

Cloning of idiotype immunoglobulin genes in B cell lymphomas by anchored PCR and production of individual recombinant idiotype vaccines in *Escherichia coli*

Bertinetti C, Simon F, Zirlik K, Heining-Mikesch K, Pfeifer D, Osterroth F, Rosenthal FM, Veelken H. Cloning of idiotype immunoglobulin genes in B cell lymphomas by anchored PCR and production of individual recombinant idiotype vaccines in *Escherichia coli*.

Abstract: *Objectives:* Individual immunoglobulins expressed by B-cell lymphomas represent tumor-specific antigens ('idiotypes'). Immunization with idiotype in follicular lymphoma patients may induce specific immune responses, sustained progression-free survival, and disappearance of minimal residual disease. Manufacturing of idiotype vaccines has mostly relied on heterohybridomas established from viable lymphoma cells. This paper describes the feasibility of production of GMP-grade idiotype vaccines as recombinant Fab fragments in *Escherichia coli*. *Methods:* IgH and IgL transcripts were analyzed by anchored PCR from 106 lymphoma and nine control biopsies. Lymphoma-derived V segments were inserted into prokaryotic expression plasmids. Recombinant idiotype Fab fragments were expressed in *E. coli* in a fermentation system. *Results:* Idiotype IgH and IgL transcripts were identified in 95% of 106 lymphoma biopsies according to stringent clonality criteria. Large-scale idiotype expression was successful in 69 of 78 cases (89%) and yielded a median of 17 mg (range: 1.2–250 mg) recombinant Fab protein. After affinity chromatography, median vaccine purity was 99% heterodimeric Fab protein (range: 72–100%). Bacterial protein contamination was detectable in one vaccine only. Fab proteins with IgL lambda chains had a tendency for inferior yield and lesser purity than kappa Fabs. Among other structural idiotype features (isotype, V family usage, somatic hypermutation pattern, novel glycosylation sites, CDR III net charge), no consistent influences on Fab yield or purity were detected. *Conclusions:* Anchored PCR cloning and subsequent protein expression in *E. coli* provides a reliable technological basis for clinical idiotype vaccination trials.

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The vast majority of malignant lymphoma cells have undergone rearrangement of antigen receptor genes and express antigen receptor molecules intracellularly or on their surface. In addition, the hypervariable regions (idiotypes) of the immunoglobulins (Ig) expressed by lymphoma cells represent lymphoma-specific tumor antigens that may be target structures for anti-lymphoma immune responses themselves (1). Purified or recombinant

lymphoma-derived Igs may be formulated into a tumor-specific vaccine and administered to individual patients to elicit a therapeutic specific host immune response (2, 3).

In multiple myeloma and many cases of lymphoplasmacytic immunocytoma, secreted idiotype (Id) paraprotein can be purified conveniently from the blood of patients (4). In the case of non-secreting lymphomas, rescue of lymphoma cells by somatic

cell hybridization with a cultured hybrid mouse-human lymphoma cell line permits production of lymphoma Ig *in vitro* (5). However, this approach requires viable and sterile lymphoma cells as well as laborious and time-consuming cell culture techniques. To circumvent these limitations, molecular cloning of the Ig genes by PCR amplification with variable (V) region family-specific primers, followed by construction of individual expression vectors for single-chain Fv (scFv) fragments of the respective Id has been proposed for DNA vaccination (6). PCR-based cloning of Id genes can also be used to express recombinant Ig in appropriate cell lines (7). Since prokaryotic expression systems have the potential for substantially reduced production times, we decided to investigate the feasibility of an bacterial expression strategy for production of individual idiotypes as recombinant Fab fragments. This report defines stringent criteria for the identification of the idiotypic genes by clonality criteria in 106 biopsies with histologically proven B-cell lymphoma, and assesses the yield and purity of such vaccines in relationship to structural immunoglobulin features.

Materials and methods

Lymphoma biopsies

Biopsies with histologically or immunocytologically confirmed lymphoma involvement were obtained from 107 patients diagnosed with B-cell non-Hodgkin's lymphomas. When sufficient material was available, a single cell suspension was prepared by mechanical dissociation and analyzed by flow cytometry with FITC-labeled murine IgG1 antibodies to CD45, CD3, CD19, CD20 (BD Biosciences, San Jose, CA, USA), and FITC-labeled goat F(ab')₂ fragments against human Ig, and μ , γ , α , κ , and λ Ig chains (Biosource, Camarillo, CA, USA). Three non-lymphomatous control biopsies,

five biopsies from patients diagnosed with lymphoma but which lacked lymphoma infiltration by histopathology, and one of the 107 lymphoma biopsies that lacked Ig expression by flow cytometry were studied as controls (Table 1). The study was approved by the local ethics committee, and conducted according to the declaration of Helsinki. All patients gave informed consent.

Anchored PCR cloning of Ig transcripts

Total RNA was isolated from biopsy material by matrix affinity chromatography (Rneasy kit; Qiagen, Hilden, Germany). Oligo dT-primed cDNA was synthesized and extended with an oligo-dG tail as described (8). Ig transcripts were amplified with an oligo-dC-containing 'anchor' primer and nested primers specific for the constant region of the respective Ig class (Table 2) by a thermostable polymerase (ThermalAce; Invitrogen, Carlsbad, CA, USA). PCR products of the expected size were cloned into pCR-Blunt II-TOPO (Invitrogen) and sequenced.

Production of Fab fragments in *Escherichia coli*

Depending on the light chain restriction, lymphoma-derived Ig heavy and light variable sequences were inserted into the periplasmic expression plasmid pFab. $\gamma\kappa$ (8) or an analogous derivative encoding the human λ constant region, pFab. $\gamma\lambda$. For analytic expression, a single colony of transformed *E. coli* JM83 were grown in RM medium to an OD of 0.5 and induced with 0.1 mg/L anhydrotetracyclin for 20–24 h. For large-scale, GMP-grade production, *E. coli* were grown in a 14l fermenter (Biostat C, Sartorius, Göttingen, Germany), and Fab expression was induced at an OD of 15.

Periplasmic fractions were prepared by osmotic shock with 0.5 M sucrose and centrifugation. Recombinant Fab fragments were purified by ion

Table 1. Type of biopsy and diagnosis of 115 biopsies with Ig transcripts analysis by A-PCR

Entity	Total	LN excision	Needle biopsy	BM aspirate	Peripheral blood	Other
Follicular lymphoma (FL)	47 ¹	31	7 (all LN)	5	4	
Multiple myeloma (MM)	22			22		
Mantle cell lymphoma (MCL)	15	6		4	5	
CLL/lymphocytic lymphoma (LL)	11	2		3	6	
Diffuse large cell lymphoma (DLCL)	6	2	2 (liver, kidney)			2 (skin biopsies)
Burkitt's and Burkitt-like lymphoma (BL)	2	2				
Non-secreting immunocytoma (IC)	2	1		1		
Waldenstrom's immunocytoma	1			1		
High-grade marginal zone lymphoma (MZL)	1			1		
Total	107	44	9	37	15	2
Reactive/uninvolved	8	4		3		1 (paracentesis)

LN, lymph node; BM, Bone marrow.

¹One FL lacked Ig expression by flow cytometry and was analyzed as a control.

Table 2. Primer sequences used for PCR amplification of γ , α , and λ immunoglobulin transcripts

Target	Primer	5'-3' Sequence	Reaction
C γ	CGA1	5'-ACCTTGGTGTGCTGGGCTTG-3'	First A-PCR
	CGA2	5'-TGTGCACGCCGCTGG-3'	Second A-PCR
	CGA3	5'-ACACCGTCACCGGTTCC-3'	Third A-PCR
C α	CA1	5'-TGGCCGGCAGGGTCAGC-3'	First A-PCR
	CA2	5'-AAGTTTCTGGCGGTCAGC-3'	Second A-PCR
	CA3	5'-CAGCGGGAAGACCTTGG-3'	Third A-PCR
C λ	CLA 1b	5'-GCAGGGGCCACTGTCTTCTCC-3'	First A-PCR
	CLA 2a	5'-CGTGACCTGGCAGCTGTAG-3'	Second A-PCR
	CLA 2b	5'-AGGTAGCTGCTGGCCGC-3'	Third A-PCR
	VL 1S	5'-AGTCGTGCTAGCACTCTTAC CGTTACTGTTTACCCCGGTGACCAAGCC...-3'	Subcloning
	VL 1AS	5'-CTCCCGGT...-3'	Subcloning

Restriction sites are underlined (VL1S: NheI; VL1AS: Xma I). Primers for μ and κ transcripts were used as described (8).

..., sequence additions designed individually for every patient.

exchange chromatography (SP sepharose; Amersham Biosciences, Uppsala, Sweden) and subsequent affinity chromatography on Zn^{2+} -charged chelating sepharose (Amersham Biosciences) eluting at 180 mM imidazole.

The purity of recombinant Fab fragments was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Fab fragments with a purity of less than 99% were subjected to Western-blot analysis with a murine peroxidase-conjugated anti-hexahistidine antibody (Roche, Mannheim, Germany) and a phosphatase-conjugated goat anti- κ or anti- λ antibody (Sigma, St Louis, MO, USA). Visualized bands were quantitated densitometrically.

Sequence analyses

All sequences from any given Ig class within a biopsy were aligned by MegAlign software (DNA-STAR, Madison, WI, USA), and the number of identical sequences was determined. The V segment of any clonal, *bona fide* lymphoma-derived Ig transcript was identified as its closest match in the Vbase (<http://www.mrc-cpe.cam.ac.uk/vbase-ok.php>), BLAST (<http://www.ncbi.nlm.nih.gov/igblast>), and IMGT/V-QUEST (imgt.cines.fr) databases.

Statistical analyses

Statistical comparisons were performed with GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Correlations between isotype and lymphoma entities vs. mutation status of heavy and light chain genes, expression yields, and purities were analyzed with the two-tailed Mann-Whitney test. Distributions of glycosylation sites (present vs. absent, sites in CDR regions vs. sites in FR regions) were analyzed by Fisher's exact test for follicular lymphoma in comparison to all other lymphoma entities.

Results

Clonality analyses and identification of lymphoma-derived idiotype transcripts

The Ig isotype expressed by the malignant clone was identified by flow cytometry or immunofixation of serum (in MM and Waldenström's macroglobulinemia) for 82 biopsies with confirmed lymphoma involvement. Forty-six tumors expressed IgM, 30 IgG, and six IgA. In 81 of these cases, sequencing of numerous clones obtained by A-PCR of the identified isotype heavy chain yielded at least three identical sequences (Fig. 1A).

In 24 lymphoma biopsies in which the paucity of material did not permit identification of the expressed isotype *a priori* by flow cytometry (including all needle biopsies), isotype transcripts of both μ and γ class were amplified to identify a *bona fide* lymphoma-derived isotype. In 10 cases, no functional sequences were obtained for either μ or γ . The A-PCR for the second isotype yielded at least 88% identical sequences in nine of these cases. In the 10th case, a DLCL with renal involvement, the μ A-PCR product was composed of two different sequences, which represented five and six clones of a total of 11 sequences, respectively. In one additional case, a leukemic follicular lymphoma (FL, case A16), no functional sequences were obtained for either μ and γ . Therefore, α chain transcripts were analyzed, and all five sequences (100%) were identical and functional. In 13 cases without immunological identification of the expressed isotype, functional transcripts were present in μ and γ A-PCR products. In 11 of these biopsies, identification of the *bona fide* lymphoma transcript was unequivocal because of at least 50% identical sequences in one reaction and only polyclonal sequences in the other reaction. In one FL biopsy (A104), the μ reaction comprised only three identical of 12 clones (25% clonality), but the nine γ sequences obtained were polyclonal. In contrast, all

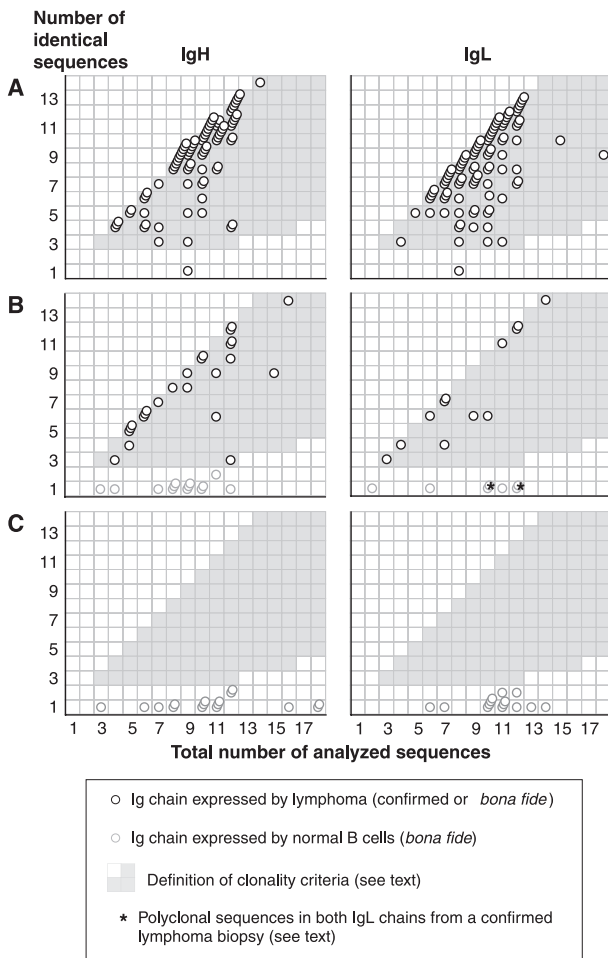


Fig. 1. Pseudo-three-dimensional dot plots depicting number of identical V_H sequences vs. number of total sequences for any cloned and sequenced isotype A-PCR product. Each circle represents one PCR product. (A) Biopsies with confirmed lymphoma involvement and with identification of the isotype expressed by the lymphoma prior to A-PCR by flow cytometry or immunofixation. Only the identified lymphoma isotype transcripts were amplified and cloned. (B) Biopsies with confirmed lymphoma involvement and without prior information on the isotype expressed by the lymphoma. Both μ and γ transcripts (in one case α), and IgL κ and λ were amplified and cloned if the PCR yielded sufficient product. (C) Biopsies without lymphoma infiltration or documented absence of Ig expression by lymphoma cells according to flow cytometry. IgH μ and γ , and IgL κ and λ transcripts were amplified and cloned if the PCR yielded sufficient product.

12 γ sequences were identical in the final FL biopsy (A170), but three different μ sequences were represented two to three times each in a total of 12 clones.

In the nine morphologically uninvolved control biopsies, most IgH sequences were polyclonal. In two cases, two identical sequences were observed among a total of 12 clones (Fig. 1C).

Similar results were obtained with respect to light chain analysis: In 92 of 93 biopsies with known

light chain restriction (53 κ and 40 λ), three or more identical sequences were obtained for the lymphoma-associated light chain (Fig. 1A). In 13 biopsies without data on light chain restriction, IgL transcripts of both types were analyzed. In 12 of these cases, *bona fide* clonal transcripts were clearly identifiable because of lack of PCR product or polyclonal sequences from the other light chain (Fig. 1B). In one case only, no repeat sequences were obtained for either IgL chain (marked with an "*" in Fig. 1B). In non-lymphoma biopsies, most sequences were polyclonal, and occasional repeat sequences were only observed when more than 10 sequences were analyzed (Fig. 1C).

In summary, the following difficulties in cloning the lymphoma Id transcripts were identified in this series:

- A low percentage of clonal, *bona fide* lymphoma-derived sequences in the absence of other repeat sequences. This scenario must be distinguished with sufficient certainty from serendipitous repeat sequences which occur with low frequency even in non-lymphoma controls and thus presumably represent oligoclonally expanded, albeit non-neoplastic B cells.
- A low percentage of repeat sequences in the presence of a distinct predominant *bona fide* lymphoma sequence. Here, the degrees of clonality must be sufficiently different to exclude misinterpretation of lymphoma Id and reactive B-cell clones.
- Presence of two dominant clonal sequences of similar frequency in the same A-PCR product. Apart from possible technical artifacts, this phenomenon may be because of a rare true biclonal lymphoma or functional rearrangement of both Ig alleles in a malignant B-cell clone with lack of allelic exclusion. In this scenario, identification of the expressed Id by sequence analysis alone will usually be impossible.
- Total lack of clonal sequences. This may occur even when the expressed isotype or light chain restriction is known through immunological tests. A possible explanation for this failure of the A-PCR approach in rare cases are mutations or polymorphisms in the primer sites.

Given these occasional difficulties in identifying a lymphoma Id by sequence analysis, clonality criteria were defined to minimize the likelihood of mistakenly interpreting occasional repeat sequences as a *bona fide* idiotype sequence, and to exclude cases with biallelic transcription of the same Ig class:

- If the lymphoma isotype and/or light chain restriction are identified immunologically, at

least three identical clones of the respective transcript class must be present and must represent at least 25% of all analyzed sequences. In addition, no other repeated sequences must be present.

- (b) If no prior information of the lymphoma Id class is available, μ and γ transcripts are cloned for IgH (and α transcripts if both μ and γ are non-clonal), and κ and λ transcripts for IgL. If no repeat sequences are present in the *bona fide* non-clonal isotype/IgL PCR product, the *bona fide* lymphoma-derived transcript must represent at least three identical clones of the respective transcript class and at least 50% of all analyzed sequences. If, however, repeat sequences are present in the *bona fide* non-lymphoma-derived transcript class, they must not exceed 30% and are acceptable only if the clonal transcript represents at least 90% of all sequences.

According to these clonality criteria, sequences for both IgH and IgL Id chains could be identified in 101 of 106 lymphoma cases (95.3%).

Idiotype expression as Fab fragments in *Escherichia coli*

After insertion of lymphoma derived idiotype V segments into the appropriate expression vector, large-scale production of recombinant idiotype Fab protein was attempted in 78 lymphoma cases who fulfilled inclusion criteria for clinical idiotype vaccination trials at Freiburg University (9) but who were otherwise unselected. After expression in a 14 l-fermentation system under a GMP-compatible standard protocol, the yield of recombinant idiotype was highly variable (Fig. 2). Nine cases (11%) failed to yield sufficient amounts of protein (<1.0 mg) for the subsequent purification steps;

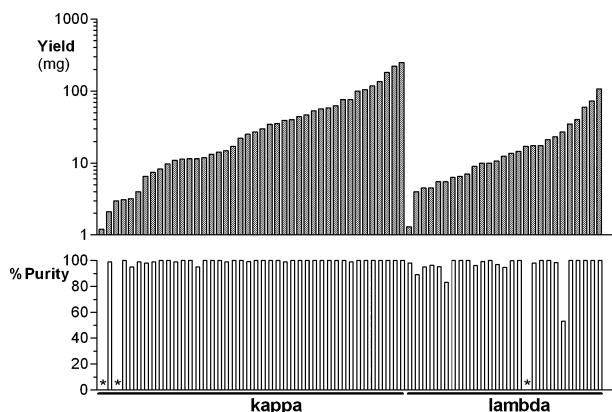


Fig. 2. Yield of recombinant Fab protein after expression in *Escherichia coli* (upper panel) and purity as assessed by SDS-PAGE under reducing conditions (lower panel). Cases are grouped according to Ig light chain type and ordered according to yield. Asterisks: not performed.

the range of protein yield in the remaining 69 cases was 1.2–220 mg with a median of 17.0 mg in a single fermentation run. Multiple myelomas had a trend for higher Fab yields than FL ($P = 0.053$), CLL/LL ($P = 0.045$), or all other NHL types combined ($P = 0.037$) (Fig. 3A).

The median content of complete Fab fragment in the final recombinant idiotype vaccine as assessed by SDS-PAGE was 99% (range: 23–100%) under non-reducing and 100% (range: 53–100%) under reducing conditions. Among the seven vaccines in which full-size Fab fragment amounted to less than 95% by SDS-PAGE under non-reducing conditions, Western-blot analysis revealed contamination of only one vaccine (A129) by 17% host cell protein (Fig. 4). In all other cases (example: A88), the protein bands other than full-size Fab fragment represented truncated Ig fragments (Fig. 4).

Influence of structural idiotype features on Fab yield and purity

To identify factors that affect the production of a given recombinant Id Fab fragment, various

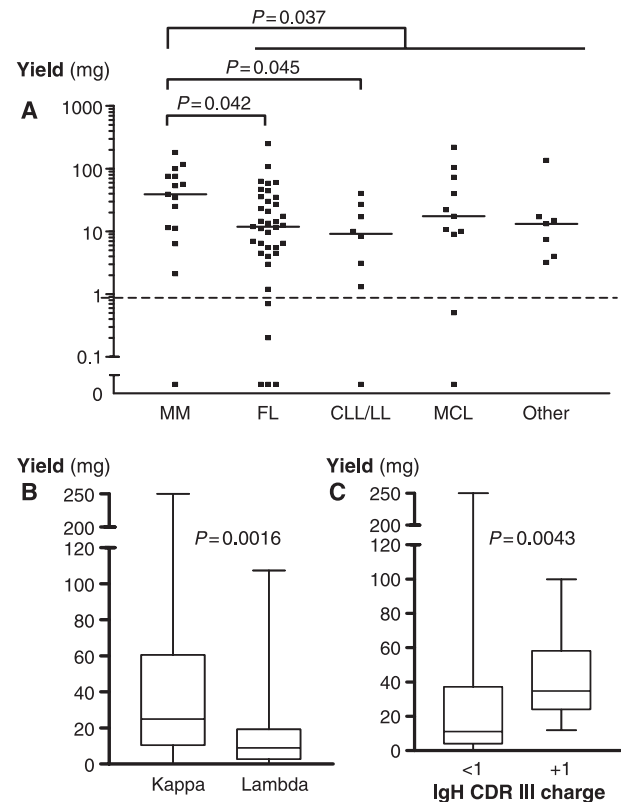


Fig. 3. Influence of selected parameters on Fab protein yield. (A) Fab yield according to lymphoma type. The dashed line represents the lowest yield permitting idiotype purification. Solid lines represent medians. (B) Fab yields according to IgL type. Boxes represent medians, 25th and 75th percentiles. Whiskers represent range. (C) Fab yields according to net charge of the IgH CDR III region. Boxes represent medians, 25th and 75th percentiles. Whiskers represent range.

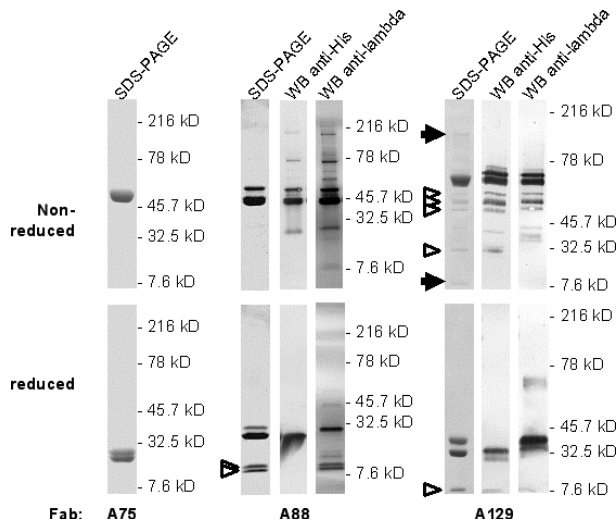


Fig. 4. Representative purity assessments of recombinant idiotype Fab fragments (cases A75, A88, and A109) by SDS-PAGE. Upper panel: Non-reducing conditions. Lower panel: Reducing conditions. SDS-PAGE analyses were performed with silver staining. Western-blot analyses were performed on replica SDS-polyacrylamide gels with an anti-hexahistidine or anti-lambda antibody. Case A75 is a typical example for idiotype vaccines with only full-length Fab fragment and full-length heavy and light chain detectable by SDS-PAGE (purity 100%). Case A88 has the highest presence of variant products by SDS-PAGE (purity 53% under reducing conditions) among all Fab products analyzed. However, all aberrant bands (white triangles) are immunoreactive by Western analysis and thus represent product-related impurities. Case A129 is the only case with non-immunoreactive impurities (black arrowheads).

parameters were analyzed for association with Fab yield and purity as continuous variables. Fabs with κ IgL chain had higher yields than λ -type Fabs (median 46 mg vs. 16 mg; $P = 0.0016$; Fig. 3B). In contrast, the lymphoma isotype and the mutation status of the IgV_H segment had no apparent influence on Fab expression (IgM vs. IgA/G: $P = 0.56$; mutated vs. unmutated: $P = 0.81$). With respect to V gene usage, Fabs containing V κ III-A27 had a trend toward superior expression yields ($P = 0.02$). Otherwise, no other over-represented V gene nor any particular V_H or V_L family was associated with superior or inferior expression levels.

Acquisition of novel glycosylation sites within lymphoma Id V segments through somatic hypermutation may have a potentially important impact on Fab expression levels. In accordance to the original observation (10), this phenomenon was observed predominantly in FL: 27 of 37 FL cases had acquired a total of 50 amino acid residues that could undergo N-linked glycosylation, whereas 36 of 41 other lymphomas lacked such sites ($P < 0.0001$). Compared with non-FL cases, where four of a total of six novel glycosylation sites were

located in FR regions, the position of such sites was strongly biased toward CDR regions in FL (40 of 50 sites; $P < 0.03$). Nevertheless, the presence of novel glycosylation sites had no influence on the yield of recombinant Id vaccines ($P = 0.52$).

Lastly, we searched for influences of net charges on Fab yields. A positively charged IgH CDR III region appeared to positively influence the Fab yield (Fig. 3C; $P = 0.0043$). However, no significant association was detectable when the combined IgH and IgL CDR III charge was considered (data not shown).

When the influence of all structural features of the idiotype on the final purity was analyzed (Fig. 2), IgL λ chain was associated with a slightly lesser degree of purity than IgL κ chain (median 99.8% vs. 100% under reducing conditions, $P < 0.03$).

Discussion

This study was undertaken to investigate a strategy relying on A-PCR and bacterial expression for wide-spread application as a method for production of individual idiotype vaccines. According to clonality criteria defined to avoid misinterpretation of occasional repeat sequences derived from non-malignant B cells in a given biopsy, the A-PCR strategy permitted successful cloning of both IgH and IgL Id chains in 95.3% of biopsies involved by malignant lymphoma. Idiotype identification failed in four biopsies because of lack of molecular clonality. Possible reasons are insufficient lymphoma infiltration in the biopsy or potential failure of primer binding to the C regions of the Ig transcripts. In addition, the expressed idiotype could not be identified in one patient because of true biclonality or biallelic expression of functional transcripts. Whereas the success rates for cloning of full-length Id IgL genes with conventional PCR protocols has rarely been addressed in B-NHL, the success rate of our approach for identification of IgH Id genes compares favorably with rates of 45–85% reported with primer pairs annealing to the 5' terminus of V_H segments and to the C or J regions (11–14). In fact, we have now adopted A-PCR as the routine diagnostic method to analyze the mutation status of Ig genes in CLL as a prognostic marker. In an independent series of 62 CLL cases with IgL chain restriction, we obtained clonal IgV_H sequences in 61 cases (98.4%; D. Pfeifer and H. Veelken, unpublished).

Expression of recombinant Id Fab fragments in *E. coli* yielded sufficient protein for vaccination in 89% of cases. Repeated fermentation or further scale-up with a larger fermentation system would allow higher yields with relative ease for cases with

poor expression levels. The trend for better Fab yields in MM compared with other NHL types may simply reflect the restriction of cases to myelomas secreting complete Ig paraproteins. This MM subgroup is probably biased for Ids whose IgH and IgL chains easily attain functional conformation and display good solubility in aqueous solutions. These properties may also favor expression in *E. coli*, which is dependent on correct protein folding. On the other hand, no particular lymphoma type was associated with consistently inferior expression levels. Therefore, our strategy is applicable to every major lymphoma entity, whereas the classical somatic hybridization approach may be difficult to apply to non-follicular NHL (15).

The adverse influence of λ -type IgL on Fab yields and homogeneity could be because of the existence of four functional C λ genes as opposed to only one C κ gene. While the influence of the C λ sequence is currently under investigation, the purity of the Id vaccine could be readily improved with additional purification steps: for example, the C γ 1 domain common to all Fab fragments in this format permits affinity chromatography with protein G (8). Furthermore, it appears unlikely that presence of aberrant or truncated Ig fragments will have important effects on the efficacy as an Id vaccine. Vaccine impurities because of host cell protein is a far more serious concern with respect to GMP criteria, but such contaminations were detectable in one vaccine only (1.5%). All other structural idiotype features analyzed, V gene usage, mutation status, glycosylation pattern, and charge, had only marginal effects on Fab yields and purities.

The high frequency of potential glycosylation sites in the idiotypes of FL, which will not be glycosylated in *E. coli*, and their presumed significance for FL pathogenesis merits further investigation with respect to the use this type of vaccine for active immunotherapy. A non-glycosylated vaccine may fail to induce therapeutically important anti-Id antibodies *in vivo* if the surface Id on FL cells were indeed actually glycosylated. However, absence of N-glycosylation enhances the immunogenicity of MHC-restricted peptides in certain experimental systems (16, 17). Therefore, it appears possible that recombinant Id vaccines produced in prokaryotes might be superior in comparison to Id secreted by eukaryotic cells for the induction of lymphoma-specific T-cell responses. Indeed, T cells from lymphoma patients exhibited specific lytic activity against Id-expressing target cells when stimulated *in vitro* with recombinant idiotype produced by the method evaluated here (1). Furthermore, intradermal vaccination with these Fab fragments in

conjunction with GM-CSF induced idiotype-specific cellular immune responses in advanced lymphoma patients (9).

In summary, A-PCR cloning of Ig transcripts has a high success rate for the identification and analysis of Id genes in all major types of B-cell lymphoma. A-PCR with expression of idiotype protein in *E. coli* as a recombinant Fab fragment provides a solid basis for production of individual Id vaccines of all major IgH and IgL classes. The short culture time required for Id expression in bacteria in comparison to eukaryotic cell cultures may facilitate clinical trials and reduce production costs. Based on the reproducibility of the expression strategy and the high degree of purity of the recombinant Id protein, a production license has been issued by the Competent Authorities.

Acknowledgements

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