

Supplemental Figure 1

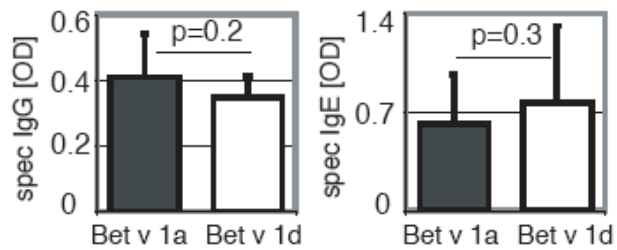


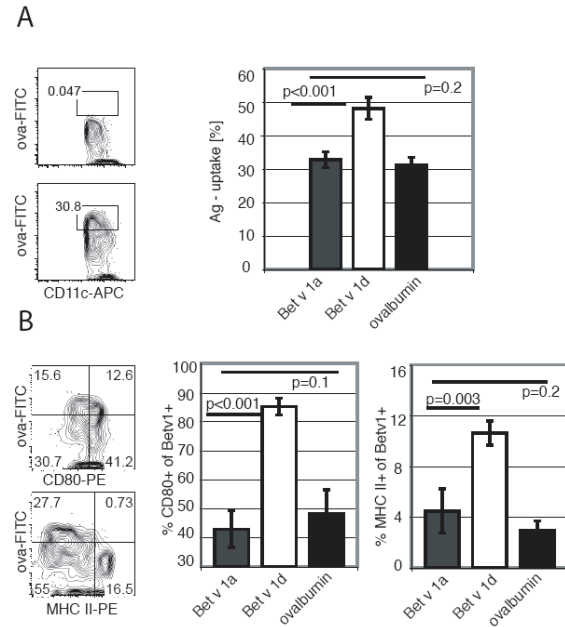
FIGURE S1. The Sera from birch pollen allergic individuals are highly cross-reactive for Bet v 1a and Bet v 1d.

Specific serum IgG and IgE titers from four allergic individuals were analyzed for reactivity towards Bet v 1a (dark grey) or Bet v 1d (white) by ELISA. Individual antibody titers were determined and given as mean values (bars) \pm SD. Values are shown as arbitrary units.

Supplemental Figure 2

FIGURE S2. Internalization of Bet v 1a and Bet v 1d by BMDCs in comparison with uptake of ovalbumin as a model antigen.

(A) BMDCs were incubated with FITC-labeled ova, FITC-Bet v 1a or FITC-Bet v 1d and uptake of FITC-conjugated antigen was determined after 26h by flow cytometry. A representative FACS profile of FITC-ova uptake by BMDCs is shown. Cells were pre-gated for live cells and the percentage of ova-FITC positive cells among the CD11c positive population is indicated within each plot. The graph to the right shows the percentage of Bet v 1a, Bet v 1d and ova positive cells at 26h post incubation with specific antigen (n=6).



(B) BMDCs were incubated with FITC conjugated Bet v 1a, FITC conjugated Bet v 1d or FITC-ova. After 26h, cells were stained for CD80 and MHC II using specific PE-conjugated antibodies in combination with APC-conjugated anti-mouse CD11c antibody. A representative FACS profile is shown and the percentage of cells within each quadrant is indicated. The graph on the right shows the percentage of CD80 and MHC II expressing cells within the Bet v 1a/d and ova-FITC positive BMDCs at 26h post incubation with specific FITC-conjugated antigen (n=6). Mean \pm SD; p values (unpaired students t-test) are indicated in graph.

Supplemental Figure 3

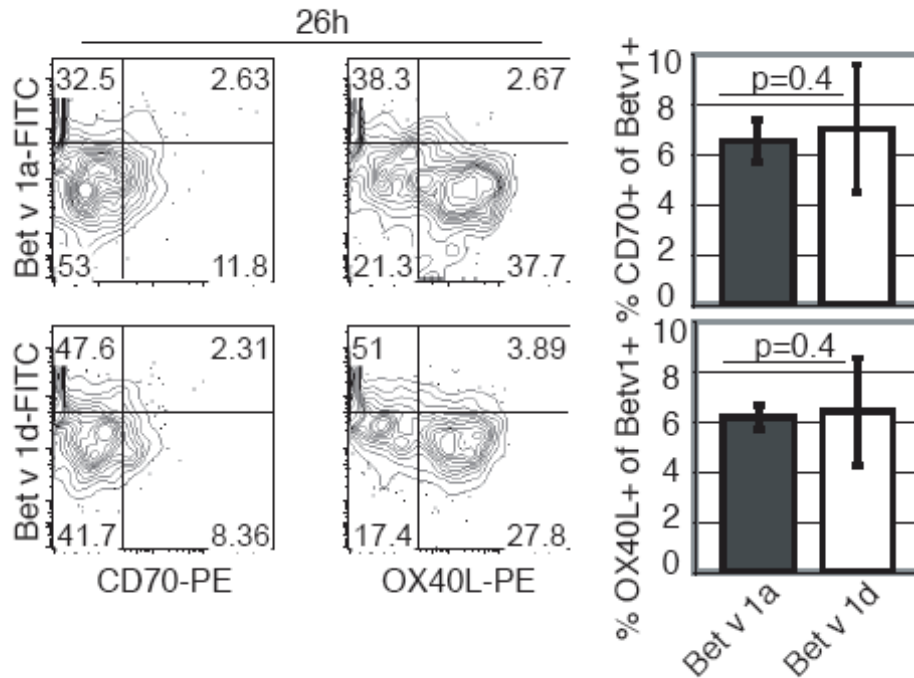


FIGURE S3. OX40L and CD70. BMDCs were incubated with FITC conjugated Bet v 1a or Bet v 1d. After 26h, cells were stained for OX40L and CD70 using specific PE-conjugated antibodies in combination with APC-conjugated anti-mouse CD11c. Cells were gated for CD11c expression and the amount of OX40L and CD70 production was determined. (A) A representative FACS profile is shown. The percentage of cells within each quadrant is indicated. (B) Statistical analysis of three independent experiments. Shown are the percentage of OX40L and CD70 expressing cells within the Bet v 1a/d-FITC positive BMDCs at 26h post incubation with specific FITC-conjugated antigen. Mean \pm SD; p values are indicated in graph (unpaired students t-test).

Supplemental Table 1

¹ Supplemental Table I. Mouse hybridomas raised against Bet v 1a and Bet v 1d ^a

clone	isotype ^b	Bet v 1a reactivity ^c	Bet v 1d reactivity ^c
9H8	IgG1	8.6	9.6
9B12	IgM	5.2	3.9
5D8	IgM	1.1	2.1
5D9	IgM	5.7	4.2
3G7	IgM	1.6	3.0

¹ ^aMice were immunized with a mixture of Bet v 1a and Bet v 1d and hybridomas were generated from the spleen. Bet v 1a and Bet v 1d specific hybridoma-derived antibodies were identified by ELISA from cell culture supernatants. ^bIsotype of the monoclonal antibodies were determined by ELISA. ^cThe reactivity of the respective clones towards Bet v 1a and Bet v 1d was determined by ELISA and is given as fold above background.