In-Cell Assembly of scFv from Human Thyroid-Infiltrating B Cells

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ABSTRACT

The construction of a large library of single-chain Fv (scFv) antibody fragments involves a random assortment of heavy and light chains. Although useful for the production of recombinant antibodies, this method is not adapted to the study of the autoantibody repertoire formed in vivo during autoimmune diseases. To attain this objective, we describe the use of the in-cell PCR together with Cre-recombination applied, to our knowledge, for the first time to human B cells to obtain in situ pairing of the variable (V) region genes of the immunoglobulin heavy (H) and light (L) chains. Our method is based on amplification and recombination of the VH and VL genes within CD19⁺ B cells isolated from human thyroid tissue. Nested primers were designed to amplify the known major human VH and VL gene families. After reverse transcription PCR and three rounds of PCR including recombination between VH and VL using the Cre-loxP system, we obtained a unique 800-bp band corresponding in size to scFv fragments. We provide evidence that recombination between VH and VL genes occurred inside the same cell. This in-cell amplification and association procedure is a potentially useful tool for the study of autoantibody gene families and the VH/VL pairing that occurs during the autoimmune process.

INTRODUCTION

The combinatorial assortment of immunoglobulin genes has permitted the creation of extremely large naive libraries (19). This approach has also been used to clone organ-specific high-affinity IgG autoantibodies (AAb) (3,14). Information on heavy (H) and light (L) chain variable gene usage of AAb obtained from random combinatorial libraries has been provided, in particular, by studies on autoimmune thyroid disease (5,7). The majority of these human organ-specific AAb are derived from VH1 and VH3 gene families (15). Most evidence suggests that the VH and VL chain pairs isolated from these libraries are combined at random and do not reflect the pairings formed in vivo (15), which result from the combination between VH and VL chains expressed within the same B cell. Recently, Lagerkvist et al. (8) proposed an alternative approach to obtain human antibody fragments that retained the original VH and VL pairing by antigen-specific selection of single human B cells followed by cell culture and mRNA extraction.

In this study, we describe the use of the polymerase chain reaction (PCR) (16) to directly amplify the VH and VL genes within human B cells. This technique allows recombination between the original VH and VL gene segments expressed in each individual B cell and minimizes cross-recombination between B cells. Thyroid-infiltrating B lymphocytes (TIBL) of patients with Graves' disease were purified using anti-CD19-coated magnetic beads. After fixation and permeabilization of the B cells, mRNA was reverse-transcribed directly inside the cell. cDNA encoding for all VH and VL chains was subsequently PCR-amplified using specific

nested primers. The two chains were linked within the B cells using the Cre-loxP site-specific recombination system of bacteriophage P1 (6), and two other rounds of PCR were performed. Using this system, a unique 800-bp band, corresponding to a single-chain Fv (scFv) gene segment, was obtained. This result demonstrates that the construction of scFv fragments can be performed within B cells, completely bypassing the necessity to extract the mRNA. Furthermore, this procedure results in a repertoire that reflects the original pairing of VH and VL, a critical feature for studying the autoimmune antibody repertoire.

MATERIALS AND METHODS

Isolation and Purification of B Cells

TIBL were obtained from patients suffering from Graves' disease. B lymphocytes were isolated from 10 g of thyroid tissue. The gland was minced with scissors and subsequently digested by using collagenase/dispase (Boehringer Mannheim, Meylan, France) at 25 mg/g of tissue, for 3 h at 37°C. The digested tissue was then passed through a filter to remove remaining tissue fragments. After washing in phosphate-buffered saline (PBS; pH 7.2, supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin), the cell suspension was incubated with 6.4×10^6 anti-CD19 magnetic beads/g of tissue (Dynal, Oslo, Norway) for 90 min at 4°C to purify the B cells. We obtained 5×10^5 purified B cells from 10 g of thyroid tissue.

Cell Fixation and Permeabilization

Cells were fixed and permeabilized as previously described (4), except that

Table 1. Primers Designed for RT and PCR Amplification

RT-PCR	External part of constant regions
CH1 IgG For	5' GCT GCT GAG GGA GTA GAG 3'
Cκ Ext For	5' C AGA TTT CAA CTG CTC ATC AGA TGG 3'
Cλ Ext For	5' C CTT GTT GGC TTG RAG CTC CTC AGA 3'
PCR1 VH	Leader peptide
VH1-ldr Back	5' ATG GAC TGG ACC TGG AG 3'
VH2-ldr Back	5' ATG GAC ATA CTT TGT TCC AC 3'
VH3-ldr Back	5' ATG GAG TTK GGG CTG ARC TGG 3'
VH4-ldr Back	5' ATG AAR CAY CTG TGG TTC TT 3'
VH5-ldr Back	5' GG TCA ACC GCC ATC CTC GCC 3'
VH6-ldr Back	5' ATG TCT GTC TCC TTC CTC AT 3'
VH7-fr1 Back	5' CAG GTG CAG CTG GTG CAR TCT GGG 3'
JH1 lox For	JH region with the loxP site, Nhe I site, and linker
JH2a lox For	5' TCA TTC TCG ACA TAA CTT CGT ATA GCA TAC ATT ATA CGA AGT TAT GCT AGC AGA GCC GCC GCC GGC ACT GGC ACC ACC TGA CGA GAC GGT GAC CAG GGT GCC 3'
JH2b lox For	5' ..... TGA CGA GAC TGT GAC GAG GGT GCC 3'
JH3 lox For	5' ..... TGA CGA GAC TGT GAC GAG GGT GCC 3'
JH4-5 lox For	5' ..... TGA CGA GAC GGT GAC CAT TGT TCC 3'
JH6 lox For	5' ..... TGA CGA GAC GGT GAC CAT TGT TCC 3'
PCR1 VA	5' ..... TGA CGA GAC GGT GAC GGT GCC 3'
VA1a lox Back	VA region with the loxP site and Nhe I site (Primers in the constant region are the same as those in RT-PCR)
VA1b lox Back	5' CTC GCA ACT GCA TAA CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT GCT AGC GCC ATG GCC GAC TCT GTG TTG ACG CAG CCG CC 3'
VA1c lox Back	5' ..... CAG TCT GTG CTG ACT CAG CCR YC 3'
VA2a lox Back	5' ..... CAG TCT GTG CTG ACG CAG CCG CC 3'
VA2b lox Back	5' ..... CAC GTT ATA YTG ACT CAA CCG CC 3'
VA2c lox Back	5' ..... CAG TCT GCC CTG ACT CAG CCT SC 3'
VA3a lox Back	5' ..... CAG TCT GTT CTG ACT CAG CCT CC 3'
VA3b lox Back	5' ..... TCC TAT GTG CTG ACT CAG CCA CC 3'
VA3c lox Back	5' ..... TCC TAT GAG CTG ACW CAG CCA C 3'
VA4 lox Back	5' ..... TCC CGT AAG CTG ACT CCA GAG C 3'
VA6 lox Back	5' ..... CAG CTT GTG CTG ACT CAA TCG CC 3'
VA7/8 lox Back	5' ..... AAT TTT ATG CTG ACT CAG CCC CA 3'
VA9 lox Back	5' ..... CAG DCT GTG GTG ACY CAG GAG CC 3'
VA10a lox Back	5' ..... CAG CCT GTG CTG ACT CAG CCA CC 3'
VA10b lox Back	5' ..... CAG GCA GGG CAG ACT CAG CAG CT 3'
PCR1 Vκ	5' ..... CAG GCA GGG CTG ACT CAG CCA CC 3'
Vκ1a lox Back	Vκ region with the loxP site and Nhe I site (Primers in the constant region are the same as those in RT-PCR)
Vκ1b lox Back	5' CTC GCA ACT GCA TAA CTT CGT ATA ATG TAT GCT ATA CGA AGT TAT GCT AGC GCC ATG GCC GAC ATC CAG ATG ACC CAG TCT CC 3'
Vκ1c lox Back	5' ..... GTC ATC TGG ATG ACC CAG TCT CC 3'
Vκ1d lox Back	5' ..... RAT TTC CAG ATG ACT CAG TCT CC 3'
Vκ1e lox Back	5' ..... GAC ATC CAG ATG ACT CAG TCT CC 3'
Vκ2a lox Back	5' ..... GAT ATT GTG ATG ACY CAG WCT CC 3'
Vκ2b lox Back	5' ..... GAC ATT CTG TTG ACC CAG ACT CC 3'
Vκ2c lox Back	5' ..... GAT GCT GYG AWG ACC CAA CCT CC 3'
Vκ2d lox Back	5' ..... GAT GCT GTG TGA MCC AGC CTC YA 3'
Vκ3a lox Back	5' ..... GAA ATT GTG TTG ACG CAG TCT CC 3'
Vκ3b lox Back	5' ..... GAA ATT GTA TTG ACA CAG TCT CC 3'
Vκ3c lox Back	5' ..... GAA ATA GTG ATG ACG CAG TCT CC 3'
Vκ3d lox Back	5' ..... GAA CTT GTG ATG ACA GTG TCC AG 3'
Vκ3e lox Back	5' ..... GAA GTT GTG CTG ACA TGG TCC CC 3'
Vκ3f lox Back	5' ..... GAA ACA GTG ATG ACA CAG TCT CC 3'
Vκ4 lox Back	5' ..... GAC ATC GTG ATG ACC CAG TCT CC 3'

The Cre-loxP site is indicated in bold, the linker is underlined and restriction sites (*NheI*, *SfiI* and *NotI*) are in italics.

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Table 1. Primers Designed for RT and PCR Amplification (Continued)

PCR2	Internal part of constant regions (Primers in the leader peptide are the same as those in PRC1)	
Cλ Int For	5' GAG GAS GGY RGG AAC AGA GTG AC 3'	
Cκ Int For	5' TGG CGG GAA GAT GAA GAC AGA 3'	
PCR3	VH region with the Sfi I site	
VH1a Sfi Back	5' GTC CTC GCA ACT GCG GCC CAG CCG <i>GCC TTG GCC</i>	CAG GTG CAG CTK GTG CAR TCT GGG 3'
VH1b Sfi Back	5'	CAG GTG CAG CTG GTG CAG TCT GGA 3'
VH1c Sfi Back	5'	CAG GTM CAG CTG GTG CAG TCT GGG 3'
VH1d Sfi Back	5'	CTG GTG CAG CTG GTG CAG TCT GGG 3'
VH1e Sfi Back	5'	CAR ATG CAG CTG GTG CAG TCT GGG 3'
VH1f Sfi Back	5'	CAG GTT CAG CTG TTG CAG CCT GGG 3'
VH2 Sfi Back	5'	CAG RTC ACC TTG ARG GAG TCT GGT 3'
VH3a Sfi Back	5'	GAG GTG CAG CTG KTG GAG TCT GGG 3'
VH3b Sfi Back	5'	GAG ATG CAR CTG GTG GAG TCT GGG 3'
VH3c Sfi Back	5'	GAG GTG CAG CTG GTG GAG TCT GCG 3'
VH3d Sfi Back	5'	GAG GAT CAG CTG GTG GAG TCT GGG 3'
VH3e Sfi Back	5'	CAG GTG CAG CAG CAG GAG TGA GGC 3'
VH3f Sfi Back	5'	CAG GTG CAG CTG GTG GAG TCT GGG 3'
VH4a Sfi Back	5'	CAG GTG CAG CTG CAG GAG TCG GGC 3'
VH4b Sfi Back	5'	CAG GTG CAG CTA CAR CAG TGG GGC 3'
VH4c Sfi Back	5'	CAG STG CAG CTG CAG GAC TCG GGC 3'
VH5a Sfi Back	5'	GAG GTG CAG CTG GTG CAG TCT GGA 3'
VH5b Sfi Back	5'	GAG GTG CAG CTG TTG CAG TCT GCA 3'
VH5c Sfi Back	5'	GAA GTG CAG CTG GTG CAG TGY GGA 3'
VH6 Sfi Back	5'	CAG GTA CAG CTG CAG CAG TCA GGT 3'
VH7 Sfi Back	5'	CAG GTG CAG CTG GTG CAR TCT GGG 3'
	Jκ region with the Not I site	
Jκ1 Not For	5' GAG TCA TTC TCG ACT TGC <i>GGC CGC</i>	WCG TTT GAT TTC CAC CTT GGT CCC 3'
Jκ2 Not For	5'	WCG TTT GAT CTC CAG CTT GGT CCC 3'
Jκ3 Not For	5'	WCG TTT GAT ATC CAC TTT GGT CCC 3'
Jκ4 Not For	5'	WCG TTT GAT CTC CAC CTT GGT CCC 3'
Jκ5 Not For	5'	ACG TTT AAT CTC CAG TCG TGT CCC 3'
	Jλ region with the Not I site	
Jλ1 Not For	5' GAG TCA TTC TCG ACT TGC <i>GGC CGC</i>	CTA GGA CGG TGA CCT TGG TCC CAG 3'
Jλ2a Not For	5'	GGG ACC TAG GAC GGT CAG CTT GGT 3'
Jλ2b Not For	5'	CTA GGA CGG TCA GCT TGG TCC CTC 3'
Jλ4 Not For	5'	CTA AAA TGA TCA GCT GGG TTC CTC 3'
Jλ5 Not For	5'	CTA GGA CGG TCA GCT CGG TCC CCT 3'
Jλ6a Not For	5'	CGA GGA CGG TCA CCT TGG TGC CAC 3'
Jλ6b Not For	5'	CGA GGA CGG TCA CTT GGT CCA TGC 3'
Jλ7a Not For	5'	GGG ACC GAG GAC GGT CAG CTG GGT 3'
Jλ7b Not For	5'	CGA GGA CGG TCA GCT GGG TGC CTC 3'

The Cre-loxP site is indicated in bold, the linker is underlined and restriction sites (*NheI*, *SfiI* and *NotI*) are in italics.

the B cells were bound to magnetic beads. Briefly, the purified B cells were washed three times in cold PBS, suspended in 50 μL of an ice-cold 4% formaldehyde solution in 0.15 M NaCl and passed through a 1-mL syringe equipped with a 20- or 21-gauge needle to disperse clumps caused by aldehyde-induced cross-linking. Cells were incubated on ice for 1 h with shaking every 10 min, pelleted by attraction with a magnet and suspended for washing in 50 μL ice-cold PBS containing 0.1 M glycine using the same syringe and needle. The washing step was repeated twice. The cells were permeabilized by suspending them in 50 μL of 0.5% vol/vol Nonidet® P-40 (BDH, Essex,

Figure 1. B cells bound to magnetic beads (4.5 μm in diameter) are visualized by rosette formation before (a) and after (b) PCR rounds.

England, UK) in distilled water, using the same syringe and needle. After incubation for 1 h on ice with occasional shaking, the cells were washed three times in PBS-0.1 M glycine, resuspended using the same syringe and a 26-gauge needle to disperse the clumps visible under a light microscope (40× magnification). The cells were adjusted to a final density of 10^7 cells/mL in PBS-0.1 M glycine and used immediately or frozen at -70°C .

Oligonucleotides

Upstream primers were designed by analysis of the V BASE of human Ig genes [Medical Research Council (MRC), Cambridge, England, UK; World Wide Web address <http://www.mrc-cpe.cam.ac.uk>]. Four sets of primers were designed (see Table 1). The first one was used for the reverse transcription (RT) of mRNA of heavy and light chains into cDNA. It hy-

bridizes with the constant regions (CH1, C λ and C κ). The second set of primers used for PCR1 allowed the amplification of VH and VL regions. They match with all the VH leader sequences and the JH, V κ , V λ , C κ and C λ regions. These oligonucleotides introduce the loxP recombination site and the linker to obtain scFv fragments. The third set of primers was used after the recombination catalyzed by the Cre recombinase (1). The last set of primers was designed to limit the amplification to an scFv fragment and to introduce the restriction sites (SfiI and NotI) needed for the cloning step.

RT, Cre-Recombination and PCR Amplification

The reverse transcription was performed on the purified B cells (5×10^5 cells), using SUPERSCRIPT™ II RNase H⁻ Reverse Transcriptase (Life Technologies, Cergy Pontoise, France). Cre-recombination was performed according to the manufacturer's instruction (Novagen, Abdington, England, UK). After PCR amplification of the variable domain genes of the heavy and light chains, the Cre-protein mixture was added to the cell pellet to allow recombination between the loxP sites. We carried out the PCR in a total volume of 50 μL containing 5×10^2 to 5×10^4 cells bound to anti-CD19-coated magnetic beads; 5 pmol of each primer were used as a mixture with 10 μM dNTP, 5 μL of HiTaq™ buffer and 2.5 U of HiTaq Polymerase (Bioprobe Systems, Montreuil, France). Thirty cycles of DNA amplification were carried out at 95° (1 min), 65° (1 min) and 72°C (1 min) for PCR1 and PCR2, and 30 cycles at 95° (1 min) and 72°C (2 min) for PCR3. The cells were pelleted using a magnet and washed twice with 100 μL of PBS-0.1 M glycine after RT, Cre-recombination and each PCR amplification to eliminate all the amplification products that had diffused outside of the cells.

RESULTS AND DISCUSSION

Isolation and Purification of B Lymphocytes

The digestion of thyroid tissue from

Figure 2. Schematic overview of the different steps leading to scFv amplification. 1) cDNA synthesis, 2) first PCR for amplification of the VH and VL immunoglobulin genes, 3) a further round of PCR performed on the scFv product after Cre-recombination and 4) nested PCR and introduction of restriction sites. The hybridization position of primers are indicated by arrows, and the size of the amplified fragments are shown.

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Graves' disease patient is a crucial step in the isolation of infiltrating B cells. McLachlan et al. (11) have shown that enzymatic digestion is required to separate the thyrocytes and to provide access to the lymphocytes that produce AAb. We obtained the best yields of purification of lymphocytes from thyroid tissue after digestion for 2–3 h with collagenase/dispase (5×10^5 cells/10 g biopsy). After a shorter digestion period (1 h), only 1×10^2 cells were obtained, and digestion of thyroid tissue for more than 5 h did not lead to efficient CD19⁺ lymphocyte purification.

Anti-CD19 antibodies covalently bound to magnetic beads were used to purify the TIBL. As shown in Figure 1, isolated B cells can be clearly visualized by rosette formation. After cell fixation, permeabilization and three PCR steps inside the cells, the morphology of the cells did not appear to be modified (Figure 1b), suggesting that the B cells were unaltered.

Design of Oligonucleotides

To cover the widest possible range of VH and VL genes, we designed primers by analysis of available human sequences of antibody genes of the V BASE (MRC Cambridge). Eighty-four oligonucleotides were defined for their ability to amplify V gene families (Table 1). Primers were designed for RT-PCR and the three PCR steps.

Figure 3. Amplification of VL and VH domains TIBL and PBL mRNA using a mixture of primers. Lanes 1 and 6, 100-bp ladder; lanes 2 and 4, VL domain (450-bp) from TIBL and PBL, respectively; lanes 3 and 5, VH domain (470-bp) from TIBL and PBL, respectively.

PCR1 amplified the VH and VL genes separately; the primers contained the loxP site necessary for Cre-recombination. PCR2 amplified a combination of VH/VL, and PCR3 introduced the restriction enzyme sites. The hybridization positions of the primers involved in each step of the PCR protocol are described in Figure 2. The loxP site nucleotide sequence used for the recombination between the VH and VL immunoglobulin genes was chosen on the basis of work reported by Waterhouse and coworkers (20).

As described by Campbell et al. (2), the primers used for amplification of the VH genes hybridize with the leader sequences of the VH1 to VH6 human gene families. To amplify the VH7 gene family, we used a primer that hybridizes with the first framework region. In contrast to the use of degenerate primers for PCR3 as described by other groups (9,10), we designed new oligonucleotides that took into account the representation of all the V genes in each family (18,22). This should favor representative amplification of the expressed V gene families during the PCR protocol. In addition, we used nested primers to reduce primer bias and to obtain specific amplification products.

The ability of these primers to amplify the VH and VL immunoglobulin genes separately was checked on TIBL and peripheral blood lymphocytes (PBL). Electrophoresis on a 1% agarose gel showed 470- and 450-bp

PCR products corresponding in size to VH and VL, respectively (Figure 3). We observed a second band of about 620 bp. By alignment of primers and CL genes, we found a second site of potential hybridization (65% matching) of one primer that could be responsible for this amplification. The hybridization efficiency of oligonucleotides was tested on total RNA of PBL. Primers amplified the most represented VH families (VH1 and VH3 but also VH5 and VH7) (data not shown). The bias in the representation of VH subgroups obtained with our primer set corresponded to the frequency of gene usage in peripheral B cells (22).

Validation and Optimization of In-Cell PCR

As shown in Figure 4, the supernatant of in-cell PCR contained amplified material that had diffused throughout the cell membrane. Particularly, after the first PCR, 450/470-bp products were found in the supernatant (Figure 4, lane 2). Using a model of in-cell amplification of two hybridoma cell lines, Embleton et al. (4) previously showed that 70% of the total amplification products diffuse into the supernatant. For this reason, PCR products that had diffused outside of the cells were eliminated by washing the cells twice after each round of PCR, thereby preventing recombination of gene products from different cells as shown in Figure 4, lane 3. After PCR3, we

Figure 4. In-cell PCR. Lanes 1 and 8, 100-bp ladder; lane 2, PCR1 supernatant (450/470 bp); lane 3, PCR1 cell-pellet wash; lane 4, scFv 800-bp product of PCR3 performed on the washed cell pellet (5×10^4 B cells); lanes 5, 6 and 7, scFv 800-bp product of PCR3 performed on PCR2 supernatant from 5×10^2 , 5×10^3 and 5×10^4 B cells, respectively.

observed an 800-bp PCR product on a 1% agarose gel (Figure 4, lanes 4–7), corresponding in size to scFv fragments resulting from amplification and combination within the B cells.

Figure 5. In-cell PCR control. Lane 1, 100-bp ladder; lane 2, PCR1 supernatant after amplification of VL genes only; lane 3, PCR1 supernatant after amplification of VH genes only; lane 4, Cre-recombination, PCR2 and PCR3 performed on the mixture of VH and VL gene-amplified cells.

To confirm that the scFv products generated corresponded to pairing within the B cells, we amplified the VH and VL genes separately in different B cells (Figure 2, lanes 2 and 3). The PCR1 cell pellets of the VH-amplified cells and the VL-amplified cells were subsequently mixed, and then Cre-recombination, PCR2 and PCR3 were performed. No product was observed after recombination, PCR2 and PCR3 (Figure 5, lane 4), indicating that the Cre-recombination in Figure 4 clearly occurred between VH and VL genes inside the same cell. Furthermore, Embleton et al. (4) showed in their 1992 paper, in which the in-cell PCR technique was described for the first time, that there was no cross-recombination when the technique was applied to a mixture of hybridoma cells lines, even when a 1:9 mixture of the two hybridomas was used. This finding was later confirmed using the Cre-loxP system to

link the VH and VL genes, using the same hybridoma cell mixture (13).

PCR2 consisted of the amplification of VH and VL genes combined inside the cells. Thus, after PCR2, further amplification (PCR3) could be done either on the cell pellet or on the PCR2 cell supernatant. An 800-bp band, corresponding in size to an scFv fragment, was observed with both the cell pellet (Figure 4, lane 4) and the cell supernatant (Figure 4, lanes 5–7). The best apparent yield of amplification was obtained when the PCR3 was performed on the supernatant corresponding to an amplification product obtained from 5×10^2 cells (Figure 4, lane 5), whereas PCR3 with 5×10^3 or 5×10^4 cells led to lower amplification (Figure 4, lanes 6 and 7). This indicates that the PCR efficiency is highly dependent on the number of B cells and apparently decreases as it does with increasing cells number. We think that the cells bound

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to the magnetic beads sediment to the bottom of the tubes during the PCR steps, thereby preventing the reagents from reaching all the cells. To improve PCR efficiency, we could dissociate the cells from the beads, but in this case, we would be deprived of a rapid way to wash the cells. Or, we could vortex mix the PCR mixture more frequently.

We report an in-cell PCR protocol associated with Cre-recombination applied to human TIBL. By this approach, we demonstrated the amplification and combination of VH and VL antibody genes within individual B cells, thus allowing natural VH and VL gene pairing. This approach offers a critical new alternative to the random combinatorial library technology. A major application of this technique is the cloning of polymorphic immunoglobulin genes to study V gene combinations of natural and autoimmune B cells. Furthermore, this in-cell PCR procedure can be performed with a relatively small number of purified B cells. The source of the B cells is critical for the successful cloning of organ-specific AAb. Fortunately, the TIBL population is enriched in autoimmune B cells secreting specific AAb. Thus, the antigen-specific selection of B cells can be avoided.

In autoimmune diseases, several reports have shown a biased usage of V gene families (12,17) and a relationship between constant gene shift and the manifestation of the disease (21). The in-cell PCR described here, followed by phage display of antibody fragments, should allow access to the true autoimmune antibody repertoire in terms of gene usage and epitopic specificity.

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