

FIG E1. IgE binding to pollen extract and purified Jun a 1 IgE. Serum IgE antibodies against whole mountain pollen extracts and purified Jun a 1 for 35 patients with mountain cedar pollinosis were quantified by using ImmunoCap assays (A). The 7 sera selected for further analyses (a-g) are shown by *bold line* and (■) in Fig E1, A, and individually in B. The relationship of the IgE binding to whole pollen and Jun a 1 is expressed as % Jun a 1 reactivity (Fig E1, B) for each serum.

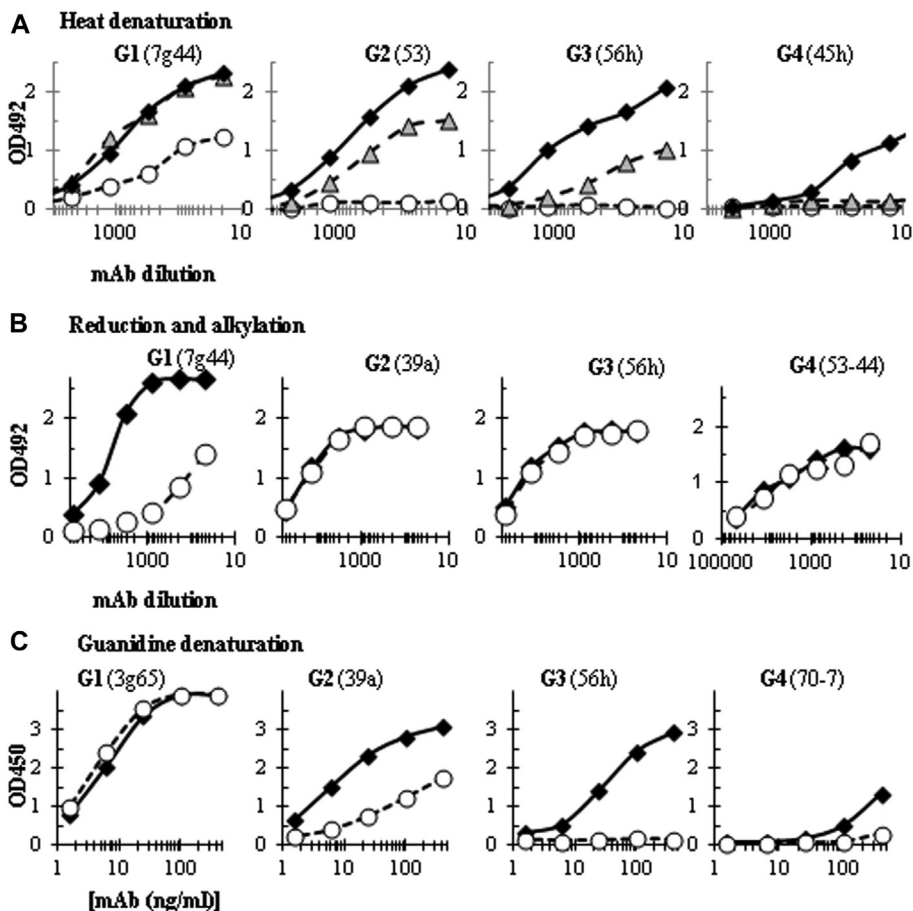


FIG E2. mAb binding pattern to native (◆) and denatured (○) Jun a 1. ELISA testing of the relative binding of 4 groups of mAbs (G1-G4) to wells coated with native Jun a 1 (◆) and Jun a 1-coated wells denatured by heating (: 56°C, ○: 75°C, **A**), wells coated with reduced and alkylated Jun a 1 (○, **B**), wells coated with Jun a 1 and then treated with 6 mol/L guanidine (○, **C**). The results indicate that epitopes recognized by mAbs G2 to G4 were disrupted by heat (56°C and 75°C), while the epitope for mAb G1 was only partially lost after exposure to 75°C, but was completely disrupted after reduction and alkylation, which breaks disulfide bonds.

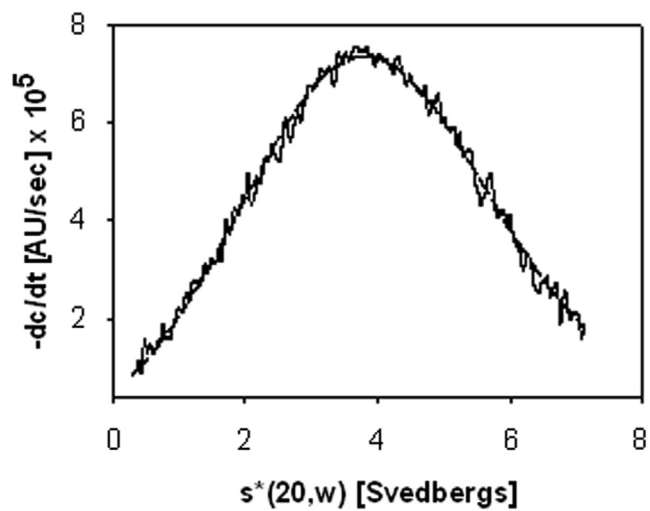


FIG E3. Molecular mass of Jun a 1. Ultracentrifugation data indicate that Jun a 1 is globular and exists as a monomer with a molecular mass of approximately 42 kDa in a non-denaturing solution. The mass and shape are consistent with glycosylation at several sites.

TABLE E1. Classification of anti-Jun a 1 mAb by ELISA inhibition

Unlabeled mAb	Biotinylated mAb							
	Group 1		Group 2		Group 3		Group 4	
	7g44	3g65	39a80	84e78	56h58	58g	70-7	53-44
Group 1								
7g44	<i>83 ± 5</i>	<i>80 ± 2</i>	3 ± 3	5 ± 4	0 ± 0	3 ± 5	2 ± 1	2 ± 2
3g65	<i>81 ± 4</i>	<i>79 ± 2</i>	3 ± 2	3 ± 4	4 ± 6	5 ± 5	0 ± 0	2 ± 3
Group 2								
39a80	2 ± 2	1 ± 2	<i>84 ± 5</i>	<i>80 ± 7</i>	7 ± 8	14 ± 10	1 ± 1	4 ± 2
84e78	1 ± 2	3 ± 3	<i>81 ± 6</i>	<i>88 ± 6</i>	3 ± 3	5 ± 4	2 ± 1	2 ± 3
Group 3								
56h58	6 ± 5	3 ± 3	2 ± 2	2 ± 3	<i>92 ± 6</i>	<i>79 ± 3</i>	6 ± 9	7 ± 3
58g	0 ± 0	3 ± 6	6 ± 2	5 ± 4	<i>85 ± 12</i>	<i>81 ± 4</i>	0 ± 1	4 ± 7
Group 4								
70-7	4 ± 6	0 ± 0	1 ± 2	2 ± 4	2 ± 3	5 ± 4	<i>78 ± 4</i>	<i>75 ± 9</i>
53-44	6 ± 4	1 ± 2	1 ± 2	3 ± 2	6 ± 5	7 ± 3	<i>77 ± 2</i>	<i>73 ± 10</i>

The values are expressed as % inhibition of the binding of biotinylated mAb (columns) to Jun a 1 by the preincubation with excess unlabeled mAb (rows). Values in italics and boldface indicate the autologous inhibition (labeled antibody inhibited by the same antibody without label). The remainder of the numbers indicates that the members of each of these 4 groups do not cross-react with other groups.

TABLE E2. Characteristics of the study population (n = 35)

No.	Age (y)	Sex	Medication	Cosensitization	Diseases	Total IgE (kU/L)
1	54	F	Diphenhydramine, loratadine	Trees, grasses, dust mites		110
2	66	F	Fexofenadine, fluticasone, olopatadine	Mold, dust mite	Hypertension	127
3	46	M		Oak		73.5
4	45	F		Dust mite, cat, dog		241
5	59	M		Trees, grasses, ragweeds, cat, dog	Hypertension	88.4
6	29	F		Grasses, ragweed, mold, dust mite, cat, dog		690
7	41	M				226
8	42	M				151
9	35	F	Fexofenadine, fluticasone	Trees, grasses, ragweeds, mold, dust mite		234
10	10	M	Mometasone, desloratadine	Trees, grasses, ragweed, dust mite, mold		1,209
11	54	M		Grasses, mold, dust mite		265
12	25	F	Mometasone, desloratadine	Pine, grasses, ragweed, mold, dust		290
13	28	M	Mometasone, desloratadine		Arthritis, scoriasis, asthma	50.8
14	65	M	Allergy shots	Elm, ragweed, dust, mold	Arthritis, hypertension	27.8
15	38	F	Diphenhydramine, pseudoephedrine	Trees, grasses, ragweed		465
16	28	M		Elm, grasses, ragweed	Asthma	17.7
17	45	F	Loratadine	Oak, grasses, ragweed, dust		72.9
18	54	F		Oak, ragweed, grasses, dust		65.2
19	33	M		Oak, grasses, ragweed, mold		175
20	15	F	Loratadine, fluticasone	Pecan, pine, ragweed		137
21	46	F		Oak, elm, ragweed, dust mite, mold	Arthritis, hypertension	205
22	48	F	Fluticasone, diphenhydramine, loratadine	Oak, grasses, ragweed, mold, dust mite		144
23	46	M	Fluticasone	Pecan, oak, ragweed, dust mite	Hypertension	73.5
24	32	F		Ragweed		18.7
25	38	M		Oak, elm, grasses, ragweed		174
26	38	F		Grasses, ragweed		20.6
27	39	M		Ragweed, dust mite		490
28	27	F				159
29	42	F				691
30	21	F				254
31	45	F				56.4
32	24	F				284
33	47	F				1,020
34	22	F				376
35	49	F				247

F, Female; M, male.