CHROMOSOMAL TRANSLOCATIONS ACTIVATING *myc* SEQUENCES AND TRANSDUCTION OF v-*abl* ARE CRITICAL EVENTS IN THE RAPID INDUCTION OF PLASMACYTOMAS BY PRISTANE AND ABELSON VIRUS

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The cells from plasmacytomas induced in BALB/c and susceptible BALB/c congenic strains of mice by pristane (2,6,10,14-tetramethylpentadecane) (1) have non-random chromosomal translocations, rcpt (6;15) or rcpt(12;15) (2, 3). To date, the karyotypes of 27 early transfer generation or primary pristine–induced plasmacytomas have determined, and 26 of the tumors have either rcpt(6;15) or rcpt(12;15) (3–5). The translocation breakpoints on these chromosomes occur in the same chromosome bands in all the plasmacytomas studied to date. The breakpoints in chromosomes 12 and 15 have been studied by cloning and DNA sequencing. The breakpoint in chromosome 12 occurs in the immunoglobulin heavy chain gene (IgH)[1] complex, frequently, but not always, in the switch region of the alpha heavy chain gene (6–11). In chromosome 15 the breakpoint occurs close to or within the c-*myc* oncogene (8–12). The breakpoints in chromosome 6 have not yet been determined. Expression of c-*myc* RNA, often in relatively large amounts, characterized most of the plasmacytomas (11, 13, 14), and rearrangement of c-*myc* DNA was frequently seen.

The development of plasma cell tumors in BALB/c mice injected with pristane alone requires a minimum of 120 d, but usually more than 200 d (15). In contrast, plasmacytomagenesis can be greatly accelerated (5, 16) by infecting pristane-injected mice with Abelson virus. This murine acute leukemia virus contains two retroviral elements, a replication competent Moloney leukemia virus helper and a defective component, A-MuLV (17). The defective element has lost part or all of the viral *gag*, *pol*, and *env* genes and has acquired sequences from the mouse c-*abl* gene (for review see reference 18). Adult mice of only a

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[1] *Abbreviations used in this paper:* ABPC, plasmacytomas induced by Abelson virus plus pristane; A-MuLV, Abelson murine leukemia virus; IgH, immunoglobulin heavy chain; i.p., intraperitoneal; LTR, long terminal repeats of retroviruses; rcpt, reciprocal chromosome translocation.

few inbred strains, including BALB/c, are susceptible to tumor induction by Abelson virus. They generally develop pre–B cell lymphosarcomas (16, 17, 19, 20) that appear 3–4 wk after infection with the virus. Although most of the mice developed lymphosarcomas, 5–25% of the mice developed plasmacytomas (16). Unlike the plasmacytomas that arose 5 or more months after injection of pristane alone, the plasmacytomas induced in the presence of Abelson virus arose after relatively short latent periods (4–15 wk when determined from the time pristane was injected, or 3–9 wk when the latent period was determined from the time virus was injected).

Whereas pristane-induced plasmacytomas characteristically contained chromosome translocations, it has been previously shown that the lymphosarcomas induced by Abelson virus had normal karyotypes (21). Further, it had been shown that certain types of tumors induced by Abelson virus lack integrated v-*abl* sequences and v-*abl* transcripts (22, 23). Thus, investigation of the mechanism of carcinogenesis in plasmacytomas induced by pristane plus Abelson virus (ABPC) prompted an examination of the chromosomes of 18 transplanted ABPC for evidence of translocations of chromosome 15. In addition, appropriate molecular hybridization studies were used to determine whether c-*myc* and v-*abl* sequences were being actively transcribed in these tumors.

Materials and Methods

Plasmacytomas, Abelson Virus. The BALB/c plasmacytomas were induced in BALB/cAn or BALB/c π female mice, by first injecting 0.5 ml pristane (2,6,10,14-tetramethylpentadecane, Aldrich Chemical Co., Milwaukee, WI 53233) intraperitoneally (i.p.) followed 23–56 d later by 0.1 ml of an Abelson virus preparation. Our stock of Abelson virus was originally obtained from Dr. Wallace P. Rowe, NIAID (16). New preparations of virus were prepared from the transplantable Abelson virus–induced lymphosarcoma ABLS 19. 10% (wt/vol) extracts of subcutaneous ABLS 19 tumor tissue contained $\sim 10^6$ plaque-forming units/ml of viral activity (as measured in the XC test). Such preparations contained $\sim 10^3$ lymphosarcoma-inducing units/ml. The tumors (listed in Table I) in the present study were derived from several different experiments carried out over the last 10 years and were examined after transplatation.

Detection of v-abl and c-myc. Standard methods were used to prepare high molecular weight DNA (13, 24) and total RNA (25) from solid tissue excised from subcutaneous or intraperitoneal tumors. The presence of absence of an integrated A-MuLV proviral genome in the cellular DNA of a tumor was determined by hybridization of Southern blots of nuclear DNA after digestion with Kpn I, which cleaves A-MuLV only in the LTR (26). 25 μg of DNA was fragmented by Kpn I endonuclease, electrophoresed in 0.7% agarose gels, and transferred to nitrocellulose paper. A subcloned 1.2-kbp *abl*-containing Bgl II fragment of cloned A-MuLV (26) was labeled with ^{32}P by nick-translation (27) and hybridized with the tumor DNA. The presence of an intensely hybridized band at 6.7 kbp indicated that A-MuLV had been integrated into the genome as a provirus. Electrophoresis of 5 μg of poly(A)$^+$ RNAs on 1% agarose gels containing 5 mM methyl mercury hydroxide, transfer to diazotized paper, and hybridization to the ^{32}P-labeled v-*abl* probe described above were performed as presented elsewhere (13). A-MuLV RNA appeared as an intensely hybridizing band of 6.6-kb RNA, while c-*abl* RNA transcripts took the form of 5.8-kb bands.

Hybridization of blots of RNA and EcoRI digested DNA was also performed using a ^{32}P-labeled mouse c-*myc* probe. This probe was a 5.5-kbp BamHI fragment that had been isolated from a bacteriophage library constructed from the plasmacytoma S107 and subcloned into pBR322 (28).

Chromosome Analysis. Metaphase spreads were performed from ascitic plasmacytomas

TABLE I

Characteristics of A-MuLV-Plus-Pristane–induced Plasmacytomas

Tumor		No. of day tumor arose		Heavy chain class	v-*abl*		Rearranged EcoRI *myc* DNA fragment	*myc* RNA	Chromosome translocation rcpt
		After A-MuLV	After pristane		DNA	RNA			
ABPC	4	?	?	α	+	+	−	++	6; 15
	17	58	99	α	+	+	+	++	6; 15
	18	62	101	γ2b	+	+	+	++*	12; 15
	20	21	78	γ2b	+	+	−	++	6; 15
	22	52	98	μ	+	+	−	++	None
	24	32	88	α	+	+	+	++*	12; 15
	26	?	?	α	+	+	−	++	None‡
	33	55	111	α	+	+	+	++	12; 15
	45	46	69	α	+	+	+	++	None‡
	47	42	78	α	+	+	−	++	12; 15
	48	28	84	α	+	+	+	++	12; 15
	52	45	72	α	+	+	−	++	12; 15
	60	62	98	γ1	+	+	−	++	12; 15
	65	32	72	ND§	+	+	ND	++	12; 15
	72	39	76	α	+	+	+	++*	12; 15
	89	29	49	α	+	+	−	++	12; 15
	103‖	32	88	γ1	+	+	−	++	6; 15
	105	72	102	α	+	+	−	++	6; 15

* Shortened *myc* RNA present.

‡ Internal deletion within chromosome 15 can be detected by high resolution banding (39).

§ ND, not determined.

‖ The ABPC 103 was derived from an experiment done in collaboration with Dr. Charles D. Scher, Sidney Farber Cancer Institute, Boston, MA. It originated from spleen cells from the BALB/c C.B20 congenic mouse. This mouse was injected with pristane on day 0, and 40 d later spleen cells were cultured in RPMI 1640 medium containing BALB/c cells, 5×10^{-5} M mercaptoethanol, and 50 μg/ml lipopolysaccharide. The spleen cells were incubated with Abelson virus stock NIH/PCV-22/MLV-C with a titer of 8×10^5 FFU/ml and 1.5×10^6 PFU/ml and 2 μg/ml polybrene for 2 h before culture. 9 d later 1.6×10^6 cells were injected into each of 82 BALB/c mice that had been conditioned with 0.5 ml pristane 30 d before. One BALB/c recipient developed a plasmacytoma 30 d later. Upon transplantation it was shown that the tumor, designated ABPC 103, secreted an IgG_1 myeloma protein of C.B20 origin. This datum suggested that ABPC 103 arose in a C.B20 lipopolysaccharide blast spleen cell in vitro.

without colcemid treatment. Banded metaphase plates were prepared by using a slightly modified method of Wang and Federoff (29). High quality promethaphase and prophase chromosome plates were prepared according to the method of Yu et al. (30), adapted to mouse chromosomes. Chromosome identification followed the recommendations of the Committee on Standard Genetic Nomenclature for Mice (31). Breakpoints of translocated chromosomes were located according to Nesbitt and Francke (32).

Results

The 18 plasmacytomas utilized in the present study were taken at random from a series of 60 that had been induced in BALB/c mice that had first been given a single 0.5 ml i.p. injection of pristane, followed by an i.p. injection of Abelson virus 20–60 d later (usually 40–50 d after pristane). The mean latent period of these 60 plasmacytomas was 89 d (range 49–149 d). The latent period determined from the time of virus injection was 43 d (range 21–93 d). The mean

latent period of 77 plasmacytomas induced in BALB/c mice by a single injection of 0.5 ml pristane alone was 221 d (range 140–360 d). A comparison of the latent periods is shown graphically in Fig. 1, and the individual latent periods of the tumors used in this study are presented in Table I.

Hybridization Studies. As summarized in Table I, A-MuLV RNA was detected as a 6.6-kb v-*abl* hybridizing RNA in all the ABPC. Similarly, A-MuLV provirus was detected in Kpn I digested DNA from all ABPC as a 6.7-kbp *abl* hybridizing band. Fig. 2 shows representative examples of *abl*-hybridized RNA and DNA blots of ABPC, normal tissues, and ABPL 1, a lymphosarcoma tumor that arose after Abelson virus-plus-pristane injection but, which contains neither RNA nor DNA from A-MuLV (22). The only ABPC tumor without the 6.7-kbp *abl* proviral Kpn fragment is ABPC 60, which has a smaller, 5.8-kb, v-*abl* RNA and a correspondingly smaller, 5.9-kbp, proviral DNA fragment.

Hybridization with the c-*myc* gene probe revealed that 7 ABPC (ABPC 17, 18, 24, 33, 45, 48, and 72) showed altered EcoRI *myc* fragments, indicating that rearrangements had occurred at the *myc* gene locus. Three of these seven tumors (ABPC 18, 24, and 72) contained 1.8-kb *myc* RNA indicating that some of the rearranged *myc* genes gave rise to altered mRNA transcripts. These results are summarized in Table I, and representative examples of *myc*-hybridized RNA and DNA blots of ABPC and normal tissues are shown in Fig. 3. The altered (1.8 kb) *myc* RNA has been found so far only in tumors with rcpt(12;15) translocations. It is important to note that rearrangements at the *myc* gene locus could be seen in one tumor with no translocation (ABPC 45) as well as in one tumor containing rcpt(6;15) (ABPC 17) (Table I).

Karyotypes. The karyotypes of 18 ABPC are summarized in Table II. ABPC 4, 17, 47, 48, and 72 had near-tetraploid chromosome constitutions, while ABPC 20 and ABPC 33 showed near-diploid populations. The other 11 ABPC (ABPC 18, 22, 24, 26, 45, 52, 60, 65, 89, 103, and 105) had both near-diploid cells and near-tetraploid cells. In spite of this wide variation in chromosome number,

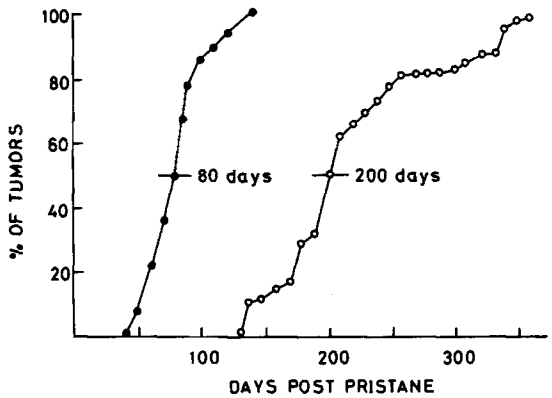

FIGURE 1. Comparison of the latent periods of 77 plasmacytomas induced with 0.5 ml pristane with 18 plasmacytomas induced with 0.5 ml pristane plus A-MuLV. The A-MuLV–associated plasmacytomas appeared on an average of 80 d before plasmacytomas induced with pristane alone. (●) percent of tumors induced with A-MuLV plus pristane, (○) percent of tumors induced with pristane alone.

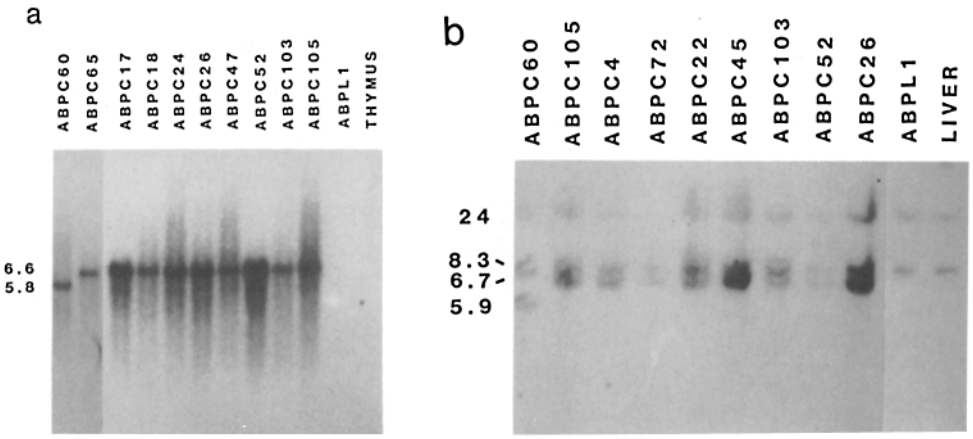

FIGURE 2. RNA and DNA blots hybridized with v-*abl*. (*a*) 5 μg poly(A)$^+$ RNA from tumors and tissues indicated were separated by electrophoresis on 1% agarose gels containing 5 mM methyl mercury hydroxide. After blotting onto diazotized paper, the RNAs were hybridized (13) with a v-*abl* probe and exposed to film. Typical A-MuLV RNA of 6.6 kb was abundant in all ABPC's except ABPC 60, which contained a presumed deletional mutant of A-MuLV. Faint bands of c-*abl* RNA transcripts of 5.6 kb can also be seen in some ABPC tumors. Similar c-*abl* RNAs can be seen in tumors such as ABPL 1 that lack v-*abl* expression and in normal thymus after longer exposures. (*b*) 25 μg of DNA from the tumors and tissue indicated were digested to completion with Kpn I, electrophoretically separated on 0.7% agarose and blotted onto nitrocellulose. The blot was hybridized with a v-*abl* probe (13) and exposed to film. Normal liver and ABPL 1 DNA showed 2 hybridizing bands of 8.3 and 24 kbp. All ABPC's showed one additional hybridizing band of 6.7 kbp, except ABPC 60 which demonstrated the two normal bands and one additional band of 5.9 kbp. The additional band is presumed to be the A-MuLV provirus, which has undergone a deletional mutation in ABPC 60.

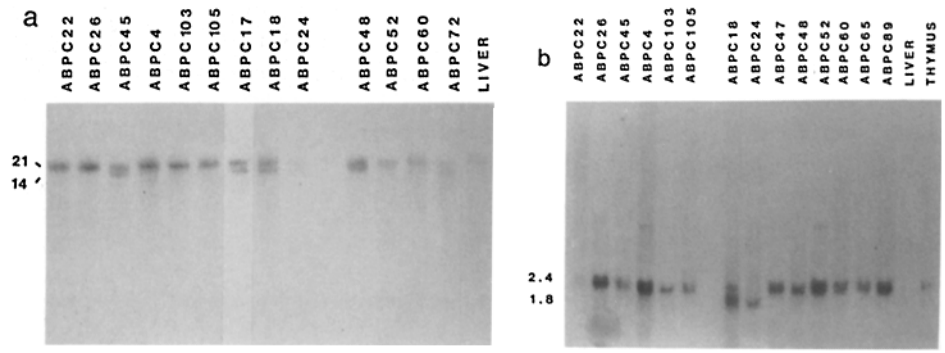

FIGURE 3. DNA and RNA blots hybridzed with c-*myc*. (*a*) Southern blots of 25 μg of DNA from the tumors and tissue indicated processed as in Fig. 2 *b* and hybridized with c-*myc* probe. Most tumors and the normal liver have a single hybridizing band at ~21 kbp, but some ABPC's have an additional smaller hybridizing band at ~14 kbp. (*b*) Blot of 5 μg poly(A)$^+$ RNA from tumors and tissues indicated processed as in Fig. 2 *a* and hybridizing with c-*myc* probe. Most tumors have a broad, intensely hybridizing band of RNA of ~2.4 kb. Normal tissues also contain *myc* RNA of this size, but normal tissues have less of this *myc* RNA than most ABPC's. Some ABPC's also have a *myc* RNA band of ~1.8 kb which is not found in normal tissues.

banding analysis revealed a remarkable uniformity in chromosome aberrations, specifically the non-random reciprocal translocation involving one of the Ig gene–bearing chromosomes 6 or 12 and chromosome 15. Of 18 ABPC analyzed, 5/18 showed rcpt(6;15) (ABPC 4, 17, 20, 103, and 105) (Fig. 4) and 10/18 had rcpt(12;15) (ABPC 18, 24, 33, 47, 48, 52, 60, 65, 72, and 89) translocation (see Fig. 6). Specific breakpoints associated with these translocations were at 15D2/3, 6C2, and 12F2, respectively. It should be noted that three ABPC (ABPC 22, 26, and 45) had no chromosome translocations.

In 7 of the 11 tumors with both tetraploid and diploid chromosome numbers, ABPC 18, 24, 45, 60, 65, 89, and 103, the diploid cells far outnumbered the near-tetraploid cells (Table II). In tumors showing 41 chromosomes the additional chromosome was usually chromosome 11. Trisomy 11 occurred in cells from 2/5 tumors with rcpt(6;15) (ABPC 103 and 105), 6/10 tumors with rcpt(12;15) (ABPC 18, 24, 33, 60, 65, and 89), and all 3 tumors with no translocations (ABPC 22, 26, and 45) (Fig. 5).

A few plates of 41 or 42 chromosomes were equivocal for the presence of trisomy 11. This was due to the inability to identify each chromosome in certain preparations with poor G-banding quality, rather than the absence of the trisomy 11.

A variety of other structural aberrations was also present in occasional tumors. The trisomy 11 in ABPC 60 was due to a single No. 11 and a copy of isochromosome of No. 11 [Rb(11;11)]. ABPC24 showed 1 to 2 copies of a long chromosome, possibly resulting from a single translocation of chromosome 18 onto the telomeric end of chromosome X (terminal addition). The isochromosome of No. 6 [Rb(6;6)] was identified in ABPC 47. Of 16 plates analyzed from this tumor, 10 cells were positive for Rb(6;6) while the others (6/16) were negative. Finally, a number of metaphase plates with 39 chromosomes were found in ABPC 52. Karyotype analysis revealed that a copy of chromosome X has been deleted (Fig. 6).

Discussion

A striking feature of pristane-induced plasmacytomas in BALB/c mice is the occurrence of the reciprocal translocations rcpt(6;15), rcpt(12;15) (references 2–5) or interstitial deletions of chromosome 15 (del 15qD1qE) (references 2, 3). One of these three chromosome-15 abnormalities has been found in all pristane-induced plasmacytomas so far studied (2, 5). The breaksite in chromosome 15 in all of the tumors examined occurs within or near the *c-myc* oncogene (8, 11, 12, 14) and is associated with *myc* gene transcription (9, 13, 14), often in relatively large amounts. Similar forms of chromosomal abnormalities involving the *myc* gene chromosome-8 in man (33) and chromosome-7 in the rat (34) have been found. The importance of *myc* gene activation by retroviral promoter insertion has been demonstrated in Bursal lymphomatosis in the chicken (35, 36). It would appear that *myc* gene activation is a critical event in many forms of B cell neoplasia; however, it is not clear that it is the only oncogenic event.

In the present study we have examined another experimental form of plasmacytomagenesis in BALB/c mice, the short-latent period plasmacytomas that are induced by infecting pristane-treated mice with Abelson virus. The mean

TABLE II

Karyotype and Protein Analysis of A-MuLV-Plus-Pristane–induced Plasmacytomas with Near-diploid and Near-tetraploid Chromosome Constitution

Plasmacytoma		Transplant generation	Myeloma protein	Chromosome constitution (no. of plates/total no. of plates analyzed)	No. of metaphase plates analyzed	Type of chromosome aberration	Trisomy 11 (no. of plates/total no. of plates analyzed)
ABPC	4	18	IgA, κ	65 (Range: 44–68)	26	rcpt (6; 15),* 6q−, 15q+	?‡
ABPC	17	14	IgA, κ	65 (Range: 45–68)	26	rcpt (6; 15), 6q−, 15q+	?
ABPC	18	4	IgG_{2b}, κ	41 (8/29) 42 (19/29) 83 (1/29) 85 (1/29)	29	rcpt (12; 15),§ 12q+, 15q−	41 (8/8) 42 (16/19)
ABPC	20	9	IgG_{2b}, κ	40 (7/10) 41 (2/10) 42 (1/10)	10	rcpt (6; 15), 6q−, 15q+	No trisomy 11
ABPC	22	11	IgM, κ	41 (3/26) 42 (9/26) 43 (1/26) 81 (Range: 60–85) (13/26)	26	No translocations	41 (3/3) 42 (9/9) 43 (1/1)
ABPC	24	8	IgA, κ	39 (1/24) 40 (3/24) 41 (12/24) 42 (5/24) 78–85 (3/24)	24	rcpt (12; 15), 12q+, 15q−, Xq+	39 (1/1) 41 (11/12) 42 (5/5)
ABPC	26	5	IgA	40 (3/20) 41 (5/20) 76 (Range: 71–81) (12/20)	20	No translocations‖	41 (4/5)
ABPC	33	16	IgA, κ	38 (1/9) 40 (2/9) 41 (6/9)	9	rcpt (12; 15), 12q+, 15q−	38 (1/1) 41 (6/6)
ABPC	45	7	IgA, κ	40 (4/25) 41 (11/25) 80 (Range: 56–82) (10/25)	25	No translocations‖	41 (8/11)
ABPC	47	20	IgA, κ	85 (Range: 77–85)	16	rcpt (12; 15), 12q+, 15q−, Rb (6; 6)¶	?
ABPC	48	11	IgA, κ	78 (Range: 54–81)	14	rcpt (12; 15), 12q+, 15q−	?
ABPC	52	15	IgA, κ	39 (13/19) 79 (Range: 64–80) (6/19)	19	rcpt (12; 15), 12q+, 15q−	No trisomy 11
ABPC	60	7	IgG_1	40 (1/14) 41 (10/14) 65–82 (3/14)	14	rcpt (12; 15), 12q+, 15q−, Rb (11; 11)**	41 (10/10)

TABLE II—*Continued*

Plasmacytoma		Transplant generation	Myeloma protein	Chromosome constitution (no. of plates/total no. of plates analyzed)	No. of metaphase plates analyzed	Type of chromosome aberration	Trisomy 11 (no. of plates/total no. of plates analyzed)
ABPC	65	7	ND‡‡	40 (7/17) 41 (7/17) 74 (1/17) 80 (1/17) 82 (1/17)	17	rcpt (12; 15), 12q+, 15q−	41 (7/17) 2 plates: RB (11; 11)
ABPC	72	8	IgA	76 (Range: 65–80)	14	rcpt (12; 15) 12q+, 15q−	?
ABPC	89	7	IgA	40 (1/21) 41 (18/21) 79 (1/21) 81 (1/21)	21	rcpt (12; 15), 12q+, 15q−	41 (17/18)
ABPC	103	2	IgG_1, κ	40 (12/31) 41 (14/31) 80 (Range: 47–83) (5/31)	31	rcpt (6; 15) 6q−, 15q+	41 (13/14)
ABPC	105	2	IgA, κ	40 (1/24) 41 (3/24) 42 (1/24) 78 (Range: 54–80) (19/24)	24	rcpt (6; 15), 6q−, 15q+	41 (2/3) 42 (1/1)

* Reciprocal translocation between chromosomes 6 and 15.
‡ The metaphase plastes were polyploid. Therefore, in these plasmacytomas it was not possible to define which chromosome was specifically duplicated first.
§ Reciprocal translocation between chromosomes 12 and 15.
‖ The possible deletion of D1 and D2 regions on chromosome 15 in the translocation-negative plasmacytomas (ABPC 22, 26, and 45) has been found by the high resolution banding technique.
¶ Robertsonian translocation between two chromosomes 6.
** Robertsonian translocation between two chromosomes 11.
‡‡ Not determined.

latent period for plasmacytoma development following a single injection of 0.5 ml pristane alone is 221 d (range 140 to 360 d). Introducing A-MuLV 20 to 40 d after an i.p. injection of 0.5 ml pristane reduces the mean latent period to 89 d (range 49 to 149 d determined from the time of the pristane injection).

Abelson virus alone has not yet been observed to induce plasmacytomas in normal BALB/c mice (16). Rather, plasmacytoma induction requires the formation of a pathological peritoneal granulomatous tissue by such agents as mineral oils, pristane, or solid plastics. This tissue is important for the development (37) and maintenance (38) of the primary plasmacytomas. The chronic inflammatory tissue in the peritoneum may be an essential factor in generating cells with chromosome 15 translocations; however, this has not yet been demonstrated.

The karyotypes of ABPC were of particular interest because of the possibility that the A-MuLV transduction of v-*abl* sequences into the genomes of plasma cells would be sufficient to transform the cells. We found, however, the nearly universal presence of another genetic alteration, i.e., 15 of the 18 tumors had

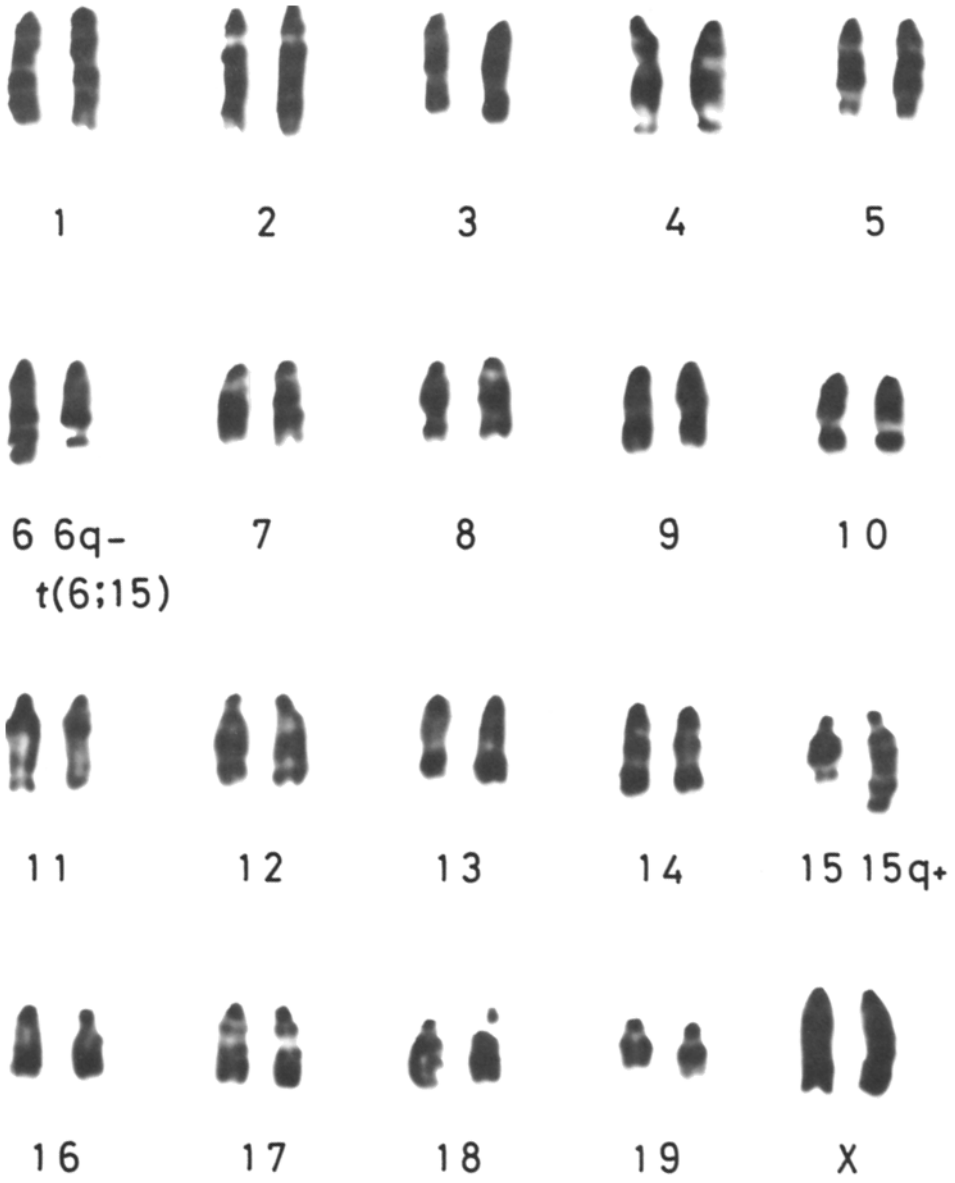

FIGURE 4. G-banded karyotype of a diploid plasmacytoma cell (ABPC 103, generation 2). Note the presence of one copy of rcpt(6;15) representing 6q- and 15q+.

translocations of chromosome 15, and 2 had interstitial deletions of chromosome 15 (Table I, reference 39). Only one tumor (ABPC 22) lacked a detectable chromosome 15 anomaly (39). All of the ABPC actively transcribed *myc* gene sequences.

Hybridization studies revealed that all of the ABPC transcribe large amounts of v-*abl* and contain stable inserts of v-*abl* in their chromosomes (Fig. 2). Abelson virus has been associated with the induction of neoplasms that have lost both the

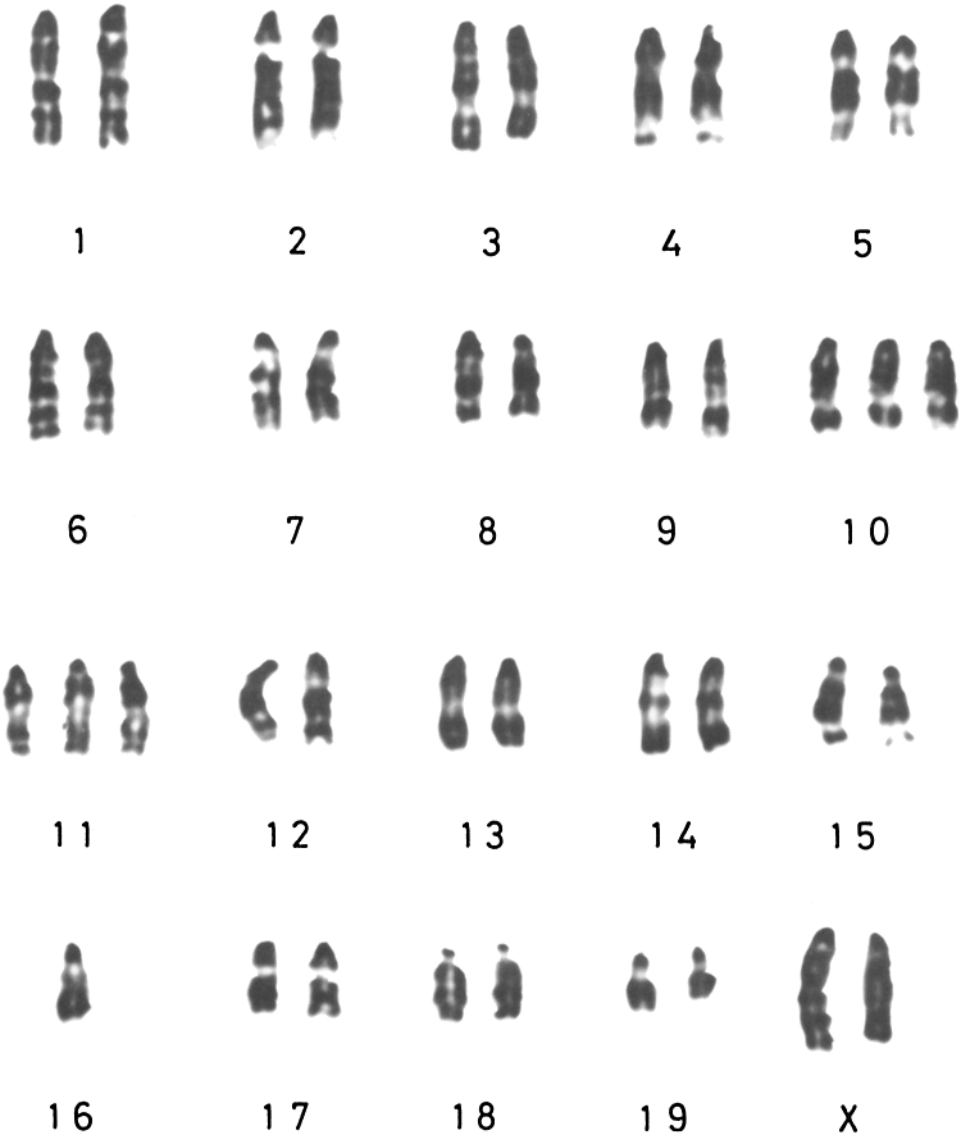

FIGURE 5. G-banded karyotype of a near-diploid plasmacytoma cell (ABPC 22, generation 11). Neither rcpt(6;15) nor rcpt(12;15). Not the presence of trisomy 11. Trisomy 10 and monosomy 16 are also seen. However, the latter two changes occur in a random manner and without any consistency between different tumors.

virus and the v-*abl* inserts (22, 23). In Abelson virus–induced plasmacytoid lymphosarcomas that appear to have lost v-*abl*, other oncogenes are activated and rearranged, notably the *myb* oncogene. The stability of the v-*abl* inserts in the ABPC form of tumor provides strong evidence that v-*abl* plays an important role in maintaining the neoplastic state in these plasmacytomas. Further, it may be reasonable to consider that *myc* gene activation and v-*abl* transduction cooperate in completing the neoplastic transformation of plasma cells.

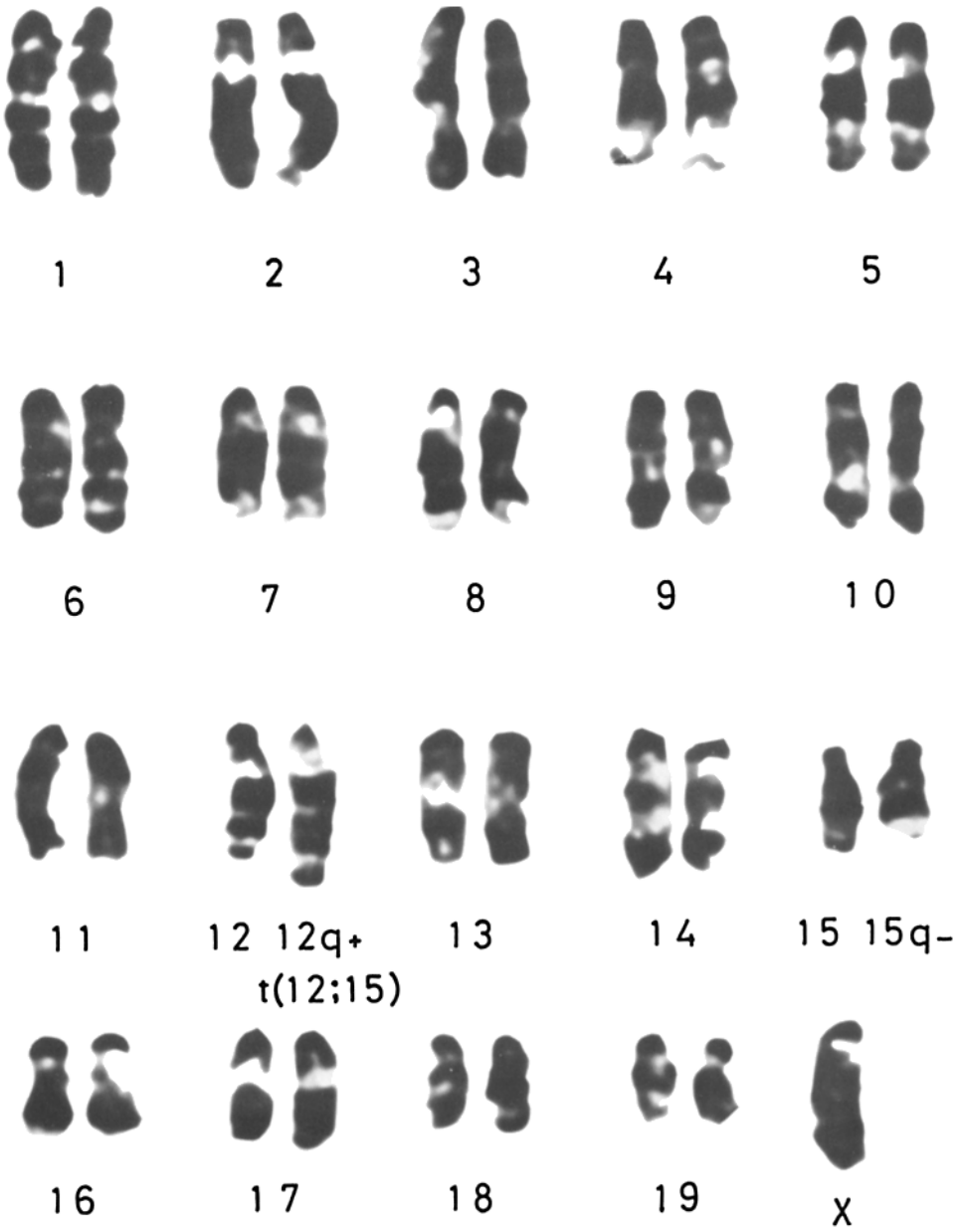

FIGURE 6. G-banded karyotype of a hypodiploid plasmacytoma cell (ABPC 52, generation 15). Note the presence of one copy of rcpt(12;15) representing 12q+ and 15q−. One chromosome X is deleted.

It is tempting to speculate that in these tumors there is a sequential pattern of expression of these oncogenes with *myc* gene activation by chromosome-15 alteration occurring first, to be followed by v-*abl* transduction. Several facts lead us to propose this hypothesis. First, chromosomal translocations probably are incurred during differentiation of the Ig genes, that is, in pre-B lymphocytes.

Identical forms of *myc* chromosome translocations occur in Burkitt's lymphoma and other pre–B cell tumors in man (11, 33, 40) and immunocytomas in rats (34). Thus, these translocations can occur at the pre–B cell stage in mammals. The effect of chromosomal translocation with its associated *myc* gene activation on pre–B cell physiology is not known; alone it may not be sufficient to transform the lymphocytes to a neoplastic state. A second mutational event is probably required. Since this would most likely occur by chance, it would entail a long latent period, such as that known for plasmacytoma development in mice injected with pristane or mineral oil alone. Transduction of v-*abl* by Abelson virus apparently can also fulfill this second event function. In Burkitt's lymphoma and "pristane-only" plasmacytomagenesis, a second oncogene probably also is involved but as yet has not been conclusively identified. Transforming genes (other than *myc*) have been isolated from plasmacytomas and Burkitt's lymphomas (41, 42), e.g., B-*lym* (43), and these may represent such a second gene. Further c-*mos* has been found to be activated in some plasmacytomas (44), but it is unlikely that c-*mos* alteration represents a commonly used second event in neoplasia.

Recently it has been proposed that in DNA viral oncogenesis (polyoma, SV40, adenoviruses) early function genes, e.g., E1A from adenovirus and large T from polyoma have properties similar to the *myc* gene (45–47). The protein products of all these genes, like *myc* (48–50), are located in the nuclear matrix and bind DNA (49).Further, in transforming experiments involving transfection with *myc* gene sequences, the *myc* gene alone is not able to transform cells to full neoplasia by itself (45), whereas it, as well as E1A, can cooperate with *ras* in inducing transformation. It is thought, though, that in in vitro systems the *myc* oncogene plays an important role in the continuous establishment of the cell lines (45, 46). The in vivo counterpart of *myc* gene function may be to keep cells that have been triggered for immunoglobulin secretion in continuous mitosis, thus blocking the normal mechanism of cell elimination via terminal differentiation and creating an anomalous cell. The second oncogenic event may then lock the proliferative activity of these cells into a state where they can no longer be regulated in vivo.

There were several points of interest in the karyological studies of the ABPC. The distribution of the two types of translocations was different, e.g., the number of tumors with rcpt(6;15) is higher (22%) than the pristane-only group (7%). Further, there were many more near-diploid chromosome numbers in ABPC cells than in tumors produced by pristane alone. The high frequency of near-diploid cells made it possible to show that trisomy of chromosome 11 was a potentially important abnormality. Trisomy 11 was found in 11 of the 14 tumors in which diploid cells were present. It is of interest to note that ABPC 22, 26, and 45 had the trisomy 11 in the absence of the usual translocations found in plasmacytomas. It is unlikely that the trisomy 11 supplants or substitutes for a rearrangement of the *myc* locus in chromosome 15 in the 3 ABPC lacking chromosome-15 translocation, because in a few tumors, e.g., ABPC 65, only half of the diploid cells had trisomy 11, and in other tumors we found some purely diploid metaphase plates (40 chromosomes) that showed rcpt(6;15) (ABPC 103, 105) or rcpt(12;15) (ABPC 24, 33, 60, 65, 89) without trisomy 11. The significance of the trisomy 11 in plasmacytomagenesis is not known, but it is of interest that the gene encoding the P53 transformation–associated protein is located on

chromosome 11 (51). Thus, trisomy 11 may amplify the amounts of this gene product.

Rcpt(12;15) was consistently present in plates with 39 chromosomes from which one copy of chromosome X was deleted. Additional chromosome aberrations such as Rb(11;11), Rb(6;6), deletion of one chromosome X, possible translocation between chromosomes 18 and X, and trisomies of No. 3, 6, or 10 were also observed. They occurred in an apparently random manner and without any consistency between different tumors. It is, therefore, likely that they represent secondary changes.

Summary

Plasmacytomas with short latent periods can be induced in BALB/c mice by a single intraperitoneal (i.p.) injection of 0.5 ml pristane followed 20–40 d later by an injection of Abelson virus. The karyotypes of 18 such tumors were determined; 10 of these had rcpt 12;15, 5 had rcpt 6;15 and 3 had no translocations, but two of these have been shown to have interstitial deletions of chromosome 15. The specific breakpoints were the same as described in pristane-induced plasmacytomas, i.e., at 15D2/3, 6C2, and 12F2. Near diploid karyotypes and trisomy of chromosome 11 were frequently seen. All of the Abelson-plus-pristane–induced plasmacytomas (ABPC) were studied as transplanted tumors, contained integrated v-*abl* sequences, and actively transcribed v-*abl* mRNA. All but one of these tumors contained abundant *myc* RNA transcripts. The shortness of the latent periods of the ABPC suggests that the rcpt 12;15 and rcpt 6;15 occur soon after pristane administration and are present at the time Abelson virus is introduced. In this form of plasmacytomagenesis, activated v-*abl* genes appear to bypass other genetic changes that require a much longer period of time in pristane plasmacytomagenesis. Nonetheless, the consistent finding of chromosome-15 alterations and abundant *myc* expression in these plasmacytomas emphasize the apparent need for multiple events even in the genesis of some tumors induced by rapid transforming viruses.

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