

mina, Sanofi-Synthelabo OTC, Milan, Italy) spores (2 billion spores/vial) at the dosage schedule of three vials a day for 3 weeks.

The study was performed during the pollen season, when patients were symptomatic, and was approved by the Institutional Review Board. Informed consent was obtained from the parents of the children. Nasal total symptoms score (TSS), nasal eosinophils, and number of days associated with antihistamine assumption were evaluated. Symptoms and eosinophils were assessed in all subjects at baseline and after 3 weeks according to validated criteria (6).

The data collected were subjected to statistical analysis by MANOVA and *post hoc* comparison by Duncan's test. All results with *P*-values < 0.05 obtained were considered statistically significant.

Children treated with *B. clausii* spores, showed significant reduction (*P* = 0.049) of TSS without any side-effect: 7 ± 0.8 at baseline, and 3.7 ± 0.7 after treatment. Nasal eosinophils significantly (*P* = 0.048) diminished: 10.4 ± 2.9 at baseline, and 6.3 ± 1.9 after treatment.

Children treated only with levocetirizine on demand did not show significant (*P* = 0.051) reduction of TSS: 6.9 ± 0.9 at baseline, and 4 ± 0.8 after 3 weeks. Moreover, nasal eosinophils were not significantly (*P* = 0.69) diminished: 10.2 ± 2.8 at baseline, and 7.7 ± 2.3 after 3 weeks.

The intergroup analysis showed that symptom relief was not significantly different in two groups (*P* = 0.061), whereas eosinophils were significantly less in children treated with probiotic (*P* = 0.042). Finally, children treated with *B. clausii* spores significantly (*P* = 0.034) assumed less levocetirizine tablets than other subjects: 8.1 ± 1.7 vs 11.1 ± 2.2 days (Fig. 1).

In conclusion, this pilot study provides evidence that *B. clausii* may exert a modulatory effect on allergic response as documented by reduced eosinophil infiltration. Moreover, this probiotic may

synergize with antihistamine in relieving nasal symptoms. In addition, the symptomatic use of antihistamine does not allow adequate control of both symptoms and inflammation. The possible mechanism of action of *B. clausii* may be attributed to the previous documented activity on immune response at the nasal level (4).

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Flow-assisted diagnosis of anaphylaxis to patent blue

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Key words: anaphylaxis; CD63; flow cytometry; methylene blue; patent blue.

A 20-year-old man was referred because of an anaphylactic reaction with angioedema, bronchospasm with cyanosis and profound hypotension (systolic pressure 120 mmHg to immeasurable) during anaesthesia for combined

We report a case of patent blue-induced anaphylaxis during fistulography in which flow cytometry confirmed diagnosis.

excision of a dermal cyst in the neck and a multifistulated sacral cyst. History revealed no previous allergies. Revision of his anaesthetic report disclosed that the reaction had started 30 min after induction with propofol, sufentanyl and cisatracurium, but within minutes after fistulography with patent blue (Blue patente V[®], Guerbet, Aulnay, France).

Laboratory analysis showed a normal blood count, complement profile and protease inhibitor concentrations. Total immunoglobulin E (IgE) was 11 kU/l and IgE for latex, suxamethonium and ethylene oxide were negative (< 0.35 kUa/l; Immuno-CAP FEIA method; Pharmacia Diagnostics, Brussels, Belgium). Skin tests included latex (Stallergenes, Genval, Belgium), chlorhexidine-digluconate, serial dilutions of five muscle relaxants: suxamethonium (Myoplegine[®]), pancuronium (Pavulon[®]), rocuronium (Esmeron[®]), atracurium (Tracrium[®]) and cisatracurium (Nimbex[®]), the analgesic sufentanyl (Sufenta[®]), the anaesthetic propofol (Diprivan[®]), and the dyes patent and methylene blue. Except for patent blue (Table 1) skin tests were negative.

Table 1. Skin tests and basophil activation tests. Results in patient and control individuals

Variable	Patient	Control 1	Control 2	Control 3	Normal
Skin prick test*					
Patent blue 10 ⁻²	3/7	ND	ND	ND	< 3 mm
Patent blue 10 ⁻¹	5/30	ND	ND	ND	< 3 mm
Methylene blue 10 ⁻³ up to neat solution	0/0	ND	ND	ND	< 3 mm
Intradermal test*					
Methylene blue 10 ⁻⁴ up to neat solution	0/0	ND	ND	ND	< 5 mm
Basophil activation test†					
Spontaneous CD63 expression	2	2	2	2	< 10
Anti-IgE	94	87	94	89	> 25
Patent blue (µg/ml)					
5 × 10 ⁻⁵	4	0	5	2	< 10
5 × 10 ⁻³	52	0	4	2	< 10
5 × 10 ⁻¹	86	0	6	0	< 10
5	85	0	4	2	< 10
Methylene blue (µg/ml)					
5 × 10 ⁻⁴	1	0	5	2	< 10
5 × 10 ⁻³	1	0	5	1	< 10
5 × 10 ⁻¹	4	0	2	1	< 10
5	4	0	1	1	< 10

ND, not done; IgE, immunoglobulin E.

*Results are shown as wheal/flare size in millimetres read after 15 min.

†Results are shown as percentage CD63-positive basophils.

Flow cytometric quantification (FACSCalibur, BD, Immunocytometry Systems, San Jose, CA) of activated basophils was performed using phycoerythrin (PE) anti-CD123, peridinin chlorophyll protein (PerCP) anti-human leucocyte antigen (HLA) DR and fluorescein isothiocyanate (FITC) anti-CD63 conjugated antibodies (provided by BD, Biosciences, Erembodegem, Belgium). Basophil activation implied, a negative control, a positive control (anti-IgE), suxamethonium, pancuronium, rocuronium, atracurium and cisatracurium, and a serial dilution of the dyes patent and methylene blue in the patient and patent blue in controls. Basophil activation with muscle relaxants induced almost no increased CD63 expression (< 4%). Results of basophil activation with the dyes are summarized in Table 1.

Patent blue is a dye with ubiquitous application in textile and paper industry, agriculture and cosmetics. Patent blue is also employed in medical settings. Beside its former use as antibacterial and antifungal agent, it has long been applied for lymphography. Today, it is the dye of choice for intraoperative sentinel lymph node mapping for breast cancer and melanoma. Hypersensitivity reactions

towards patent blue have been reported since 1966 (1). Most patients have shown reactions to the intralymphatic administration of the dye. As in our patient, anaphylaxis may result from alternative routes of administration. Reactions occurred at various doses and are frequently characterized by cutaneous and mucosal symptoms such as pruritus, rash, urticaria, and angio-oedema. However, potentially life-threatening reactions with bronchospasm, hypotension and shock have been described (2–4). Symptoms may be delayed in onset and tend to progress. Currently, skin tests constitute the only confirmatory procedure for patent blue anaphylaxis.

Upon activation, basophils up-regulate the expression of certain membrane markers that can be detected by flow cytometry. Flow cytometry-assisted allergy diagnosis has been proven reliable for the diagnosis of different IgE-mediated allergies including inhalant allergens, food, latex, *Hymenoptera venom* and drugs (review: 5). One could argue that the technique does not allow to determine whether an allergic reaction was due to the bridging of allergen-specific membrane-bound IgE rather than complement activation, short-sensi-

tizing IgG antibodies or even nonimmunological direct mediator release. This should, however, not be considered as a drawback of the assay. The technique might even offer a confirmatory test in cases where no allergens for specific IgE quantification are available (6). In our patient, flow cytometric analysis completely paralleled skin test reactivity. Both tests not only identified patent blue as the offending compound but also designated methylene blue as a potential safe alternative dye.

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