

Accepted for publication 19 January 2004
Allergy 2004; 59:1021–1022
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Dendritic cells present neuromuscular blocking agent-related epitopes to T cells from allergic patients

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Key words: allergen presentation; allergy; anaphylactic reaction; dendritic cells; NMBA.

Anaphylactic reactions to neuromuscular blocking agents (NMBA) remain a major

life-threatening and unpredictable complication during anaesthesia (1).

Dendritic cells (DC) are considered to be the most powerful antigen-presenting cells (2).

They can skew CD4⁺ T-cell differentiation towards a Th2 cytokine secretion pattern, which induces humoral responses dominated by an enhanced IgE production such as in allergic diseases.

We undertook a case-control study to assess whether DC from patients having presented an anaphylactic reaction to an NMBA during anaesthesia were able to present the incriminated drug and to stimulate T cells.

Ten patients sensitized to an NMBA and eight control subjects were involved in the study (Table 1). Monocytes and T lymphocytes were purified from fresh heparinized blood. Monocyte-derived DC (3) were generated in a 5-day culture with GM-CSF and IL-4, then matured for 2 additional days with TNF- α and PGE₂ in the presence or absence of NMBA. Both NMBA-pulsed and control DC were co-cultured (ratio 1 : 10) with T lymphocytes from the same donor. After 5 days, proliferative responses were quantified by the mean [³H]-thymidine incorporation. Stimulation indices (SI) were calculated as 'cpm in coculture with NMBA-pulsed DC/cpm in coculture with unpulsed DC' and were considered as

Dendritic cells from patients allergic to neuromuscular blocking agents are able to present the allergen and stimulate autologous T cells *in vitro*.

positive when SI > 2. In addition, lymphocyte transformation tests (LTT) were performed in the presence of the incriminated NMBA for each patient and in the presence of succinylcholine for healthy donors.

No proliferative response was observed using LTT either in patients or in healthy donors. In contrast, NMBA-pulsed DC induced a significant proliferative response of T cells in two patients sensitized to succinylcholine and in one patient sensitized to atracurium. These specific responses demonstrate for the first time the capacity of DC to present NMBA-related epitopes that are recognized by T cells.

Several mechanisms could explain how DC might process and present the incriminated NMBA (4). The drug might undergo covalent binding to soluble proteins processed by DC and consequently modified peptide might be expressed in association with MHC molecules. Another possibility is a covalent or noncovalent interaction of NMBA with the MHC-peptide complex directly at the surface of the DC. Nevertheless, experimental conditions for optimal NMBA presentation by DC remain to be defined, and this could be of particular importance for the development of DC-based assays for the characterization of NMBA-specific T-cell clones and for the *in vitro* diagnosis of an allergy to these drugs.

This work was supported in part by a grant from the Société française d'Anesthésie et de Réanimation, France.

Table 1. Clinical and immunological characteristics of allergic patients

Patient	Birth year	Sex (M/F)	Incriminated NMBA	Time since last exposure	Skin test response	LTT (SI)	DC-T (SI)
1	1944	F	Atracurium	20 years	+	0.3	2.1
2	1964	F	Atracurium	3 months	+	0.4	0.7
3	1941	F	Atracurium	1 year	+	0.9	0.7
4	1940	M	Succinylcholine	22 years	+	0.7	3.2
5	1948	F	Succinylcholine	10 months	+	ND	2.4
6	1932	M	Rocuronium	6 years	+	1.0	1.4
7	1966	F	Rocuronium	2 years	+	0.8	0.9
8	1956	F	Rocuronium	10 months	+	0.8	0.8
9	1930	M	Pancuronium	15 years	+	1.5	1.1
10	1969	F	Mivacurium	1 year	+	0.3	0.8

NMBA, neuromuscular blocking agent; LTT, lymphocyte transformation test; DC, dendritic cell; SI, stimulation index; ND, not done.

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Accepted for publication 3 December 2003
Allergy 2004; 59:1022–1023
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Adverse reactions to vitamin B12 injections due to benzyl alcohol sensitivity: successful treatment with intranasal cyanocobalamin

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Key words: benzyl alcohol; cyanocobalamin; drug reaction; hydroxycobalamin; vitamin B12.

A 16-year-old female was referred for assessment of possible anaphylactic reactions following vitamin B12 injections. In March 2001, the patient received an intramuscular injection of vitamin B12 (cyanocobalamin 1000 µg) and experienced pain at the injection site. In May 2001, 1 h after her second injection, she reported a sensation of substernal burning and pleuritic pain that resolved over days. Within minutes of her third injection in June 2001, she felt similar chest discomfort and developed pruritus of the arms and legs, but no rash.

As the reactions to cyanocobalamin injections were becoming progressively

more serious, she was referred for assessment. A number of allergic reactions following parenteral administration of vitamin B12 have been described (1–4). Cobalamin is synthesized for therapeutic use as either cyanocobalamin or hydroxycobalamin. There are reports of patients with reactions to one form of cobalamin who have been able to tolerate the alternate formulation (4–7). Unfortunately, this management strategy was not available to us as the hydroxycobalamin preparation is not available in the United States.

She was skin tested to three commercial preparations of injectable cyanocobalamin and to the benzyl alcohol (0.9%) preservative found in each. Prick testing was negative (Table 1a), but intradermal testing to all cyanocobalamin preparations and benzyl alcohol was positive (Table 1b).

These results indicated that the patient was likely reacting to the benzyl alcohol preservative present in the cyanocobalamin injections. However, the data did not eliminate the possibility of sensitization to both the benzyl alcohol and cyanocobalamin. Hypersensitivity reactions to benzyl alcohol have been documented in patients treated with a variety of medications containing benzyl alcohol as a preservative (8, 9).

Although there was no commercially available parenteral formulation of vitamin B12 lacking the benzyl alcohol preservative, we were fortunate to identify Nascobal® (Questcor Pharmaceuticals

Intranasal administration of cyanocobalamin is an option for vitamin B12-deficient patients sensitive to benzyl alcohol.

Table 1. Skin test results

	Patient	Healthy control
(a) Skin prick testing results		
Cyanocobalamin brand no. 1 (1 mg/ml)	Negative	Negative
Cyanocobalamin brand no. 2 (1 mg/ml)	Negative	Negative
Cyanocobalamin brand no. 3 (1 mg/ml)	Negative	Negative
Benzyl alcohol (0.9%)	Negative	Negative
Histamine (6 mg/ml)	10 mm wheal + flare	10 mm wheal + flare
Normal saline	Negative	Negative
(b) Intradermal testing results		
Cyanocobalamin brand no. 1 (0.01 mg/ml)	7 mm wheal + flare	Negative
Cyanocobalamin brand no. 2 (0.01 mg/ml)	6 mm wheal + flare	Negative
Cyanocobalamin brand no. 3 (0.01 mg/ml)	10 mm wheal + flare	Negative
Benzyl alcohol (0.009%)	9 mm wheal + flare	Negative
Normal saline	Negative	Negative