Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 6067–6071, August 1988
Immunology

Rapid induction of IgM-secreting murine plasmacytomas by pristane and an immunoglobulin heavy-chain promoter/enhancer-driven c-*myc*/v-Ha-*ras* retrovirus

(RIM retrovirus/Southern blotting/S1 nuclease mapping)

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Contributed by Michael Potter, March 14, 1988

ABSTRACT **A retroviral vector, RIM, containing murine c-*myc* under the control of immunoglobulin heavy-chain gene promoter and enhancer elements and v-Ha-*ras* driven by a Moloney murine leukemia virus long terminal repeat induced IgM-secreting plasmacytomas in 28% of adult and 83% of 3-week-old pristane-conditioned mice with mean latency periods of 60–70 days. In contrast, the same vector only harboring c-*myc* or v-Ha-*ras* was virtually ineffective. RIM-induced plasmacytomas expressed retroviral *myc* and *ras* genes while their endogenous c-*myc* alleles were unrearranged and transcriptionally inactive. These plasmacytomas were clonal as each possessed a unique immunoglobulin heavy-chain joining region rearrangement and a single recombinant provirus. Moloney murine leukemia helper virus did not play an obligatory role in tumorigenesis since insertions of Moloney murine leukemia proviruses were found in only 6 of 24 plasmacytomas induced in adult mice. Taken together, these findings support the view that the v-Ha-*ras* oncogene can cooperate with an activated *myc* gene in pristane plasmacytomagenesis.**

Plasmacytomas (PCTs) induced in BALB/cAn mice by pristane appear after minimal latent periods of 120 days and mean latent periods of 210–220 days (1). Over 95% of PCTs induced in BALB/cAn mice have chromosomal translocations that either directly or indirectly involve the c-*myc* gene and deregulate its transcription (reviewed in ref. 2). PCTs can be induced with shorter latent periods in mice that are given a single dose of pristane followed by injection of specific kinds of oncogenic retroviruses [e.g., J3 (3) or Abelson murine leukemia virus (4)] along with a wild-type Moloney murine leukemia helper virus (Mo-MuLV).

In the present study, we describe the properties of a highly efficient PCT-inducing retrovirus, RIM, that contains the coding exons of a normal murine c-myc cDNA linked to the immunoglobulin heavy-chain enhancer and an immunoglobulin heavy-chain variable region (V_H) promoter in addition to a long terminal repeat (LTR)-driven v-Ha-*ras* gene. RIM virus consistently induced a high incidence of IgM-secreting PCTs with short latent periods in pristane-treated mice. Another retroviral construct harboring only v-Ha-*ras* induced a few PCTs, whereas another containing only the immunoglobulin heavy-chain promoter/enhancer-driven *myc* cassette did not induce PCTs.

MATERIALS AND METHODS

Animals and Tumor Induction. Mice were 3- to 16-week-old BALB/cAnPt and (BALB/cAnPt × DBA/2N)F_1 (designated CDF_1) strains that were raised in a conventional mouse facility at Hazelton Laboratories, Rockville, MD. Animals were examined 2 or 3 times a week for the presence of tumors beginning 30 days and ending 150 days after pristane injection. Diagnoses of PCTs and other tumors were made from peritoneal smears stained with Wright–Giemsa. Mice 4 weeks or older were given a single 0.5-ml intraperitoneal injection of pristane (2,6,10,14–tetramethylpentadecane; Aldrich), whereas 3-week-old mice received 0.2- or 0.3-ml doses.

Production of Retroviruses. Retroviruses containing v-Ha-*ras* and c-*myc* either alone or in combination were constructed in which v-Ha-*ras* and c-*myc* were placed under the transcriptional control of an Mo-MuLV LTR and immunoglobulin heavy-chain transcriptional enhancer and promoter sequences, respectively (see Fig. 1 for vector constructions). These retroviral constructs [designated pRN(S), pN(S)IM1, and pRN(S)IM1] were cotransfected along with pZAP, a wild-type Mo-MuLV proviral clone (6), by the calcium phosphate technique (11) into NIH 3T3 cells. High titer retrovirus stocks were prepared from tissue culture supernatants of neomycin-resistant 3T3 clones. Viruses were designated R (LTR-driven v-Ha-*ras*), IM (immunoglobulin heavy-chain promoter/enhancer driven c-*myc*) and RIM (combined version of R and IM), and their titers and structures were verified by infecting NIH 3T3 cells and performing Southern analysis on the integrated proviruses in neomycin-resistant colonies. Blot-hybridization analysis revealed LTR-driven spliced and unspliced genome length retroviral transcripts in these neomycin-resistant fibroblasts, whereas subgenomic V_H promoter-driven myc transcripts were not apparent in the IM- and RIM-infected clones. Titers of R, IM, and RIM were 1×10^6, 1×10^7, and 5×10^5 neomycin focus-forming units per ml, respectively. R- and RIM-expressing colonies were morphologically transformed; and as expected IM-infected clones were of normal morphology.

Heavy-Chain Typing. Immunoglobulin heavy-chain classes were determined by a particle concentration fluorescence immunoassay (12), which was read in a Pandex Screen Machine (Baxter, Round Lake, IL). All samples were reacted with goat anti-mouse sera [Southern Biotechnology Associates, (Birmingham, AL), Kirkegaard and Perry (Gaithersburg, MD), and Pandex] that was specific for IgM, IgA, or IgG.

DNA and RNA Analysis. DNA and total cellular RNAs were extracted from pulverized frozen samples by a modifi-

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Abbreviations: PCT, plasmacytoma; Mo-MuLV, Moloney murine leukemia virus; LTR, long terminal repeat; V_H, heavy-chain variable region; nt, nucleotide(s).

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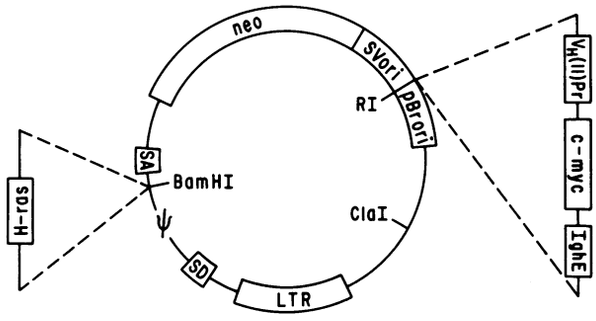

FIG. 1. Retroviral constructs. A smaller derivative of the ZIPNeoSVX(1) retroviral vector (5) was prepared by cleavage at the *Sst* I sites in each LTR followed by ligation, bacterial transformation, and selection in kanamycin medium. A 483-bp *Cla* I–*Sst* I fragment containing the ZIP LTR enhancer was replaced by a 555-bp DNA segment isolated from pZAP, a wild-type Mo-MuLV proviral clone (6), to generate pN(S). The latter manipulation was performed due to the absence of one 72-bp repeat in the original ZIP(Neo) vector. pRN(S) contains the v-Ha-*ras* gene (H-*ras*) of the Harvey murine sarcoma retrovirus (7) on a 700-bp *Sac* I–*Pst* I fragment inserted into the pN(S) *Bam*HI site by linker ligation. pN(S)IM1 and pRN(S)IM1 contain the coding exons of a normal murine c-myc cDNA (8) on a 1.4-kb *Xho* I fragment flanked by immunoglobulin heavy-chain gene transcriptional control elements inserted into the *Eco*RI sites of pN(S) and pR(N)S, respectively. The subgenomic *myc* expression cassette in pN(S)IM1 has the MPC-11 heavy-chain variable-region gene promoter [V_H(11)Pr] on a 204-bp *Eco*RI–*Nco* I fragment (9) at its 5′ end and the immunoglobulin heavy-chain enhancer (IghE) on a ≈700-bp *Xba* I–*Eco*RI fragment (10) at its 3′ end. All constructions were performed by linker ligation in an M13mp18 polylinker, and the completed *myc* expression cassette was inserted into either pN(S) or pRN(S) as a 2.3-kb *Eco*RI fragment. SA, splice acceptor; SD, splice donor; SVori and pBrori, SV40 and pBr322 origins of replication.

cation of the method of Walker and McLaren (13) and by the hot phenol/guanidinium thiocyanate technique (14), respectively. DNA and RNA gel blot hybridizations and S1 nuclease mapping were performed as described in previous publications (8, 15, 16).

RESULTS

Biological Effects of RIM, R, and IM Viruses: Pristane-Dependent Induction of PCTs by RIM Virus. RIM virus injected into normal 3-week-old to 3-month-old mice produced no PCTs in 58 BALB/cAnPt mice (Table 1). Zero percent and 10% of adult and 3-week-old BALB/c mice, respectively, developed thymic lymphocytic neoplasms. In contrast, in single dose pristane-conditioned BALB/cAn mice infected with RIM virus, PCTs were induced with mean latency periods of 60–70 days in 28% of 2- to 4-month old mice and 83% of 3-week-old mice, whereas R virus only induced a few PCTs. RIM virus also induced several myeloid tumors in pristane-conditioned mice. No PCTs were obtained in mice injected with the IM virus (data not shown). The pristane could be administered either 15 days preceding or up to 20 days following viral infection, but the optimal time was 1–3 days before virus infection. CDF_1 mice were also highly susceptible to PCT induction by RIM virus (Table 1). In contrast to PCTs induced by pristane alone, those induced by the RIM and R viruses were readily established in transplant without additional pristane priming. Neither sequential nor simultaneous infection with the R and IM viruses gave rise to PCTs, but 22% of the latter mice developed lymphocytic tumors due to their higher Mo-MuLV dosage (data not shown).

Heavy-Chain Class Secretion. To determine heavy-chain isotype expression, serum or ascites fluid from primary and secondary hosts was electrophoresed on thin-layer agarose gels and found to contain myeloma proteins with a range of electrophoretic mobilities (data not shown). Diluted ascites fluid or serum was reacted with polystyrene beads coated with class-specific goat anti-heavy-chain serum and was developed with a fluorescein-conjugated class-specific goat anti-heavy-chain antibody. Ascites fluid or serum from mice with tumors displayed IgM values 10- to 12-fold above normal serum controls, and most IgM levels were 7 mg/ml or more, while the IgG and IgA values were background. Seventy-nine out of 94 sera (84%) from animals with RIM-induced PCTs contained elevated IgM. One had an IgG and two had both IgM and IgG elevations, while only nine (<10%) contained elevated IgA.

Structure and Expression of Proviral Oncogenes and Endogenous c-*myc* Genes. DNAs were extracted from a representative sample of RIM-induced PCTs, digested with *Eco*RI, *Bam*HI, and *Sst* I, and hybridized to c-*myc*, v-Ha-*ras*, and *neo* specific probes, respectively (Fig. 2). As expected, the viral *myc* gene was found on a 2.3-kilobase (kb) *Eco*RI fragment in all RIM-induced PCTs, and the endogenous c-*myc* alleles remained unrearranged on a 21-kb *Eco*RI fragment. All tumors in Fig. 2 contain a v-Ha-*ras* 700-base-pair (bp) *Bam*HI fragment in addition to endogenous BALB/cAn *ras* hybridizing sequences. Southern hybridization of *Sst* I digestions to a *neo*-specific probe revealed the expected size of the entire recombinant provirus since *Sst* I only cleaves the integrated provirus once in each LTR. Nine RIM-induced PCTs in Fig. 2 contain an intact 7.4-kb provirus, whereas two, 2177 and 2274, show altered sizes. However, our other Southern data indicate that the rearrange-

Table 1. Induction of neoplasias by RIM virus: Frequency and latency of PCTs depend on pristane, animal age, and genetic background

Mouse strain	Pristane, ml	Virus, day	Mice		Tumors			PCT latency period†
			No.	Age	% myeloid	% PCT	% LN*	
BALB/cAn	—	0	28	2–4 mo	0	0	0	—
	—	0	30	3–4 wk	0	0	10	—
	0.5	3–15	122	2–4 mo	1	28	1	76 (60–118)
	0.2–0.3	0–1	76	3–4 wk	0	83	0	60 (49–91)
	0.5	(−10)‡	23	3 mo	0	22	0	77 (72–111)
	0.5	(−20)‡	23	3 mo	0	22	0	62 (62)
CDF_1	—	0	14	3 wk	0	0	7	—
	0.5	4	38	2 mo	5	74	0	81 (63–118)
	0.2	0	50	3 wk	3	63	0	80 (55–119)

*Lymphocytic neoplasms were characterized by enlargements of the thymus glands and/or spleen and mesenteric and peripheral lymph nodes.
†Numbers in parentheses are the range of time (in days) after pristane administration in which PCTs appeared.
‡Virus was inoculated 10 or 20 days prior to pristane.

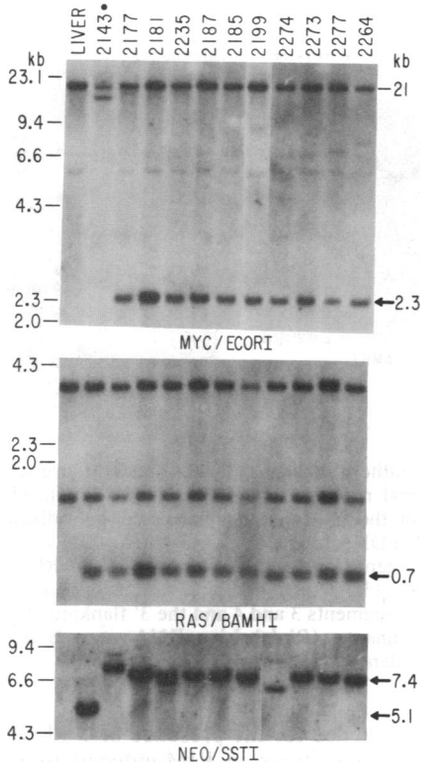

FIG. 2. Contexts of retroviral *myc*, *ras*, and *neo* genes in PCTs. Genomic DNAs of RIM and R virus-induced PCTs (indicated by numbers across the top of the gel) were digested with the indicated enzymes and subjected to Southern hybridization with random-primer labeled restriction fragments. The c-*myc* probe was a 1.4-kb *Xho* I fragment derived from the pMc-myc54 cDNA clone (8). The v-Ha-*ras* probe was a 700-bp *Bam*HI fragment, and the *neo* probe was a 1.3-kb *Hin*dIII–*Sma* I segment. The latter two probes were both isolated from the pRN(S) vector in Fig. 1. Liver DNA was of BALB/c origin. The lane denoted by a closed circle represents a Southern blot of an R virus-induced PCT. Arrows indicate retroviral-specific bands.

ments in the latter two recombinant proviruses occur outside of the *ras* and *myc* genes. PCT 2143 was obtained with R virus and contains the expected 5.1-kb provirus as well as a typical endogenous c-*myc* rearrangement caused by a chromosome translocation.

Transcription of the v-Ha-*ras* and the viral and cellular *myc* genes were evaluated by S1 nuclease mapping of total cellular RNAs prepared from frozen tumor samples. A 5′ end-labeled 410-bp *Eco*RI–*Eco*RV fragment, derived from the 2.3-kb c-*myc* expression cassette within the RIM virus, was used to detect transcripts initiating from its subgenomic V_H promoter. In Fig. 3 *Upper*, we observe a minimum of six transcription initiation sites in the V_H promoter that are differentially utilized in the various RIM-induced tumors. Transcripts originating from the 5′ LTR protected the entire 410-nucleotide (nt) nuclease probe from S1 nuclease digestion. The absence of a 206-nt protected band indicates that the endogenous c-*myc* alleles are not expressed. Because the RIM virus *myc* gene possesses only 46 bp of exon 1 at its 5′ end, we were also able to distinguish viral and endogenous c-myc transcripts by using another S1 mapping probe, 320-4 (19), which contains the 3′ 161 bp of exon 1 spliced to the 5′ 137 bp of exon 2 (see Fig. 3 *Lower*). Transcripts from the endogenous c-*myc* genes would protect a 298-nt segment of the 320-4 probe, which is seen for the RNA of 230-23-8 cells, a pre-B line with normal c-*myc* alleles (16, 20). However, RNAs from six RIM-induced tumors displayed a single band of 183 nt with the 320-4 probe as expected for viral myc transcripts. Taken together, these results indicate that the endogenous c-*myc* alleles are inactive and the V_H promoter of the RIM provirus drives c-*myc* transcription in these PCTs.

Viral *ras* gene expression was detected by S1 nuclease protection with a 5′ end-labeled rat v-Ha-*ras* probe (Fig. 4). Hybrids between the rat v-Ha-*ras* probe and endogenous murine ras RNAs would not be protected from S1 digestion. The presence of comparable amounts of a 503-nt protected band in a large panel of RIM-induced tumors indicates that the viral *ras* gene is expressed. The level of v-Ha-*ras* expression in R virus-induced PCTs (2143, 2186, and their functional equivalent, 2145, a RIM virus-induced PCT that deleted viral c-*myc*) is similar to the levels seen in RIM-induced PCTs.

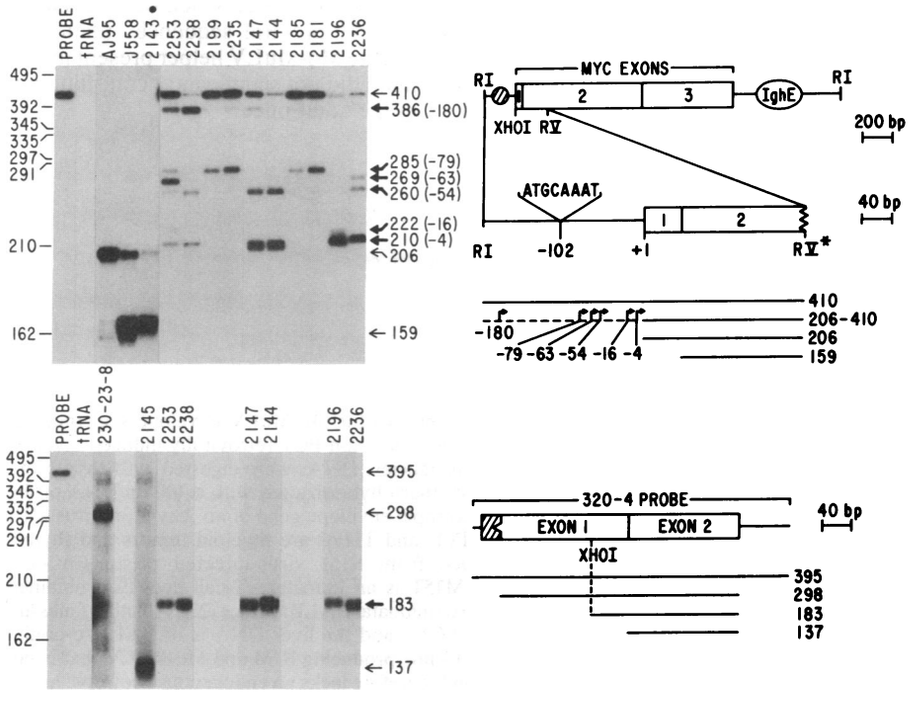

FIG. 3. Nuclease S1 analysis of proviral and endogenous c-myc RNA transcription. (*Upper*) A 5′ end-labeled S1 nuclease mapping probe was derived from the 2.3-kb *Eco*RI *myc* expression cassette as indicated. Samples include AJ95 (mature B-cell line with an amplified c-*myc* locus) (17), J558 (pristane-induced PCT with a translocated *myc* gene) (8), and 2143 (R virus-induced PCT with a rearranged cellular *myc* gene, see Fig. 1). All other samples are RIM-induced PCTs. Bold arrows indicate V_H promoter-driven myc RNAs, and light arrows are *myc* RNAs that initiated upstream of the expression cassette (410 nt), within exon 1 (206 nt) or intron 1 (160 nt) of the cellular *myc* gene. Hatched circle represents the ATGCAAAT octamer motif (18). IghE, immunoglobulin heavy-chain enhancer. (*Lower*) S1 mapping with 320-4 probe (derived from an MPC-11 c-myc cDNA clone) (19). Samples include 230-23-8 [an Abelson-MuLV pre-B-cell line with normal *myc* alleles (16, 20)], 2145 (RIM-induced PCT lacking the viral *myc* gene but possessing an endogenous c-*myc* rearrangement). All other samples are RIM-induced PCTs. myc transcripts originating from the RIM provirus would all migrate at 183 nt.

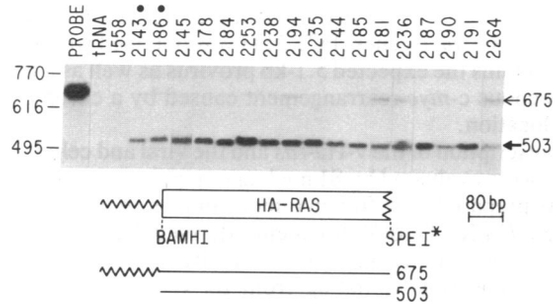

FIG. 4. Nuclease S1 analysis of proviral v-Ha-*ras* transcription. A ^{32}P 5′ end-labeled v-Ha-*ras*-specific probe was prepared from the pRN(S) retrovector, which was hybridized to 40 μg of total cellular RNA and, after exhaustive digestion with S1 nuclease, was analyzed on an 8 M urea/polyacrylamide gel. PCTs 2143 and 2186 are R virus-induced PCTs, and the other lanes contain RIM-induced PCTs. Arrows indicate the full-length probe (675 nt) and bands protected from S1 nuclease digestion by v-Ha-ras RNAs (503 nt). The wavy line in the probe map represents plasmid sequences.

A large number of other tumors were examined by Southern and S1 nuclease analysis (data not shown), and in total, 48 out of 50 RIM-induced tumors retained the viral *myc* gene and lacked rearrangements of their endogenous c-*myc* loci. Nuclease S1 analysis of the two exceptional RIM tumors indicated that 2213 has a rearranged endogenous c-*myc* gene but only expresses viral myc RNAs, whereas 2323 produces similar amounts of viral and cellular myc transcripts. Therefore, out of 22 RIM-induced tumors that were subjected to S1 nuclease analysis and were found to express viral *myc*, only 1, 2323, harbors a transcriptionally active, endogenous c-*myc* rearrangement. All except 1 of 53 RIM-induced PCTs retained their viral v-Ha-*ras* genes, which were found to be transcriptionally active in all tumors subjected to S1 analysis (Fig. 4 and data not shown). Two R virus-induced PCTs (2143 and 2186) and their functional equivalent, 2145, contained endogenous c-*myc* rearrangements.

Immunoglobulin Heavy-Chain Locus Rearrangements and Proviral Integrations in RIM-Induced Tumors. RIM virus-induced PCTs were shown to be monoclonal by two criteria. First, Southern analysis with a heavy-chain specific joining region probe (15) revealed one or at most two rearranged immunoglobulin heavy-chain alleles in addition to a germ-line-sized 6.2-kb band (due to the presence of contaminating normal cells in the tissue samples) and a 2.3-kb band corresponding to the viral *myc* expression cassette (Fig. 5). The latter band was revealed by the heavy-chain joining region probe because both contain immunoglobulin heavy-chain enhancer sequences. Similar results were obtained with 24 other RIM-induced tumors (data not shown). These findings strongly suggested that the RIM-induced PCTs were an outgrowth of a single malignant B-cell clone. To confirm this conclusion, a viral-specific *neo* probe was hybridized to *Bam*HI digests of tumor DNA (Fig. 5). The 5′ *Bam*HI sites of fragments that hybridize to the *neo* probe are within the proviruses, whereas the 3′ *Bam*HI sites are unique to the flanking genomic DNA of each proviral integration site. The presence of a single *neo*-positive band in each of the 10 tumors in Fig. 4 indicated that they developed from a B-cell clone containing a single integrated provirus. The additional submolar band of higher molecular weight in 2143 is due to an incomplete digestion. Single proviral integration sites were found in 20 of 25 other RIM-induced PCTs (data not shown). Therefore, of 35 virally induced PCTs, only 5 contained more than one provirus, which presumably resulted from independent retrovirus insertion events within the same malignant clone.

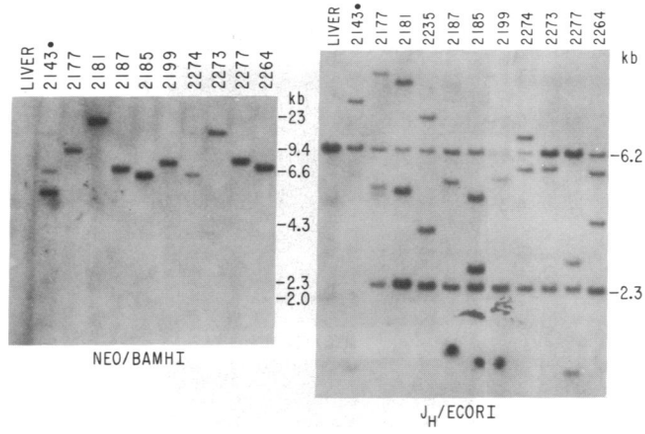

FIG. 5. Southern analysis indicates that RIM virus-induced PCTs are monoclonal malignancies. DNAs of RIM-induced PCTs were digested with the indicated enzymes and hybridized to random primer-labeled DNA fragments: a 1.3-kb *Hin*dIII–*Sma* I segment of the pN(S) *neo* gene (*Left*) and a 1.6-kb *Bam*HI–*Eco*RI fragment of the pj11 plasmid (15), which contains immunoglobulin heavy-chain joining region segments 3 and 4 and the 3′ flanking immunoglobulin heavy-chain enhancer (*Right*). Liver DNA was of BALB/c origin. A closed circle denotes an R virus-induced PCT.

We next screened for the presence of wild-type Moloney helper virus integrations in RIM-induced tumors with an ecotropic-specific viral envelope probe derived from AKR mice (Fig. 6) (21). This probe hybridizes to a single ecotropic retroviral locus in BALB/cAn on a 4.3-kb *Pvu* II fragment (23) as well as to a 3.0-kb *Pvu* II fragment of Mo-MuLV. The presence of the 3.0-kb Mo-MuLV-specific band in two thymocytic and two myeloid neoplasms obtained from Mo-MuLV infected, pristane-primed mice indicated that these malignancies may in part have resulted from Moloney provirus insertional activation of cellular protooncogenes. The presence of other less prevalent, smaller hybridizing bands in the M15T T-cell neoplasm are due to mink cell focus-forming (MCF) provirus insertions (22). In contrast, 18 out of 24 RIM-induced PCTs in adult mice lacked the Moloney-specific 3.0-kb band (Fig. 6 and data not shown). The latter result was confirmed with the use of a Moloney LTR probe, which only revealed bands corresponding to RIM proviral sequences (data not shown). We conclude that the Mo-MuLV helper present in the recombinant viral inoculum did not significantly contribute to plasmacytomagenesis in the adult mice.

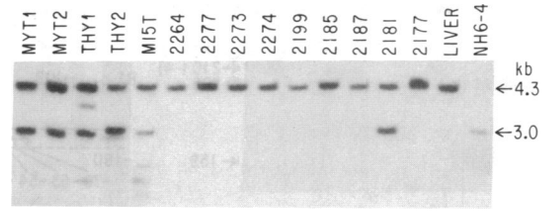

FIG. 6. Southern analysis for Mo-MuLV envelope sequences in RIM-induced PCTs and other non-PCT retrovirally induced myeloid and T-lymphoid malignancies. DNAs were digested with *Pvu* II and were analyzed by Southern hybridization with a 400-bp DNA probe derived from the ecotropic envelope gene of an Akv-1 provirus (21). MYT1, MYT2, THY1, and THY2 are myeloid tumors and thymic lymphomas obtained from RIM virus-infected pristane-primed BALB/cAn mice. M15T is an immature T-cell neoplasm obtained from IM virus-infected neonatal BALB/c mice (22). All other lanes are RIM virus-induced PCTs, and the liver DNA is of BALB/c origin. NH6-4 is an NIH 3T3 line producing RIM and Mo-MuLV that is not of BALB/c origin and therefore lacks an endogenous ecotropic locus.

DISCUSSION

RIM, a c-*myc*/v-Ha-*ras* retrovirus, has been shown to induce short latency, monoclonal, IgM-secreting PCTs in pristane-primed BALB/cAnPt mice with incidences of 28% in adult and 83% in 3-week-old animals. CDF_1 mice, which are genetically resistant to PCT induction by pristane alone, were susceptible to PCT induction by pristane and RIM virus, indicating that the virus circumvented normal pristane-resistance mechanisms. Furthermore, our findings support the view that an activated *myc* gene and a second oncogene of the *ras* class can collaborate to induce PCTs. This is the first direct evidence for a role of an activated *ras* gene in plasmacytomagenesis.

The inability of IM, our c-*myc* virus, to induce PCTs or any other malignancy was somewhat surprising. *In vitro* studies demonstrated that IM virus productively infects pre-B- and mature B-cell lines and expresses copious amounts of V_H promoter-driven myc RNAs (data not shown). Other workers have reported that the identical coding segments of our murine c-myc cDNA, under LTR control in an MuLV retrovector, exclusively induced myeloid tumors in pristane-conditioned BALB/c mice (24). One plausible explanation for these seemingly contrasting results would be that the c-myc polypeptide is not produced from the LTR-driven genome-length transcripts of the IM and RIM viruses and is only expressed from the subgenomic V_H promoter-driven transcripts in B cells.

The variable incidence (20–90%) and clonality of RIM-induced PCTs suggest that other genetic events in addition to viral infection may be necessary for malignant transformation of the target B cells. However, the very high incidence of tumors in 3-week-old mice and our recent observations of plasma cell ascites as early as 40 days after pristane conditioning make a requirement for additional genetic mutations in the target cell less appealing. Indeed much of the time required for tumor development may be a consequence of the kinetics of the infection and subsequent expansion of susceptible target cells. The latter process may require antigenic and/or other proliferative stimuli within the oil granuloma.

We favor the possibility that RIM virus integration takes place in a dividing B-cell precursor population (i.e., at the pre-B stage) and that neoplastic transformation manifests after the cell is activated by antigen or other mitogens. Osmond and coworkers (25) have shown that the i.p. injection of paraffin oils dramatically increased the size of the pre-B-cell population. Mature B lymphocytes may be another vulnerable target cell for RIM-induced transformation since a variety of morphological forms of proliferating plasma cells are known to exist in pristane-conditioned mice (26, 27). RIM virus integration causes these cells to continue in a proliferative phase, ultimately transforming them and thereby blocking their ability to become postmitotic. The very high proportion of IgM-secreting RIM-induced PCTs is an unexpected finding since BALB/cAn mice injected with pristane alone develop PCTs that predominantly secrete IgA (28). This observation would likely be discordant with the primary target of the virus being a proliferating plasma cell in the oil granuloma.

Moloney helper virus integrations and c-*myc* chromosome translocations are not required for the genesis of RIM/pristane-induced PCTs. We find, with only a few minor exceptions, that RIM-induced tumors contain transcriptionally silent, unrearranged endogenous c-*myc* alleles. This observation extends the results of a previous study involving a v-*myc* retrovirus (3) by showing that a retrovirally transduced normal *myc* gene can also substitute for a c-*myc* translocation in plasmacytomagenesis. In addition to lacking c-*myc* rearrangements, the majority (18 of 24) of RIM-induced PCTs in adult mice lacked Moloney proviruses, implying that the helper virus inoculum does not contribute to the genesis of these B-cell malignancies by the insertional activation of other cellular protooncogenes.

We thank Chris Helmke and Joyce Schirmer for preparation of the figures and Elizabeth Mushinski for mouse serum electrophoretic analyses. K.B.M. gratefully acknowledges the hospitality of Prof. Francois Cuzin of the Universite de Nice for allowing him to complete the pRN(S)IM1 vector in his laboratory. The *myc* 320-4 S1 probe was kindly provided by Dr. Martin Julius. This work was supported by grants from the National Cancer Institute (CA36246) and the New York State Science and Technology Foundation awarded to K.B.M., who is a Catacosinos Cancer Research Professor. R.C. was partially supported by predoctoral training grant GM08065 awarded to the Department of Biochemistry (State University of New York at Stony Brook).

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