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Quantitation of immunoglobulin μ–γ1 heavy chain switch region recombination by a digestion–circularization polymerase chain reaction method

(IgG1/interleukin 4/isotype)

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ABSTRACT B lymphocytes expressing surface IgM with or without IgD may switch to the expression of other isotypes (IgG, IgA, or IgE) in the course of immune responses. Analyses of genomic DNA from cloned myelomas and hybridomas have shown that the isotype switch is accompanied by a rearrangement characterized by deletion of DNA between the switch (S) region of the μ gene and that associated with the new isotype, resulting in the formation of a composite S region. Measurement of this deletional rearrangement has been difficult in populations of normal B cells but would be useful for investigating the mechanism of the rearrangement and determining whether deletional rearrangement is responsible for all instances of class switching. We have developed a sensitive assay for deletional rearrangement that we designate the digestion–circularization polymerase chain reaction (PCR). In this assay, genomic DNA is digested with a restriction enzyme that recognizes sites that flank the recombined composite S region. The digested DNA is then ligated at low concentrations to favor the formation of circles. The ligation joins the 5′ and 3′ ends of each restriction fragment, making it possible to amplify by PCR across the ligated restriction site by using appropriate primers. From DNA that has undergone deletional rearrangement, a single-sized PCR product is produced and can be quantitated. We demonstrate here that the digestion–circularization PCR assay can detect Sμ–Sγ1 rearrangements in B cells cultured with lipopolysaccharide and interleukin 4. The assay is sensitive enough to quantitate switched cells constituting only 1–2% of the population.

Mature naive B lymphocytes bear receptors of the IgM and IgD classes. Some of these cells undergo immunoglobulin class switching leading to their expression of immunoglobulins of other classes. In IgM-secreting cells, the functionally rearranged VDJ (variable–diversity–joining) gene encoding the heavy chain V region lies upstream of the gene encoding the μ constant region (Cμ), whereas the C regions encoding the other isotypes lie in a cluster downstream of Cμ. In plasmacytomas and hybridomas that express an isotype other than IgM, the corresponding C region gene has been rearranged to a position downstream of the VDJ. This DNA rearrangement is a consequence of deletional recombination involving regions of repeated sequence known as switch (S) regions that are found upstream of every C gene except Cδ (1, 2).

Mechanisms other than deletional recombination may explain the expression of non-IgM isotypes under certain circumstances. For example, a "switched" C region (Cx) could be expressed without the deletion of DNA by (*i*) alternative splicing of a long transcript that includes VDJ, Cμ, and Cx (3–6) or (*ii*) trans-splicing between separate transcripts encoding VDJ and Cx (7, 8).

Evaluation of the importance of these alternatives to deletional recombination requires an assay capable of quantitating recombination in normal B cells. Class switching to IgG1 occurs with high frequency in murine B cells stimulated with lipopolysaccharide (LPS) and the lymphokine interleukin 4 (IL-4) (9). Although immunoglobulin protein and mRNA of the switched isotype can be sensitively detected, the measurement of DNA rearrangement in these cells has been difficult (see *Discussion*).

We report here a technique that allows the quantitation of the frequency of immunoglobulin regions in which deletional recombination between Sμ and Sγ1 has occurred. This technique, the digestion–circularization polymerase chain reaction (DC-PCR), measures the amount of composite Sμ–Sγ1 regions by a technique that involves restriction digestion at sites flanking the composite S region, ligation under limiting DNA concentration to form circular DNA, and subsequent amplification of a single-sized fragment spanning the ligation site that is unique to loci that have formed such composite S regions. We show that this assay is quantitative and capable of detecting composite S regions that occur at a frequency of 1–2% of the entire population. Although we describe this technique in detail for measuring rearrangement to Sγ1, the method can be modified to allow measurement of deletional recombination to any of the S regions.

MATERIALS AND METHODS

Cell Culture. Splenic B cells were prepared from 8- to 12-week-old female C57BL/6 mice and were cultured with LPS (20 μg/ml) and recombinant IL-4 (10,000 units/ml) as described (10), except that 5% (vol/vol) fetal bovine serum was used.

P3X63AG8, here called P3, a tissue culture line derived from the BALB/c myeloma MOPC21 (11), was obtained from American Type Culture Collection and maintained in Dulbecco's modified Eagle's medium with glucose (Biofluids, Rockville, MD; 4.5 g/liter) and 10% fetal bovine serum.

DNAs. BALB/c liver DNA was a gift from Toshio Tanaka, Zheng-Sheng Ye, and David Margulies (National Institute of Allergy and Infectious Diseases, Bethesda, MD). MOPC21 DNA was a gift from Fred Mushinski (National Cancer Institute, Bethesda, MD). DNA from B cells and P3 was prepared by standard methods (12). Briefly, the cells were lysed in SDS and proteinase K. After phenol/chloroform

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Abbreviations: LPS, lipopolysaccharide; IL-4, interleukin 4; DC-PCR, digestion–circularization polymerase chain reaction; C, constant; S, switch; nAChRe, nicotinic acetylcholine receptor β subunit gene; V, variable; J, joining; D, diversity; Cμ, Sμ, etc., μ C region, μ S region, etc.

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extraction, the samples were treated with RNase at 37°C for several hours and then reextracted. DNA concentration was determined by ultraviolet absorption at 260 and 280 nm. Plasmid DNAs were prepared on Quiagen (Studio City, CA) columns according to the manufacturer's instructions and quantitated by fluorometry (Hoefer).

DC-PCR Method. Genomic DNAs were digested with the restriction endonuclease *Eco*RI (New England Biolabs) under conditions suggested by the manufacturer. Usually, 2 μg of DNA in a final volume of 100 μl was digested with 5 units of *Eco*RI at 37°C overnight. The *Eco*RI enzyme was then inactivated by heating at 70°C for 20 min.

Ligation was performed under dilute conditions (1.8 μg of DNA per ml) to produce 90% circularization (13) in standard buffer (12) with 2 units of T4 DNA ligase (BRL) or 400 units of T4 DNA ligase from New England Biolabs in a final volume of 20–500 μl and incubated at 16°C overnight.

For the PCR, the 5′μ primer (see Fig. 1) was 5′-GGCCGGTCGACGGAGACCAATAATCAGAGGGAAG-3′, where the first 11 nucleotides contain a *Sal* I site and the last 23 nucleotides represent the mouse genomic sequence on the antisense strand (GenBank code, MUSIGCD07 3680-3658; GenBank accession nos. J00440, J00480, and V01524) located 94 nucleotides downstream of the *Eco*RI site 3′ of the immunoglobulin heavy chain enhancer (14). The 3′γ1 primer was 5′-GCGCCATCGATGGAGAGCAGGGTCTCCTGGGTAGG-3′, where the first 12 nucleotides contain a *Cla* I site and the last 23 nucleotides represent the mouse genomic sequence on the sense strand (GenBank code, MUSIGHANB 8893-8915; accession no. M12389) located 51 nucleotides 5′ of the *Eco*RI site that is immediately downstream of Sγ1 (15).

Usually, 5 ng of ligated DNA was amplified in 20 μl of 1.5 mM $MgCl_2$/10 mM Tris·HCl, pH 9.0/50 mM KCl/each primer at 0.5 μM/all four dNTPs (each at 200 μM; Pharmacia)/0.5–2.5 units of *Taq* DNA polymerase (Digene Diagnostics, Silver Spring, MD) or recombinant *Taq* DNA polymerase (AmpliTaq, Perkin–Elmer/Cetus) overlaid with mineral oil (Sigma). For quantitation, 0.5 μl of [α-^{32}P]dCTP (3000 Ci/mmol; 10 mCi/ml; 1 Ci = 37 GBq; Amersham) was added to the reaction mixture. This mixture was amplified in a Perkin–Elmer/Cetus DNA thermal cycler under the following conditions: denaturation at 94°C for 6 min, incubation for 5 cycles at 94°C for 1 min, 65°C for 1 min, and 72°C for 2 min; incubation for 30 cycles at 94°C for 1 min, 68°C for 1 min, and 72°C for 2 min; extension at 72°C for 7 min; and final incubation at 4°C. PCR products were analyzed by standard PAGE (12). When radioactive dCTP was incorporated, the gels were also dried and autoradiographed.

Quantitation of DC-PCR. As a control for variations in the amount of DNA in the starting material, the efficiency of digestion, and the efficiency of ligation, primers were used that would generate a DC-PCR product from an unrelated gene. For this purpose, primers from the published sequence of a 4281-base-pair (bp) *Eco*RI fragment of the nicotinic acetylcholine receptor β subunit gene (nAChRe; GenBank code, MUSACHRBB; GenBank accession no. J04699) (16) were chosen, because the *Eco*RI fragment (4281 bp) would be similar in size to that generated by Sμ–Sγ1 rearrangement. The nAChRe primers (5′-GGCCGGTCGACAGGCGCGCACTGACACCACTAAG and 5′-GCGCCATCGATGGACTGCTGTGGGTTTCACCCAG) produce a 753-bp fragment in the DC-PCR assay.

A competitive substrate method was used to quantitate the PCR amplification (17). Two modified plasmid templates were constructed: one (designated p4AP) contains a Sμ–Sγ1 DC-PCR product with a 46-bp DNA fragment inserted at the internal *Eco*RI site, cloned into the *Cla* I–*Xho* I site of pBSXR(3AF), a pBluescript KS(+) (Stratagene) derivative that lacks *Eco*RI sites. The modified plasmid template for nAChRe DC-PCR (p2AO) contains the corresponding DC-PCR product with a 263-bp deletion (created by opening the insert with *Nhe* I and religating) cloned into the *Cla* I–*Xho* I site of pBSXR(3AF). A PCR was performed on mixtures containing various amounts of modified plasmid templates added to a constant amount of genomic DNA. The amount of modified plasmid template that produced a PCR product equal in amount to that of the genomic template DNA provided an estimate of the starting concentration of genomic template.

To quantitate the amount of PCR product, [α-^{32}P]dCTP was added to the reaction mixture as described above. The gels containing the radioactive PCR product were dried and radioactivity in bands on the gel corresponding to products of plasmid and genomic DNA was measured with an AMBIS Mark II radioanalytic imaging system (AMBIS Systems, San Diego; courtesy of Ida Owens, National Institute of Child Health and Human Development). Background cpm was subtracted from these values. In calculating the relative molar yields of the DC-PCR products, the number of cpm incorporated in the products was corrected for the differing cytosine content of the products. Specifically, in Sμ–Sγ1 DC-PCR, the radioactivity of the genomic product was multiplied by 108/89, since the genomic product contains 89 cytosines, whereas the p4AP product contains 108. Similarly, in nAChRe DC-PCR, the cpm incorporated by the genomic product was multiplied by 238/342. The logarithm of the ratio of genomic to plasmid product was calculated and plotted versus the logarithm of the plasmid product alone. The point where the plasmid and genomic product are equal was determined graphically by a best fit linear curve by MICROSOFT EXCEL (Microsoft, Redmond, WA) and CRICKET GRAPH (Cricket Software, Malvern, PA) Macintosh programs.

RESULTS

DC-PCR Assay. Composite S regions are formed as a result of deletional recombination. We developed DC-PCR to quantitate such composite S regions. The strategy is shown in Fig. 1 for the murine Sμ–Sγ1 switch. Genomic DNA is cut with *Eco*RI at sites flanking both the Sμ and Sγ1 regions in areas of known DNA sequence. The resulting fragments are ligated under conditions that favor their circularization and the

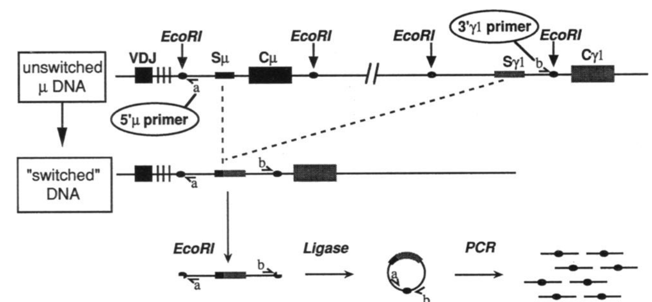

FIG. 1. DC-PCR strategy. The top line shows a schematic (not to scale) map of B-cell genomic DNA prior to the isotype switch; the Sμ and Sγ1 segments are intact and separated by ≈100 kilobase pairs. *Eco*RI sites are represented by small solid ovals. The middle line shows the genomic DNA after Sμ–Sγ1 rearrangement; the composite Sμ–Sγ1 region lies on a single *Eco*RI fragment with ends close to the oligonucleotide sequences marked 5′μ (arrow a) and 3′γ1 (arrow b). The bottom line shows the DC-PCR strategy. The switched genomic DNA digested by *Eco*RI yields the linear fragment shown at left. This fragment is circularized by ligation and the circularized fragment is subjected to a PCR using the 5′μ and 3′γ1 oligonucleotides as primers. In unswitched DNA, corresponding to the top line of the figure, *Eco*RI digestion and ligase-induced circularization would leave the primer targets of 5′μ and 3′γ1 on separate circles, so no template for the PCR would be generated.

region spanning the circle "joint" is amplified using primers complementary to sequences near the *Eco*RI site 5′ of Sμ and the *Eco*RI site 3′ of Sγ1, designated 5′μ and 3′γ1, respectively (Fig. 1). The PCR amplification product can be visualized by gel electrophoresis and quantitated by amplifying in the presence of [α-^{32}P]dCTP and measuring the incorporation of radioactivity into the product. This technique yields a PCR product only from rearranged DNA containing composite Sμ–Sγ1 regions; in unrearranged DNA subjected to the digestion–circularization protocol, the 5′μ and 3′γ1 primer targets would be on two separate DNA circles and thus no PCR amplification product would be obtained.

Sμ–Sγ1 Rearranged DNA Is Detectable by DC-PCR. The ability of DC-PCR employing the 5′μ and 3′γ1 primers to detect Sμ–Sγ1 rearrangement was tested using DNA from the IgG1-secreting myeloma MOPC21 (Fig. 2). A PCR amplification product of the expected size (219 bp) was produced from samples of MOPC21 DNA but no such product was obtained from BALB/c liver. The PCR-amplified product was shown to be the correct product by cloning and sequencing (data not shown).

Sμ–Sγ1 Rearranged DNA Appears in B Cells Stimulated with LPS and IL-4. To investigate heavy chain class switching in normal B cells, B cells were cultured with LPS in the presence or absence of IL-4. Surface IgG1 was first detected at 2.5 days of culture and peaked at 4.5–5 days (data not shown). The results of DC-PCR on DNA samples obtained from B cells cultured in LPS with or without IL-4 are shown in Fig. 2. With the 5′μ and 3′γ1 primers, no amplification product was observed from normal resting B-cell DNA (day 0) or from DNA from B cells cultured in LPS alone for up to 5 days, in keeping with the lack or paucity of cells expressing membrane-bound IgG1 or secreting IgG1 in such cultures. A PCR product of the correct size was observed in samples of DNA obtained from B cells cultured in LPS plus IL-4 for 3 days or longer. No such product was observed with samples from cells cultured for 2 days or less with LPS plus IL-4. These results demonstrate that IL-4 stimulation is required both for the appearance of membrane IgG1 and for Sμ–Sγ1 rearrangement in LPS-cultured B cells.

Quantitation of DC-PCR. To quantitate the DC-PCR assay, we used controls for both variation in template preparation (e.g., variation in DNA isolation, *Eco*RI digestion, and ligation) and for tube-to-tube variation in PCR amplification. To control for template preparation, primers that would generate a DC-PCR product from an unrelated gene, not requiring any prior rearrangement, were utilized. For this purpose, the nAChRe gene was chosen. Each sample of digested and circularized DNA was tested for both Sμ–Sγ1 rearrangement and nAChRe gene content by amplification with the appropriate primer pairs.

As a control for thermocycler variation and as a method of quantitation, modified templates, p4AP and p2AO, engineered to yield electrophoretically distinguishable PCR products, were titrated into both the Sμ–Sγ1 and the nAChRe PCRs. This quantitative DC-PCR method was used to measure the number of Sμ–Sγ1 rearranged chromosomes relative to nAChRe chromosomes in an IgG1-producing cell line, P3. Results of DC-PCR using 5′μ and 3′γ1 primers on a constant amount of P3 genomic DNA mixed with various amounts of p4AP plasmid DNA are shown in Fig. 3*A*. The radioactivity in the relevant bands was measured and the logarithm of the ratio p4AP/genomic PCR product cpm was plotted against the logarithm of the number of p4AP copies. By using a best-fit linear line (Fig. 3*B*), the number of p4AP copies at the equivalence point [log(p4AP/genomic) = 0] was calculated to be 102, which provides a raw estimate of the number of genomic Sμ–Sγ1 templates. Results of a DC-PCR using nAChRe primers on a constant amount of P3 DNA with a titration of p2AO plasmid DNA are shown in Fig. 3*C*. A similar calculation (Fig. 3*D*) revealed the amount of nAChRe templates to be equal to 160 copies of p2AO. The ratio of the estimated number of copies of Sμ–Sγ1 to that of nAChRe (102/160 in this case) was averaged over four experiments to give a raw Sμ–Sγ1/nAChRe ratio of 0.631 for P3 (Table 1). Because the actual ratio of Sμ–Sγ1/nAChRe in P3 is 0.333, as determined by quantitative Southern blot analysis (data not shown), the raw ratio estimated by DC-PCR was divided by a normalization factor of 1.89 to yield an accurate measure of the actual template ratio (see *Discussion*).

Quantitation of Sμ–Sγ1 Rearrangements in Mixtures of P3 and BALB/c Liver DNA. To demonstrate the precision of DC-PCR quantitation of Sμ–Sγ1 rearrangement events, we mixed various amounts of P3 DNA with BALB/c liver DNA and performed a DC-PCR on these mixtures. After determining the equivalence points as above, a PCR was performed on a series of 1:2 dilutions of DNA near the equivalence point to ensure that quantitation was determined within the exponential phase of the PCR (18). A log–log plot of the cpm incorporated in the PCR product bands versus dilution showed a linear relationship, allowing an estimate, using best fit linear line, of the number of template copies for both Sμ–Sγ1 and nAChRe and of the ratio of the two. The results of such quantitation at various ratios of P3 to BALB/c DNA are shown in Table 1. The normalized DC-PCR estimates accurately quantitate the expected number of Sμ–Sγ1 rearrangements from expected values of 1–33%.

DISCUSSION

In cloned IgG1-expressing cell lines, the immunoglobulin locus has undergone a recombination event in which about 100 kilobase pairs of DNA is deleted from the expressed chromosome, joining the Sμ and Sγ1 sequences into a composite S region (1, 2). In cells switching in culture, a direct assessment of deletional recombination would be useful; strategies that infer genetic events on the basis of γ1 RNA or protein expression might be misled by the potential

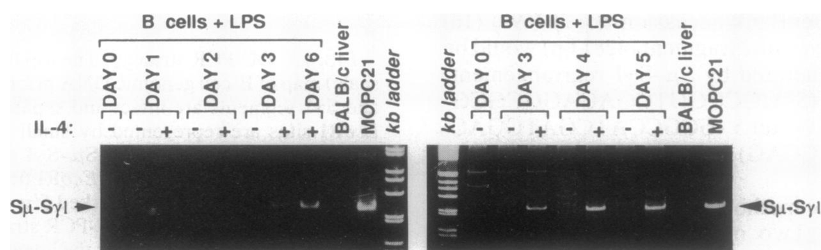

FIG. 2. Detection of Sμ–Sγ1 rearrangement by DC-PCR. Results of a DC-PCR using 5′μ and 3′γ1 primers on DNA samples from BALB/c liver, MOPC21, or B cells cultured in LPS with (+) or without (−) IL-4 are shown. Days of culture are indicated above each lane. A 10-μl sample of the amplification mixture was electrophoresed through an 8% polyacrylamide gel (*Left*) or a 20% polyacrylamide gel (*Right*). DNA size standard (1 kb DNA ladder, GIBCO/BRL; 1 μl) was electrophoresed in lanes marked kb ladder. All samples produced a DC-PCR product using a primer pair specific for a germ-line template.

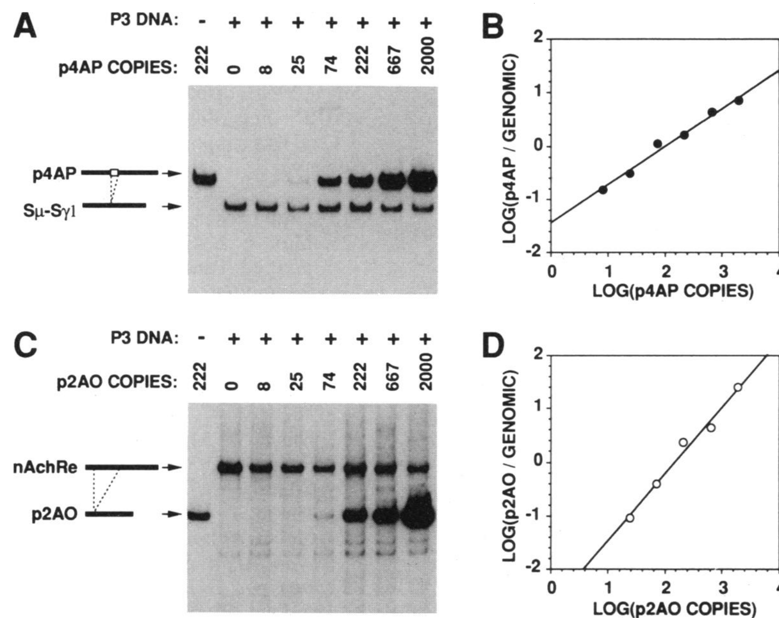

FIG. 3. Quantitation of DC-PCR by titration with competitive modified plasmid substrate. (*A* and *C*) Quantitative DC-PCR. Product (10 μl) amplified from a mixture of 0 (−) or 5.4 ng (+) of P3 DNA (digested and circularized) plus various amounts of the competitive substrates p4AP (*A*) or p2AO (*C*) were loaded in each lane of an 8% polyacrylamide gel and electrophoresed. The limit of detection is <8 copies for Sμ–Sγ1 DC-PCR and between 8 and 25 copies for nAChRe. (*B* and *D*) The logarithm of the ratio of radioactivity of the PCR products generated from modified plasmid substrates to that from the genomic templates was plotted against the logarithm of the number of p4AP or p2AO copies. The equivalence point [log(modified plasmid substrate/genomic template) = 0] is determined from the best fit linear line (for Sμ–Sγ1, $y = 1.4461 + 0.72008x$, $R^2 = 0.982$; for nAChRe, $y = 2.7470 + 1.2464$, $R^2 = 0.985$). Points where PCR amplification is not in the exponential range are not considered in the best-fit linear line determination.

participation of trans-splicing or other mechanisms that could regulate γ1 mRNA or protein expression independently of switch recombination.

Table 1. Quantitative Sμ–Sγ1/nAChRe DC-PCR on mixtures of BALB/c liver and P3 DNAs

DNA ratio	Sμ–Sγ1/nAChRe template ratio		
BALB/P3	Added	Raw DC-PCR ratio	Observed
0/1	0.333	0.631	0.333
1/1	0.159	0.277	0.146
2/1	0.104	0.172	0.091
9/1	0.031	0.069	0.036
29/1	0.010	0.012	0.006

A total of 5.4 ng of DNA was used per reaction mixture. The ratio of BALB/c liver to P3 DNA on a weight basis is indicated. The added Sμ–Sγ1/nAChRe template ratio was calculated from estimates of the BALB/c liver and P3 cell equivalents added and the content of the two templates per cell. The ratio of cell equivalents was based on karyotypic analysis of our P3 line indicating 66 chromosomes per cell (data not shown) rather than the normal 40 chromosomes per cell expected of BALB/c liver; by assuming a roughly equal weight for all chromosomes, the number of P3 cells per ng of DNA was calculated to be 40/66 of that of BALB/c liver. BALB/c liver was assumed to contain two copies of nAChRe and zero copies of Sμ–Sγ1 template per cell. For P3, the template content was estimated from the following considerations. Our P3 cell line was determined by karyotype analysis to have an average of three copies each of the chromosomes bearing the immunoglobulin heavy chain locus (chromosome 12) and the nAChRe gene (chromosome 11) (data not shown). Southern blot analysis demonstrated that all copies of the Cγ1 gene in P3 had the same rearrangement, with no unswitched copies present. Quantitative Southern blots showed that the ratio of Cγ1/nAChRe content in P3 was 0.29 of the ratio in BALB/c, consistent with the assumption of one rearranged chromosome 12 containing a Sμ–Sγ1 template per three chromosomes 11 containing nAChRe templates. The observed ratio was calculated by dividing the raw DC-PCR Sμ–Sγ1/nAChRe ratio by a normalization factor of 1.89 (see *Results*).

Two approaches have previously been used to document DNA switch rearrangement in cultured cells, each having its own limitations. Southern blots are capable of assessing switch rearrangement in switched cells representing a clonal (or perhaps pauciclonal) population by demonstrating a new rearranged band. However, in polyclonal cell populations, Southern blot measurement of switching relies upon the loss of the germ-line configuration of the involved S regions, making it difficult to obtain precise measurements when only a minor fraction of the cells have undergone the switch event.

Recently, we (unpublished observations) and others (19) have developed a PCR-based strategy that has detected switch rearrangements in populations of human B lymphocytes that synthesize IgE under the influence of IL-4. That strategy, "direct PCR," differs from the one described here in several respects. In direct PCR, the composite Sμ–Sε region is amplified using a sense primer 5′ of Sμ and an antisense primer 3′ of Sε. Although direct PCR has the advantage that the product amplified includes the composite S region, it cannot easily be adapted to quantitate rearrangement events since the amplified products vary considerably in length and, therefore, in amplification efficiency. In addition, the tandem repeat sequences of the S regions could contribute to difficulties in amplifying large composite S regions.

To circumvent the difficulties of both the Southern blot and direct PCR techniques, we designed the DC-PCR strategy. In this approach (Fig. 1), the PCR template is generated *in vitro* by a restriction endonuclease digestion and a ligation step, but template formation can occur only when the two primer sequences have been previously rearranged *in vivo* so that they fall on the same restriction fragment. By appropriate choice of primers, the DC-PCR product can be designed to be arbitrarily small and thus easily amplified. The size of the product is invariant, regardless of the position of the recombination junction in the composite S region. Finally, the difficulties that tandem repeats may present for the direct

PCR amplification process are avoided with DC-PCR since the S regions are not part of the amplification template.

Accurate and reproducible DC-PCR quantitation requires efficient restriction enzyme digestion and ligation. The ligation conditions favoring circularization have been analyzed (13) both to optimize the construction of "chromosomal-jumping" libraries and to facilitate an inverse PCR, a technique used to amplify DNA flanking a region of known sequence (20, 21). We chose to monitor the consistency of digestion and ligation steps by amplifying a control DC-PCR template, nAChRe, that does not require prior *in vivo* rearrangement and contains an *Eco*RI fragment within the size range expected for the *Eco*RI fragments bearing the composite $S\mu$–$S\gamma1$ region.

To quantitate the number of starting templates ($S\mu$–$S\gamma1$ or nAChRe) in terms of the measured yield of PCR products, we titrated increasing amounts of modified plasmid templates into a constant amount of the digested/circularized genomic DNA. Tube-to-tube variation in PCR efficiencies was controlled by basing our calculations on the ratio of the genomic and modified product and on the assumption that most causes of variation in PCR efficiency would affect amplification from both templates equivalently. To validate this assumption, we demonstrated that the ratio of the two products remained constant over a broad range of cycle numbers (data not shown). After comparison of a quantitative Southern blot analysis with a quantitative DC-PCR, a normalization factor was introduced. The reasons underlying the requirement for a normalization factor were not established but could include differences in amplification in the initial cycles of our supercoiled plasmid templates versus the relaxed circular ligated genomic DNA.

The experiments presented here have demonstrated that DC-PCR can detect $S\mu$–$S\gamma1$ rearrangement in mouse spleen cells cultured with LPS and IL-4; cells cultured in the absence of one or the other of these agents show no rearrangement. In preliminary experiments (data not shown), this technique has also been applied to detect murine $S\mu$–$S\alpha$ and human $S\mu$–$S\varepsilon$ rearrangements in cultured populations of lymphocytes; presumably, it has general applicability for detecting any DNA rearrangements that occur within specific target sequences. Because the technique is quantitative, it should be useful in addressing questions relating to the switch mechanism. In addition, the DC-PCR technique can be applied to detect the reciprocal circles that are formed as a byproduct of the switch rearrangement.

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