IgE-binding and mast cell–activating capacity of the homologue of the major birch pollen allergen and profilin from Cannabis sativa

IgE-binding and mast cell activating capacity of the homologue of the major birch pollen allergen and profilin from Cannabis sativa.

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Clinical Implication: Both the cannabis homologue of the major birch pollen allergen Bet v 1 and cannabis profilin could play a role in cannabis allergy in our Northwestern European country where birch is endemic. Sensitization to the Bet v 1 homologue does not necessitate a prior birch pollen allergy with sensitization to Bet v 1.

Cannabis is a flowering plant in the family of *Cannabaceae*, and the most widespread species is *Cannabis sativa*. In the past decade, cannabis allergy (CA) has been recognized as an increasing health issue and its prevalence is likely underestimated because of cannabis' illegal status in many countries and the absence of reliable diagnostics. The clinical presentation of CA is very heterogeneous varying from mild rhinoconjunctivitis, urticaria and angioedema to life-threatening anaphylaxis. Not seldom, CA is associated with a broad cross-reactivity syndrome involving fruits, vegetables, nuts, cereals and latex [1, 2].

In Europe, most studies point to Can s 3, the nonspecific lipid transfer protein (nsLTP) of *Cannabis sativa* as a major allergen [3]. However, Can s 3 does not cover the entire IgE-reactivity profile and it appeared that the oxygen-evolving enhancing protein (Can s 4) plays only a limited role in CA in our regions [4]. In the United States of America, the prevalence of sensitization to Can s 3 is unclear and approximately one-third of the patients with CA seem to be sensitized to Can s 4 [5]. Consequently, molecular diagnosis of CA could likely benefit from the identification of new allergenic components.

Here we aim at exploring the IgE-binding properties and clinical relevance of the cannabis homologue of Bet v 1, the major allergen from birch (*Betula verrucosa*) pollen, and *Cannabis sativa* profilin (henceforth called Cs-Bet v 1 homologue and Cs-profilin, respectively).

Patients and control individuals were included as detailed elsewhere [1, 2]. The recombinant (r) protein synthesis is described in the online repository of this article. Total and specific IgE antibodies (sIgE) to hemp, different pollen and birch pollen components (rBet v 1 and birch profilin, i.e. rBet v 2) were quantified by a FEIA ImmunoCAP technique (Thermo Fisher Scientific, Uppsala, Sweden). Specific IgE to rCan s 3, rCs-Bet v 1 homologue, rCs-profilin and MBP were quantified using a cytometric bead array (CBA, BD Biosciences, Franklin Lakes (NJ), a technique previously standardized for rBet v 1, rCan s 3, rCan s 4 and rHev b 12, the nsLTP from *Hevea brasiliensis* [1, 2, 4, 6]. The CBA technique is detailed in the repository. Specific IgE results were considered positive if $\geq$ 0.10 kUA/L. IgE-reactivity to rCs-Bet v 1 homologue and rCs-profilin was evaluated in a functional assay, a mast cell activation test (MAT), in order to reflect the *in vivo* situation more closely. As detailed in the repository file, in the MAT, healthy donor mast cells (MCs) are passively sensitized with patient's sIgE antibodies and subsequently incubated with relevant allergens. Mast cell degranulation is measured by the quantification of the net up-regulation of the lysosomal degranulation marker CD63 [7]. Control

MC activation experiments with maltose binding protein (MBP) were carried-out to exclude non-specific stimulation by the carrier protein.

Forty-five CA participants were included; 25 patients experienced immediate symptoms on exposure to cannabis. Six of them (24%) reported likely anaphylaxis as defined in [2]. Briefly, patients with generalized symptoms in two or more organ systems were categorized as likely-anaphylaxis. The remaining 19 (76%) CA patients reported localized respiratory and/or cutaneous symptoms on cannabis exposure. For initial evaluation, sera from 5 healthy control individuals (HC) were studied. As the majority of the CA patients in our region are pollen allergic [1, 2], we studied 20 atopic birch pollen allergic individuals sensitized to Bet v 1 and/or birch profilin (Bet v 2) (henceforth called atopic control individuals (AC)) to assess the effect of cross-reactivity on the outcome of the IgE-binding and MATs. Note that all 20 AC had uneventful exposure to cannabis. The local ethics committee approved the study (B300201524055), and all participants provided informed consent in accordance with the Declaration of Helsinki.

Demographics of CA patients, HC and AC are shown in the Table 1. In terms of cannabis diagnostics, 23/25 (92%) of CA patients demonstrate a positive sIgE hemp and 13/25 (52%) were rCan s 3 sensitized. In HC, no sIgE antibodies to hemp nor rCan s 3 were demonstrable, except in one case demonstrating IgE-reactivity to hemp. In AC, sIgE to hemp was positive in 17/20 (85%) and to rCan s 3 in 4/20 (20%). Furthermore, 22/25 (88%) and 10/25 (40%) of CA patients were sensitized to rBet v 1 and rBet v 2, respectively. All 20 AC were sensitized to rBet v 1 and 9 (45%) also demonstrated IgE-reactivity to rBet v 2. Table 1 shows the individual positive sIgE results by CBA for the rCs-Bet v 1 homologue and rCs-profilin. In CA patients, sensitization to rCs-Bet v 1 homologue and rCs-profilin was demonstrable in 20/25 (80%) and 4/25 (16%), respectively. Of note, in two CA patients (#22, 25) sensitization to rBet v 1, as depicted by the traditional rBet v 1 ImmunoCAP was clinically irrelevant. In patient #22 no sensitization to other cannabis components was demonstrable. In HC, no sIgE-reactivity to the cannabis components was demonstrable. In AC, sIgE to rCs-Bet v 1 homologue and rCs-profilin was demonstrable in 15/20 (75%) and 1/20 (5%) of the patients, respectively.

To study the effector cell activating capacity of the recombinant proteins, sera from 8 CA patients reactive to the rCs-Bet v 1 homologue with overt birch pollen allergy and sensitized to rBet v 1 were studied in the MAT. MAT, unlike basophil activation tests, enables analysis of historical samples and is not hampered by current high demands set by EU-imposed GMP and

GCP for in vivo testing such as skin testing [8]. Three of these sera were also reactive to rCs-profilin and to rBet v 2. As shown in figure 1 (left panel), passive sensitization with sera reactive to rCs-Bet v 1 homologue resulted in a dose-dependent MC degranulation in response to the cannabis homologue of Bet v 1. As illustrated in figure 1 (right panel), passive sensitization with rCs-profilin reactive sera also resulted in a dose-dependent MC degranulation in response to the cannabis homologue of birch pollen profilin. MATs with healthy control sera obtained from 3 HC did not trigger degranulation in response to the rCs-Bet v 1 homologue nor rCs-profilin. Sensitization with sera from 2 AC patients reactive to rCs-Bet v 1 homologue and rBet v 1 also triggered MC degranulation in response to the rCs-Bet v 1 homologue. However, MC responses were less prominent and demonstrated a shifting of the dose-response curve. Moreover, the rCs-Bet v 1 homologue concentrations of 0.01 and 0.1 µg/mL were found discriminative between CA and AC patients sensitized to the cannabis homologue of Bet v 1. Interestingly, MCs passively sensitized with sera from 2 CA patients without overt birch pollen allergy and negative skin tests to birch but sensitized to the rCs-Bet v 1 homologue, did only respond to rCs-Bet v 1 homologue but not to rBet v 1 from birch (data not shown). As indicated in table 1 in the repository, both these -patients had a positive sIgE to rBet v 1. Three sera reactive to rCs-Bet v 1 homologue but not to Bet v 1, presumed to indicate primary sensitization to rCs-Bet v 1 homologue, were pre-incubated with serial dilutions of rCs-Bet v 1 homologue and rBet v 1 from birch pollen. Pre-incubation with the rCs-Bet v 1 homologue resulted in a complete inhibition at 0.001 µg/mL. In contrast, pre-incubation with rCs-Bet v 1 showed a clear dose-response shift with an inhibition of only 36% at 10 µg/mL. Taken together, the sIgE-binding and MC activation experiments indicate that sensitization to the cannabis homologue from Bet v 1 can originate from distinct sources. In CA and AC patients sensitized to rCs-Bet v 1, this sensitization could result from cross-reactivity to Bet v 1 from birch pollen. However, as revealed by the absence of clinical symptoms in AC patients and the distinct MC responses to rCs-Bet v 1 homologue between CA and AC, this cross-reactivity is not necessarily clinically relevant. In contrast, in CA patients without overt birch pollen allergy, sensitization to the Bet v 1 homologue likely reflects a primary sensitization via *Cannabis sativa*. Whether this reasoning also applies to sensitization to Cs-profilin needs more experiments to substantiate this statement but our results seem to point in that direction.

In conclusion, this study shows that both the cannabis homologue of the major birch pollen allergen Bet v 1 as well as cannabis profilin could play a role in cannabis allergy in our

Northwestern European country where birch is endemic. Sensitization to the Bet v 1 homologue does not necessitate a prior birch pollen allergy with sensitization to Bet v 1.

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Figure 1: mast cell activation responses

Mast cell activation responses for the cannabis homologue of the major allergen from birch pollen Bet v 1 (rCs-Bet v 1 homologue) and cannabis profilin (rCs-profilin) in patients with cannabis allergy (CA) with clinically relevant sensitization to birch, atopic controls (AC) with clinically sensitization to birch, healthy controls (HC) and AC without overt birch pollen allergy. Results are expressed as mean and standard deviation.

Table 1: demographics and laboratory findings in patients with cannabis allergy (CA), atopic control individuals (AC) and healthy controls (HC).

N°	Diagnosis	Age/ Sex	Total IgE (kU/L) (1)	Specific IgE (kUA/L)					
				rBet v 1 (1)	rBet v 2 (1)	Hemp (1)	rCan s 3 (2)	rCs-Bet v 1 hom (2,3)	rCs-profilin (2,3)
1	CA	29/M	285	19.5	0.52	2.04	1.8	3.84	0.69
2	CA	35/F	16	3.67	<0.10	4.65	0.2	5.88	<0.10
3	CA*	22/M	382	47.2	1.99	0.43	<0.10	1.53	0.69
4	CA	22/M	1,577	30.9	<0.10	17.5	6.5	57	<0.10
5	CA	26/M	7,900	34.7	0.33	42.95	12.9	21.18	<0.10
6	CA	26/M	902	0.85	0.42	12.3	38.28	8.94	<0.10
7	CA*	24/M	360	27.6	<0.10	10.97	1.11	4.06	<0.10
8	CA*	22/F	165	8.77	<0.10	3.72	2.05	2.44	<0.10
9	CA	26/F	494	100	<0.10	2.8	<0.10	9.99	<0.10
10	CA	34/M	132	31.3	1.18	1.13	1.86	0.44	<0.10
11	CA*	28/M	274	21.81	3.91	6.8	5.15	23	2.44
12	CA	35/M	21,400	64.12	1.55	37.98	<0.10	35.37	<0.10
13	CA*	39/M	227	6.65	0.1	5.95	<0.10	9	<0.10
14	CA	38/M	162	1.2	<0.10	0.22	<0.10	<0.10	<0.10
15	CA*	31/M	368	4.38	<0.10	8.54	3.37	27	<0.10
16	CA	25/F	79	0.21	<0.10	2.78	<0.10	2.43	<0.10
17	CA	27/M	5,000	58.6	0.47	77.1	11.97	89	<0.10
18	CA	18/F	69	14.5	2.94	<0.10	<0.10	0.38	0.38
19	CA	20/M	71	0.56	<0.10	0.15	<0.10	<0.10	<0.10
20	CA	28/M	527	27.4	<0.10	8.63	<0.10	1.19	<0.10
21	CA•	18/F	77	<0.10	<0.10	1.09	<0.10	<0.10	<0.10
22	CA*,•	31/M	73	0.82	<0.10	0.39	<0.10	2.59	<0.10
23	CA•	29/F	328	<0.10	<0.10	10.68	34.49	<0.10	<0.10
24	CA•	26/F	754	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
25	CA*,•	32/F	476	0.26	<0.10	5.26	2.47	6.31	<0.10
26	AC	28/F	332	15.1	<0.10	0.52	<0.10	0.49	<0.10
27	AC	37/M	73	3.1	<0.10	<0.10	<0.10	<0.10	<0.10
28	AC*	31/M	99	7.67	0.42	0.34	<0.10	0.54	<0.10
29	AC	41/M	175	37.6	<0.10	0.91	<0.10	2.2	<0.10
30	AC	25/F	492	70.7	<0.10	4.37	<0.10	15.61	<0.10
31	AC	46/F	67	3.19	<0.10	0.1	<0.10	<0.10	<0.10
32	AC	22/F	28	4.06	0.15	0.12	<0.10	<0.10	<0.10
33	AC	32/M	1,983	48.1	0.14	0.48	<0.10	4.54	<0.10
34	AC	30/F	1,632	14.1	0.9	3.14	5.25	14.83	<0.10
35	AC	46/M	135	8.65	<0.10	1.48	3.6	1.98	<0.10
36	AC	25/F	2.054	63.5	0.11	0.87	<0.10	3.41	<0.10
37	AC*	18/M	6,500	7.86	24	1.38	<0.10	3.02	27
38	AC	28/M	69,700	84.9	0.51	7.55	<0.10	100	<0.10
39	AC	26/F	204	2.69	<0.10	9.12	5.43	3.16	<0.10
40	AC	22/M	889	100	<0.10	4.42	<0.10	42	<0.10
41	AC*	18/M	84	1.63	2.61	3.75	4.7	1.24	<0.10
42	AC	18/F	348	1.6	<0.10	<0.10	<0.10	<0.10	<0.10
43	AC	37/M	4,810	0.52	0.31	1.48	<0.10	0.67	<0.10
44	AC	30/F	342	2.24	<0.10	0.1	<0.10	<0.10	<0.10
45	AC	22/F	180	13.9	<0.10	<0.10	<0.10	0.13	<0.10
46	HC*	27/F	46	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
47	HC	53/F	4	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
48	HC*	28/M	506	<0.10	<0.10	0.19	<0.10	<0.10	<0.10
49	HC	32/M	101	1.03	<0.10	<0.10	<0.10	<0.10	<0.10
50	HC*	28/F	5	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10

[1] ImmunoCAP FEIA method, [2] Cytometric Bead Assay, [3] all positive samples had an additional test for maltose-binding protein (MBP) that turned out to be negative, excluding false positive results because of IgE binding to the MBP frame. No symptoms of seasonal rhinoconjunctivitis and negative skin test to birch pollen. M: male, F: male, rCs-Bet v 1 hom: rCs-Bet v 1 homologue

* denote the individuals studied in the mast cell activation test, • patients without overt birch-related seasonal rhinoconjunctivitis and/or asthma. Note that none of the patients used in the MAT experiments was sensitized to Can s 4 (oxygen-evolving enhancing protein (OEEP2).

196