METHODS

Construction of linker DNA for the assembly of VH and VL fragments

For the construction of linker DNA, 24 separate PCR reactions (Platinum PCR Supermix; Invitrogen, Life Technologies, Carlsbad, Calif) were performed in which all possible combinations of the 4 VH- and 6 VL-specific primers[E1] were used to amplify a DNA sequence coding for a 15-amino-acid linker $(Gly_4Ser)_3$. Twenty-four linker products were purified on a 2% agarose gel, eluted as described, and ligated into the precut pSTBlue-1 AccepTor Vector (Novagen, Inc, Madison, Wis) that allows direct ligation of a PCR product with 3′-dA overhangs produced by Taq-Polymerase that anneal with 3′-dU on the vector. Sequences were confirmed by means of double-strand sequencing (Microsynth, Balgach, Switzerland) performed with the standard T7 and SP6 primers and were further used as templates for another PCR step, followed by purification, as previously described. Concentrations of the 24 separate linkers were determined by means of Nanodrop (Peqlab, Erlangen, Germany), and an equimolar mix was produced.

Analysis of expression of constructed ScFvs

ScFvs were tested for reactivity with an antibody directed to the C-terminal peptide tag (E tag) by means of immunoblotting (Schleicher & Schuell, Dassel, Germany).[E2] Membrane-bound ScFvs were traced with a monoclonal mouse anti–E tag antibody (Amersham Biosciences), followed by a horseradish peroxidase–labeled sheep anti-mouse antiserum (Amersham Biosciences). Blots were developed with the ECL Plus Western Blotting Detection System (GE Healthcare, Fairfield, Conn), followed by exposure to Kodak XOMAT films with intensifying screens (Kodak, Heidelberg, Germany).

Cross-reactivity of light chain–shuffled ScFvs with nitrocellulose-blotted natural grass pollen allergens

SDS extracts were prepared from pollen of 3 different grass species (*P pratense*: timothy grass; *P pratensis*: Kentucky bluegrass; and *L perenne*: ryegrass), as previously described.[E3] The extracts were subjected to SDS-PAGE under reducing conditions and blotted onto nitrocellulose membranes (Schleicher & Schuell). *E coli* lysates containing the soluble ScFvs (dilution 1:2) were exposed to nitrocellulose-blotted natural allergens. Natural Phl p 5 was detected with rabbit anti-sera specific for Phl p 5.[E4] Bound rabbit antibodies were traced with a horseradish peroxidase–labeled donkey anti-rabbit antiserum (Amersham Biosciences). Blots were developed as described previously.

REFERENCES

E1. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G. By-passing immunization. Human antibodies from V-gene libraries displayed on phage. J Mol Biol 1991;222:581-97.

E2. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci U S A 1979;76:4350-4.

E3. Madritsch C, Flicker S, Scheiblhofer S, Zafred D, Pavkov-Keller T, Thalhamer J, et al. Recombinant monoclonal human immunoglobulin E to investigate the allergenic activity of major grass pollen allergen Phl p 5. Clin Exp Allergy 2011;41:270-80.

E4. Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. Clin Exp Allergy 2008;38:1400-8.

A Comparison of isolated heavy chains with germline genes

	Isolated VH	IGHV gene*allele_01	Nucleotide sequence Identity	Accession No	IGHD gene*allele_01	Nucleotide sequence Identity	Accession No	IGHJ gene*allele_01	Nucleotide sequence Identity	Accession No
Phl p 5-specific VH	5VH	**IGHV3-30-3*01**	**87.50%**	X92283	**IGHD1-1*01**	**88.24%**	X97051	**IGHJ3*01**	**94.00%**	J00256
		IGHV3-30*01	**86.81%**	M83134	IGHD1-14*01	82.35%	X13972	IGHJ4*01	75.00%	J00256
		IGHV3-33*01	**86.11%**	AB019439	IGHD1-20*01	82.35%	X97051	IGHJ1*01	69.23%	J00256
		IGHV3-NL1*01	**83.33%**	HM855939	IGHD1-7*01	82.35%	X13972	IGHJ5*01	68.63%	J00256
		IGHV3-7*01	81.25%	M99649	IGHD7-27*01	72.73%	J00256	IGHJ2*01	67.92%	J00256
Phl p 2-specific VH	94VH	**IGHV4-31*01**	**92.47%**	L10098	**IGHD5-18*01**	**80.00%**	X97051	**IGHJ4*01**	**85.11%**	J00256
		IGHV4-30-4*01	**91.76%**	Z14238	**IGHD5-5*01**	**80.00%**	X13972	**IGHJ5*01**	**82.00%**	J00256
		IGHV4-30-2*01	**89.96%**	L10089	IGHD5-24*01	70.00%	X97051	IGHJ3*01	75.51%	J00256
		IGHV4-61*01	**87.81%**	M29811	IGHD2-15*01	60.00%	J00234	IGHJ1*01	74.51%	J00256
		IGHV4-39*01	87.10%	AB019439	IGHD3-16*01	60.00%	X93614	IGHJ6*01	72.41%	J00256
	60VH	**IGHV4-30-4*01**	**91.40%**	Z14238	**IGHD1-26*01**	**63.64%**	X97051	**IGHJ4*01**	**85.11%**	J00256
		IGHV4-31*01	**91.40%**	L10098	**IGHD5-24*01**	**63.64%**	X97051	IGHJ5*01	78.00%	J00256
		IGHV4-30-2*01	**89.25%**	L10089	**IGHD6-25*01**	**62.50%**	X97051	IGHJ1*01	76.47%	J00256
		IGHV4-61*01	**87.46%**	M29811	IGHD1-7*01	54.55%	X13972	IGHJ3*01	75.51%	J00256
		IGHV4-39*01	**87.10%**	AB019439	IGHD5-12*01	53.85%	X13972	IGHJ6*01	63.93%	J00256
	100VH	**IGHV4-30-4*01**	**94.27%**	Z14238	**IGHD1-26*01**	**60.00%**	X97051	**IGHJ4*01**	**87.23%**	J00256
		IGHV4-31*01	**94.27%**	L10098	**IGHD5-24*01**	**60.00%**	X97051	IGHJ5*01	80.00%	J00256
		IGHV4-30-2*01	**92.11%**	L10089	**IGHD6-13*01**	**60.00%**	X13972	IGHJ1*01	76.47%	J00256
		IGHV4-59*01	**90.48%**	AB019438	**IGHD5-5*01**	**60.00%**	X13972	IGHJ3*01	73.47%	J00256
		IGHV4-61*01	**90.32%**	M29811	**IGHD7-27*01**	**55.56%**	J00256	IGHJ6*01	68.97%	J00256
Phl p 1-specific VH	25VH	**IGHV3-9*01**	**90.58%**	M99651	**IGHD5-5*01**	**54.55%**	X13972	**IGHJ4*01**	**91.49%**	J00256
		IGHV3-20*01	**85.87%**	M99657	**IGHD6-13*01**	**53.85%**	X13972	IGHJ5*01	80.00%	J00256
		IGHV3-43*01	85.14%	M99672	**IGHD6-6*01**	**53.33%**	X13972	IGHJ1*01	76.47%	J00256
		IGHV3-23*01	82.97%	M99660	**IGHD2-15*01**	**52.94%**	J00234	IGHJ3*01	69.39%	J00256
		IGHV3-48*01	82.97%	M99675	**IGHD4-23*01**	**52.94%**	X97051	IGHJ2*01	69.23%	J00256
	43VH	**IGHV3-9*01**	**93.84%**	M99651	**IGHD1-26*01**	**69.23%**	X97051	**IGHJ4*01**	**82.98%**	J00256
		IGHV3-20*01	88.04%	M99657	**IGHD2-15*01**	**68.75%**	J00234	IGHJ1*01	74.51%	J00256
		IGHV3-43*01	88.04%	M99672	**IGHD2-2*01**	**68.75%**	J00232	IGHJ5*01	72.00%	J00256
		IGHV3-h*01	83.15%	AJ879484	IGHD6-25*01	61.54%	X97051	IGHJ3*01	71.43%	J00256
		IGHV3-48*01	82.61%	M99675	IGHD3-10*01	58.82%	X13972	IGHJ2*01	69.23%	J00256
	10VH	**IGHV3-9*01**	**92.75%**	M99651	**IGHD1-26*01**	**69.23%**	X97051	**IGHJ4*01**	**85.11%**	J00256
		IGHV3-20*01	86.59%	M99657	**IGHD2-15*01**	**68.75%**	J00234	IGHJ5*01	78.00%	J00256
		IGHV3-43*01	85.51%	M99672	IGHD1-7*01	61.54%	X13972	IGHJ3*01	75.51%	J00256
		IGHV3-h*01	83.15%	AJ879484	IGHD6-25*01	61.54%	X97051	IGHJ1*01	74.51%	J00256
		IGHV3-48*01	82.61%	M99675	IGHD6-6*01	56.25%	X13972	IGHJ2*01	69.23%	J00256

FIG E1. Comparison of VH **(A)** and VL **(B)** sequences from the original IgE Fabs with germline genes (allele*01) from the ImMunoGeneTics database. The closest related germline genes and those with sequence identities of no less than 5% to the latter are displayed in boldface, together with their accession numbers and nucleotide sequence identities. Likewise, variable (IGHV), diverse (IGHD), and joining (IGHJ) genes of heavy chain sequences were identified in Fig E1, *A*, as well as variable (IGKV) and joining (IGKJ) genes of light chain sequences in Fig E1, *B*.

Comparison of isolated light chains with germline genes

B

	Isolated VL	IGKV gene*allele_01	Nucleotide sequence Identity	Accession No	IGKJ gene*allele_01	Nucleotide sequence Identity	Accession No
Phl p 5-specific VL	5VL	**IGKV1-12*01**	**96.34%**	V01577	**IGKJ2*01**	**94.12%**	J00242
		IGKV1D-12*01	**95.60%**	X17263	IGKJ1*01	85.29%	J00242
		IGKV1D-16*01	**91.94%**	K01323	IGKJ3*01	79.41%	J00242
		IGKV1-16*01	90.84%	J00248	IGKJ4*01	79.41%	J00242
		IGKV1-9*01	90.48%	Z00013	IGKJ5*01	64.71%	J00242
	14VL	**IGKV1-39*01**	**95.70%**	X59315	**IGKJ1*01**	**96.97%**	J00242
		IGKV1D-39*01	**95.70%**	X59312	IGKJ4*01	78.79%	J00242
		IGKV1-17*01	89.25%	X72808	IGKJ2*01	75.76%	J00242
		IGKV1-6*01	89.25%	M64858	IGKJ3*01	75.76%	J00242
		IGKV1-NL1*01	89.25%	Y14865	IGKJ5*01	66.67%	J00242
	28VL	**IGKV1-39*01**	**93.04%**	X59315	**IGKJ1*01**	**90.62%**	J00242
		IGKV1D-39*01	**93.04%**	X59312	IGKJ3*01	78.12%	J00242
		IGKV1-9*01	86.81%	Z00013	IGKJ4*01	75.00%	J00242
		IGKV1-6*01	86.45%	M64858	IGKJ2*01	71.88%	J00242
		IGKV1D-13*01	86.45%	X17262	IGKJ5*01	68.75%	J00242
	31VL	**IGKV1-39*01**	**97.49%**	X59315	**IGKJ2*01**	**94.12%**	J00242
		IGKV1D-39*01	**97.49%**	X59312	IGKJ1*01	73.53%	J00242
		IGKV1-12*01	91.40%	V01577	IGKJ3*01	73.53%	J00242
		IGKV1-9*01	90.68%	Z00013	IGKJ4*01	73.53%	J00242
		IGKV1D-12*01	90.68%	X17263	IGKJ5*01	73.53%	J00242
Phl p 2-specific VL	94VL	**IGKV1-39*01**	**93.77%**	X59315	**IGKJ2*01**	**94.87%**	J00242
		IGKV1D-39*01	**93.77%**	X59312	IGKJ1*01	84.21%	J00242
		IGKV1-12*01	88.64%	V01577	IGKJ4*01	84.21%	J00242
		IGKV1-9*01	88.64%	Z00013	IGKJ3*01	81.58%	J00242
		IGKV1-17*01	88.28%	X72808	IGKJ5*01	71.05%	J00242
	60VL	**IGKV1-39*01**	**92.67%**	X59315	**IGKJ3*01**	**86.84%**	J00242
		IGKV1D-39*01	**92.67%**	X59312	**IGKJ2*01**	**82.05%**	J00242
		IGKV1-12*01	86.81%	V01577	IGKJ1*01	76.32%	J00242
		IGKV1-37*01	86.81%	X59316	IGKJ4*01	73.68%	J00242
		IGKV1D-37*01	86.81%	X71893	IGKJ5*01	73.68%	J00242
	100VL	**IGKV1-12*01**	**95.97%**	V01577	**IGKJ2*01**	**94.74%**	J00242
		IGKV1D-12*01	**95.24%**	X17263	IGKJ1*01	86.84%	J00242
		IGKV1D-16*01	**91.58%**	K01323	IGKJ3*01	81.58%	J00242
		IGKV1-16*01	90.48%	J00248	IGKJ4*01	81.58%	J00242
		IGKV1-9*01	90.11%	Z00013	IGKJ5*01	68.42%	J00242
Phl p 1-specific VL	25VL	**IGKV1-39*01**	**95.24%**	X59315	**IGKJ5*01**	**97.37%**	J00242
		IGKV1D-39*01	**95.24%**	X59312	IGKJ2*01	71.05%	J00242
		IGKV1-12*01	90.11%	V01577	IGKJ4*01	71.05%	J00242
		IGKV1-17*01	89.38%	X72808	IGKJ1*01	68.42%	J00242
		IGKV1D-12*01	89.38%	X17263	IGKJ3*01	68.42%	J00242
	43VL	**IGKV1-39*01**	**90.32%**	X59315	**IGKJ4*01**	**89.47%**	J00242
		IGKV1D-39*01	**90.32%**	X59312	IGKJ2*01	76.32%	J00242
		IGKV1-17*01	**85.66%**	X72808	IGKJ3*01	76.32%	J00242
		IGKV1-9*01	**85.66%**	Z00013	IGKJ1*01	73.68%	J00242
		IGKV1-12*01	84.95%	V01577	IGKJ5*01	65.79%	J00242
	10VL	**IGKV1-39*01**	**91.76%**	X59315	**IGKJ4*01**	**97.37%**	J00242
		IGKV1D-39*01	**91.76%**	X59312	IGKJ1*01	81.58%	J00242
		IGKV1-12*01	86.38%	V01577	IGKJ3*01	81.58%	J00242
		IGKV1-27*01	86.02%	X63398	IGKJ2*01	78.95%	J00242
		IGKV1-9*01	86.02%	Z00013	IGKJ5*01	71.05%	J00242

FIG E1. *(Continued)*

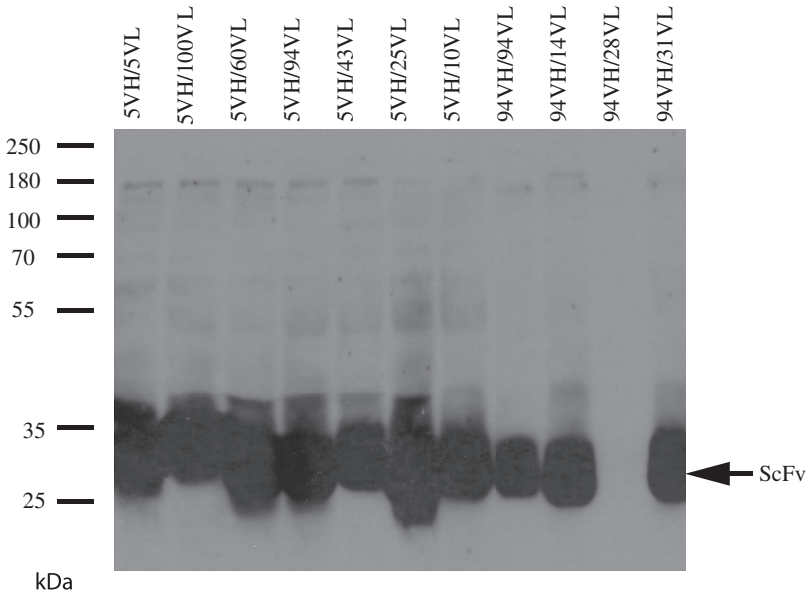

FIG E2. Detection of complete ScFvs in nitrocellulose-blotted bacterial lysates with an anti–E tag antibody. The analysis of a representative number of bacterial clones expressing original and shuffled ScFvs *(top)* is shown. Molecular weight markers (in kilodaltons) are indicated on the *left margin*.

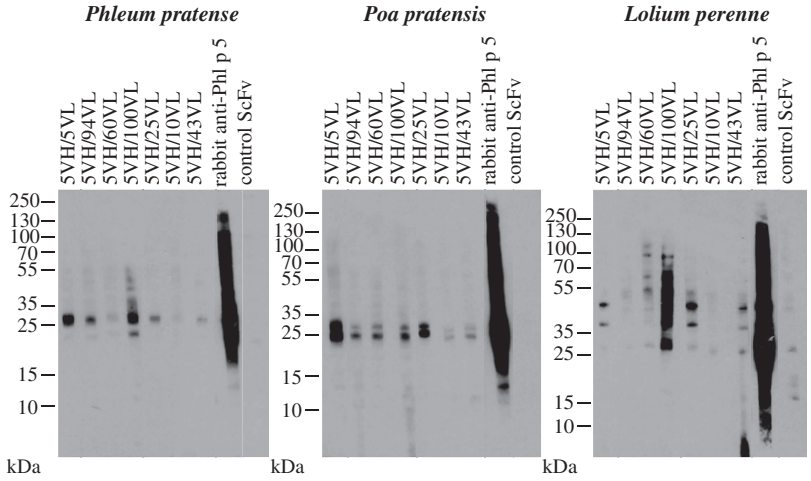

FIG E3. Detection of cross-reactivity of original and shuffled ScFvs and a control ScFv *(top)* to nitrocellulose-blotted natural grass pollen extracts from *P pratense (left)*, *P pratensis (middle)*, and *L perenne (right)*. Rabbit anti–Phl p 5 antiserum was used to identify group 5 allergens in the extracts. Molecular weight markers (in kilodaltons) are displayed on the *left margin*.

TABLE E1. Amino acid sequence identities (in percentages) and mean percentage sequence identities between the analyzed VL regions are displayed

	Phl p 1-specific				Phl p 2-specific				Phl p 5-specific				
	25VL	**43VL**	**10VL**	**Mean**	**94VL**	**60VL**	**100VL**	**Mean**	**5VL**	**28VL**	**31VL**	**14VL**	**Mean**
Phl p 1 specific													
25VL	100%			82.7%									
43VL	82%	100%											
10VL	83%	83%	100%										
Phl p 2 specific													
94VL	83%	75%	78%	78.8%	100%			81.0%					
60VL	82%	79%	79%		82%	100%							
100VL	83%	74%	76%		81%	80%	100%						
Phl p 5 specific													
5VL	83%	74%	76%	82.2%	81%	80%	100%	85.2%	100%				86.0%
28VL	88%	81%	84%		83%	84%	82%		82%	100%			
31VL	90%	82%	82%		86%	85%	87%		87%	89%	100%		
14VL	87%	79%	80%		88%	83%	83%		83%	86%	89%	100%	