

Original article

Anaphylaxis to Patent Blue V. II. A unique IgE-mediated reaction

Background: Patent Blue V (PBV) is injected in order to map sentinel nodes during cancer staging procedures. Anaphylactic reactions, allegedly IgE antibody mediated, have been reported. The aim of the study was to explore the immunological mechanism of anaphylaxis to PBV.

Methods: PBV allergen threshold basophil sensitivity, CD-sens, was performed on cells from nine patients diagnosed as having had adverse reactions to PBV. The mechanisms of the CD-sens were studied by immunological and immunochemical methods.

Results: Five of the nine patients had a positive CD-sens to PBV which was completely eliminated by washing the cells in phosphate buffered saline before allergen challenge. However, the positive CD-sens was completely reconstituted by incubating the cells in plasma or serum of that patient or the other PBV-anaphylactic patients for 15 min at room temperature. In some patients the factor mediating CD-sens was completely or partially destroyed by heating at +56°C for 30 min or being exposed to the low pH used for elution from anti-Ig columns. A 1000-fold excess of monoclonal IgE blocked the reconstitution by approximately 50%.

Conclusion: Anaphylactic reactions to PBV are mediated by IgE antibodies giving a classical CD-sens reaction. However, the allergenic configuration seems to constitute a structure completely dependent on PBV, as a hapten, linked to a, so far, unknown carrier that seems to be unique for patients having experienced a PBV-induced reaction. Further studies are needed to characterize the postulated carrier.

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Anaphylaxis in relation to anaesthesia and operative procedures is rather uncommon but often serious. Thus the prevalence of severe anaphylaxis to neuromuscular blocking agents (NMBAs) has been reported to approximately 1/5000 in France and Norway and 1/80 000 in Sweden (1, 2). Other agents are chlorhexidine (3, 4), latex (5), antibiotics and opioids (1). In the case of the opioids the anaphylaxis is probably not allergic since the reaction has not been proven to be mediated by immunological mechanisms, in contrast to the allergic anaphylaxis initiated by NMBAs, chlorhexidine and latex.

The prerequisite for an allergic anaphylaxis is that the patient must be sensitized at the time of exposure and reaction, and have IgE antibodies or, in special cases, IgG antibodies or sensitized lymphocytes. The IgE-sensitization is the result of prior exposure to either the agent causing the anaphylaxis or a substance sharing the allergenic epitopes with the agent. Thus, pholcodine in cough syrup seems to be the important immunogenic agent sensitizing individuals to the allergenic epitope of NMBA, the quaternary ammonium ion (QAI), although

the QAI can be found on a large variety of environmental chemicals (6).

Patent Blue V (PBV) is used to identify the primary draining lymph nodes, the so called sentinel nodes. Severe adverse reactions have been reported and recently a compilation of nine patients investigated in Norway was reported (7). Six of seven patients with anaphylaxis had increased serum tryptase in relation to their reaction. All skin prick tested patients, six with anaphylaxis and two with skin reactions only, had positive reactions to PBV.

The aim of this study was to characterize the mechanism of PBV anaphylaxis by using CD-sens (8) as a model of clinical reactivity.

Material and methods

Patients

Blood for cell analysis was collected in heparinized tubes from nine Norwegian patients diagnosed as having had adverse reactions to PBV, (7) following the routines of the Norwegian National Network for

Reporting and Investigating Allergic Reactions during Anaesthesia. The diagnosis was based on clinical findings, a significant increase in serum tryptase during the reaction in six anaphylactic patients, and positive skin prick testing (SPT) with PBV in the eight tested (7). All patients gave their informed consent allowing the use of their basophils in studies of allergic inflammation. The study was approved by the Regional Committee for Medical Research Ethics, Western Norway.

Basophil allergen threshold sensitivity, CD-sens

CD-sens was performed as previously described (8). Aliquots of 100 µl heparinized blood were incubated with 100 µl RPMI-hepes (negative control) (Gibco Ltd, Paisley, Renfrewshire, UK), fMLP 10^{-7} M (positive control) (Sigma Chemical Co., St Louis, MO, USA), 25 µl α -Fc ϵ RI (positive control) (Bühlmann Laboratories, Basel, Switzerland) or 100 µl PBV 0.1–100 µg/ml (CAS 20262-76-4; Laboratoire Guerbet, Cedex, France). After stimulation cells were incubated with CD203c-PE (Immunotech, Marseille, France) for basophil identification and CD63-FITC (Immunotech) for detection of basophil activation. After antibody staining the red blood cells were haemolysed by adding isotonic NH_4Cl -EDTA lysing solution. The leukocyte suspensions were washed once with 2 ml of phosphate buffered saline (PBS), centrifuged and re-suspended in PBS and analysed in an EPICS XL flow cytometer (Beckman Coulter, Inc., Fullerton, CA, USA).

CD-sens (8) describes allergen-specific sensitivity and is defined as the inverted value for the allergen concentration giving a 50% of maximum CD63% up-regulation multiplied by 100. Thus, high CD-sens means high basophil allergen sensitivity. The intra assay coefficient of variation (CV) was 13.1% and the inter assay CV 5.4% (8). In some experiments the cellular fraction, both leukocytes and erythrocytes, was washed twice with 50-fold excess of PBS before CD-sens was measured.

Other, potentially cross reactive colours, were also tested: Iso-sulfan Blue (CAS 68238-36-8), Alphazurine A (CAS 3486-30-4), Xylencyanol FF (CAS 2650-17-1), Sulforhodamine B sodium (CAS 3520-42-1), Rhodamine B (CAS 81-88-9), Coomassie Brilliant Blue R (CAS 6104-59-2), Brilliant Green (CAS 633-03-4), Victoria Pure Blue (CAS 2390-60-5), Indigo Carmine (CAS 860-22-0) and Methyl Blue (CAS 28983-56-4).

Calculation of IgE and Fc ϵ RI receptors on basophils

The number of IgE molecules and Fc ϵ RI receptors per basophil was calculated using a FITC-conjugated antibody to IgE (Dako Cytomation, Copenhagen, Denmark) or Fc ϵ RI (eBioscience, San Diego, CA, USA), compared to a standard curve of Calibration Beads (Dako Cytomation) and arbitrarily multiplied by ten. With assigned molecules of equivalent fluorochrome (MEF) values for the fluorescent bead populations, arbitrary units of mean fluorescence intensity (MFI) could be transformed into absolute units.

IgE and IgE antibodies in serum

The serum concentrations of IgE (kU/l) were determined by ImmunoCAP™ Total IgE (Phadia AB, Uppsala, Sweden) and of IgE antibodies (kU_A/l) to a mixture of common allergens by ImmunoCAP™ Specific IgE (Phadia AB) according to the manufacturer's instructions.

Passive sensitization of normal basophils

The test was performed as previously described (9). Basophils in the buffy coat of blood from healthy, nonallergic donors were stripped

from Fc ϵ RI bound IgE by incubation in acid buffers and then used for sensitization by plasma from a patient. One volume of plasma of Case 6 was incubated with one volume cells for 180 min at +37°C. Subsequently 25 µl samples of the passively sensitized cells were incubated with 25 µl samples of PBV in the following six concentrations in duplicate: 300, 100, 30, 10, 3 and 1 µg/ml dissolved in plasma of Case 6. The specificity of the passive sensitization was tested using in-house control plasma from a nonallergic healthy person and a house dust allergic patient. Passive sensitization results are expressed as % histamine release of the total cellular histamine content, determined by the HR-test system (RefLab, Copenhagen, Denmark). Identical experiments were performed on buffy coat from four different donors. Assay sensitivity is 5 ng/ml and variation on duplicates is $\leq 4.9\%$.

An additional experiment was performed, where passively sensitized cells were acid treated. Thus IgE originating from plasma and previously bound to Fc ϵ RI was obtained. The eluted IgE solution was adjusted to pH 7.4 with 1 M Tris base and used to sensitize stripped basophils. Cells sensitized with released IgE were challenged with (i) PBV in plasma of Case 6 and (ii) house dust mite allergen extract and histamine release was determined.

Results

Patient characteristics

The nine patients studied were diagnosed as having had allergic reactions during injection of PBV. The diagnosis was based on clinical findings, increased serum tryptase and positive SPT with PBV at 2.5%.

Five of the patients had serum IgE levels below the geometric mean of healthy adults, 13.2 kU/l (18) (Table 1). Only one, Case 7, had an increased level, 230 kU/l, and a positive Phadiatop indicating atopy. Case 6, who had the highest CD-sens to PBV, had an extremely low IgE, i.e. 0.3–0.5 kU/l.

CD-sens to PBV

The structure of PBV is quite complex with quaternary ammonium ions and sulphite groups (Fig. 1). However, no toxic effects on the basophils were seen and five of the patients had a positive CD-sens to PBV. Challenge of their basophils with decreasing concentrations of PBV showed a typical dose–response pattern, as illustrated by Case 6 (Fig. 2). The CD-sens of Case 6, at 13 months after the anaphylaxis, was 52. After another 4 months it levelled out at 130–140 and stayed there, i.e. up to 31 months after the anaphylactic shock (Table 1).

The cells of Case 6 responded to PBV and Isosulfan Blue but not to other colours with similar structure, e.g. Alphazurine A, Xylencyanol FF, Sulforhodamine B sodium, Rhodamine B, Coomassie Brilliant Blue R, Brilliant Green, Victoria Pure Blue, Indigo Carmine and Methyl Blue. Three additional PBV-positive patients were tested with Isosulfan Blue. One patient, Case 7, was positive and two, Case 1 and Case 8, were negative.

When the cell fraction of Case 6 was washed twice with PBS and re-challenged with the same dilutions of PBV,

Table 1. CD-sens, serum IgE concentrations and IgE and FcεRI on basophils before and after the cells were washed in PBS, in samples from the nine cases with adverse reactions to Patent Blue V taken at different times after the clinical reaction

Patient	Months after reaction	CD-sens	Tryptase	Serum IgE (kU/l)	IgE per cell Before	IgE per cell After	FcεRI per cell Before	FcεRI per cell After
Case 1	92	7.4	22.1	5.6	136 000	113 000	98 000	72 000
Case 2	47	neg	58.1	12.2	180 000	nt	nt	nt
Case 3	40	neg	34.6	10.7	133 000	nt	nt	nt
Case 4	39	4.6	7.7	29.4	318 000	nt	nt	nt
Case 5	71	neg	nt	19.2	309 000	nt	nt	nt
Case 6	13	52	75.4	0.5	nt	nt	nt	nt
idem	31	140	nt	0.3	54 000	200 000	21 000	23 000
Case 7	25	6.0	11.4	230	157 000	161 000	86 000	100 000
Case 8	43	38	25.4	3.9	157 000	181 000	51 000	47 000
Case 9	43	neg	19.1	40.0	406 000	nt	nt	nt

The tryptase values (μg/l) are from the day of the reaction (7). nt, not tested.

no up-regulation of CD63 was obtained (Fig. 2). The four other patients with a positive CD-sens to PBV (Table 1) also became negative after washing. However, the basophil PBV allergen sensitivity was fully restored when the washed cells from Case 6 were re-incubated with her own undiluted serum or plasma during 5–20 min at approximately +20°C. There was a dose-response relation between the volume of serum used and the CD-sens obtained (Fig. 3). Similarly, her washed cells could be sensitized with all the other seven PBV cases available at the time (Cases 1–5, 7 and 9), thus even the four that had nonreactive cells. In addition, serum from Case 6 could sensitize washed basophils from (i) two CD-sens positive patients (Cases 1 and 7), whose CD-sens actually became higher than their own, and (ii) one CD-sens negative patient (Case 5). This 'passive sensitization' was reproduced using serum from Cases 1, 6 and 8 and basophils from Case 7.

Washed basophils from Case 6 could not be passively sensitized for PBV by serum from normal or atopic individuals and basophils from those persons could not be sensitized for PBV sensitivity by serum from Case 6 or other PBV-patients.

Histamine release

In parallel to the up-regulation of CD63, histamine was released by incubation of passively sensitized cells, using

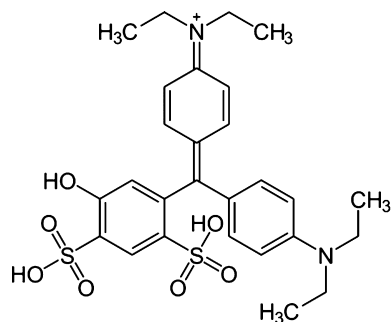


Figure 1. The structure of Patent Blue V.

plasma from Case 6, with PBV at a concentration of 1 μg/ml. The sensitization was specific, since cells sensitized with plasma from a healthy nonallergic person and from a mite allergic patient did not respond to PBV. The histamine release results were almost identical when passive sensitization was performed with surface IgE removed from previously sensitized cells (Table 2).

IgE on basophils

The effects of added IgE on the sensitization of basophils of Case 6 followed by stimulation with PBV were studied. Purified E-myeloma proteins, from patient ND or patient SB, at doses ranging from 100 to 10 000 kU/l, were added for 15 min at approximately +20°C to cells washed in PBS and the cells were then washed again before

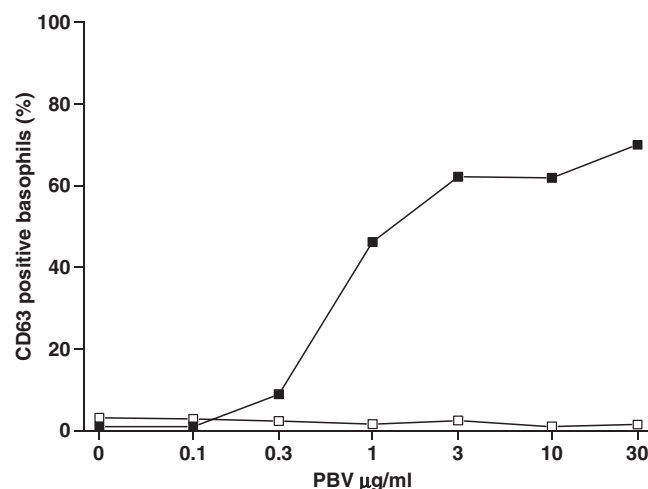


Figure 2. CD-sens was estimated on Case 6 whole heparinized blood (■) and buffy coat cells washed in PBS (□). For the whole blood the CD-sens of this particular sample was 73 while the washed cells did not respond at any PBV concentration. The positive control anti-FcεRI was 93.3% and fMLP was 33.7% CD63 positive cells for whole blood and 88.0% and 44.8%, respectively, for the washed cells.

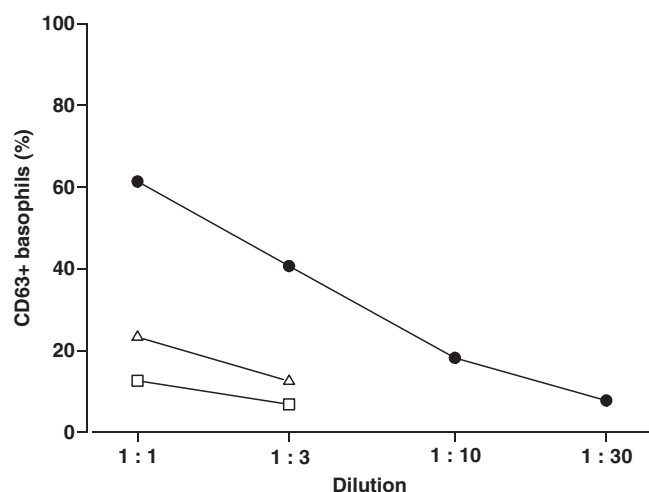


Figure 3. When serial dilutions in PBS of Case 6 plasma, sampled 13 months after reaction, were used for reconstitution of her cells a dose-response curve was obtained (●). Serum samples from other PBV patients, e.g. Case 1 (Δ) and Case 7 (□), showed parallel slopes. The negative control, RPMI was in all cases <2% CD63.

re-sensitization. A step-wise reduction of CD-sens was seen. At 100 and 1000 kU/l the CD-sens was 88, compared to 152 for the nontreated sample. The 10 000 kU/l dose of IgE pressed CD-sens down to 33, i.e. 22% of maximum.

Washed cells of Case 6 were reconstituted with equal volumes of serum of Case 6 and tenfold increasing dosages, 500–50 000 kU/l, of monoclonal IgE (Fig. 4). CD-sens then dropped from 152 at the 500 kU/l dosage to 88 at the 5000 kU/l dosage. However, the highest dose of IgE (50 000 kU/l) made the cells nonresponsive. This experiment was repeated with another blood sample from Case 6 using IgE-doses of 1–1000 kU/l. The CD-sens dropped from 115 at the lowest dose to 52 at the highest dose of IgE, i.e. approximately 50% at a 1000 fold excess of IgE.

The number of IgE molecules per basophil was estimated in all cases (Table 1), and found to have a median of 170 000 molecules, which could be compared to basophils from allergic individuals ($n = 56$) with a median of 500 000 molecules per cell (unpublished data from Ref. (18)).

Table 2. Percent histamine release upon challenge of buffy coat cells from non-allergic individuals passively sensitized with serum from a house dust mite allergic patient, Case 6, and a normal, nonallergic control, with PBV and house dust mite allergen extract

	Passive sensitization with plasma		Passive sensitization with surface IgE	
	PBV 1 (μg/ml)	Mite	PBV 1 (μg/ml)	Mite
Mite allergic	0	62	0	38
Case 6	24	4	21	0
Nonallergic	5	6	0	5

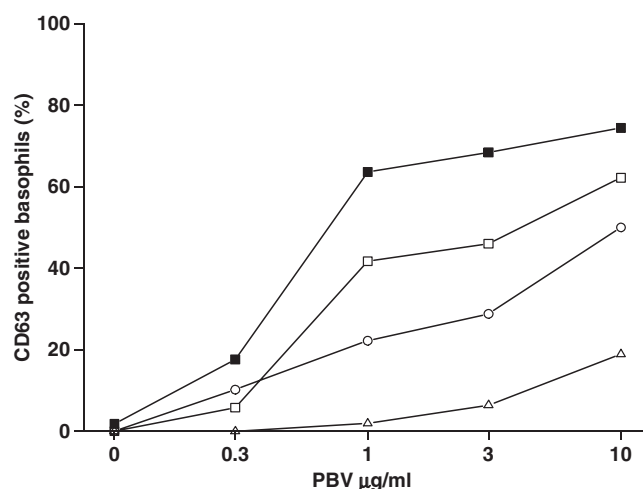


Figure 4. The buffy coat of Case 6 was washed in PBS and then incubated for 15 min at room temperature with PBS (■), monoclonal IgE (patient SB) of 500 kU/l (□), 5000 kU/l (○) or 50 000 kU/l (Δ), and then washed again before challenge with PBV at concentrations 0.3–10 μg/ml. CD-sens was calculated to be 152, 88, 88 and 33, respectively.

Washing the basophils did not change the numbers with one exception; a fourfold increase in Case 6. The number of FcεRI receptors per basophils in the four tested patients had a median of 70 000 molecules per basophil, which could be compared to 200 000 in allergic individuals ($n = 56$) (19). However, no increase was seen after washing in Case 6.

Characterization of a CD-sens mediating serum factor

Basic physico-chemical properties of the factor in serum mediating the CD-sens reaction to PBV were studied. No loss of activity was seen after repeated freezing and thawing or lyophilization. Some activity, usually in the order of 30–50%, was lost when serum of Case 6 was preincubated with sugar-polymers like agarose, dextran and cellulose gels, but not lost after passage through a sterilization filter. Also, mixing serum from Case 6 with acid buffer completely destroyed the activity, as did heating the serum to +56°C for 30 min. Heating serum of Case 1 and Case 8 also resulted in a loss of approximately 50% of the activity, while all activity was preserved after heating the serum of Case 9. Addition of fresh, normal serum did not reconstitute the activity. Stimulation with a mixture of PBV and human serum albumin, a possible carrier of PBV as a hapten, resulted in decreased CD63 up-regulation.

Discussion

Adverse, presumably allergic, reactions to PBV is a significant clinical problem (10), especially as many patients will be exposed more than once. Positive SPT

and basophil allergen stimulation tests (11–13) indicate that at least the anaphylactic type of reactions is IgE-mediated. If that is the case the patient must have been IgE-sensitized before being exposed to PBV, in a similar way as with anaphylaxis to NMBAs (6).

Nine cases of severe reactions to PBV have been diagnosed (7) through the Norwegian National Network for Reporting and Investigating Allergic Reactions during Anaesthesia (14). The anaphylaxis was documented by increased concentrations of tryptase in serum and the clinical link to PBV, supported by positive SPT to PBV.

Fresh blood was obtained from those nine cases and a basophil allergen threshold sensitivity test, CD-sens, (8) using PBV was performed. Five of the nine patients had a positive CD-sens, 4.6–38, and one, Case 6, was extremely sensitive with a CD-sens of 140. The hypothesis that the patients have been IgE-sensitized from a house hold chemical to which they are exposed in daily life is supported by the finding that the patients had had their anaphylactic reaction as long as 25–92 months before the CD-sens was performed. Actually Case 6 had a lower CD-sens closer to the anaphylactic incident, i.e. 52 only 13 months after, than later when she levelled out at 130–140, a value she had also in the latest tested sample, obtained 31 months after the reaction.

In our laboratory CD-sens is routinely performed both on basophils in the patients' plasma and basophils washed in PBS. Many patients with a classical IgE-mediated allergy (15), and especially those that have undergone allergen specific immunotherapy (ASIT), have a higher CD-sens after elimination of the plasma, indicating some allergen binding activity (ABA), e.g. allergen specific IgG4 antibodies (16). When the basophils of the PBV-allergic patients were washed in PBS and then challenged with PBV the basophils did not respond. However, the cell suspensions reconstituted with plasma from the autologous patient, followed by addition of PBV, resulted in the same CD-sens as before elimination of the plasma. The washed cells could be re-sensitized by plasma from other PBV-patients, also from those that had a negative CD-sens to PBV, but not by plasma from other atopic or nonatopic individuals.

A complete elimination of CD-sens, by washing the buffy coat in PBS, has never been seen to any classical allergen. There are several possible explanations for this loss of response. One is that the reaction is not mediated by IgE antibodies but some other factor that is not as firmly linked to the basophil membrane as IgE is to its high affinity receptor, FcεRI. However, after being challenged by PBV, this factor can trigger the basophils to expose the CD63 structure in a similar way to a classical allergen/IgE-antibody complex. Although the factor seems to be at least partially heat sensitive, which could indicate a complement dependent reaction, heat-destroyed activity could not be reconstituted by fresh, normal serum.

Another possibility would be that the factor is IgE but an IgE with a different Fc-structure which prohibits a firm binding to the FcεRI receptor (17). Several findings argue strongly against such an alternative. A 1000-fold excess of monoclonal IgE during re-sensitization reduced the CD-sens only with approximately 50%. Incubating basophils from cat- or timothy allergic patients with a similar excess of monoclonal IgE resulted in a 50% or higher reduction in CD-sens (unpublished observation), indicating interference of high concentrations of IgE with the basophil allergen triggering mechanisms. Also, after passive sensitization of IgE-stripped basophils from nonallergic individuals (9) with serum from Case 6, the basophils behaved, after stimulation with PBV, identical to a classical allergen/IgE-antibody system. This procedure could be repeated by stripping the passively sensitized cells and used to sensitize cells from a new donor. Thus, our conclusion is that the anaphylactic reaction to PBV is, indeed, mediated by regular IgE antibodies.

PBV and many other colours are common in our environment both in textiles and plastics but also in food. Some of the patients were tested against quite a large panel of colours in addition to PBV, and most, but not all, reacted to the structurally similar Isosulfan Blue, but no other, indicating a high specificity of the IgE antibodies involved.

The tested PBV-allergic patients are different to ordinary atopic individuals. Their serum IgE levels were very low, with the exception of the one atopic patient; five of them were below geometric mean of healthy, nonatopic adults (19). Especially Case 6 with the highest CD-sens had an extremely low, 0.3–0.5 kU/l, IgE value. As a consequence they had low numbers of IgE molecules on their basophils. The finding of essentially the same number before and after the PBS washing step also support our conclusion that the reaction is mediated by a classical IgE antibody. The same is true for the number of FcεRI receptors on the cells.

The most likely explanation for our findings is that the allergen, to which the IgE antibody binds, is not PBV as such but a structure consisting of PBV, as the hapten, bound to a carrier that could be a protein, sugar or lipid. The structure of PBV with quaternary ammonium ions with ion-binding capacity and sulphite groups, possibly cell-surface affecting, probably plays a role in the reaction, but how remains to be elucidated. Interestingly, so far, the proposed carrier has only been found in serum of patients that have experienced an IgE-mediated anaphylaxis to PBV. If the carrier is related to the disease that motivated the PBV injection study or has developed as part of, or as the necessity for, the PBV IgE-sensitization, can be speculated.

Attempts to characterize a PBV carrier were not successful. Gel filtration, ion exchange chromatography, specific immune-adsorption and gel diffusion have all failed, since the carrier seems to adhere to the solid phases used (unpublished observations). However, during further elution and isolation procedures the activity was lost.

In conclusion, anaphylactic reactions related to injection of PBV are IgE-mediated, but the allergen seems to constitute a unique structure created by the binding of the hapten PBV to a carrier in serum of the IgE-sensitized individual. The proposed carrier seems to be unique to these individuals. The nature of this carrier is not known. If this mode of action is common also to other drugs our approach to diagnosis of drug allergy must be reconsidered.

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References

1. Laxenaire MC. Drugs and other agents involved in anaphylactic shock occurring during anaesthesia. A French multicenter epidemiological inquiry. *Ann Fr Anesth Reanim* 1993; **12**:91–96.
2. Harboe T, Guttormsen AB, Irgens A, Dybendal T, Florvaag E. Anaphylaxis during anesthesia in Norway: a 6-year single-center follow-up study. *Anesthesiology* 2005; **102**:897–903.
3. Cheung J, O'Leary JJ. Allergic reaction to chlorhexidine in an anaesthetised patient. *Anaesth Intensive Care* 1985; **13**:429–430.
4. Garvey LH, Roed-Petersen J, Husum B. Anaphylactic reactions in anaesthetised patients – four cases of chlorhexidine allergy. *Acta Anaesthesiol Scand* 2001; **45**:1290–1294.
5. Bousquet J, Flahault A, Vandenplas O, Ameille J, Duron JJ, Pecquet C et al. Natural rubber latex allergy among health care workers: a systematic review of the evidence. *J Allergy Clin Immunol* 2006; **118**:447–454.
6. Florvaag E, Johansson SG, Oman H, Vennemalm L, Degerbeck F, Dybendal T et al. Prevalence of IgE antibodies to morphine. Relation to the high and low incidences of NMBA anaphylaxis in Norway and Sweden, respectively. *Acta Anaesthesiol Scand* 2005; **49**:437–444.
7. Hunting AS, Nopp A, Johansson SGO, Wilhelmsen V, Anderssen F, Guttormsen AB. Anaphylaxis to Patent Blue V. I. Clinical aspects. *Allergy* 2009; [E-pub ahead of print, DOI:10.1111/j.1398-9995.2009.02192.x].
8. Johansson SGO, Nopp A, van Hage M, Olofsson N, Lundahl J, Wehlin L et al. Passive IgE-sensitization by blood transfusion. *Allergy* 2005; **60**:1192–1199.
9. Garvey LH, Kroigaard M, Poulsen LK, Skov PS, Mosbech H, Vennemalm L et al. IgE mediated allergy to chlorhexidine. *J Allergy Clin Immunol* 2007; **120**:409–415.
10. Lyew MA, Gamblin TC, Ayoub M. Systemic anaphylaxis associated with intramammary isosulfan blue injection used for sentinel node detection under general anaesthesia. *Anesthesiology* 2000; **93**:1145–1146.
11. Ebo DG, Wets RD, Spiessens TK, Bridts CH, Stevens WJ. Flow-assisted diagnosis of anaphylaxis to patent blue. *Allergy* 2005; **60**:703–704.
12. Dewachter P, Mouton-Faivre C, Benhajjoub A, Abel-Decollogne F, Mertes PM. Anaphylactic reaction to patent blue V after sentinel lymph node biopsy. *Acta Anaesthesiol Scand* 2006; **50**:245–247.
13. Scherer K, Studer W, Figueiredo V, Bircher AJ. Anaphylaxis to isosulfan blue and cross-reactivity to patent blue V: case report and review of the nomenclature of vital blue dyes. *Ann Allergy Asthma Immunol* 2006; **96**:497–500.
14. Guttormsen AB. Allergic reactions during anaesthesia – increased attention to the problem in Denmark and Norway. *Acta Anaesthesiol Scand* 2001; **45**:1189–1190.
15. Nopp A, Johansson SGO, Ankerst J, Bylin G, Cardell LO, Grönneberg R et al. Basophil allergen threshold sensitivity. A useful approach to anti-IgE treatment efficacy evaluation. *Allergy* 2006; **61**:298–302.
16. Nopp A, Cardell LO, Johansson SGO, Öman H. CD-sens: a biological measure of immunological changes stimulated by ASIT. *Allergy* 2009; **64**:811–814.
17. MacDonald SM, Lichtenstein LM, Proud D, Plaut M, Naclerio RM, MacGlashan DW, Kagey-Sobotka A. Studies of IgE-dependent histamine releasing factors: heterogeneity of IgE. *J Immunol* 1987; **139**:506–512.
18. Johansson SGO, Nopp A, Öman H, Ankerst J, Cardell LO, Grönneberg R et al. The size of the disease relevant IgE antibody fraction in relation to “total-IgE” predicts the efficacy of anti-IgE (Xolair®) treatment. *Allergy* 2009; in press.
19. Zetterström O, Johansson SGO. IgE concentrations measured by PRIST in serum of healthy adults and in patients with respiratory allergy. *Allergy* 1981; **36**:537–547.