RESEARCH ARTICLE

Identification of two aberrant transcripts derived from a hybridoma with amplification of functional immunoglobulin variable genes

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Murine monoclonal antibodies (mAbs) are widely used but have limitations if administered in humans. The use of chimeric or humanized mAbs can reduce immunogenicity. The first step in producing such mAbs is to clone murine variable genes from a hybridoma, but it is possible to amplify both functional and aberrant variable genes, as they coexist in the hybridoma. During the development of a murine–human chimeric antibody, we have cloned from a hybridoma the functional heavy chain variable region (V_H) and light chain variable region (V_L) genes of a mAb that blocks the binding of anthrax lethal factor to protective antigen. In this study, we report the detection of two aberrant transcripts from a hybridoma produced using myeloma cell line OUR-1, the development of a method to distinguish between the functional and abundant aberrant V_L transcripts, and the origins of these aberrant genes. The aberrant V_L gene is derived from OUR-1 cells, while the aberrant V_H gene might derive from antibody repertoires in B cells or from gene rearrangement in the hybridoma cells. The aberrant V_H and V_L genes in this study may facilitate discrimination between the functional and aberrant variable genes from hybridoma cells.

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INTRODUCTION

Hybridoma technology is widely used to produce monoclonal antibodies (mAbs) by fusion of murine B cells and myeloma cells. The medical applications of mAbs are numerous in both fundamental research and clinical diagnosis.¹ Murine mAbs administered into the human body may result in an immune response to the foreign immunoglobulin epitopes, mainly because of the development of a human anti-mouse antibody response.² Therefore, it is necessary to develop mAbs with low immunogenicity. There are some solutions to this problem, such as generating chimeric, humanized or human mAbs.³ Murine mAbs have been modified by genetic engineering into chimeric or humanized antibodies, which have a high proportion of human components and the original target specificity of the murine precursors.⁴ Furthermore, human mAbs without murine components have been raised by other approaches including phage display, transgenic mice and human–human hybridomas.⁵

The first step in generating a chimeric or humanized mAb is to clone the murine variable genes from hybridoma cells. This is complicated if there are any aberrant variable genes similar to the functional ones in the cells. It has been reported that the myeloma cell line P3-X63-Ag8 (derived from clone MOPC21 and used in early hybridoma technology) secretes immunoglobulin, while its sublines, such as Sp2/0, NS-0, OUR-1 and P3-X63-Ag8.653, are considered non-immunoglobulin-secreting fusion partners.⁶ It is well known that most myel-

oma fusion partners still contain a large amount of aberrant kappa chain transcripts with a frame shift at the beginning of the joining region. This transcript cannot be translated into a fully functional immunoglobulin due to a non-functional rearrangement, resulting in a premature termination codon.⁷

Several strategies have been applied to overcome the problem of aberrant light chain variable region (V_L) gene. First, if the light chain peptide was sequenced before the gene-specific primers were designed, the aberrant gene would not be amplified at all.⁸ Second, Nicholls *et al.*⁹ generated single-chain variable fragment clones by expression in a rabbit reticulocyte lysate-based, coupled transcription–translation system *in vitro*, and distinguished between the functional and aberrant V_L genes on the basis of the sizes of the proteins produced. Third, a peptide nucleic acid clamp specific for the aberrant V_L gene during reverse transcription PCR was applied to suppress the amplification of the aberrant one.¹⁰ Lastly, enzymes have been used to treat the aberrant mRNA or DNA: an antisense-directed RNase H digesting the aberrant V_L mRNA was used when antibody variable genes were cloned from a hybridoma;¹¹ a ribozyme specific for the aberrant V_L gene was developed and packaged in a retroviral expression vector system to eliminate the endogenous aberrant V_L mRNA;¹² several restriction endonucleases cutting the aberrant V_L products rather than the functional ones were applied after PCR amplification of the antibody variable genes.¹³

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In contrast to the aberrant V_L transcript, aberrant heavy chain variable region (V_H) genes are less often reported but are more diverse and complicated, because more than one V_H aberrant transcript may exist in a single hybridoma.¹⁴ Furthermore, some aberrant V_H genes have no stop codon in the reading frame of V_H , which may easily be confused with the functional genes.^{15,16} For these reasons, it is hard to discriminate between the functional and non-functional V_H genes, and much more difficult to avoid cloning the aberrant ones before the functional sequences are clarified. Two solutions have been proposed for this problem. One is to use phage display technology and selective panning against the antigen to enrich the functional single-chain variable fragment clones,¹⁷ but it is laborious with low efficiency. The other uses multiplex PCR screening and specific primers for the aberrant CDR3 to isolate the aberrant V_H gene, but this may not work if the aberrant sequences still remain unknown.¹⁸ In fact, because of the diversity and unpredictability of the aberrant V_H genes, those two methods are not effective and rarely used.

In this study, during the development of a murine-human chimeric antibody, the functional V_H and V_L genes were cloned from the hybridoma produced using the myeloma cell line OUR-1, and two aberrant V_H and V_L transcripts were also found. Repeated use of restriction endonuclease *Bci*VI on PCR product and then again on TA plasmids was developed to distinguish between the functional and abundant aberrant V_L transcripts. The origins of these two aberrant genes were identified. The aberrant V_L gene was confirmed as arising from OUR-1 cells, while the aberrant V_H gene may derive from antibody repertoires in the B cells or from gene rearrangement in the hybridoma cells. The method used to identify the aberrant genes may facilitate the cloning of functional antibody variable genes and avoid the amplification of aberrant ones.

MATERIALS AND METHODS

Cell lines

The murine myeloma cell lines Sp2/0 and OUR-1 were maintained in our lab. A murine hybridoma cell line secreting antibody against anthrax lethal factor (named LF8) was developed by fusion of B cells and OUR-1 cells, as described previously.¹⁹ Sp2/0, OUR-1 and LF8 cells were all cultured in OPTI-MEM I supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA, USA). The cells were maintained at 37 °C in a humidified 5% CO₂ incubator.

RNA isolation and cDNA synthesis

Total RNA was isolated from LF8 cells by TRIzol reagent (Invitrogen), and cDNA was synthesized using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer's protocols. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was amplified as an internal control (5'-ATGGTGAAGGTCGGTGT-3' and 5'-TTCACACCCATCA-CAAAC-3'). The cDNAs from myeloma cell lines OUR-1 and Sp2/0 were obtained as well.

PCR amplification of V_H and V_L genes from framework region (FR) 1 to FR4

The murine V_H and V_L genes were amplified from LF8 cDNA by two approaches. The first primer sets and conditions (described by Andris-Widhopf *et al.*²⁰) were used to amplify fragments from FR1 to FR4. An unequal mixture of 19 primers served as the V_H sense primer and an equal mixture of four primers as the antisense one. For V_L amplification, 17 and 3 primers were used as sense and antisense primers, respectively. PCR reactions were performed in a volume of 100 µl with 2 µl cDNA and 1 µl Taq DNA polymerase (Invitrogen). PCR was

carried out for 30 cycles in a PTC-200 thermal cycler (MJ Research, Watertown, MA, USA) with 30 s denaturing (94 °C), 30 s annealing (56 °C), 1 min extension (72 °C) and a final extension of 10 min (72 °C). The sizes of the PCR products were verified by gel electrophoresis in a 1% Tris-acetate-EDTA agarose gel stained with ethidium bromide.

The PCR product of V_H was directly TA cloned by ligation of purified PCR product with a pGEM-T Easy vector (Promega, Madison, WI, USA). For V_L , the aberrant gene has a *Bci*VI site while the functional one does not. Therefore, the PCR product of V_L was digested at 37 °C overnight by the restriction endonuclease *Bci*VI (New England Biolabs, Ipswich, MA, USA) and only non-cut fragments were gel extracted prior to ligation to a pGEM-T Easy vector. TA plasmids containing V_L were verified by digestion again with *Bci*VI before sequencing. As there are two *Bci*VI sites in the pGEM-T Easy vector, the TA plasmids inserted with functional V_L would be cut into two fragments, whereas the TA plasmids containing aberrant V_L would be cut into three.

PCR amplification of V_H and V_L genes from signal sequence to FR4

The second primer sets and conditions (according to Coloma *et al.*²¹) were used to amplify the fragments from the signal sequence to FR4. Three V_H sense primers (named MHALT1 to MHALT3) were mixed equally or applied individually with the same V_H antisense primer. Correspondingly, five V_L sense primers (named MLALT1 to MLALT5) were mixed equally or applied individually with the same V_L antisense primer. PCR was carried out for 30 cycles with annealing at 60 °C. Purified PCR products were cloned into a pGEM-T Easy Vector directly as mentioned above.

Sequence analysis

TA plasmids containing inserted murine V_H and V_L genes were sequenced using an ABI 3700-capillary electrophoresis DNA sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were assembled and assessed for functional translation using DNAMAN software (version 4.0; Lynnon Corporation, Pointe-Claire, Que., Canada) and were analyzed in VBASE2 database (<http://www.vbase2.org>) and GenBank database using BLAST (<http://blast.ncbi.nlm.nih.gov>). Accordingly, aberrant genes with the corresponding sense and antisense primers were identified, as well as the functional genes with the corresponding primers.

Identification of the origins of aberrant variable genes

Aberrant variable gene could arise from an unproductive transcript in myeloma cells, a rearranged transcript in the B cells, or a gene mutation in the fusion process of the two kinds of cells. To identify the origins of the two aberrant genes in this study, variable genes were also amplified using cDNA synthesized from the myeloma cell lines OUR-1 and Sp2/0. According to the sequencing results, sense primers for the functional V_H (MHALT1) and the aberrant V_H (MHALT3) were used in V_H amplification while sense primers for the functional V_L (MLALT2) and the aberrant V_L (MLALT1) were used in V_L amplification. PCR products were sequenced after TA cloning.

RESULTS

Amplification of V_H and V_L genes from FR1 to FR4

Murine V_H and V_L genes were amplified using cDNA from LF8 hybridoma cells as the template, and the primer sets and conditions described by Andris-Widhopf *et al.* GAPDH was amplified as an internal control. Agarose gel electrophoresis demonstrated that the

Figure 1 Agarose gel electrophoresis of PCR products of variable genes. Murine V_H and V_L genes were amplified from FR1 to FR4 by reverse transcription PCR from hybridoma cells. GAPDH was an internal control. Lane M: DNA marker; lane 1: GAPDH; lane 2: V_H ; lane 3: V_L . FR, framework region; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

PCR products were about 380 bp (V_H), 370 bp (V_L) and 400 bp (GAPDH), as expected (Figure 1).

Six TA plasmids containing V_H were sequenced and all inserts were the same which had the functional V gene features. For V_L , purified PCR product was digested by *Bci*VI to isolate the functional gene. After incubation overnight, most fragments were cut, forming two bands of approximately 180 and 190 bp (Figure 2). Non-cut product was rare. Eight TA plasmids containing V_L were digested again by *Bci*VI. Figure 3 shows that only three of eight were cut into two fragments, indicating no cut in V_L . Sequencing analysis indicated that those three inserts were the same which had the functional V gene features. The other five plasmids cut into three fragments were sequenced and proven to carry the same aberrant gene.

Amplification of V_H and V_L genes from signal sequence to FR4

Using the primer sets and conditions according to Coloma *et al.*, the expected PCR products were about 400 bp. As shown in Figure 4, V_H genes were successfully amplified by PCR using the sense primers mixture, MHALT1 and MHALT3 with the antisense primer, respectively. No product was obtained with sense primer MHALT2. The sequencing results identified that MHALT1-amplified V_H had the functional V gene features, and that MHALT3-amplified V_H was an aberrant one (named abVH-LF8, with Genbank accession no. HM046413). Similarly, V_L genes were successfully amplified by PCR using the sense primers mixture, MLALT1 and MLALT2 with the antisense primer. No product was obtained with sense primers MLALT3 to MLALT5 (Figure 5). The sequencing results showed that MLALT2-amplified V_L had the functional V gene features, and that MLALT1-amplified V_L was an aberrant one (named abVL-LF8) which was identical to the aberrant V_L gene amplified from FR1 to FR4, except for an upstream signal sequence.

Figure 2 Agarose gel electrophoresis of the V_L PCR product before and after *Bci*VI digestion. Purified PCR product of the V_L gene was digested by *Bci*VI to isolate the functional V_L gene. After incubation overnight at 37 °C, most of the product was cut to form two bands of approximately 180 and 190 bp. Non-cut products were rare.

Figure 3 Agarose gel electrophoresis of eight TA plasmids containing V_L digested again by *Bci*VI. Eight TA plasmids containing V_L were digested by *Bci*VI. Three (nos. 2, 5 and 7) were cut into two fragments, indicating no cut in V_L (functional V_L gene). Five plasmids (nos. 1, 3, 4, 6 and 8) cut into three fragments carried the same aberrant gene.

Figure 4 Agarose gel electrophoresis of PCR products of V_H genes using different sense primers. Murine V_H genes were amplified from signal sequence to FR4 by reverse transcription PCR using different sense primers and the same antisense primer. Lane M: DNA marker; lane 1: mixture of three MHALT primers; lane 2: MHALT1; lane 3: MHALT2; lane 4: MHALT3. FR, framework region.

Sequencing analysis

The V_H and V_L genes of LF8 with functional V gene features were cloned into eukaryotic vectors to express chimeric immunoglobulin G (IgG) against lethal factor according to the method of Roque-Navarro *et al.*²² Chimeric IgG protein expression (both heavy and light chains) was confirmed by ELISA and SDS-polyacrylamide gel electrophoresis. Binding of the chimeric IgG to lethal factor was demonstrated by ELISA and confirmed the preservation of the antigen-binding specificity of the parental antibody LF8 (data not shown). The functional variable genes of LF8 were registered in GenBank with accession nos. GU290193 and GU290194.

According to analysis of the GenBank database using BLAST, the abVL-LF8 gene showed about 97% homology to the well-known MOPC21 V_L aberrant gene derived from myeloma cell line P3-X63-Ag8.653 (GenBank accession no. M35669). The abVH-LF8, if truncated the upstream signal sequence, was homologous to several aberrant V_H genes released in GenBank: 98 and 96% homology to aberrant V_H genes derived from fusion partner P3-X63-Ag8.653 (GenBank accession nos. EU121635 and X58634, respectively); 96% homology to an aberrant V_H genes derived from fusion partner P3U1 (GenBank accession no. D50398). The main differences located at 5' and 3' ends, and all genes had no upstream signal sequence reported except the abVH-LF8. Interestingly, the abVH-LF8 had 94% identity to a

Figure 5 Agarose gel electrophoresis of PCR products of V_L genes using different sense primers. Murine V_L genes were amplified from signal sequence to FR4 by reverse transcript PCR using different sense primers and the same antisense primer. Lane M: DNA marker; lane 1: mixture of five MLALT primers; lane 2: MLALT1; lane 3: MLALT2; lane 4: MLALT3; lane 5: MLALT4; lane 6: MLALT5. FR, framework region.

functional V_H gene (GenBank accession no. U60820) with a few different nucleotides, especially an inserted 'A' in CDR3 (at 365 bp in abVH-LF8). The abVH-LF8 had 82% identity to another functional V_H gene derived from the hybridoma generated using myeloma cell line Sp2/0 (GenBank accession no. AY646832), with an inserted 'G' (at 363 bp in abVH-LF8) and 21 base pairs deleted in CDR3. The inserted 'A' and 'G' were confirmed by several sequencing results and these two inserts also showed up in all aberrant V_H genes listed above. Alignment of the DNA sequences of the abVH-LF8 is shown in Figure 6 with the most homologous aberrant V_H gene (GenBank accession no. EU121635) and two functional ones.

Identification of the origins of aberrant variable genes

Variable genes were also amplified using cDNA synthesized from the myeloma cell lines OUR-1 and Sp2/0 to identify the origins of the two

aberrant genes in this study. MHALT1 and MHALT3 with corresponding antisense primer were used in V_H amplification. MLALT1 and MLALT2 with corresponding antisense primer were used in V_L amplification. As shown in Figure 7, the functional V_H and V_L genes were only in the LF8 cells, while the aberrant V_L genes were in OUR-1, LF8 and Sp2/0 cells. According to sequencing results, the aberrant V_L genes in OUR-1 and Sp2/0 were identical to abVL-LF8 in LF8 cells. However, abVH-LF8 was only found in LF8 cells, not OUR-1 or Sp2/0 cells.

DISCUSSION

Today, fully human antibodies are desired for application in humans. However, there are difficulties, because it is generally not ethical to immunize humans with antigen in order to produce a human antibody. In addition, the lack of a human cell line that has high fusion efficiency and that does not secret immunoglobulin has hindered the

abVH-LF8	<u>ATGGCTGTCTGGGGCTGCTCTTCTGCCTGGTGACTTTCCCAAGCTGTGCTCTGTCCAG</u>	60
EU121635	-----GAG	3
U60820	<u>ATGGCTGTCTGGGGCTGCTCTTCTGCCTGGTGACTTTACCAAACTGTGCTCTGTCCAG</u>	60
AY646832	<u>ATGGCTGGTTTGGGGCTGCTCTTCTGCCTGGTTGCATTTCGAAGCTGTGCTCTGTCCAG</u>	60

abVH-LF8	GTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACA	120
EU121635	GTGCAGCTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACA	63
U60820	GTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACA	120
AY646832	GTGCAGTTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACT	120
	***** **	
abVH-LF8	TGCACCGTCTCAGGGTTCTCATTAAGTATAGTATGGTGTAAGTGGTTTCGCCAGCCTCCA	180
EU121635	TGCACCGTCTCAGGGTTCTCATTAAGTATAGTATGGTGTAAGTGGTTTCGCCAGCCTCCA	123
U60820	TGCACCGTCTCAGGGTTCTCATTAAGTATAGTATGGTGTAAGTGGTTTCGCCAGCCTCCA	180
AY646832	TGCACTGTCTCTGGATTTTCATTAACCAATATGGTTTACACTGGGTTTCGCCAGCCTCCA	180
	***** **	
abVH-LF8	GGAAAGGGTCTGGAGTGGCTGGTAGTGATATGGAGTGATGGAAGCACAACTATAATTCA	240
EU121635	GGAAAGGGTCTGGAGTGGCTGGTAGTGATATGGAGTGATGGAAGCACAACTATAATTCA	183
U60820	GGAAAGGGTCTGGAGTGGCTGGTAGTGATATGGAGTGATGGAAGCACAACTATAATTCA	240
AY646832	GGAAAGGGTCTGGAGTGGCTGGGAGTAATATGGGCTGGTGGAGCACAAATATAATTCTG	240
	***** **	
abVH-LF8	GCTCTCAAATCCAGACTGAGCATCAGCAAGGACAACCTCCAAGGGCCAAGTTTCTTAAAA	300
EU121635	GCTCTCAAATCCAGACTGAGCATCAGCAAGGACAACCTCCAAGAGCCAAGTTTCTTAAAA	243
U60820	GCTCTCAAATCCAGACTGAGCATCAGCAAGGACAACCTCCAAGAGCCAAGTTTCTTAAAA	300
AY646832	GCTCTCATGTCCAGACTGAGCATCAACAAAGACAACCTCCAAGAGCCAAGTTTCTTAAAA	300
	***** **	
abVH-LF8	ATGAACAGTCTCCAACTGATGACACAGCCATGTACTACTGTGCCAGAGAGCCTCCACG	360
EU121635	ATGAACAGTCTCCAACTGATGACACAGCCATGTACTACTGTGCCAGAGAGCCTCCACG	303
U60820	ATGAACAGTCTCCAACTGATGACACAGCCATGTACTACTGTGCCAGAGAGCCTCCACG	360
AY646832	ATGAAAAGTCTGCAACTGATGACACAGCCATGTACTACTGTGCCAGAGAGCCCCAATTC	360
	***** **	
abVH-LF8	ACGTACGTTTGCT-----TACTGGGGCCAAGGGACTCTGGTCAC	399
EU121635	ACGTACGTTTGCT-----TACTGGGGCCAAGGGACTCTGGTCAC	342
U60820	<u>ACGT-CGTTTGCT-----TACTGGGGCCAAGGCACCACTGTTAC</u>	398
AY646832	<u>AT-TACTTCGGCTACGAGATTTCGATGGTATGGACTACTGGGGTCAAGGAACCTCAGTCAC</u>	419
	* * * * * **	
abVH-LF8	CGTCTCCGCAGCTAGC	415
EU121635	TGTCTCTGCG-----	352
U60820	CGTTAAGCTT-----	408
AY646832	CGTCTC-----	425
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Figure 6 Alignment of the DNA sequences of abVH-LF8 (HM046413) with other V_H genes. The DNA sequence of aberrant abVH-LF8 was aligned with an aberrant V_H gene (EU121635) and two functional V_H genes (U60820 and AY646832). Signal sequences are underlined and complementarity-determining regions of functional genes are shadowed. Gaps are represented by dashes, identical bases by *.

Figure 7 Agarose gel electrophoresis of functional and aberrant V_H and V_L genes using different sense primers and corresponding antisense primer. Lane M: DNA marker; lane F: functional variable genes (sense primer MHALT1 for V_H and sense primer MLALT2 for V_L); lane A: aberrant variable genes (sense primer MHALT3 for V_H and sense primer MLALT1 for V_L).

development of human–human hybridomas.²³ Consequently, production of a chimeric mAb and a humanized mAb are often carried out.²⁴ The first step in creating these antibodies is to clone the functional immunoglobulin variable genes by PCR amplification from the cDNA of a hybridoma. The major problem in rapidly obtaining heavy and light chain variable genes from a hybridoma is the occurrence of aberrant mRNAs that can be transcribed but that have no function.²⁵ If the aberrant gene is used in the construction of the chimeric or humanized antibody, it may produce immunoglobulin that has low affinity for the antigen and is completely non-functional. Several strategies may decrease the amplification of aberrant variable genes, but they have limitations primarily because the aberrant genes may be highly homologous to the functional ones.²⁶ As a result, the more information we have about the aberrant genes, the higher efficiency of functional gene amplification we can obtain and the better we can avoid the aberrant genes in antibody engineering.

An antibody repertoire is generated from a large number of antibody genes. Gene rearrangements lead to antibody diversity.²⁷ Primer design is very important for the amplification of variable genes. The sense primer can be set to pair with either FR1 or the upstream signal sequence, while the antisense primer pairs with either FR4 or the beginning of the constant region. As the signal sequence and the constant region are usually conserved in the immunoglobulin genes, few primers paired to those regions might be required.²⁸ Although these primer sets have proven to be successful and are used widely in antibody variable gene extraction, the aberrant genes can also be amplified. In this study, four primer sets were used for immunoglobulin variable gene cloning. For amplification from FR1 to FR4, 19 primers were used for V_H sense and 4 primers for antisense (as described by Andris-Widhopf *et al.*). For V_L , 17 and 3 primers were used for sense and antisense, respectively. It would be laborious to use these primers individually to distinguish the functional genes from aberrant ones, so other measures are needed to reduce aberrant amplification. For amplification from the conserved signal sequence to FR4, as reported by Coloma *et al.*, only three primers were used for V_H sense and five for V_L sense, with only one antisense primer for V_H and one for V_L . Accordingly, the sense primers could be applied individually with an antisense primer to isolate the functional and aberrant genes.

Because amplification of an aberrant V_L gene from the cDNA of a hybridoma is very common, two methods were employed to reduce aberrant amplification from the MOPC21κ allele. In one approach, the PCR product of V_L was digested by *Bci*VI to cut the aberrant gene and isolate the functional gene. Most fragments were cut, and an uncut band was hardly seen, suggesting that the aberrant gene has much greater abundance than the functional one. A second digestion of

TA plasmids was used to verify functional gene isolation. To our surprise, only three out of eight plasmids contained a functional gene, resulting from the relative low efficiency of the first digestion by *Bci*VI, which is not accordant with the results of Juste *et al.* As far as we are concerned, it is necessary to do the second digestion.

In the other approach, five sense primers were used individually to amplify from the V_L signal sequence. The sequencing results of the amplification products were then analyzed in the VBASE2 database to distinguish the functional from aberrant V_L genes. In our results, it was shown that the functional and the aberrant V_L genes were amplified by two separate sense primers: MLALT2-amplified V_L gave the functional gene, while MLALT1-amplified V_L gave the aberrant one. If the functional V_L had shared the same sense primer with the aberrant V_L , further steps would have been required for identification. According to analysis in the GenBank database using BLAST, the abVL-LF8 showed about 97% homology to the well-known MOPC21 V_L aberrant gene derived from the myeloma cell line P3-X63-Ag8.653; only a few nucleotides located in primer regions are different. This indicates that there might be only one V_L aberrant gene existing in several myeloma cell lines, and the sequences reported are essentially identical.

For amplification of the V_H genes, there was no difficulty in using the primer set described by Andris-Widhopf *et al.* Six TA plasmids containing V_H were identified to be inserted with the same functional gene, and no aberrant V_H gene was found. While using the primer set described by Coloma *et al.*, two transcripts were found with different sense primers. Sequencing showed that MHALT1-amplified V_H had the functional V gene features while MHALT3-amplified V_H was an aberrant one. This unexpected abVH-LF8 showed high homology to both aberrant genes and functional ones, according to the GenBank database. The results confirm the homology of P3-X63-Ag8.653 and OUR-1, an ouabain-resistant subclone of the former,²⁹ and verify that some aberrant V_H genes have high homology to the functional ones. It could be confusing to distinguish functional V_H genes from aberrant ones. The results also suggest that abVH-LF8 may be a mutation and rearrangement of a functional transcript existing in the B-cell genome.

The aberrant variable gene may arise from an unproductive transcript in myeloma cells, a rearranged transcript in B cells, or a gene mutation in a fusion of the two kinds of cells.³⁰ To identify the origins of the two aberrant genes, variable genes were also amplified using cDNA synthesized from myeloma cell line OUR-1. Sp2/0 myeloma cells were also used because abVH-LF8 showed high homology to a functional V_H gene derived from the hybridoma generated using Sp2/0. The two fusion partners, OUR-1 and Sp2/0, do not secrete immunoglobulin but have the same abVL-LF8 as LF8 does. However, abVH-LF8 was only amplified in LF8 cells, not in OUR-1 or in Sp2/0 cells, which indicates that the abVH-LF8 might originate from the antibody repertoire in B cells or from gene rearrangement in the hybridoma cells.

In conclusion, we have identified two aberrant variable genes when cloning functional genes to develop a murine–human chimeric antibody. The abVL-LF8 reported here in LF8, OUR-1 and Sp2/0 is identical to the MOPC21 V_L aberrant gene, and the origin of this aberrant V_L gene is also confirmed to be accordant with the known research results. Further, digestion twice by *Bci*VI can reduce the chance of TA cloning of the aberrant V_L gene and effectively isolate the functional one. Although aberrant V_L gene has high abundance, there might be only one V_L aberrant gene existing in several myeloma cell lines and many methods have been proposed to avoid it. On the contrary, the aberrant V_H genes have seldom been reported; they are more complicated

and diverse than the aberrant V_L gene. There is no better strategy than sequencing and analysis in databases to avoid the aberrant V_H genes. The abVH-LF8 reported in this study may facilitate discrimination between the functional and aberrant V_H genes from hybridoma cells, especially those developed using OUR-1 cells as a fusion partner.

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