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Avian v-*myc* Replaces Chromosomal Translocation in Murine Plasmacytomagenesis

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Deregulation of c-*myc* expression in association with chromosomal translocations occurs in over 95% of murine plasmacytomas, rat immunocytomas, and human Burkitt lymphomas. Infection with a murine retrovirus (J-3) containing an avian v-*myc* rapidly induced plasmacytomas in pristane-primed BALB/cAn mice. Only 17% of the induced plasmacytomas that were karyotyped showed the characteristic chromosomal translocations involving the c-*myc* locus. Instead, all of the translocation-negative tumors demonstrated characteristic J-3 virus integration sites that were actively transcribed. Thus, the high levels of v-*myc* expression have replaced the requirement for chromosomal translocation in plasmacytomagenesis and accelerated the process of transformation.

PLASMACYTOMAS CAN BE REGULARLY induced in inbred BALB/cAn mice by the intraperitoneal (i.p.) injection of pristane (2,6,10,14-tetramethylpentadecane) (*1*). The incidence of plasmacytomas varies from 20% to 60% depending on the dose of pristane used (*1*). More than 95% of pristane-induced plasmacytomas have chromosomal translocations involving chromosome 15 (*2*) and an associated deregulation of *myc* gene transcription (*3*). Plasmacytomas can be induced with much shorter latent periods when the mice are given 0.5 ml pristane followed by infection with Abelson murine leukemia virus (A-MuLV) (*4*), and these plasmacytomas also have chromosome 15 translocations (*5*). In an analogous manner, deregulation of *myc* expression occurs in 100% of human Burkitt lymphomas along with translocations involving the *myc* chromosome (*6, 7*). In spite of this nearly universal association of plasmacytomas and Burkitt lymphomas with *myc* gene deregulation, this oncogene's causative role in either tumor has never been directly demonstrated.

The present study was designed to determine whether an activated avian v-*myc* gene can participate in plasmacytomagenesis. Thus, we conditioned BALB/c mice with a single i.p. injection of 0.5 ml pristane and then infected them with defective J-2 or J-3 viruses and Moloney murine leukemia virus (Mo-MuLV) as helper. J-2 and J-3 are murine retroviral constructs that contain a hybrid avian v-*myc* and a hybrid avian and mouse v-*raf* oncogene between long terminal repeats (LTRs) and other elements from Mo-MuLV (*8*). The J-3 virus contains the same elements as J-2 but has a 200-bp deletion from the 3′ end of *gag* and the 5′ end of *raf*, creating a frame shift that prevents synthesis of complete *raf* protein.

During an observation period of 153 days no tumors developed in the 45 mice infected with J-3 virus that had not been primed previously with pristane or in 13 mice injected with pristane alone. Helper virus alone injected intraperitoneally did not produce any tumors in primed mice in 150 days (*9*). By comparison, 22% of 114 BALB/cAn mice injected with pristane and then infected with J-3 developed plasmacytomas, about the same frequency as plasmacytomas induced by a single dose of 0.5 ml pristane and no virus (*1*). One significant difference is that in the presence of J-3 virus, plasmacytomas developed in less than half the latent period (mean of 68 days) for mice treated with pristane alone (*1*). Another significant difference resulting from including J-3 virus in the induction regimen is that a few plasmacytomas were also induced in BALB/cJ and (BALB/cAn × DBA/2)F_1 (CDF_1) mice, which are resistant to plasmacytoma induction by pristane alone (*1*).

Myeloid tumors were the next most common neoplasms, arising in 8% of BALB/cAn, in 25% of BALB/cJ, and in 2% of CDF_1 mice within 153 days after pristane and J-3 virus. Myeloid tumors were very common (57% of the animals) in BALB/cAn mice injected with J-2 virus 8 or 33 days postpristane, but no mice on this regimen developed plasmacytomas (Table 1, experiment IB). Three pristane-treated mice (10%) developed lymphocytic neoplasms af-

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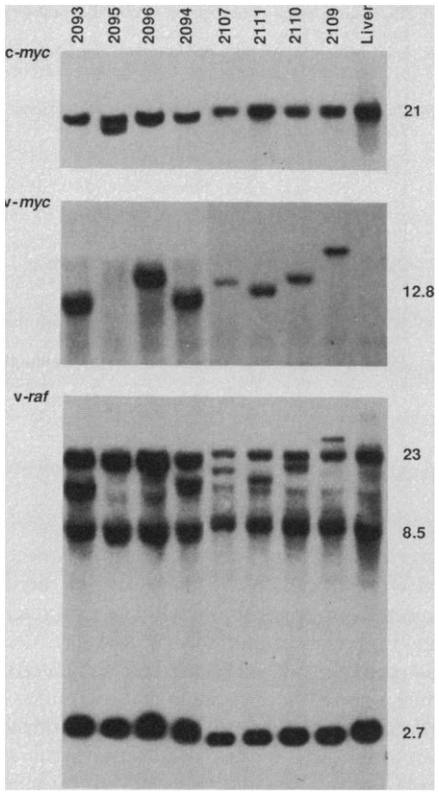

Fig. 1. DNA was prepared from tumors and liver by standard methods (*15*). Southern blots of Eco RI digests of 15 μg of each DNA were prepared and hybridized with nick-translated probes as indicated. The sizes of selected hybridizing bands in kbp are indicated on the right. Probes included v-*myc* [a 1.5-kbp Pst I fragment from the cloned chicken virus MC29 (*16*)], c-*myc* [a 2.7-kbp Hind III fragment from pM*myc* 54, a mouse *myc* complementary DNA (*14*)], and v-*raf* [a 1.3-kbp Xba I–Sst II fragment from the cloned mouse virus 3611 (*17*)]. Under the conditions used here the v-*myc* and c-*myc* probes do not cross-hybridize, whereas the v-*raf* probe hybridizes with both the viral and the three Eco RI bands of the c-*raf* proto-oncogene (23, 8.5, and 2.7 kbp).

Table 1. Induction experiments.

Experiment	Strain*	Treatment†				Number of mice with tumors		
		Pris	Virus	Day	Mice	PCT‡ (mlp)§	MYT‡ (mlp)	LN‡ (mlp)
IA	B/C	–	J-3	0	15	0	0	0
	B/C	+	J-3	8	15	3 (78)	3 (79)	0
	B/C	+	J-3	33	15	3 (103)	2 (82)	0
IB	B/C	–	J-2	0	15	0	0	2 (51)‖
	B/C	+	J-2	8	15	0	7 (54)	2 (54)
	B/C	+	J-2	33	15	0	10 (69)	1 (65)
II	B/C	+	–	–	13	0	0	0
	B/C	+	J-3	24	30	3 (83)	2 (83)	0
	CDF_1	+	J-3	24	30	2 (136)	0	0
III	B/C	+	J-3	7	25	7 (81)	2 (105)	1 (90)
	B/J	+	J-3	7	24	2 (115)	6 (77)	0
	DBA/2	+	J-3	7	24	0	1 (71)	3 (87)
	CDF_1	+	J-3	7	25	2 (87)	1 (77)	0
IV	B/C	–	J-3	0	30	0	0	0
	B/C	+	J-3	17	29	9 (68)	0	0

*B/C, BALB/cAnPt; CDF_1, (BALB/cAnPt × DBA/Pt)F_1; BALB/cJPt mice raised in our conventional mouse colony maintained at Hazleton Laboratories, Rockville, MD, under NCI contract NO1 CB2-5584. †Nonproducer NIH 3T3 cells containing the J-2 and J-3 viruses were prepared as described (*8*). Pseudotype virus stocks were prepared by infection of nonproducer cell lines with Mo-MuLV helper virus and cocultivation with NIH 3T3 cells. Transforming activity of virus pools was measured by titration in SC-1 cells (*12*), and ranged from $10^{4.2}$ to $10^{4.9}$ transforming units per milliliter for different pools of the J-2 and J-3 viruses used in these studies. Mice were inoculated with 0.1 ml of virus stocks at the indicated number of days after a single i.p. dose of 0.5 ml of pristane (Pris). ‡PCT, plasmacytoma; MYT, myeloid tumor; LN, lymphocytic neoplasm. Tumors were classified by the morphology of cells found in Wright-Giemsa stained smears of the ascites. Mice were examined weekly for development of ascites for a period of 150 days. §mlp, mean latent period in days post pristane. ‖Latent period after virus.

Table 2. Characterization of transplantable plasmacytomas from pristane-primed BALB/cAn mice infected with J-3 virus.

Tumor	Latent period (days)		Ig class*	Translocation	Rearranged† c-*myc*	Integration† and expression of avian v-*myc*
	Post-virus	Post-pristane				
J3PC2093	46	54	A(κ)	0	–	+
J3PC2094	46	54	M	0	–	+
J3PC2095	46	79	A+M	rcpt(12;15)	+	–
J3PC2096	46	79	A	0	–	+
J3PC2107	77	91	M	0	–	+
J3PC2109	59	83	none	0	–	+
J3PC2110	83	97	A(κ)	0‡	–	+
J3PC2111	59	83	A	0	–	+
J3PC2114	59	83	λ	rcpt(12;15)	+	–
J3PC2116	71	78	A	0	–	+
J3PC2117	71	78	A(κ)	0	–	+
J3PC2118	71	78	A	0	–	+

*The Ig class was determined by immunoperoxidase staining (*13*) of paraffin-embedded tissue sections previously fixed in ethanol/acetic acid/Formalin. †An Eco RI digest of DNA from the tumors was hybridized with a mouse *myc* complementary DNA probe (*14*) and scored as negative when they had only the germline 21-kb band and positive when an additional band was present (Fig. 1). Metaphase spreads were examined for rcpt(12;15) and rcpt(6;15) translocations. ‡J3PC2110 at generation 4 had some cells with an rcpt(6;15) in one mouse, but in a second mouse all of the metaphases examined lacked chromosome 15 translocations, suggesting that this translocation represented an unstable minor population.

ter J-2 virus, as did two mice that received J-2 virus and no pristane.

The plasma cells in many of the J-3 virus/pristane-induced plasmacytomas contained less cytoplasm than the plasmacytomas induced by pristane alone, and the cytoplasm stained more intensely blue with Wright-Giemsa stain. Nonetheless, cytoplasmic immunoglobulin (Ig) typical of plasma cells was detected by immunoperoxidase staining in 11 of the 12 tumors so far examined (Table 2). Thus, the tumor cells resembled plasmablasts and were probably less mature than the tumors induced by pristane alone (*1*).

These 12 tumors were analyzed cytogenetically for chromosomal translocations (Table 2). Nine of these showed no rcpt(12;15) or rcpt(6;15); two, J3PC2095 and J3PC2114, contained rcpt(12;15) translocations; and one, J3PC2110, had some cells with rcpt(6;15), but these were only observed in one mouse.

All the plasmacytomas except J3PC2095 and J3PC2114 showed Southern blot evidence of J-3 provirus sequences integrated in their genomes, namely an Eco RI band that hybridized with both v-*myc* and v-*raf* probes (Table 2 and Fig. 1). The size of the provirus-containing band varied among tumors, but there appeared to be only one such band in every tumor except J3PC2116, which had three v-*myc*/v-*raf*–containing Eco RI bands. This suggests that with one exception only one provirus has integrated in each tumor cell, that each of these tumors was clonal, and that the site of integration was not critical. DNA from J3PC2095 and J3PC2114 did not hybridize with v-*myc*. Thus, J-3 virus had not integrated in either of these tumors. All the tumors except J3PC2095 and J3PC2114 were shown to have only a germline, 21-kb Eco RI band that could hybridize with the c-*myc* probe. In J3PC2095 and J3PC2114 the molecular

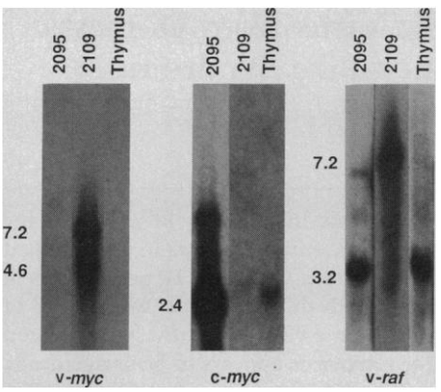

Fig. 2. Polyadenylated RNA was prepared from tumors and thymus, and 10 μg of each sample were fractionated by electrophoresis in 5 m*M* methyl mercuric hydroxide–1% agarose gels and blotted onto diazotized paper for hybridization, as described (*15*). The same probes were used as in Fig. 1. Sizes of hybridizing RNA bands were determined by comparison with RNAs from tobacco mosaic virus and bromegrass mosaic virus and are indicated (in kilobases) to the left of each panel.

consequences of the aforementioned rcpt (12;15) were seen, that is, the rearrangement of one of the c-*myc* genes to a 17-kbp (Fig. 1) or a 16-kbp Eco RI fragment, respectively.

A blot of RNA from J3PC2109 (Fig. 2) shows abundant 4.6- and 7.2-kb RNAs that hybridized with v-*myc* and a 7.2-kb v-*raf*–hybridizing species. This result was found in all of the J-3 plasmacytomas, except J3PC2095 and J3PC2114, and is typical of the expression of J-3 proviruses integrated in other tumors (*8*). When the c-*myc* probe was used, a large amount of 2.4-kb c-*myc* RNA (and lesser amounts of larger precursors) was seen in J3PC2095 and J3PC2114, while only barely detectable amounts of c-*myc* RNA were found in the other J-3 plasmacytomas, as exemplified by J3PC2109. Normal-sized 3.2-kb c-*raf* messenger RNA (and a larger presumed precursor) was present in J3PC2095 and J3PC2114, while the levels of this RNA and the normal 2.4-kb c-*myc* RNA appeared markedly lower in all the other tumors.

The data from this study demonstrate two major points. First, plasmacytomas can be induced with short latencies in BALB/cAn mice treated with a single dose of pristane and infected with J-3, a murine retroviral construct containing functional avian v-*myc* sequences, in contrast to the long latent periods observed in such tumors induced by pristane alone. Second, most of the J-3/pristane plasmacytomas contained integrated J-3 virus and expressed v-*myc* RNA, but lacked translocations involving the c-*myc* locus, which are characteristic of virtually all plasmacytomas induced by pristane alone or pristane and an unrelated transforming virus containing the *abl* oncogene (*5*). Two plasmacytomas in the present series lacked integrated J-3 and v-*myc* RNA, and both had undergone rcpt(12;15) translocation and c-*myc* activation. These findings provide the most direct evidence to date that deregulated expression of *myc* is critical to plasmacytoma induction. The J-3/pristane tumors reported here represent the only series of plasmacytomas that lack both deregulated c-*myc* transcripts and translocations involving chromosome 15. Without exception, this c-*myc* alteration has been replaced by abundant expression of v-*myc* under the control of the strong promoting and enhancing J-3 LTRs.

These findings indicate that deregulated expression of *myc* in appropriate target cells is necessary to achieve the outgrowth of malignant plasma cells, but is it sufficient by itself? Two aspects of our results suggest that this is not the case and that an additional alteration of normal gene function is required for target cells to exhibit the fully transformed phenotype. First, all but one of the plasmacytomas induced by the J-3 virus appeared to be monoclonal as indicated by single J-3 integration sites in these tumors (Fig. 1). However, this may only be a marker for an initial clonal event, and the relatively long postviral latent periods strongly suggest this event per se is insufficient to bring the cells to an autonomous state. Other critical mutational changes in these cells are required for full autonomy. Second, mice infected with the J-2 virus that contains and expresses the same v-*myc* gene as the J-3 virus do not develop plasmacytomas. The reason for this dramatic difference is not clear, but there are several possibilities amenable to testing: (i) expression of an intact v-*raf* protein may interfere with plasmacytoma induction, (ii) the deleted v-*raf* gene may play some as yet undetected role, and (iii) our current working hypothesis, the rapid development (mean latent period of 64 days) of lethal myeloid tumors induced by J-2 may preclude the appearance of the later-arising plasma cell tumors (mean latent period in J-3 experiments of 79 days). We cannot completely rule out some contribution from the deleted v-*raf* gene in J-3 to plasmacytomagenesis. This is unlikely, since the deletion changes the v-*raf* reading frame and thus creates a premature terminator codon close to the 5′ end. Apparently the predicted terminator is being used, inasmuch as the p15 *gag* protein in J-3–infected cells is no larger than the size expected for *gag* alone, that is, this is not a *gag-raf* fusion protein, and an antibody to the carboxyl terminal portion of v-*raf* does not precipitate any protein from these cells. Other studies have shown that pristane-primed BALB/c mice infected with murine retroviral constructs containing mouse c-*myc* genes develop myeloid tumors but do not develop plasmacytomas (*9, 10*). The basis for the differences between these reports and the present study are unknown, but future studies must investigate whether they could be due to quantitative differences in *myc* expression or qualitative differences in the *myc* sequences employed.

Analyses of the requirements for transformation of primary cells in vitro have also suggested that more than one defect in normal gene function is needed and that these defects may involve the activities of oncogenes (*11*). The results of the present study support this concept and provide direct evidence that deregulated expression of *myc* is a crucial feature of plasmacytoma induction. This is probably not the only critical change necessary for the development of these tumors, and the requirement for multiple events to occur stochastically in a single cell must be responsible for the long latencies of the plasmacytomas induced by pristane alone.

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