Detection of Immunoglobulin/c-myc Recombinations in Mice That Are Resistant to Plasmacytoma Induction

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ABSTRACT

Interchromosomal recombinations between c-myc and immunoglobulin sequences can be found in preneoplastic lesions (oil granuloma) during pristane-induced plasmacytoma development in susceptible BALB/cAn mice. In this study we used a more sensitive approach, hybridization-enriched templates with nested PCR, to detect microclones with Igα/c-myc recombinations in oil granuloma of susceptible and resistant mice. Recombinations were detected in as many as 73% (32/44) of plasmacytoma-susceptible BALB/cAn mice 30 days after an injection of pristane. Mice that are resistant to plasmacytoma induction can also harbor recombination-positive cells, but these are less frequent [2/20 DBA/2N, 8/20 (BALB/cAn × DBA/2N)F1, hereafter called CDF2]. The clones in DBA/2N mice were small (<400 cells), whereas in BALB/cAn, the oil granuloma harbored up to several thousand of these cells. We conclude that the molecular machinery for generating characteristic interchromosomal recombinations can be found in all strains of mice. Both the frequency of generating Igα/c-myc recombinations and the expansion of recombination-positive cells are greater in susceptible mice than in resistant strains.

INTRODUCTION

Transchromosomal recombinations juxtaposing c-myc and immunoglobulin sequences characterize Burkitt’s lymphoma and oil-induced murine plasmacytoma (1–5). Although these mutations are found in nearly 100% of these tumors, it is not known whether tumor resistance can be caused by a failure to generate these recombinations. In the mouse model, plasmacytomas can be induced in BALB/cAn mice by the i.p. injection of the isoalkane pristane. However, DBA/2N mice and F1 hybrids between BALB/cAn and DBA/2N (CDF2) are solidly resistant to plasmacytoma induction (reviewed in Ref. 4). Cells with Igα/c-myc recombinations are found in preneoplastic lesions (OGs)3 in BALB/cAn mice at a high frequency (6, 7). The size of the clones detected suggests that some of the recombination-positive clones must have undergone substantial expansion in the OG. Here we describe the supplementation of nested PCR with an enrichment of specific DNA sequences. Applying this method, we compared the frequency and size of preneoplastic recombination-bearing cell clones with the susceptibility to plasmacytoma induction by pristane in mice with different genetic backgrounds.

MATERIALS AND METHODS

Preparation of DNA from OG. Mice were given i.p. injections of 0.5 ml pristane and sacrificed after 30 days. The small intestine, beginning at the ileocecal junction and proceeding proximally from the ilium to the distal duodenum, was stripped away from the mesentery. The remaining intestinal mesentery was then dissected out in a single fragment. This tissue contained a large portion of the 30-day OG, and to this sample the omentum was added. The tissue was then disrupted into a single-cell suspension by passing through wire meshes. The cells liberated from the sieved tissues were centrifuged, and the pellet was then lysed in buffer consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl2, 0.5% Tween 20, and 100 μg/ml proteinase K. The DNA preparation continued with phenol/chloroform extraction and ethanol precipitation, yielding a total of 250–500 μg DNA/mouse OG. The precipitate was rehydrated in 400 μl TE buffer (10 mM Tris-HCl (pH 8.3) and 1 mM Na2EDTA), and 270 μl of this preparation were digested with EcoRI (500 ng or >2 X 106 copies): a 7-kbp HindIII fragment that spans c-myc exons 1–3 and a 5.4-kbp EcoRI fragment that spans the Igα switch region to exon 2 of Ca. Prior to use, the probes were biotinylated by nick translation with biotinylated α-UTP (according to the supplier BRL, Gaithersburg, MD), and excess biotin was removed by washing twice with 1 ml water and recentrifugation using Centricon-30 columns. The hybridization was performed in 1.5 ml 50% formamide, 5× SSPE (750 mM NaCl, 50 mM NaH2PO4, and 5 mM EDTA), 0.1% SDS, and 5× Denhardt’s solution.

To isolate specific DNA hybridized to the biotinylated probes, 300 μl avidin-coated polystyrene beads (Baxter, Mundelein, IL) were mixed with the probe-DNA solution and rocked gently for 30 min at room temperature. Ten ml water were added, and the beads were pelleted for 10 min at 9000 × g. The pellet was washed once with 1.5 ml 0.2× SSC, 0.1% SDS at room temperature, and recentrifuged. Washed beads were resuspended in 1 ml 0.1 M NaOH and shaken at 37°C for 30 min to denature the hybridized molecules. The denatured DNA in the supernatant was washed twice with water using Centricon-30 columns and used as a template for nested PCR.

PCR. The PCR reaction mixture contained a final concentration of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.1 mg/ml gelatin, 0.5 μM each primer, and 200 μM each deoxynucleotide triphosphate in a volume of 50 μl. Cycling conditions involved a 5-min initial denaturation at 95°C with the reaction subsequently being held at 80°C for the addition of 1.25 units Taq polymerase. This was followed by 40 cycles of denaturation (15 s at 95°C), annealing (15 s at 65°C), and extension (between 30 s and 2 min at 72°C). For nested PCR, 1 μl reaction mixture from round one was used as a template for an additional round of the same 40 cycles. Amplification products were subsequently gel purified and sequenced directly using cycle sequencing with Taq polymerase (Promega, Madison, WI) or were sequenced using the Sequenase kit (United States Biochemical, Cleveland, OH) after cloning into pBluescript vector (Stratagene, La Jolla, CA). We classified products as nonartificial when two independent PCR amplifications of the same DNA preparation resulted in identical and unique recombination sequences.

All templates were analyzed using six different primer combinations that have been successfully used to amplify Igα/c-myc recombinations on the Chr 12 and with two different pairs that were successful in detecting the reciprocal chromic Chr 15 (7). The sequences and locations of the oligonucleotides used for PCR amplification were derived from published sequences (GeneBank entries MUSCMYC1, MUSIALPHA, and MUSIGCDO7) and named as follows: mycl2+1a: 5’-TAAAA-GGGGA-GGGGG-TGTCA-AATAA-TAAGA-3’; mycl2+1b: 5’-GAGAGGCTCC-TTGGTGAGC-TCAACA-3’; mycl2+2a: 5’-CCGGT-TTTCC-CTTCTC-CCTTCT-CCTTGT-GCAT-CTT-3’; mycl2+2b: 5’-ACTCCA-GATCT-ACCCACC-GACCT-ACTCC-ACTA-CTT-3’; mycl2+3a: 5’-AAGG-GGGGG-TGGGG-GGGGA-TTATG-3’; mycl2+3b: 5’-TTTC-GTCTC-TGTCG-TGCTT-GCCCT-GCCGT-CTTGT-3’; αl2+1a: 5’-CGTGA-ATCA-GACG-GCACG-GCTATT-GATCC-3’; αl2+1b: 5’-GAGCT-GACCA-ACAGT-TCTGG-CTGTA-TAGAC-3’; αl2+2a: 5’-ATTGT-AACCA-GCCCA-GCCCA-CTTTC-3’; αl2+2b: 5’-CATCCC-C...
This DNA was 35S-labeled by nick translation, and 2.5 ng (approximately 10^8 copies) were mixed with 300 μg of sheared herring sperm DNA for each reaction. This mixture was hybridized under different conditions to the c-myc and Iga hybridization probes, followed by the separation step with avidin-coated beads. The enrichment efficiency was assessed by measuring the 35S label.

Varying the following parameters affected the efficiency of the enrichment: (a) denaturing the genomic DNA/hybridization probe mixtures by heat was slightly more efficient than incubation in 0.2 N NaOH for 15 min; and (b) different concentrations of the avidin beads during the binding step to the biotinylated hybridization probes had the most dramatic effect on the enrichment efficiency. For a given amount of beads, increasing the reaction volume decreased the yield of labeled DNA.

Changing other reaction conditions that did not affect the yield of 35S label was as follows: (a) Biotinylation of the hybridization probes according to the instructions of the supplier proved to be sufficient. Using the probes at lower concentrations during the biotinylation did not improve the efficiency of enrichment. (b) Neither the use of higher concentrations of hybridization probes nor the use of inserts rather than of entire plasmids during the hybridization to the genomic DNA increased the recovery of 35S label. (c) The binding of avidin-coated beads to the biotinylated probes was equally efficient whether the reaction was performed in hybridization buffer or in TE buffer. (d) During the washing step, <5% of label was lost and <5% remained bound to the beads after treatment with NaOH. (e) To exclude mouse strain-specific differences in the efficiency of the hybridization-enriched-template-PCR protocol, the IGA hybridization probe was replaced by 3.0-kbp probes of the IGA switch regions from either BALB/cAn or DBA/2N mice. The use of either probe along with the c-myc hybridization probe did not effect the yield of 35S label.

The procedure of template enrichment for IGA and c-myc sequences was optimized using a cloned 15.5-kbp EcoRI fragment that includes the IGA/c-myc rearrangement of the plasmacytoma TEPC609 (7) between 12.5 ng in Lane 1 and 20 pg in Lane 5; Lane 6, liver DNA only). These mixtures were processed as described above. The plasmacytoma-specific recombination could be amplified with 100 pg DNA as the minimum (Lane 4). Four independent PCR reactions were performed with identical results. Lane 7, 500 ng 1-kbp ladder size marker (BRL).

RESULTS

Sensitivity of Detecting Iga/c-myc Recombination Fragments. The procedure of template enrichment for IGA and c-myc sequences was optimized using a cloned 15.5-kbp EcoRI fragment that includes the IGA/c-myc rearrangement of the plasmacytoma TEPC1165 (10). This DNA was 35S-labeled by nick translation, and 2.5 ng (approximately 10^8 copies) were mixed with 300 μg of sheared herring sperm DNA for each reaction. This mixture was hybridized under different conditions to the c-myc and IGA hybridization probes, followed by the separation step with avidin-coated beads. The enrichment efficiency was assessed by measuring the 35S label.

Using the optimized protocol, 60–70% of the 35S-labeled specific IGA/c-myc chimeric fragment of TEPC1165, but <1% of the total DNA, was consistently recovered in the enriched samples (data not shown). Tumor-specific recombinational fragments could be detected by PCR in an artificial mixture that contained as little as 100 pg genomic DNA from the plasmacytoma TEPC609 (Ref. 7; equivalent to about 20 cells) in 300 μg mouse liver DNA (Fig. 1b).
To estimate the number of Igα/c-myc recombination-positive cells that are necessary to yield products with regular PCR, i.e., using nonenriched DNA template, we carried out the following dilution experiments. We mixed defined numbers of recombination-positive plasmacytoma cells [XRPC24 (11)] with BALB/cAn 30-day-old OG and performed five PCR amplifications with each dilution. Approximately 400 tumor cells had to be present in an OG to yield one successful amplification of the plasmacytoma-specific recombination in five PCR experiments. More than 2000 cells resulted in the appropriate product in nearly 100% of amplifications (data not shown).

Frequency of Igα/c-myc Recombinations in Preneoplastic Lesions. We amplified recombinations between Igα and c-myc in mesenteric OG by PCR using DNA that had undergone enrichment for c-myc- and Igα-specific EcoRI fragments (Table 1). In all mouse strains the incidence of mice harboring Igα/c-myc recombined cells exceeded the strain-specific tumor incidence (Table 2). The highest frequency of rearrangements (73%) was detected in plasmacytoma-susceptible BALB/cAn mice. In contrast, recombination-positive cells were rarely found in DBA/2N mice (10%) which are resistant to plasmacytoma induction by pristane. The number of CD2F1, hybrids with recombinations at day 30 was intermediate (40%), and it exceeded by far the strain-specific plasmacytoma incidence (<1%).

Size of Cell Clones with Igα/c-myc Recombinations. Recombination-positive OGs were divided into three different groups (Table 2): The sole amplification with hybridization-enriched template-PCR indicated the presence of microclones only (<400 cells). If Igα/c-myc junctions could be detected by PCR in nonenriched DNA, we estimated the clonal size to be between 400 and 2000 recombination-positive cells. If PCR products of identical size were generated in all five or more PCR reactions, then we assumed the presence of at least 2000 cells with immunoglobulin/c-myc recombination in the OG. These samples were further analyzed by competitive PCR.

The occurrence of substantially expanded Igα/c-myc recombination-positive clones (>400 cells) is restricted almost exclusively to susceptible BALB/cAn mice (Table 2). Approximately one third of these mice bore expanded clones, and in two specific cases (cases 5 and #23) we found as many as 5000 Igα/c-myc-positive cells in the OG (Fig. 2). In contrast, none of the 40 DBA2/N mice was positive for an expanded cell clone; we found 1 in 20 CD2F1 mice.

DISCUSSION

In previous articles, we described a nested PCR amplification procedure for detecting Igα/c-myc recombinations, the most prevalent form of transchromosomal recombinations in pristane-induced plasmacytomas (6, 7). In the present study, the method was supplemented by a simple hybridization-based enrichment of specific EcoRI restriction fragments. This addition permitted us to detect microclones of aberrant cells carrying transchromosomal recombinations in a large mass of uninvolved tissue. The high sensitivity of this method may be useful in detecting low numbers of genetically aberrant cells in other situations, particularly in those in which it is desirable to detect minimal residual disease.

This technique allowed us to detect interchromosomal recombin-
ACKNOWLEDGMENTS

We thank Dr. J. Shaughnessy for providing genomic probes and for technical advice. We thank Dr. J. F. Mushinski for many helpful discussions throughout these studies.

1 E. Shacter, personal communication.
DETECTION OF Igα/c-myc RECOMBINATIONS IN MICE

REFERENCES

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