focus [70]. That is why several ways of inhibiting IL-4 are under investigation. Transcription factors involved in IL-4 signaling pathway such as STAT-6 and c-maf are also attractive molecular therapeutic targets as far as they interfere with FcεRI function. The results of advanced studies on specific inhibitor blocking interactions between IgE and FcεRI can be applied in therapy in the future [52].

Basophils are also a source of sulfidoleukotrienes (sLT) which are involved in the initiation of acute bronchoconstriction. A high generation of LTC4 and histamine release are detected upon activation by specific or nonspecific stimuli.

Sulfidoleukotrienes cause smooth muscle contraction, increased vascular permeability, vasodilatation and mucous hypersecretion when they bind to specific cys-LTs receptors [71].

Tryptase is a significant mediator released by basophils, often attributed as specific only for mast cells. It is a trypsin-like, serine protease with proinflammatory activity. Tryptase activates the protease-activated receptors (PAR-2) on endothelial and epithelial cells, which initiates a cascade of events, such as up-regulation of adhesion molecules selectively attracting eosinophils and basophils [52]. Basophil tryptase may play an essential role in the pathogenesis of asthma and may explain its heterogeneity in high-expressing donors [40]. Li et al. [72] identified these tryptase-expressing peripheral blood leukocytes as a mast cell-basophil hybrid [27, 72]. Other researchers observed the presence of neoplastic basophils or immature basophil-like cell lines with the expression of a high level of tryptase [25, 40, 73].

Basophil participation in allergic diseases

Basophils have long been considered effector cells in some acute IgE-associated responses, particularly anaphylaxis, and other allergic disorders like hay fever and asthma. Current studies on the role of basophils in allergic diseases are focused on their presence or their mediators at the site of LPR allergic inflammation or their contribution to chronic allergic inflammation. Basophils are involved in severe asthma, which has been suggested on the basis of their presence in airways from subjects dying of asthma [2-3, 74].

Mukai K et al. reported that the presence of basophils is required in a newly identified type of IgE-chronic allergic inflammation (IgE-CAI) [75]. This study revealed that the basophil-enriched fraction of bone marrow cells from normal mice models can reconstitute the delayed-onset inflammation in FcεRI-deficient mice. Such findings suggest a novel mechanism by which basophils mediate CAI [50, 75]. There are also studies which confirm the hypothesis that circulating basophils may be recruited from the blood at the

sites of inflammation. Urticarial weals in urticaria patients can be the example, which t is associated with the reduction in peripheral blood basophils [76]. Corticosteroids improve urticaria and this mechanism may include the inhibition of migration of basophils into the skin. Sustained histamine release from tissue basophils might explain why urticarial weals are more prolonged than after histamine injection in healthy subjects [76-77].

Basophilia has been associated with chronic inflammation, endocrine disorders, infections, and some types of neoplasia. However, basopenia has only been linked with anaphylaxis and urticaria [77]. Basophils are found to accumulate in asthmatic human airways during episodes of bronchospasm induced by different stimuli and they release spasmogenic mediators influencing the local inflammatory reaction [31, 63].

A group of patients with chronic idiopathic urticaria (CU) is characterized by circulating autoantibodies against IgE molecule and/or particularly the α chain of high-affinity receptor (FcεRI α). The sera from patients with autoimmune CU induced histamine release *in vitro* from basophils isolated from both normal and atopic donors [78]. Basophils also play a role in the pathogenesis of seasonal allergic rhinitis and this fact has been utilized in immunotherapy, after which the recruitment of basophils to the nasal tissue was reduced [79]. Furthermore, increased numbers of basophils have been identified in the lower airways, blood and sputum of some patients during acute asthma attacks and in bronchial biopsy specimens from the patients with fatal asthma [54, 80].

Conclusions

Basophilic granulocytes seemed to be neglected as a trace amount of mast cells in the peripheral blood for many decades. Currently it is known that basophils are significant cells participating in the initiation and prolongation of various allergic diseases [81]. The development of knowledge and technical refinements reveal more information about the involvement of basophils

in the inflammation. It increases the possibility to achieve novel methods to improve patient condition, relieve clinical manifestations or even prevent the development of the disease. Recently, except standard anti-inflammatory treatment, other methods have been tested: therapy with cytokines (IL-2, IL-10), antibodies against cytokines (anti-IL-4), chemokines and cytokine receptor antagonists (antagonist for IL-5 receptor), antibodies anti-CD4, anti-IgE, and immunosuppression [82]. For instance, there are reports on monoclonal antibody designated Ba103 which was selected and extensively analyzed. Ba103+ cells presented in the bone marrow expressed both FcεRI and CD123 (IL-3 receptor), the same as human basophils [46]. The successful suppression of ongoing IgE-CAI by Ba103 treatment is a convincing promise as a therapeutic target for this type of CIA, at least in the skin. Targeting a small number of initiator cells would be more effective than targeting a large number of effector cells. Basophils mediating chronic allergic inflammation might contribute to the initiation, prolongation or deterioration of CAI in some forms of asthma and atopic dermatitis in humans. This fact confirms that basophils might be a good therapeutic target for these disorders [50, 75]. Thus, basophils have to be no longer neglected as regards their role in immune response.

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