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Elimination of endogenous aberrant kappa chain transcripts from sp2/0-derived hybridoma cells by specific ribozyme cleavage: utility in genetic therapy of HIV-1 infections

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ABSTRACT

The pooled degenerate-primer polymerase chain reaction (PCR) technology is now widely used in the amplification and cloning of murine hybridoma-specific immunoglobulin gene cDNAs. The design of primers is mainly based on the highly conserved 5′ terminus of immunoglobulin gene variable regions and the constant region in the 3′ terminus. Of note, most murine hybridoma cell lines are derived from the Sp2/0 cell line, which is demonstrated to express endogenous aberrant kappa chains (abVκ). This high-level endogenous abVκ mixes with specific kappa chains in the hybridomas and interferes with the efficiency of the reverse transcriptase (RT)–PCR cloning strategy. In this report, during the cloning of murine anti-human immunodeficiency virus type I (HIV-1) hybridoma immunoglobulin cDNAs, a specific primer-PCR screening system was developed, based on the abVκ complementarity-defining region (CDR), to eliminate abVκ-carrying plasmids. Furthermore, an abVκ sequence-specific derived ribozyme was developed and packaged in a retroviral expression vector system. This abVκ ribozyme can be transduced into different murine hybridomas, and expressed intracellularly to potently eliminate endogenous abVκ RNA.

INTRODUCTION

'Intracellular immunization' utilizing intracellularly expressed and modified fragments of antibodies containing the antigen binding site(s) as single-chain-variable fragments (SFv), is a relatively new and exciting development in molecular therapeutics (1–3). Utilizing specific expression of SFv proteins (single-chain mini immunoglobulins) in target cells, blockage of specific enzymatic antigens, such as alcohol dehydrogenase, has been demonstrated in yeast systems (4). As well, viral regulatory proteins, such as human immunodeficiency virus type I (HIV-1) Rev, may be targeted in mammalian cells (1). By selecting oncogene-specific antibodies, it is also possible to inhibit specific cellular oncogenes (5). With more knowledge about the function and structure of SFvs, it may be possible to further modify these moieties with different functional protein domains, such as specific cellular component domains, endoplasmic reticulum localization domains, nuclear translocation signals, or specific protein binding sites (6). As such, it would be possible to target proteins to specific subcellular locations, complex two unrelated natural proteins together to perform specific intracellular biochemical functions, or develop catalytic antibodies (7). All of these potential studies will mainly be dependent on how quickly and accurately the precise immunoglobulin genes can be isolated and cloned.

The polymerase chain reaction (PCR) is now widely used for genomic and cDNA cloning (8). Recently, PCR has been used to amplify genes encoding variable domains of immunoglobulins, utilizing degenerate oligonucleotide primers (9). Those methods are based on the observation that immunoglobulin variable chain genes are in fact a highly conserved family of sequences, from which one can design degenerate primers that will amplify most, if not all, variable chain genes utilizing PCR (10).

The well-developed PCR–phage cloning system allows the amplification of whole antibody libraries from human bone marrow B cell pools. Cloning PCR-derived cDNA fragments into a phage expression system to present specific antibodies on the surface of phage, then allows antigen-based selection via panning (11). Nevertheless, those procedures involve a large number of screening steps and, finally, most of the selected phage antibodies have to be further characterized for their antigen binding sites (11). Presently, the most well-characterized monoclonal antibodies are from murine hybridoma cells, which maintain high binding affinities, with known antigen binding domains. Recently, we have attempted to clone anti-HIV-1 antigen-specific murine monoclonal variable region cDNAs, such as anti-HIV-1 Rev, Tat, and reverse transcriptase (RT). All of our hybridomas were derived from the cellular fusion partner, Sp2/0 parent cell line.

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Of note, Sp2/0 cells carry endogenous aberrant kappa chain (abVκ) (12). Importantly, all of the PCR-based degenerate primers for kappa chain reactions will mainly amplify this endogenous aberrant kappa chain (13). This background abVκ contamination adds a significant number of cloning and screening steps to obtain functional kappa chain cDNA sequences. In this report, we present an optimized and specific ribozyme cleavage system to reduce endogenous aberrant kappa chain RNA. As well, we demonstrate an aberrant kappa chain complementarity-defining region (CDR)-specific primer set, for screening the recombinant plasmids, compatible with commercially available degenerate PCR immunoglobulin primers, for quick and efficient amplification and selection of correct kappa chain variable cDNAs.

MATERIALS AND METHODS

Cell cultures and preparation of RNA

All hybridoma cell lines were kindly provided by Intracel Inc. (Cambridge, MA) and cultured in RPMI-1640 medium supplemented using 10% fetal calf serum (FCS) with 2 mM glutamine and 50 mM 2-mercaptoethanol. For isolation of total cellular RNA, RNA was extracted with a micro-adaptation of the guanidinium isothiocyanate/cesium chloride procedure (14). Generally, $3-5\times10^6$ cells were pelleted by centrifugation at 1000 *g* for 5 min at 4°C and washed with 10 ml of cold phosphate-buffered saline (PBS) solution, without Ca^{2+} or Mg^{2+}. Cell pellets were resuspended in a solution containing 2 ml 4 M guanidinium isothiocyanate in 50 mM Tris (pH 7.5), 25 mM EDTA, 100 mM 2-mercaptoethanol, and passed through a syringe with a 21 gauge needle several times, to completely disrupt the cells and shear DNA. Two milliliters of cell lysates were layered over 4 ml of a 5 M $CsCl_2$ solution in a Beckman ultracentrifuge tube and the samples were centrifuged at 35,000 rpm for 16 h in a SW55Ti rotor. After centrifugation, all of the supernatants were aspirated without dislodging the pellet and the RNA pellet was resuspended in 400 μl of 75% ethanol at −80°C for 15 min and spun in an Eppendorf centrifuge for 30 min. The pellets were washed with 75% ethanol, then vacuum-spin dried and resuspended in 10 μl DEPC-treated H_2O.

cDNA synthesis and amplification

The standard cDNA synthesis reaction was performed based on a suggested protocol by Novogen Inc. using the Ig-prime system (1). Briefly, 0.5−1.5 μg of total RNA in 10 μl of DEPC-treated H_20 was utilized. The reaction can be scaled up proportionately, so that 5 μl aliquots can be used in separate amplification reactions with differing 5′ primers. The following components were added to a sterile, RNase-free 1.5 ml tube: 2 μl 5×murine leukemia virus (MLV)-RT buffer (250 mM Tris−HCl (pH 8.3), 250 mM KCl, 50 mM $MgCl_2$, 2.5 mM spermidine, 50 mM DTT), 0.5 μl 10 mM dNTPs, 0.5 μl oligo(dT) (1 μg/μl) (Pharmacia Inc.), 1 μl RNase inhibitor, and 1 μl immunoglobulin 3′ primer, either for heavy or light chains. RNA (0.5−1.5 μg) was then added, after it was first heated at 72°C for 5 min and cooled on ice. Finally, 0.6 ml MLV-RT (200 U/ml from BRL Inc.) were added and incubated at 37°C for 1 h. The reaction was stopped by heating at 70°C for 15 min.

The PCR reaction was performed by transferring 5 μl of the RT reaction mixture, with the immunoglobulin 5′ primer, to a fresh tube. A reaction mixture (total volume 50 μl) was then added containing: 1 μl 10 mM dNTPs, 5 μl 10×PCR buffer [100 mM Tris−HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatin], 2.5 μl immunoglobulin 5′ degenerate primer, and 0.5 μl Taq DNA polymerase. PCR was performed using the following amplification protocol: 94°C for 5 min to denature, followed by 35 cycles of 94°C for 1 min 20 s, 45°C for 2 min, and 72°C for 1 min 30 s. A final extension step of 10 min at 72°C was then performed.

PCR products were purified on 1.5% low-melting agarose gels. The cDNA fragments were ligated into the pT7 Blue(R) vector and the Novo Blue *E.coli* strain (Novogen Inc.) was transformed with these plasmids. Recombinant plasmids were selected on X-Gal plates and screened, as suggested by the manufacturer's protocol. Plasmid DNA sequencing was performed by either the ABI automatic DNA system (14,15) or using the UBS Sequenase 2.1 kit, with either T7 primer: 5′-TAATACGACTCACTAT-3′ or the U19 primer: 5′-GTTTTCCCAGTCACGACGT-3′.

PCR-derived methodology for screening aberrant kappa chains

Fifteen microliters of bacterial cultures, which carry recombinant plasmids grown overnight in TB medium (1.2% Tryptone, 2.4% yeast extract, 0.04% glycerol, 170 mM KH_2PO_4 and 72 mM K_2HPO_4) were spun in an Eppendorf centrifuge for 20 s, and the pellets were resuspended in 100 ml H_2O, and boiled for 3 min. These suspensions were then spun for 1 min, and 5 μl of supernatant were used for a PCR reaction with the T7 primer and the U19 primer, which were utilized for insert DNA screening. The same supernatants were also used for PCR amplification with oligonucleotides based on abVκ gene CDR-1 and CDR-3 regions (Figure 2), abVκ-1: 5′-TCT GGC TAT A-GT TAT ATG CAC-3′ and abVκ-2: 5′-TGT AAG CTC CCT AAT GTG CTG-3′, as these primers can only amplify sequences contained in the plasmids which contain the abVκ insert DNA. The PCR amplification was performed in 20 μl, using 35 cycles of 94°C for 1 min 20 s, 50°C for 1 min 30 s, and 72°C for 2 min. Finally, an extension reaction at 72°C for 10 minutes was performed. Ten milliliters of PCR-amplified products were assayed on a 1.3% agarose gel.

Construction of the abVκ ribozyme

Aberrant kappa chain (abVκ)-specific ribozymes were designed, based on the complementarity-defining region (CDR)-1 sequence: 5′-C AGC AAA AGT GTC AGT ACA TCT GGC TAT AGT TAT ATG CAC TG-3′. The GTA site was the specific target site for ribozyme cleavage. The ribozyme DNA fragment was synthesized by renaturation of oligonucleotide-1: 5′-CCT GGA TCC AGA TCT CAG CAA AAG TGT CAG TTT CGT CCT CGC GGA CTC-3′ and oligonucleotide-2: 5′-CTC AAG CTT CAT ATA ACT ATA GCC AGA TGC TGA TGA GTC CGC GAG GAC GA-3′. After renaturation, extension using the Klenow enzyme reaction was performed. After *Hin*dIII/*Bam*HI digestion, this ribozyme DNA fragment was inserted into the pGEM4Z vector (Promega, Inc.) at the *Hin*dIII−*Bam*HI sites, to create p4ZabVκribo. The DNA sequence was then confirmed. Of note, transcription of the ribozyme was driven by the T7 RNA polymerase promoter.

In order to clone short aberrant kappa chain RNA templates for *in vitro* cleavage reactions, the aberrant kappa chain cDNA was re-amplified by PCR with oligonucleotides Temp-1: 5′-GCG AAT TCT GAC ACA GTC TCC TG-3′ and Temp-2: 5′-ACA AGC TTC AGG GAC CCC AGA TTC-3′. After *Eco*RI/*Hin*dIII digestion, this 186 bp PCR product was inserted into the

pGEM3Z vector at the *Eco*RI−*Hin*dIII sites to create plasmid p3ZabVκ-Tem. The kappa chain RNA was transcribed using the T7 RNA polymerase promoter.

To ligate the ribozyme DNA fragment into the murine retroviral expression vector, pLXC-CMV (17), *Hin*dIII-digested p4ZabVκribo was filled in using the Klenow reaction, and then cut with *Bam*HI. The gel-purified abVκ-ribozyme fragment was inserted into the retroviral vector, pLXC-CMV, at the *Hpa*I and *Bam*HI sites, to create pLXC-CMVabVκribo. As a control, the chloramphenicol acetyl transferase (CAT) gene (726 bp *Hin*dIII−*Bam*HI) was blunted at the *Hin*dIII site and inserted into pLXC-CMV (*Hpa*I−*Bgl*II), to produce CAT expression vector, pLXC-CMV-CAT.

Ribozyme cleavage reaction *in vitro*

The p3ZabVκ-Tem construct was linearized by digestion with *Hin*dIII and p4ZabVκribo was linearized with *Bam*HI. *In vitro* transcription was performed according to the manufacturer's suggested protocol, using Promega Riboprobe Systems. RNA was labeled with [^{32}P]dUTP and purified on a 6% polyacrylamide gel. RNA was electroeluted from the gel and precipitated by ethanol and resuspended in 20 ml DEPC-treated H_2O (18).

A typical ribozyme cleavage reaction was performed as follows: the radiolabeled template RNA (1×10^7 cpm) and ribozyme RNA (1×10^7 cpm) were heated at 73°C for 3 min, and both of these RNA were then mixed together in 10 μl of a buffer containing 40 mM Tris−HCl (pH 7.5), 12 mM $MgCl_2$ and 2 mM spermidine. This reaction mixture was incubated at 37°C for 30 min. Of note, reactions were also conducted with varying concentrations of $MgCl_2$. The cleavage reactions were stopped by adding 5 μl of a specific buffer [2.2 M formaldehyde, 50% formamide, 0.1% (w/v) Bromocresol green, and 0.8% sucrose]. The cleavage products (2 μl) were resolved on 8% urea−PAGE gels, and the gels were dried and exposed on X-ray film.

Treatment of isolated cellular RNA from hybridomas with abVκ ribozyme

Cellular RNA (5 μg) from various hybridomas were treated with *in vitro* transcribed abVκ ribozyme RNA (1 μg) for 30 min in 1×RT buffer conditions. The cellular RNA was then subjected to RT−PCR for Vκ chains. The cDNAs were then cloned into the pT7 Blue (R) vector, and based on X-Gal selection 150 individual recombinant colonies were isolated and screened by PCR for the aberrant and correct Vκ chains. Five individual colonies, for each hybridoma, were also analyzed by DNA sequencing, for confirmation.

Transduction of the hybridoma cells with MLV-expressed ribozymes

Twenty micrograms of pLXC-CMVabVκribo or pLXC-CMV-CAT were transfected into a packaging cell line, PA317, as previously described (17,19). After 48 h, transfected cells were selected with 800 μg/ml of G418 for 2 weeks. Individual cellular clones were isolated and grown to 2×10^6 cells, then supernatants from these clones were assayed for viral titers, as previously described (20).

RESULTS

Identification of the aberrant kappa chain cDNA sequence and screening abVκ by primer selection

As reported by Carroll *et al.* (12) hybridoma fusion cell lines from Sp2/0 backgrounds contain one heavy chain transcript and two light chain transcripts, one of which is an aberrant kappa transcript with a four nucleotide deletion at the VJ joining site. Utilizing standard RT−PCR with degenerate immunoglobulin primers, we were able to clone 12 out of a total of 13 anti-HIV-1 antigen-specific hybridoma heavy chain variable region cDNAs (Figure 1). For heavy chain cloning, the PCR can usually amplify correct heavy chain cDNA, as Sp2/0 cells do not express endogenous heavy chain RNA. Of note, cloning kappa light

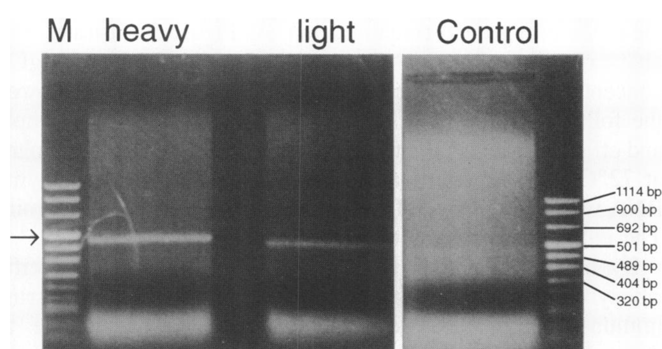

Figure 1. Polymerase chain reaction (PCR) of murine immunoglobulin variable regions. Amplification of murine immunoglobulin variable regions of an anti-HIV-1 Rev hybridoma cell line (D8), using the Mouse Ig-Primer kit (Novogen Inc.). 1.5 μg of total RNA was used for cDNA amplification with either heavy or light chain primers, in 10 μl reactions. 5 μl of a cDNA mixture were used for further PCR amplification in a 50 μl volume. The control lane represents a RT−PCR reaction for light chain cDNA, utilizing a human T lymphocytic cell line. Products were analyzed on a 1.3% agarose gel with DNA marker VIII (Boehringer-Mannheim Inc.) (lane M). The dark arrow points to specific bands representing light and heavy chain cDNA. These cDNAs were each cloned into the pT7 Blue (R) vector and analyzed by DNA sequencing.

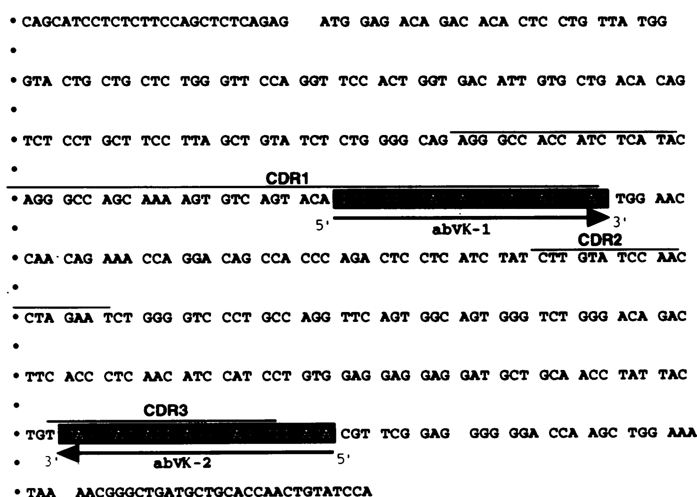

Figure 2. Nucleotide sequence of the aberrant Vκ transcript in murine myeloma fusion cell partners. The complementarity-defining regions CDR-1, CDR-2 and CDR-3 are indicated in this figure. The primers specific for aberrant Vκ chains are illustrated (abVκ-1 and abVκ-2). This is the same sequence for an aberrant endogenous murine Vκ chain as demonstrated by Carroll *et al.* (12).

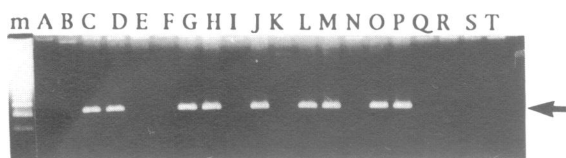

Figure 3. Screening of murine immunoglobulin light chain recombinant plasmids by abVκ-specific primers. Twenty recombinant clones from X-Gal-selected plates were isolated and lysed by boiling for 3 min in 50 μl H_2O, and 5 μl of the lysates were used for PCR amplification. Lane M is a 100 bp DNA marker. Lanes C, D, G, H, J, L, M, N, O, and P show bands containing abVκ insert DNAs. The arrow indicates these specific bands (204 bp). Those lanes which do not illustrate a specific band either contain correct Vκ chains, or had no specific amplification by RT–PCR.

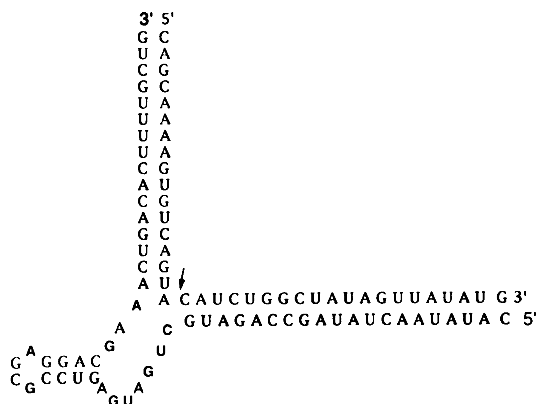

Figure 4. Cleavage structure of abVκ-specific ribozyme based on the abVκ CDR-1 region GUA site. The arrow indicates the precise cleavage site.

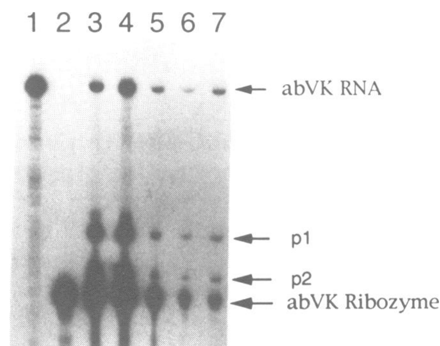

Figure 5. *In vitro* cleavage reaction using an abVκ-specific ribozyme. The abVκ RNA template was cleaved utilizing different RT buffer conditions. 191 bp RNA was transcribed using T7 RNA polymerase from a *Hin*dIII linearized plasmid, p3ZabVκ, in the presence of [^{32}P]dUTP. The 67 bp abVκ ribozyme RNA was transcribed from p4ZabVκribo, using T7 RNA polymerase. The two labeled RNAs were mixed and incubated at different concentrations of RT buffer for 30 min, and resolved using 8 M urea–6% PAGE. Lane 1: abVκ RNA template; lane 2: abVκ ribozyme RNA; lanes 3–7 are mixtures of the two RNAs at RT buffer concentrations of 1×to 5×. p1 is the 3′ abVκ cleavage RNA product and p2 is 5′ abVκ cleavage RNA product.

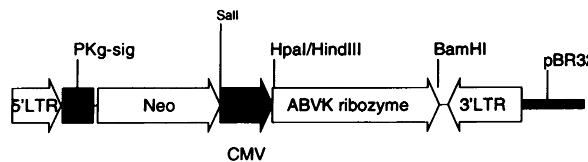

Figure 6. Map of retroviral expression vector pLXC-CMVabVκribo. The abVκ ribozyme was cloned downstream of an internal cytomegalovirus (CMV) immediate early promoter.

chains required mini-preparation of 150 clones from X-Gal-selected cultures for the anti-HIV-1 Rev hybridoma cell line D8, to obtain only 2 clones with correct anti-Rev kappa chain cDNA (1). All of the other clones contained aberrant kappa chain cDNA (Figure 2). Importantly, from the 12 screened anti-HIV-1 antigen-specific hybridoma kappa chain PCR-derived constructs, over 150 PCR-derived kappa chains have been analyzed by DNA sequencing and more than 95% of the clones carried this endogenous kappa chain cDNA.

Utilizing the DNA sequence of the Sp2/0 endogenous kappa chain variable region, we developed aberrant kappa chain CDR-1- and CDR-3-specific oligonucleotide primers (abVκ-1 and abVκ-2) (see Figure 2), for quick and efficient screening of the recombinant plasmids (Figure 3). By using these two primers, we can potently eliminate aberrant kappa chain cDNA-containing plasmids, thus dramatically reducing the DNA sequencing necessary in these studies.

Specific ribozyme cleavage of abVκ RNA *in vitro*

Of note, even by using specific PCR screening to eliminate the aberrant kappa chain cDNAs, we still had to screen more than 100 clones to obtain only a few abVκ PCR-negative clones for further DNA sequencing, to obtain correct kappa chain cDNA. In order to reduce endogenous aberrant kappa chain RNA levels to enhance specific kappa chain RNAs, we synthesized a ribozyme DNA sequence, based on the abVκ-specific CDR-1 sequence (Figure 4). Initially, we evaluated the ribozyme cleavage *in vitro*, prior to the RT–PCR reaction, which would reduce the abVκ background contamination. As Figure 5 illustrates, this abVκ ribozyme functions quite well in a wide range of $MgCl_2$ concentrations, within the buffer (21). These conditions allowed the following RT–PCR reaction to be performed in a simple and efficient manner. Firstly, heating of 1–2 μg of RNA template at 72°C for 3–5 min was performed. Then 1 μl of ribozyme RNA was added in 3×RT buffer, incubated at 37°C for 30 min. The entire mixture was then used for the RT reaction.

By first treating total cellular RNA with the abVκ-specific ribozyme, then performing RT–PCR amplification of murine immunoglobulin variable region cDNA, we were able to dramatically reduce abVκ RNA background, and potently enhance the specific kappa chain cDNA ratio, prior to the cleavage treatment (Table I).

Retroviral vector DNA delivery of the abVκ ribozyme into hybridoma cells

Although the pre-ribozyme cleavage reaction and then RT–PCR amplification can reduce the abVκ background and enhance the specific kappa chain RNA for RT–PCR amplification, this reaction occurs *in vitro*. Thus, it may sometimes cause the non-specific degradation of total cellular RNA. In order to avoid this

Table I. Comparison of cloning efficiency for Vϰ chains after abVϰ ribozyme treatment of total hybridoma RNA *in vitro*

	Correct Vϰ cDNAs isolated before treatment*	Correct Vϰ cDNAs isolated after treatment
Anti-HIV-1 Rev	2	21
Anti-HIV-1 Tat	4	25
Anti-HIV-1 Integrase	1	12

*From 150 screened recombinant colonies.

Table II. Comparison of cloning efficiency for Vϰ chains after abVϰ ribozyme-expressing retroviral vector treatment of hybridoma cells

	Correct Vϰ cDNAs isolated before treatment*	Correct Vϰ cDNAs isolated after treatment
Anti-HIV-1 Rev	3	34
Anti-HIV-1 Tat	2	32
Anti-HIV-1 integrase	3	19

*From 150 screened recombinant colonies.

Table III. Comparison of cloning efficiency for Vϰ chains after CAT-expressing retroviral treatment of hybridoma cells

	Correct Vϰ cDNAs isolated before treatment*	Correct Vϰ cDNAs isolated after treatment
Anti-HIV-1 Rev	3	5
Anti-HIV-1 Tat	2	3

*From 150 screened recombinant colonies.

potential difficulty, we developed a specific retroviral shuttle vector delivery system (Figure 6). By establishing an efficient packaging cell line, we were able to obtain a viral titer of 5.2×10^5 cfu/ml, containing the murine leukemia virus vector which carries the abVϰ ribozyme DNA (not illustrated). Based on this very efficient DNA delivery system, $2-3 \times 10^6$ hybridoma cells were centrifuged at 400 *g* for 5 min, and resuspended in 8–10 ml of packaging cell supernatant, with 8 μg/ml polybrene overnight. The cells were then cultured in 5 ml of fresh medium for another 24 h. Total cellular RNA was then isolated and RT–PCR for Vϰ cDNAs were performed. The cDNAs were then cloned into the pT7 Blue (R) vector for PCR screening and sequence analysis, after X-gal selection.

This ribozyme-transduced retrovirus vector treatment was quite efficient and reduced the abVϰ RNA background, without requiring an *in vitro* cleavage reaction (Table II). Of note, the abVϰ ribozyme-expressing retroviral vector system was even more potent than the methodology in which isolated cellular RNA was directly treated with the ribozyme (Table I). A control MLV retroviral vector, expressing chloramphenicol acetyltransferase (CAT), rather than the abVϰ ribozyme, did not alter the aberrant kappa chain background in any hybridoma analyzed (Table III).

DISCUSSION

The successful and general application of PCR to the rapid cloning of variable regions of rearranged immunoglobulin genes requires a careful design of 'universal' primers. This system functions by taking advantage of the finding that mammalian light and heavy chains of immunoglobulins contain conserved regions, adjacent to hypervariable complementarity-defining regions (CDR). Thus, appropriately designed oligonucleotide primer sets allow these regions to be specifically amplified by PCR (9,22,23). To develop a set of compatible primers for mouse antibodies, several laboratories pooled and aligned the murine antibody sequences collected in the Kabat–Wu data base (24). These primers can be based on either the immunoglobuin leader region (amino acids −20 to −13) or the framework region-1 (FR-1) (amino acids 1–7 or 8). Similar sequences were grouped and putative primer sequences were aligned from each group. All the putative primer sequences were then compared to the pooled sequences, and the 'best fit' primer sequence was selected. This process was repeated until all the sequences were evaluated. This allowed the optimization of the degenerate primers to cover as much of the possible 5′ immunoglobulin DNA sequences, for PCR amplification. Although those sets of primers will efficiently amplify cDNA of immunoglobulin genes from immunized mouse B cells to construct an antibody repertoire display library (25), this creates significant difficulties for cloning mouse immunoglobulin kappa chain cDNA sequences from established murine hybridoma cells which have Sp2/0 parent cell backgrounds. As demonstrated in this study, Sp2/0 cells vastly over-express endogenous aberrant kappa chains, and this mutation of the abVϰ gene, which consists of a 4 base pair deletion, is located downstream of the variable region. Thus, none of the published primer sets can eliminate the amplification of these contaminating abVϰ genes. Importantly, all of the immmunoglobulin primer designs are based on the immunoglobulin 5′

highly conserved secretory signal domain region or the FR-1, and this abVκ has a wild-type leader signal sequence and FR-1. As such, any effort to modify the primers to eliminate this abVκ gene will reduce the PCR amplification efficiency. Of note, in these studies our laboratory utilized primers in the conserved leader signal sequences (22,23).

In this report, we also demonstrate a strategy to eliminate this abVκ gene by taking advantage of specific CDR-1 region DNA sequence information. Each of the immunoglobulin genes have their own very specific hypervariable regions (i.e. CDR). It would be extremely rare to have completely conserved CDR-1 and CDR-3 cDNA sequences in two different immunoglobulin genes with precisely the same 5′ cDNA sequence. Based on the sequence of the specific abVκ CDR-1, we designed a pair of oligonucleotide primers (in abVκ CDR-1 and CDR-3 regions) which can specifically PCR amplify abVκ cDNA. As we now demonstrate with this methodology, one can easily determine contaminating abVκ recombinant plasmids in the selection procedure.

Due to the high level of endogenous abVκ RNA expression, the amplification efficiency of correct kappa chains is quite low and, sometimes, abVκ may actually act as an inhibitor of this PCR. Based on the CDR information of the abVκ DNA sequence, we constructed a ribozyme-based expression vector to specifically eliminate this abVκ RNA by cleavage of the abVκ in the CDR-1 region. Ribozymes are small RNA molecules capable of highly specific catalytic cleavage of RNA. Ribozyme-mediated cleavage in *trans* was first demonstrated *in vitro* by Ulhenbeck (26) and, subsequently, by Haseloff and Gerlach (27). In viroids and virusoids, the reaction is intramolecular. However, ribozymes that possess a catalytic domain and flanking sequences complementary to the target mRNA can cleave in *trans* provided that a three base sequence (GUX) occurs within the target molecule (25). Here we utilize the hammerhead-like ribozyme motif to specifically target the abVκ CDR-1 region. As such, the abVκ ribozyme only binds the abVκ CDR-1 region and specifically cleaves solely abVκ RNA. This strategy can dramatically reduce the endogenous abVκ RNA level, and increase the proper kappa chain:abVκ ratio to specifically enhance the RT−PCR amplification efficiency.

Finally, for practical convenience, we developed a retroviral vector DNA delivery system to simplify the entire system. Based on the amphotrophic retrovirus' high efficiencies in cellular transduction, this approach can be used to rapidly transduce any murine hybridoma cell line with infectious cell-free virus, or hybridoma lines can be co-cultured with a packaging cell line.

In the early 1980s, the joining of DNA-encoding mouse variable regions with gene segments encoding the human immunoglobulin constant region gave rise to so-called chimeric antibodies. Presently, the CDR humanizing technology, comprising the recombination of cDNA libraries of heavy and light chains and the expression of antibody fragments on the surface of filamentous bacteriophages is under development and further study (28−30). Antibody engineering, i.e. the construction of 'designer antibodies', is one of the fastest growing fields in molecular biology. These advances have been greatly facilitated by the introduction of techniques such as the polymerase chain reaction and the use of degenerate primers for efficient cloning of DNA-encoding immunoglobulin variable regions (29). Although mouse hybridomas derived from Sp2/0 cells carry endogenous aberrant kappa chains which interfere with the amplification procedure, the abVκ ribozyme system, which is described in this report, will eliminate this abVκ contamination.

As such, it is now demonstrated that the construction of multiple murine anti-HIV-1 immunoglobulin cDNAs can be efficiently accomplished. These findings allow the timely development of multiple murine SFv moieties, which target diverse viral proteins in the HIV-1 life cycle. Thus, genetic therapies using the intracellular expression of various murine SFv molecules can be brought to bear to combat HIV-1 infections, and other disease states.

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