

A Highly Conserved Interspecies V_H in the Human Genome

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Idiotypic conservation between human and mouse antibodies has been observed in association with various infectious and autoimmune diseases. We have isolated a human anti-idiotypic antibody to a mouse monoclonal anti-IgE antibody (BSW17) suggesting a conserved interspecies idio type associated with an anti-IgE response. To find the homologue of BSW17 in the human genome we applied the guided selection strategy. Combining V_H of BSW17 with a human V_L repertoire resulted in three light chains. The three V_L chains were then combined with a human V_H repertoire resulting in three clones specific for human IgE. Surprisingly, one clone, Hu41, had the same epitope specificity and functional *in vitro* activity as BSW17 and V_H complementarity-determining regions identical with that of BSW17. Real-time PCR analysis confirmed the presence of the Hu41 V_H sequence in the human genome. These data document the first example of the isolation of a human antibody where high sequence similarity to the original murine V_H sequence is associated with common antigen and epitope specificity.

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Introduction

Idiotypic conservation between mouse and human antibodies of the same antigen specificity has been reported in several antigen systems.^{1–4} Eilat *et al.* showed that a major cross-reactive idio type on mouse anti-DNA monoclonal antibody was also expressed on serum anti-DNA antibodies in systemic lupus erythematosus (SLE) sera.⁵ Analysis of the human influenza-specific B cell repertoire has documented the presence of shared idiotypes expressed on murine and human influenza-specific antibodies.⁶

Such shared idiotypes are referred to as public, recurrent, dominant or cross-reactive idiotypes (CR1). The importance of CR1 in the pathogenesis of disease and in the idio type anti-idio type network interaction has been demonstrated in several

systems.^{7–10} In sera of patients with different forms of clinical paracoccidioidomycosis, anti-idiotypic antibodies were found that recognize both human and monoclonal murine antibodies specific to parasite glycoprotein gp43.¹⁰ Analysis of the autoimmune response in thrombocytopenic patients has demonstrated the presence of anti-idiotypic antibodies produced against cross-reactive idiotypes shared by human and mouse anti-platelet antibodies.⁹ These findings underlie the role of shared idiotypes in idio type modulation and may imply that specific antibody responses are associated with immunoglobulin variable-region genes that are highly conserved during evolution.^{11–13}

Using a pool of non-immune human Fab phage display libraries we have previously described the isolation of two human anti-idiotypic antibodies that mimic epitopes located in the constant domain of the IgE molecule and are specific to a murine monoclonal anti-IgE antibody, termed BSW17.¹⁴ Additionally these antibodies showed functional mimicry and were able to induce *in vivo* a BSW17-like anti-IgE response after rabbit immunization.

Abbreviations used: V, variable region; H, heavy chain; L, light chain; CDR, complementarity-determining region.

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These findings suggested a conserved functional idiotype and a possible structural relationship between mouse and human associated with an anti-IgE response. Thus, we addressed of whether a human anti-IgE antibody displaying the same epitope specificity and idiotype sequence as BSW17 exists in the human repertoire.

We previously tried to isolate BSW17-like human anti-IgE from human antibody libraries using the phage technology. With this approach only two clones were isolated with an anti-isotypic specificity to IgE but not with the same epitope specificity as BSW17.¹⁵ This might be due to the limited size of the libraries, which do not allow access to the whole anti-isotypic repertoire. Indeed previous results with recombinant antibody repertoires of non-immunized donors provided evidence for a correlation between the repertoire size and the presence of specific, high-affinity antibodies.^{16,17} Thus antibody libraries have been constructed with high diversity and a size of up to 6.5×10^{10} clones from where high-affinity antibodies to many different target antigens have been selected.^{17–19} However, *in vitro* selection procedures can be subject to bias especially in the case of complex antigens and it still remains difficult to isolate antibodies recognizing a particular epitope.^{20,21}

During the last few years an alternative method termed “guided” selection has been developed for the generation of human antibodies. This technique combines chain shuffling and phage selection to derive the antibody with the designed specificities. The V_H or V_L of a parental murine antibody serve as a guiding structure for the selection of a human complementary V-region from a repertoire of cloned human V_L or V_H genes. Several studies have shown the feasibility of this method to generate human antibodies with different specificities.^{22–32}

We describe here the humanization of the mouse monoclonal anti-IgE antibody, BSW17 by guided

selection based on phage display. The V_H region of the BSW17 monoclonal antibody was first used to guide the selection of a human V_L from a V_L repertoire. In a consecutive step, three selected human V_L were used to select a human BSW17-like V_H region from a human V_H repertoire. This strategy allowed the isolation of a fully human anti-IgE antibody that retained the same binding specificity and *in vitro* functional activity as the murine monoclonal antibody BSW17. Remarkably, the selected V_H sequence shared 97% identity with the parental murine V_H . To our knowledge, this is the first time that a highly conserved human V_H sequence with the same functional specificity as the murine parental antibody has been isolated by guided selection.

Results

Light chain humanization

To obtain a human anti-IgE the strategy of guided selection, which combines chain shuffling and phage display (Figure 1), was followed using the V_H of the mouse monoclonal anti-IgE antibody, BSW17 as the guiding molecule. The V genes of BSW17 were first cloned as a Fab fragment into the phagemid vector pMVS and both V chains were sequenced (Figure 2). As the interaction of two matching human constant domains C_L and $C_{H\gamma 1}$ is preferred to stabilize the hybrid Fab, the mouse Fd was substituted by a chimeric Fd fragment composed of mouse V_H linked to the human first constant domain of the heavy chain which was then cloned into pMVS. The human light chain repertoire derived from four human combinatorial Fab libraries¹⁴ was then cloned into pMVS containing the chimeric mouse/human Fd fragment yielding a phage library of about 2.3×10^7 different clones expressing hybrid Fab. To isolate human light chain analogs of BSW17 this

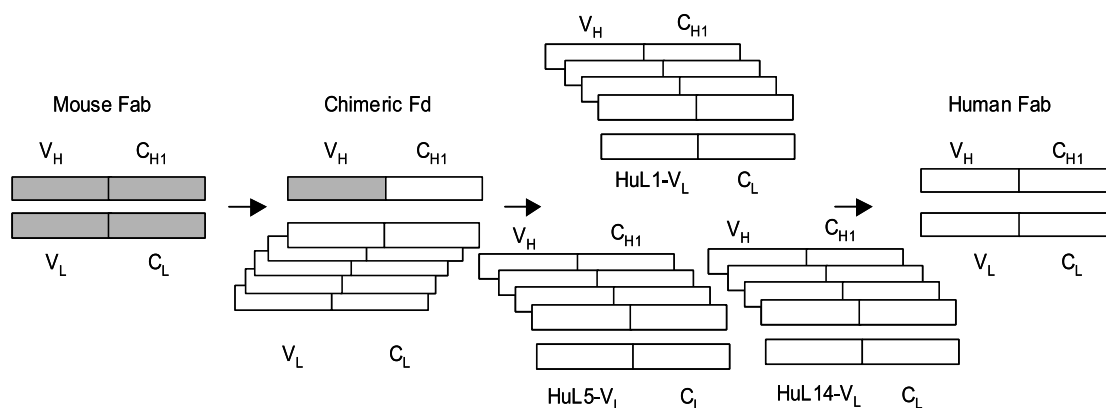


Figure 1. Schematic drawing of humanization of mouse monoclonal anti-IgE antibody, BSW17 using guided selection. Mouse sequences are indicated in gray, human sequences in white. In a first step a chimeric mouse/human Fd is used as guiding structure to select human light chains from a human light chain repertoire. In the second step three human light chain fragments were used as guiding structure for the selection of human V_H from a human V_H repertoire. The two sequential shuffling procedures were performed using phage display technology.

Light chain

	L1		L2		L3		
Clones	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
BSW17-V _L	ELVMTQSPAIMASASPGEKVTMTC	SASSS.V..TFIH	WYRQKSGTSPKGWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISTMEAEADAATYYC	QHWSG..NPL.T	FGTGTKLEL.K
HuL1-V _L	---.----GTL-L----RA-LS-	R--Q-.-NRIYLA	--Q--L-QA-RLL--	GA-NR-A	-I-D-----DFT----RL-P--F-V---	LQYGS..S-IF-	--P---VDI.-
HuL5-V _L	-----TL-L----RA-LS-	R--Q-.GS YLD	--Q--P-QA-RLL--	-A-NR-T	-I-----DFT----SL-P--F-V---	-QH-N.W-PW-.	--Q---V-I.-
HuL14-V _L	---V--P-SV.--A-Q---IS-	-G---NIGNNYVS	--Q-LP--A--LL--	-NN-RP-	-I-D----K----AT-G-TGLQTG-E-D---	GT-D-SLSAGRV	--G----TVLG

Heavy chain

	H1		H2				
Clones	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
BSW17-V _H	EVQLLESGGGFVKPGGSLKLSCVVSGFTFS	SYAMS	WVRQTPEKRLEWVA	SIS.SGNIIYYPDNVKG	RFTISRDNVRNILYQMSSLRSEDAMYYCTR	GRSTYGGFDH	WGQGTTLTVSS
Hu19-V _H	-----V-Q--R--R---AA-----	T-G-H	---A-G-G-----	V-YD--SNK--A-S---	-----SE-TM----N---A---V---A-	. .RGIY-M-V	-----V----
Hu22-V _H	-----V-Q--R--R---AA-----	N-G-H	---A-G-G-----	F--KDAYNKN-V-S---	-----SKKT-----N---A-----AK	EDRAS-AL-I	-----MV----
Hu41-V _H	-----	-----	-----V---	---.-----	-----V---A-	-----	--T---V----

Figure 2. Deduced amino acid sequence of selected human V_L and V_H genes. The sequences are segregated into CDR (complementarity-determining regions) and FR (framework regions) and aligned towards parental mouse BSW17 V_L and V_H fragments. Dashes indicate identical amino acids. Loop regions of V_L (L1, L2 and L3) and V_H (H1 and H2) are deduced using V Base.

Table 1. Classification and predicted canonical structures of the antigen binding loops of V-genes

Clones ^a	V-gene family ^b	Predicted canonical structures of antigen binding loops ^b			Human germline gene with closest protein sequence ^c	Amino acid sequence identity with murine BSW17 (%)
		Loop1 ^b	Loop2 ^b	Loop3 ^b		
BSW17-V _L	V _κ 7	1/10 ^d	1/7	1/9	B1 +	100
HuL1-V _L	V _κ 3	n.f./12	1/7	n.f./10	DPK22	65
HuL5-V _L	V _κ 3	2/11	1/7	n.f./10	Vg/38K	69
HuL14-V _L	V _λ 1	5/13	1/7	n.f.	DPL5	58
BSW17-V _H	V _H 3	1/10	1/9	n.a.	DP58	100
Hu19-V _H	V _H 3	1/10	3/10	n.a.	DP49	76
Hu22-V _H	V _H 3	1/10	3/10	n.a.	DP49	75
Hu41-V _H	V _H 3	1/10	1/9	n.a.	DP58	96

n.f., not found, canonical structure classes could not be assigned based on currently known classes; n.a., not applicable.

^a Selected human V_Ls were assigned in combination with BSW17 V_H. Selected human V_H of Hu19 and Hu41 were assigned in combination with the V_L of HuL14 whilst selected human V_H of clone Hu22 with the V_L of HuL1.

^b Canonical structures were assigned as described by Martin & Thornton.⁶¹ Loop1, loop2 and loop3 correspond to the antigen binding loops of heavy and light chains.

^c V-gene families and closest human germlines were deduced from V BASE (<http://www.mrc-cpe.cam.ac.uk>).

^d The class numbers consist of the Chothia-equivalent structural class number followed by the number of amino acids in the antigen binding loops.

library was subjected to three rounds of panning on solid phase IgE. After the last round of panning, 15 clones were tested for IgE reactivity in the competitive immunodot assay where the binding of phage clones to IgE was inhibited by BSW17. Three clones demonstrating the same IgE epitope specificity as the monoclonal antibody (mAb) BSW17 were selected and sequence analyzed. Figure 2 shows their deduced amino acid sequences, which are aligned to the mouse BSW17 sequence. Databank screening indicated that they belonged to the same V_κIII family but to two different germlines, namely DPK22 and Vg/38K (Table 1). One clone, HuL14 carried a λ chain derived from the germline DPL5 of the V_λ1 family (Table 1). As shown in Table 1, the selected V_L have different canonical structures and amino acid lengths for L1 compared to that used by BSW17 V_L. No differences in canonical loop were observed for L2. In the case of L3, loop lengths could be determined in only two clones (HuL1 and HuL5) and were different from that used by BSW17. No known canonical structure for L3 could be found in any of the three human selected V_L sequences. As the three selected clones showed the same specificity to IgE as BSW17, the light chains of all three clones were used to guide the selection of the human heavy chain.

Heavy chain humanization

For the shuffling of the heavy chain, the selected human light chains were cloned separately into pMVS, which did not carry any V_H to avoid risk of contamination with the BSW17 V_H. Human Fd fragments were amplified by PCR amplifying from phagemid DNA of four human Fab combinatorial libraries.¹⁴ These PCR products were pooled and cloned into the vectors containing the selected light chain sequences to generate three libraries of 2×10^7 clones each. The libraries were

pooled and selected by four rounds of panning on immobilized IgE. After the fourth round, 50 phage clones were tested for binding to IgE in the competitive immunodot assay described above. One clone (Hu41) was found whose binding to IgE was completely inhibited by BSW17, suggesting that this clone recognized the same IgE epitope as BSW17. Two clones (Hu19 and 22) were also selected whose binding to IgE was only partially inhibited by BSW17. Sequence analysis of the three clones revealed two different human V_L (reselected from chimeric clones HuL1 and HuL14) and three different V_H sequences. The human V_λ1 reselected from chimeric clone HuL14 was found in two clones (Hu19 and Hu41), whereas the third clone (Hu22) carried the human V_κIII reselected from the chimeric clone HuL1. The V_H amino acid sequences of the three clones (Hu19, Hu22 and Hu41) and of the parental antibody BSW17 are aligned in Figure 2. Two clones (Hu19 and Hu22) had closely related V_H fragments that derived from the same germline DP49 of the V_H3 family. The third clone (Hu41) contained a V_H fragment that derived from the germline DP58 of the V_H3 family. Amino acid sequence alignment revealed that the V_H sequence of clone Hu41 was 97% identical with the parental murine V_H gene. As shown in Table 1, the H1 canonical lengths and structures for the three clones (Hu19, Hu22 and Hu41) were identical with that of BSW17 V_H, whereas only clone Hu41 used the same canonical class for H2 as BSW17 V_H. These results imply that a murine-like V_H sequence was selected from the human genome. However, as the selected V_H sequence differed in only three amino acid residues from the parental mouse V_H a potential reason for this selection would be a carry-over PCR contamination. To rule out this possibility, the presence of the V_H sequence from the clone Hu41 in the human genome was verified by PCR.

Identification of Hu41 V_H sequence in the human genome by PCR

The presence of the clone Hu41 V_H sequence in the human genome was monitored by conventional reverse transcriptase (RT)-PCR and real-time PCR. RT-PCR was performed using cDNA of 21 randomly chosen donors. For PCR amplification a pair of primers was designed that were specific to the complementarity-determining regions (CDR2 and CDR3) regions of the V_H fragment. A PCR band of the expected size was obtained for 17 donors (data not shown) and was sequenced. All the samples showed identical sequences corresponding to the same V_H fragment found in the selected Hu41 clone, suggesting that the selected BSW17-like V_H sequence is present in the human genome. However, this result might still be a subject of controversy due to the problems associated with conventional RT-PCR, namely specificity and carryover contamination.

To circumvent these problems and reduce the potential for false-positive results we chose the real-time PCR approach using the TaqMan technology. The TaqMan assay was performed on DNA of the same donors as used for the RT-PCR. DNA was preferred to RNA because of the reproducibility and sensitivity problems related to RT-PCR. To increase sequence specificity a 3' minor groove binding (MGB) probe was used instead of a normal TaqMan probe. This probe prevented annealing with single nucleotide mismatches. To ensure that quantification of the V_H of Hu41 was not affected by differences in the quality and quantity of the DNA, the housekeeping gene,

porphobilinogen deaminase (PBGD) was chosen as reference standard. Quantification analysis was performed using the comparative C_T method. This was possible because the efficiencies of amplification of Hu41 and PBGD genes were equal.³³ The amount of Hu41 V_H gene for each donor was given by $2^{-\Delta\Delta C_T}$. As shown in Figure 3, $2^{-\Delta\Delta C_T}$ values higher than 0 were obtained in all 21 samples tested, indicating that all donors contain the Hu41 V_H gene in their genome. However, these $2^{-\Delta\Delta C_T}$ values vary considerably among the donors and show changes between 1.9 and 8051-fold, suggesting that the Hu41 V_H gene copy number differs greatly between the donors.

Affinity measurement and binding specificity of humanized BSW17

As clone Hu41 was the only clone showing the same IgE specificity as BSW17, it was produced as full-length IgG in eukaryotic cells for further characterization. The affinity of clone Hu41 for IgE was assessed by online monitoring of binding using the IAsys cuvette system. The affinity was 3.0×10^{-8} M, which is similar to the value of 6.0×10^{-8} M obtained for the parental murine antibody, BSW17.

In order to show that BSW17 recognizes an epitope on IgE, which is also responsible for the binding of IgE to the FcεRIα, we tested whether clone Hu41 can still bind to IgE bound to FcεRIα. Sequential binding of clone Hu41 to IgE and of the mouse monoclonal anti-IgE antibodies, BSW17 and Le27, was assessed by online monitoring using the IAsys system. As shown in Figure 4, a

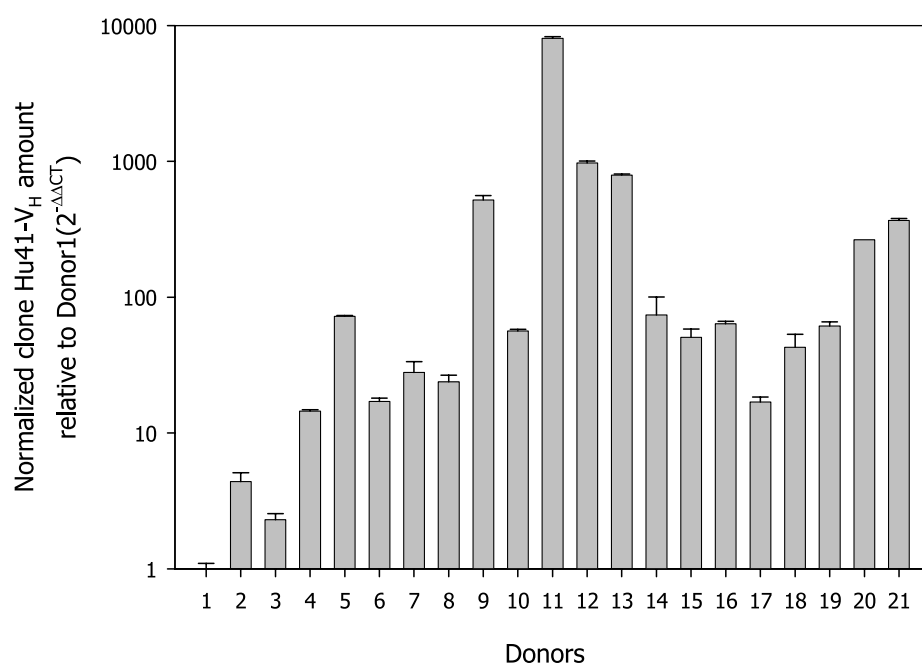


Figure 3. Relative quantification of Hu41 V_H gene using the comparative C_T method. DNA from peripheral blood mononuclear cells of 21 donors was used as template for real-time PCR reactions containing primers and probes either for Hu41V_H or for PBGD. The copy number of Hu41V_H gene normalized to PBGD and relative to the calibrator (Donor 1) was given by $2^{-\Delta\Delta C_T}$.

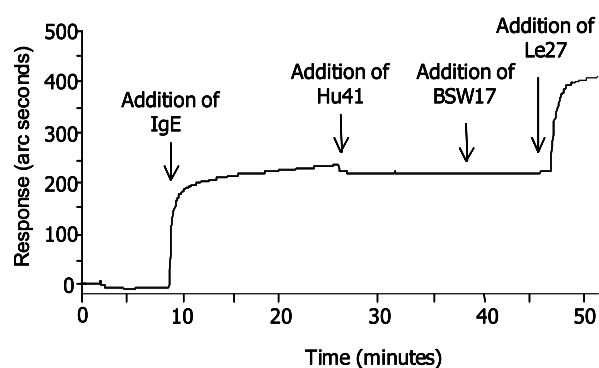


Figure 4. On-line monitoring of anti-IgE antibodies interacting with IgE bound to FcεRIα. IgE Sus11 (100 nmol) was incubated with immobilized FcεRIα for ten minutes. The volume was then replaced successively with 100 nmol of Hu41, BSW17 and Le27 and incubated for ten minutes. Finally, the cuvette was washed with $3 \times 50 \mu\text{l}$ of PBS/Tween 0.05%. Arrows indicate the addition of antibodies. Antibody binding is displayed as arc seconds of angle correction with maximal laser reflection.

binding curve was obtained after addition of IgE, whereas no enhancement of the binding curve was observed after sequential addition of clone Hu41 and then BSW17. These results indicated that Hu41 behaved like BSW17 and that both antibodies recognized overlapping epitopes on IgE that are used for binding IgE to FcεRIα. In contrast, addition of the same amount of Le27 showed an enhancement of the binding curve, indicating that this antibody reacted with a different epitope on IgE that is still exposed in FcεRIα-bound IgE.

Non-induction of IgE-mediated mediator release from human basophils

Our previous data have shown that BSW17 is non-anaphylactogenic and does not induce mediator release from basophils.³⁴ Therefore, the non-anaphylactogenic properties of Hu41 were tested using freshly isolated peripheral blood lymphocytes enriched for basophils. Figure 5A shows the effect of different monoclonal anti-human IgE antibodies on histamine release after priming with IL-3. An anaphylactogenic anti-FcεRIα monoclonal antibody, 29C6, was used as a positive control for histamine release. However, stimulation with either Hu41 or BSW17 did not trigger the cells for histamine release, whereas an anaphylactogenic anti-human IgE antibody, Le27, triggered a strong histamine release response compared to the spontaneous release in the presence of IL-3 alone.

Similar results were obtained for sulfido-leukotriene (sLT) release. As shown in Figure 5B, no sLT release was observed from cells treated with Hu41 and BSW17. Again, stimulation of the cells with Le27 induced a marked enhancement of sLT production over spontaneous release.

Non-induction of IgE-mediated hexosaminidase release from human FcεRIα-transfected rat RBL-2H3 cells

We further tested the capacity of Hu41 to modulate hexosaminidase secretion from rat basophilic leukemia cells expressing human FcεRIα (RBL-2H3E5.D12.8 cells). To monitor cellular activation, we measured the extent of degranulation of the preformed mediator hexosaminidase from RBL-2H3E5.D12.8 cells that were stimulated with an optimal amount of an anaphylactogenic anti-FcεRIα mAb, 22E7. Under these conditions the release of hexosaminidase was in the range of 18% of the total cell cellular hexosaminidase (Figure 6, 22E7) with 5% spontaneous secretion (Figure 6, buffer control). We then tested the capacity of anti-human IgE antibodies to modulate degranulation of RBL-2H3E5.D12.8 cells preincubated for 90 minutes with saturating amounts of IgE Sus11. Figure 6 shows that addition of Hu41 and BSW17 did not produce an increase of release compared with the buffer control. In contrast, the anaphylactogenic anti-human IgE antibody Le27 triggered β-hexosaminidase release in the same range as 22E7.

Discussion

Antibody humanization has become a standard procedure to reduce immunogenicity of rodent monoclonal antibodies for clinical application in humans.³⁵ The humanization strategy termed guided selection was successfully used for obtaining human antibodies with the same functionality and antigen specificity as the parental mouse antibody.^{22,25–29,36} In most of the cases the humanized antibodies showed rather low sequence similarity with the parental mouse antibody. Here, we report the cloning of a human anti-IgE antibody by guided selection that showed the same specificity but also carried a V_H sequence highly similar to the parental murine monoclonal anti-human IgE, BSW17.

In our guided selection and in contrast to other studies,^{26,37} we did not retain the V_H and V_L CDR3 region of the parental murine antibody. The rationale for this strategy was to ensure full humanization of V_H and V_L sequences. Humanization of the light chain was performed by using the murine BSW17 V_H sequence as guiding structure to select human light chains from a light chain repertoire obtained from four different human Fab libraries. Three chimeric clones containing different kappa and lambda chains that had the same epitope specificity as BSW17 were isolated. Comparison of the sequences of the original murine V_L chain with the human V_L chains revealed differences in length and sequence of the CDR1 and relative low similarity in the CDR2 and CDR3. The kappa chains were not derived from the human germline closest to the mouse V_L chain. This corresponds to

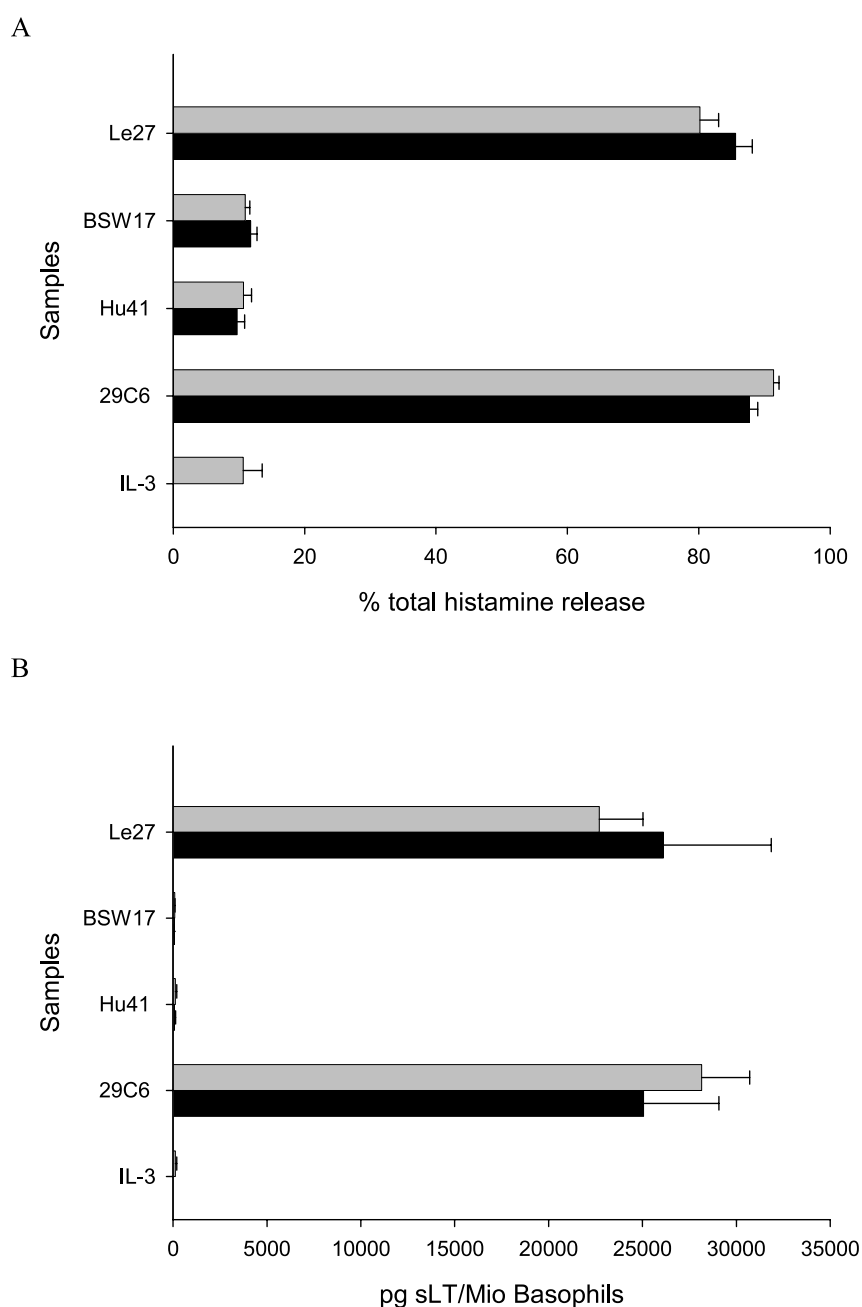


Figure 5. A, Non-induction of *in vitro* histamine release with Hu41. Freshly isolated and enriched basophils (3×10^5 cells containing 41% basophils) isolated from peripheral blood were primed with IL-3 (10 ng/ml) and then incubated with anti-human IgE antibodies (Hu41, BSW17 and Le27) at the concentrations of 10 µg/ml (black bars) and 0.1 µg/ml (gray bars). Monoclonal anti-FcεRIα antibody 29C6 was used as positive control. Results are expressed as mean percentage of total histamine cell content (TC) of triplicate cultures. B, Non-induction of *in vitro* sulfoleukotriene (sLT) release with Hu41. Basophils were isolated and treated as for the histamine release assay. Pg sLT represents the mean of triplicate assays of sLT release from 10^6 cells.

other examples of human V_L chain selection where the guided selection did not yield the most similar human counterpart sequence.^{22,26,27,29,36} For the antigen binding site, more important than sequence homology are the canonical structures defined by Chothia *et al.*^{38,39} Previous studies have shown the isolation of human antibodies by guided selection, which shared these structural elements with the parental mouse antibody. In our

case the three light chains did not share similar L1 and L3 canonical structures with the mouse antibody BSW17. This observation suggests that the light chain can be more promiscuous and not only one chain plays a role for maintaining the antigen binding specificity.

To increase the probability of obtaining a fully human Fab in the V_H selection step the three V_L chains were used to guide the selection of a V_H

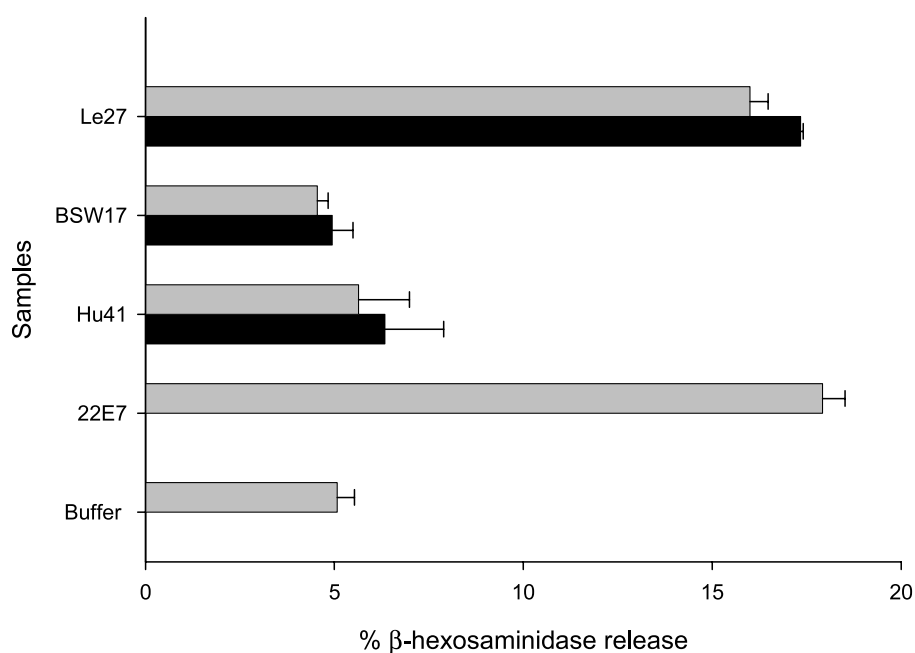


Figure 6. Non-induction of hexosaminidase release from RBL-2H3E5.D12.8 cells. Cells were pulsed with 50 $\mu\text{g}/\text{ml}$ of IgE Sus11 and incubated with anti-IgE antibodies (Hu41, BSW17 and Le27) at the concentrations of 10 $\mu\text{g}/\text{ml}$ (black bars) and 1 $\mu\text{g}/\text{ml}$ (gray bars). Anaphylactogenic monoclonal anti-Fc ϵ R1 α antibody 22E7 was used as a positive control at 1 $\mu\text{g}/\text{ml}$. Spontaneous release was measured using a buffer (0.5% BSA in Hank's balanced salt solution) control. The results are shown as mean percentage of duplicate cultures of total hexosaminidase release. The total hexosaminidase release was calculated as the sum of spontaneous release and the content of hexosaminidase present in the lysate of unstimulated cells.

chain. Three clones were selected that paired with two different V_L chains. This differs from what was found by other groups, where only one particular light chain with the best binding characteristics was reselected.^{24,26,28,36} This might be due to the fact that in our case the selected light chains did not differ in their IgE binding specificity. The heavy chains of two clones (Hu19 and 22) showed common features in the CDR1 and CDR2 and were derived from the same germline DP49 of the V_H3 family. Compared with the original murine V_H they showed minimal similarities especially in the sequences of the CDR2 and CDR3.

Surprisingly, one clone, Hu41, which demonstrated the same IgE binding specificity as BSW17, carried a V_H sequence sharing 97% identity with the original murine V_H sequence and differed in only three residues located in FR2 and FR3. For the heavy chain shuffling the selected light chains were recloned separately into a vector not carrying any V_H genes in order to prevent contamination with BSW17 V_H . Therefore the isolation of a V_H highly similar to the BSW17 V_H is highly unlikely to be due to a cross-contamination with the original or a mutated murine V_H sequence.

Interestingly Watzka *et al.*, who used a similar guided selection approach, described the isolation of a human Fab carrying V_L and V_H sequence with an overall similarity of about 90% and 80%, respectively, compared to the parental murine antibody.²³ However, this human Fab did not have the same antigen epitope specificity as the parental

mouse antibody. Nevertheless this result is in line with our present data suggesting the presence of variable immunoglobulin sequences highly similar to mouse in the human genome. In fact comparison studies of human and mouse Ig V_H have previously demonstrated strong similarity existing between some V_H families of these two mammalian species.⁴⁰ Our data indicate that Hu41 V_H was derived from the human germline gene DP58 of the V_H3 family. Interestingly there are reports showing that human and mice V_H3 genes have above 80% DNA sequence similarity, suggesting that they shared a common ancestor and have been conserved throughout mammalian evolution.^{11,41} Additionally, pairwise comparison of human and mouse V_H allowed us and others to identify human/mouse V_H pairs demonstrating similarity above 80%, suggesting some form of environmental selection for the maintenance of specific antibody gene sequences.¹³ Interestingly some of these highly similar V_H sequences have been identified in autoreactive antibody molecules of either human or mouse origin.⁴² Thus, it has been reported that sera from patients with SLE contain antibodies that compete with the mouse monoclonal antibodies for binding on DNA, suggesting structural similarities between human and mouse anti-DNA antibodies.^{3,4,43} In sera of patients with thrombocytopenia, anti-idiotypic antibodies were found against murine antibodies specific to a platelet glycoprotein, suggesting cross-reactive idiotypes shared by human and

mouse anti-platelet antibodies.⁹ In a previous study we have isolated a human monoclonal anti-idiotypic antibody against the mouse anti-IgE antibody, BSW17, implying an interspecies idio- type conservation associated with the anti-IgE response.¹⁴ Furthermore, shared idiotype specificity was also observed in non-autoreactive immune responses. Analyses of human and murine influenza responses after immunization demonstrated the existence of interspecies idio- types expressed on murine and human influenza- specific antibodies.⁶

Our data confirm and extend these findings by the isolation of a murine-like human V_H sequence in the human genome. By using RT-PCR, a trans- script whose size and sequence correspond to the V_H of clone Hu41 was found in most of the tested donors. Additionally we have performed real-time quantitative PCR that provides a highly reliable method for rapid quantification of gene copies. This method eliminates the need for post sample processing and thereby prevents potential PCR carryover contamination. Until now most real-time PCR methods have been performed using sequence-specific fluorescent probes for geno- typing, like TaqMan and molecular beacon probes. We used a novel fluorescent oligonucleotide that contains a 3' minor binding group (MGB) which binds to the minor groove of DNA with higher affinity compared to ordinary DNA probes.⁴⁴ This method reduces non-specific probe hybridization and has been successfully used for single nucle- otide polymorphism (SNP) analysis.⁴⁵ Real-time PCR analysis revealed the clone Hu41 V_H gene in all the donors tested, thus supporting the presence of a highly homologous murine sequence in the human genome.

Antigen specificity and non-anaphylactogenicity are critical parameters to be maintained during the humanization of BSW17, which binds to a dis- continuous epitope on the IgE molecule that is involved in the binding of the IgE to α chain of the high-affinity receptor for IgE (Fc ϵ RI α).⁴⁶ By binding to this epitope BSW17 prevents the bind- ing of IgE to Fc ϵ RI α and shows unique properties, as it is non-anaphylactogenic and thereby prevents the sensitization of the effector cells implicated in allergic diseases. Our data indicate that the humanized clone Hu41 binds to the same epitope as BSW17, as determined by competition studies and IAsys analysis. In addition, clone Hu41 demonstrated the same *in vitro* functional activity as the parental murine BSW17 antibody, namely it does not induce preformed and *de novo* synthesis mediator release from either human basophils or human Fc ϵ RI α transfected rat leukemia cells. Thus, the fact that the clone Hu41 retained the binding and non-anaphylactogenic properties of the murine BSW17 suggests that it could be used as a passive vaccine for immunotherapy against allergy. However, further *in vivo* experiments in animal models, e.g. in monkey, are required to investigate whether clone Hu41 will be a potent

immunotherapeutic agent to prevent the sensitiz- ation of the effector cells implicated in allergic diseases.

In summary, we have been able to obtain a human full-length anti-IgE antibody, which demonstrates the same epitope specificity and functional activity as the original murine BSW17 antibody. Moreover this antibody carries a highly conserved interspecies V_H sequence. These results demonstrate structural similarities between mouse and human immunoglobulin V_H sequences and point out the usefulness of the guided selection approach for the isolation of human antibodies with the same functional specificity and common idiotype sequence as murine antibodies.

Material and Methods

Vectors, bacterial strains and cell lines

The pMVS vector is a phagemid vector based on pComb3H which allows expression of Fab on the surface of filamentous phage M13.⁴⁷ The *Escherichia coli* strain XL-1 Blue and the VCSM13 helper phage were pur- chased from Stratagene (La Jolla, CA). The mouse hybridoma cell line BSW17 was isolated in our laboratory from mice immunized with purified myeloma IgE.⁴⁶ The human embryonic kidney (HEK-293) cells were purchased from American Type Culture Collection (ATCC No. TIB202). The cell line RBL-2H3E5.D12.8 (RBL-2H3 transfected with human Fc ϵ RI α) was a kind gift from S. Mécheri, Institut Pasteur, Paris, France.

Antibodies and reagents

The following human IgE were used: IgE PS was kindly provided by Drs T. Ishizaka and K. Ishizaka (La Jolla, CA); IgE Savazal was kindly provided by Dr V. Savazal (Pilsen, Czech Republic). IgE JW8 is a mouse/human chimeric IgE, IgE ND is a myeloma IgE and IgE Sus11 is a monoclonal IgE.^{48–50} BSW17 and Le27 are mouse anti-human IgE mAbs as described.⁴⁶ Murine anti-Fc ϵ RI mAb, 29C6, directed against the non-IgE bind- ing epitope of the high-affinity IgE receptor α -chain⁵¹ was a gift from Drs Hakimi and Chizzonite (Hoffman- La-Roche, Nutley, NJ). Recombinant human IL-3 was provided by Novartis (Basel, Switzerland). Recombinant Fc ϵ RI α , a fusion protein of human serum albumin as car- rier, flanked by two extracellular parts of the α chain of the high-affinity IgE receptor was kindly provided by Dr H. Kocher (Novartis Pharma, Basel, Switzerland).

Humanization of the light chain

In order to use the heavy chain of the mouse mono- clonal BSW17 as template for the humanization of the light chain, the V genes of BSW17 were cloned as Fab fragment into the vector pMVS^{15,37,47} and sequenced at Microsynth GmbH (Balgach, Switzerland). To stabilize the hybrid Fab the mouse C_H1 region of the cloned BSW17 Fd fragment was substituted with human C_H1 and cloned into the pMVS vector as described.²⁸ The light chain coding sequences were obtained by restric- tion enzyme digestion from four human combinatorial Fab libraries¹⁴ and cloned into the pMVS vector contain- ing the chimeric mouse/human Fd fragment. The

ligation product was electroporated into *E. coli* strain XL1-Blue resulting in a light chain library and phages were produced as described.³⁷ Three rounds of panning against hybridoma IgE Sus11 carried out in polystyrene immunotubes (Becton, Dickinson) using 10 µg/ml of protein in 50 mM NaHCO₃ (pH 9.6) for coating, blocking and washing steps were performed as described.³⁷ Phages were competitively eluted with 100 µg/ml of BSW17. After the third round of panning single phage clones were produced and tested for binding to different human IgE in a competitive phage immunodot assay using BSW17 as competitor antibody and horseradish peroxidase (HRP)-conjugated rabbit anti-phage as developing antibody. Light chain coding genes of positive clones were analyzed by DNA sequencing. The sequences are available from the EMBL Genbank under the numbers AJ633625, AJ633626 and AJ633627 for HuL1, HuL5 and HuL14, respectively.

Humanization of the heavy chain

To prevent contamination with BSW17 Fd fragment the light chains of the three selected clones were recombined into the pMVS vector. The human Fd fragments were obtained from the above-mentioned libraries by PCR amplification using five upstream primers, which hybridize to V_H families,²⁸ and the downstream IgG1 primer, which hybridizes to the hinge region of the constant domain.¹⁵ The PCR fragments encoding human Fd fragments were cloned into the pMVS vectors containing the selected human light chains, thus generating three libraries. The phage libraries were pooled and four rounds of panning on immobilized IgE were carried out as described for the light chain. After the fourth round of panning, phages were produced from single clones and were tested for binding to different IgE using competitive phage immunodot assay as described above. Light chain and Fd fragment coding sequences of positive clones were analyzed by DNA sequencing. The sequences are available from the EMBL Genbank under the numbers AJ633622, AJ633623 and AJ633624 for Hu19, Hu22 and Hu41, respectively.

Full-length IgG from clones Hu19, 22 and 41 was produced in HEK-293 cells using an integrated vector system kindly provided Dr A. Bradbury (Trieste, Italy). Antibodies in the cell supernatant were purified on protein G-Sepharose columns (Pharmacia) and purity was controlled on an SDS/6% (w/v) acrylamide gel (data not shown).

RT-PCR amplification

Total RNA was extracted from peripheral blood mononuclear (PBM) cells of 21 unselected healthy donors and synthesized into cDNA using the Superscript reverse transcriptase system with oligo(dT) priming (Invitrogen, AG, Basel, Switzerland). Analysis of the presence of Hu41 V_H in the human genome was done with specific Hu41 V_H-CDR2 forward primer (5'-TAG TGG TAA TAT CAT CTA CTA TCC AGA CAA-3') and Hu41 V_H-CDR3 reverse primer (5'-GTC AAA TCC CCC GTA GGT ACT-3'). Primers specific for the actin gene were used as control for testing the quality and quantity of RNA. PCR amplification was performed in 50 µl of PCR reaction mixture containing 6 µl of cDNA, tenfold PCR mix, 1 µl of each primer (20 pmol), 2.5 units of Platinum Pfx DNA polymerase (Invitrogen) and water. Amplifications were done at 94 °C for five minutes, 94 °C for 30 seconds,

51.8 °C for 50 seconds, 72 °C for 30 seconds for a total of 60 cycles followed by a final extension at 72 °C for ten minutes.

Primers and TaqMan probes

The primers and the TaqMan probes for real-time quantitative PCR were designed from appropriate sequences using the PrimerExpress software (Applied Biosystems). The primers and the TaqMan MGB probe for amplification of Hu41 V_H gene and the housekeeping gene porphobilinogen deaminase (PBGD) are Hu41V_H-for, 5'-GTG GTA ATA TCA TCT ACT ATC CAG ACA ATG-3'; HuV_H-rev 5'-CCG TAG GTA CTG CGG CCT C-3'; Hu41V_H-pr 5'-TGT CAG GAA CAT CCT GTA CC-3'; PBGDfor 5'-TGA CCC ACA GTT GGT AGG CAT-3'; PBGDrev 5'-ATG CCC AAG TTC TGG GCA G-3' and PBGDpr 5'-CGT AAC ATT CCA CGA GGG CCC CA-3'. The primers were synthesized by Microsynth (Balgach, Switzerland) and the TaqMan probes by Applied Biosystems (Rotkreuz, Switzerland). The Hu41V_H TaqMan MGB probe was labeled at the 5' end with 6-carboxy fluorescein (FAM) and minor groove binder (MGB)/non-fluorescent quencher at the 3' end. The housekeeping gene porphobilinogen deaminase (PBGD) TaqMan probe was labeled at the 5' end with FAM and tetramethyl rhodamine (TAMRA) at the 3' end.

Real-time quantitative PCR

DNA was extracted from the same donors as for RNA. Quantitative DNA PCR was performed using the TaqMan Universal PCR Master Mix (Applied Biosystems). All reactions were done in triplicate and in 25 µl with 1.5 µg of DNA of the 21 donors, 12.5 µl of PCR mix, 900 nM each specific forward and reverse primer, 200 nM TaqMan probe. The amplification conditions for quantification were as follows: 50 °C for two minutes; 95 °C for ten minutes; 60 cycles at 95 °C for 15 seconds and 60 °C for one minute. The amplifications were performed on an ABI prism 7700 sequence detector equipped with 96-well thermal cycler. Data were collected and analyzed with Sequence Detector software (Applied Biosystems). To ensure that quantification of the V_H of Hu41 was not affected by differences in the quality and quantity of the DNA, the housekeeping gene PBGD was taken as reference standard. The average C_T values of PBGD gene in the various DNA samples differed by less than 1.1 (data not shown), implying that the efficiency of the amplifications of DNA per sample were comparable. Quantitative analysis of gene copy number was done using the comparative C_T method, in which C_T is the threshold cycle number (the minimum number of cycles needed before the product can be detected). The arithmetic formula for the comparative C_T method is described as the copy number of the Hu41 V_H gene normalized to PBGD copy number relative to a normalized calibrator and is given by the formula $2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T = \Delta C_T(\text{sample}) - \Delta C_T(\text{calibrator})$, and ΔC_T is the C_T of the Hu41 V_H subtracted from the C_T of PBGD. Donor 1 was chosen arbitrarily as calibrator sample. The standard deviation of the difference was calculated for the Hu41 V_H and PBGD values using the following formula:

$$S(\text{std dev of difference}) = \sqrt{S_1^2 + S_2^2}.$$

IASys analysis

Affinity of full-length Hu41 IgG for IgE was determined using the IASys cuvette system as described.⁵² The subsequent binding of anti-IgE antibodies to IgE bound to FcεRIα was assayed by on-line monitoring using the IASys cuvette system. The cuvette was immobilized with 10 μg of FcεRIα as described⁵² and incubated with 100 nM IgE diluted in PBS/0.05% Tween 20. Each anti-IgE antibody was diluted in PBS/0.05% Tween 20 and added in 50 μl samples (100 nM) to the cuvette.

Mediator release from human basophils

Basophil-enriched peripheral blood leucocytes from a randomly selected healthy donor were prepared as described^{53,54} by dextran sedimentation and Percoll gradient (Pharmacia). Cells were stimulated with 10 ng of IL-3 for 15 minutes, followed by the addition of different antibodies at the concentrations of 10 μg/ml and 0.1 μg/ml for 30 minutes at 37 °C. Triggering of the basophils was stopped by incubating the cells on ice for 20 minutes. Histamine release was measured in the cell supernatants using an automated fluorimetric method⁵⁵ and calculated as percentage of total histamine. Soluble sulfidoleukotriene (sLT) was quantified in an RIA assay.^{56,57}

Hexosaminidase release from RBL-2H3-huα cells

The release of hexosaminidase from the cell line RBL-2H3E5.D12.8 was performed in a similar manner as described.^{58–60} Briefly, the cells were distributed to 24-well tissue culture plates (5 × 10⁴ cells in 0.5 ml of antibiotic-free complete Dulbecco's modified Eagle's medium (DMEM)/well). The medium was supplemented with 0.1 mM hydroxyurea to induce the expression of FcεRIα and the cells were cultured for two days. The medium was removed and cells were treated with 50 μg/ml of IgE Sus11 in prewarmed complete DMEM (100 μl/well) for 90 minutes at 37 °C. The cells were washed with Tyrode buffer (Sigma) and triggered with antibodies (at 1 μg/ml and 10 μg/ml, in 100 μl of Tyrode buffer) for 45 minutes at 37 °C. Secreted and intracellular hexosaminidase levels were measured as described.⁵⁸ The percentage of secreted hexosaminidase was calculated as the ratio of secreted to total available hexosaminidase × 100.

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References

- Takei, M., Dang, H., Wang, R. J. & Talal, N. (1988). Characteristics of a human monoclonal anti-Sm auto-antibody expressing an interspecies idiotype. *J. Immunol.* **140**, 3108–3113.
- Takei, M., Dang, H. & Talal, N. (1987). A common idiotype expressed on a murine anti-Sm monoclonal antibody and antibodies in SLE sera. *Clin. Expt. Immunol.* **70**, 546–554.
- Solomon, G., Schiffenbauer, J., Keiser, H. D. & Diamond, B. (1983). Use of monoclonal antibodies to identify shared idiotypes on human antibodies to native DNA from patients with systemic lupus erythematosus. *Proc. Natl Acad. Sci. USA*, **80**, 850–854.
- Morgan, A., Isenberg, D. A., Naparstek, Y., Rauch, J., Duggan, D., Khirya, R. *et al.* (1985). Shared idiotypes are expressed on mouse and human anti-DNA autoantibodies. *Immunology*, **56**, 393–399.
- Eilat, D., Fischel, R. & Zlotnick, A. (1985). A central anti-DNA idiotype in human and murine systemic lupus erythematosus. *Eur. J. Immunol.*, **15**, 368–375.
- Sigal, N. H., Chan, M., Reale, M. A., Moran, T., Beilin, Y., Schulman, J. L. & Bona, C. (1987). The human and murine influenza-specific B cell repertoires share a common idiotope. *J. Immunol.*, **139**, 1985–1990.
- Halpern, R., Kaveri, S. V. & Kohler, H. (1991). Human anti-phosphorylcholine antibodies share idiotopes and are self-binding. *J. Clin. Invest.*, **88**, 476–482.
- Amano, T., Nakazawa, M., Oshima, T., Bosshardt, S. C. & Colley, D. G. (1996). Cross-reactive idiotypes on rabbit anti-SEA antibodies stimulate anti-idiotype spleen and lymph node cell responses of mice infected with *Schistosoma mansoni*. *Parasite Immunol.* **18**, 21–28.
- Clofent-Sanchez, G., Laroche-Trainean, J., Lucas, S., Rispal, P., Pellegrin, J. L., Nurden, P. & Nurden, A. (1997). Incidence of anti-mouse antibodies in thrombocytopenic patients with autoimmune disorders. *Hum. Antibodies*, **8**, 50–59.
- Souza, A. R., Gesztes, J. L., del Negro, G. M., Benard, G., Sato, J., Santos, M. V. *et al.* (2000). Anti-idiotypic antibodies in patients with different clinical forms of paracoccidioidomycosis. *Clin. Diagn. Lab. Immunol.* **7**, 175–181.
- Tutter, A. & Riblet, R. (1989). Conservation of an immunoglobulin variable-region gene family indicates a specific, noncoding function. *Proc. Natl Acad. Sci. USA*, **86**, 7460–7464.
- Barre, S., Greenberg, A. S., Flajnik, M. F. & Chothia, C. (1994). Structural conservation of hypervariable regions in immunoglobulins evolution. *Nature Struct. Biol.* **1**, 915–920.
- Schroeder, H. W., Jr, Hillson, J. L. & Perlmutter, R. M. (1990). Structure and evolution of mammalian VH families. *Int. Immunol.* **2**, 41–50.
- Vogel, M., Miescher, S., Kuhn, S., Zurcher, A. W., Stadler, M. B., Ruf, C. *et al.* (2000). Mimicry of human IgE epitopes by anti-idiotypic antibodies. *J. Mol. Biol.* **298**, 729–735.
- Vogel, M., Miescher, S., Biaggi, C. & Stadler, B. M. (1994). Human anti-IgE antibodies by repertoire cloning. *Eur. J. Immunol.* **24**, 1200–1207.
- Marks, J. D., Hoogenboom, H. R., Bonnert, T. P., McCafferty, J., Griffiths, A. D. & Winter, G. (1991). By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J. Mol. Biol.* **222**, 581–597.
- Vaughan, T. J., Williams, A. J., Pritchard, K., Osbourn, J. K., Pope, A. R., Earnshaw, J. C. *et al.* (1996). Human antibodies with sub-nanomolar affinities isolated from a large non-immunized phage display library. *Nature Biotechnol.* **14**, 309–314.
- Nissim, A., Hoogenboom, H. R., Tomlinson, I. M.,

- Flynn, G., Midgley, C., Lane, D. & Winter, G. (1994). Antibody fragments from a "single pot" phage display library as immunochemical reagents. *EMBO J.* **13**, 692–698.
19. Hoogenboom, H. R. & Winter, G. (1992). By-passing immunisation. Human antibodies from synthetic repertoires of germline VH gene segments rearranged *in vitro*. *J. Mol. Biol.* **227**, 381–388.
20. Persic, L., Horn, I. R., Rybak, S., Cattaneo, A., Hoogenboom, H. R. & Bradbury, A. (1999). Single-chain variable fragments selected on the 57–76 p21Ras neutralising epitope from phage antibody libraries recognise the parental protein. *FEBS Letters*, **443**, 112–116.
21. Hoogenboom, H. R., Lutgerink, J. T., Pelsers, M. M., Rousch, M. J., Coote, J., Van Neer, N. *et al.* (1999). Selection-dominant and nonaccessible epitopes on cell-surface receptors revealed by cell-panning with a large phage antibody library. *Eur. J. Biochem.* **260**, 774–784.
22. Figini, M., Obici, L., Mezzanzanica, D., Griffiths, A., Colnaghi, M. I., Winter, G. & Canevari, S. (1998). Panning phage antibody libraries on cells: isolation of human Fab fragments against ovarian carcinoma using guided selection. *Cancer Res.* **58**, 991–996.
23. Watzka, H., Pfizenmaier, K. & Moosmayer, D. (1998). Guided selection of antibody fragments specific for human interferon gamma receptor 1 from a human VH- and VL-gene repertoire. *Immunotechnology*, **3**, 279–291.
24. Schmidt, A., Muller, D., Mersmann, M., Wuest, T., Gerlach, E., Garin-Chesa, P. *et al.* (2001). Generation of human high-affinity antibodies specific for the fibroblast activation protein by guided selection. *Eur. J. Biochem.* **268**, 1730–1738.
25. Mersmann, M., Schmidt, A., Rippmann, J. F., Wuest, T., Brocks, B., Rettig, W. J. *et al.* (2001). Human antibody derivatives against the fibroblast activation protein for tumor stroma targeting of carcinomas. *Int. J. Cancer*, **92**, 240–248.
26. Klimka, A., Matthey, B., Roovers, R. C., Barth, S., Arends, J. W., Engert, A. & Hoogenboom, H. R. (2000). Human anti-CD30 recombinant antibodies by guided phage antibody selection using cell panning. *Br. J. Cancer*, **83**, 252–260.
27. Beiboer, S. H., Reurs, A., Roovers, R. C., Arends, J. W., Whitelegg, N. R., Rees, A. R. & Hoogenboom, H. R. (2000). Guided selection of a pan carcinoma specific antibody reveals similar binding characteristics yet structural divergence between the original murine antibody and its human equivalent. *J. Mol. Biol.* **296**, 833–849.
28. Rader, C., Cheresch, D. A. & Barbas, C. F., 3rd (1998). A phage display approach for rapid antibody humanization: designed combinatorial V gene libraries. *Proc. Natl Acad. Sci. USA*, **95**, 8910–8915.
29. Jespers, L. S., Roberts, A., Mahler, S. M., Winter, G. & Hoogenboom, H. R. (1994). Guiding the selection of human antibodies from phage display repertoires to a single epitope of an antigen. *Biotechnology (NY)*, **12**, 899–903.
30. Figini, M. & Canevari, S. (2002). Isolation of human monoclonal antibodies using guided selection with mouse monoclonal antibodies. *Methods Mol. Biol.* **178**, 207–217.
31. Figini, M., Green, A., Colotta, F. & Canevari, S. (2003). Isolation of human Fab fragments against ovarian carcinoma using guided selection. *Methods Mol. Biol.* **207**, 145–159.
32. Osbourn, J. K. (2002). Proximity-guided (ProxiMol) antibody selection. *Methods Mol. Biol.* **178**, 201–205.
33. PE-Applied-Biosystems. Relative quantitation of gene expression. User Bulletin 2, 11–14.
34. Rudolf, M. P., Furukawa, K., Miescher, S., Vogel, M., Kricek, F. & Stadler, B. M. (1996). Effect of anti-IgE antibodies on Fc epsilonRI-bound IgE. *J. Immunol.* **157**, 5646–5652.
35. Clark, M. (2000). Antibody humanization: a case of the "Emperor's new clothes"? *Immunol. Today*, **21**, 397–402.
36. Figini, M., Marks, J. D., Winter, G. & Griffiths, A. D. (1994). *In vitro* assembly of repertoires of antibody chains on the surface of phage by renaturation. *J. Mol. Biol.* **239**, 68–78.
37. Barbas, C. F., 3rd, Kang, A. S., Lerner, R. A. & Benkovic, S. J. (1991). Assembly of combinatorial antibody libraries on phage surfaces: the gene III site. *Proc. Natl Acad. Sci. USA*, **88**, 7978–7982.
38. Chothia, C., Lesk, A. M., Gherardi, E., Tomlinson, I. M., Walter, G., Marks, J. D. *et al.* (1992). Structural repertoire of the human VH segments. *J. Mol. Biol.* **227**, 799–817.
39. Chothia, C., Lesk, A. M., Tramontano, A., Levitt, M., Smith-Gill, S. J., Air, G. *et al.* (1989). Conformations of immunoglobulin hypervariable regions. *Nature*, **342**, 877–883.
40. Andersson, E. & Matsunaga, T. (1995). Evolution of immunoglobulin heavy chain variable region genes: a VH family can last for 150–200 million years or longer. *Immunogenetics*, **41**, 18–28.
41. Ollio, R., Sikorav, J. L. & Rougeon, F. (1983). Structural relationships among mouse and human immunoglobulin VH genes in the subgroup III. *Nucl. Acids Res.* **11**, 7887–7897.
42. Dersimonian, H., Schwartz, R. S., Barrett, K. J. & Stollar, B. D. (1987). Relationship of human variable region heavy chain germ-line genes to genes encoding anti-DNA autoantibodies. *J. Immunol.* **139**, 2496–2501.
43. Shoenfeld, Y., Isenberg, D. A., Rauch, J., Madaio, M. P., Stollar, B. D. & Schwartz, R. S. (1983). Idiotypic cross-reactions of monoclonal human lupus autoantibodies. *J. Expt. Med.* **158**, 718–730.
44. Kutyavin, I. V., Afonina, I. A., Mills, A., Gorn, V. V., Lukhtanov, E. A., Belousov, E. S. *et al.* (2000). 3'-minor groove binder-DNA probes increase sequence specificity at PCR extension temperatures. *Nucl. Acids Res.* **28**, 655–661.
45. de Kok, J. B., Wiegeler, E. T., Giesendorf, B. A. & Swinkels, D. W. (2002). Rapid genotyping of single nucleotide polymorphisms using novel minor groove binding DNA oligonucleotides (MGB probes). *Hum. Mutat.* **19**, 554–559.
46. Knutti-Muller, J. M., Stadler, B. M., Magnusson, C. M. & de Weck, L. (1986). Human IgE synthesis *in vitro*. Detection with monoclonal antibodies. *Allergy*, **41**, 457–467.
47. Pachlopnik, J. M., Horn, M. P., Fux, M., Dahinden, M., Mandallaz, M., Schneeberger, D. *et al.* (2004). Natural anti-Fc epsilonRIalpha autoantibodies may interfere with diagnostic tests for autoimmune urticaria. *J. Autoimmun.* **22**, 43–51.
48. Nilsson, K., Bennich, H., Johansson, S. G. & Ponten, J. (1970). Established immunoglobulin producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient. *Clin. Expt. Immunol.* **7**, 477–489.
49. Watson, N., Bodtke, K., Coleman, R. A., Dent, G.,

- Morton, B. E., Ruhlmann, E. *et al.* (1997). Role of IgE in hyperresponsiveness induced by passive sensitization of human airways. *Am. J. Respir. Crit. Care Med.* **155**, 839–844.
50. Zurcher, A. W., Lang, A. B., Aebischer, I., Miescher, S. & Stadler, B. M. (1995). IgE-producing hybridomas established after B-cell culture in the CD40 system. *Immunol. Letters*, **46**, 49–57.
51. Riske, F., Hakimi, J., Mallamaci, M., Griffin, M., Pilson, B., Tobkes, N. *et al.* (1991). High affinity human IgE receptor (Fc epsilon RI). Analysis of functional domains of the alpha-subunit with monoclonal antibodies. *J. Biol. Chem.* **266**, 11245–11251.
52. Horn, M. P., Gerster, T., Ochensberger, B., Derer, T., Kricek, F., Jouvin, M. H. *et al.* (1999). Human anti-Fc epsilon RI alpha autoantibodies isolated from healthy donors cross-react with tetanus toxoid. *Eur. J. Immunol.* **29**, 1139–1148.
53. Brunner, T., Heusser, C. H. & Dahinden, C. A. (1993). Human peripheral blood basophils primed by interleukin 3 (IL-3) produce IL-4 in response to immunoglobulin E receptor stimulation. *J. Expt. Med.* **177**, 605–611.
54. Eglite, S., Pluss, K. & Dahinden, C. A. (2000). Requirements for C5a receptor-mediated IL-4 and IL-13 production and leukotriene C4 generation in human basophils. *J. Immunol.* **165**, 2183–2189.
55. Burgi, B., Otten, U. H., Ochensberger, B., Rihs, S., Heese, K., Ehrhard, P. B. *et al.* (1996). Basophil priming by neurotrophic factors. Activation through the trk receptor. *J. Immunol.* **157**, 5582–5588.
56. Kurimoto, Y., De Weck, A. L. & Dahinden, C. A. (1991). The effect of interleukin 3 upon IgE-dependent and IgE-independent basophil degranulation and leukotriene generation. *Eur. J. Immunol.* **21**, 361–368.
57. Bischoff, S. C., Brunner, T., De Weck, A. L. & Dahinden, C. A. (1990). Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. *J. Expt. Med.* **172**, 1577–1582.
58. Nechansky, A., Robertson, M. W., Albrecht, B. A., Apgar, J. R. & Kricek, F. (2001). Inhibition of antigen-induced mediator release from IgE-sensitized cells by a monoclonal anti-Fc epsilon RI alpha-chain receptor antibody: implications for the involvement of the membrane-proximal alpha-chain region in Fc epsilon RI-mediated cell activation. *J. Immunol.* **166**, 5979–5990.
59. Alber, G., Kent, U. M. & Metzger, H. (1992). Functional comparison of Fc epsilon RI, Fc gamma RII, and Fc gamma RIII in mast cells. *J. Immunol.* **149**, 2428–2436.
60. Roa, M., Paumet, F., Le Mao, J., David, B. & Blank, U. (1997). Involvement of the ras-like GTPase rab3d in RBL-2H3 mast cell exocytosis following stimulation via high affinity IgE receptors (Fc epsilon RI). *J. Immunol.* **159**, 2815–2823.
61. Martin, A. C. & Thornton, J. M. (1996). Structural families in loops of homologous proteins: automatic classification, modelling and application to antibodies. *J. Mol. Biol.* **263**, 800–815.

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