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Identification of Two Genes on Chromosome 4 that Determine Resistance to Plasmacytoma Induction in Mice

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ABSTRACT

BALB/cAn mice are highly susceptible to the induction of plasmacytomas (PCTs) by the i.p. injection of paraffin oils, whereas DBA/2 mice are solidly resistant. To search for genes that control the dominant resistant phenotype of DBA/2, BALB/c.DBA/2 (C.D2) congenic strains were constructed, and the susceptibility and resistance to PCT development were determined. PCT formation takes place over an extended period of 365 days but begins morphologically in focal proliferations of atypical plasma cells (foci) in the reactive oil granuloma that forms on mesenteric surfaces. Cells from some of these foci spread to other locations in oil granuloma tissue, forming new foci. Mice that develop six or more foci appear to be progressing towards eventual overgrowth and replacement of all peritoneal tissues with PCT cells. From Days 100 to 250, between 28 and 56% of PCT-susceptible BALB/cAn mice had 6 or more foci, whereas less than 5% of resistant DBA/2, BALB/c × DBA/2 F_1 (hereafter called $CD2F_1$), C57BL/6, and BALB/cJ mice had 6 or more foci. Four C.D2 congenic strains carrying D2 alleles of genes on chromosomes other than chromosome 4 were highly susceptible. Between 0 and 20% of the mice in C.D2-Chr 4 congenic strains C.D2-MIA, C.D2-TF3, C.D2-Fv-$1^{n/n}$, C.D2-Pnd7, C.D2-Lgm-1A, C.D2-Lgm-1B, C.D2-Lgm-1C, and C.D2-Lgm-1H developed 6 or more foci from 125 to 260 days, indicating resistance. The segments of DBA/2 chromosome 4 chromatin in C.D2-Fv-$1^{n/n}$ and C.D2-Pnd7 were discontinuous with those in C.D2-TF3, C.D2-Lgm-1A, C.D2-Lgm-1B, C.D2-Lgm-1C, and C.D2-Lgm-1H, indicating there are at least two genes (*Pct*^{r1} and *Pct*^{r2}) in the distal half of this chromosome that confer resistance. *Pct*^{r1} is located between *Ifa* and *D4Rck41*, and *Pct*^{r2} is between *Tnfr-1* and *Pkcz*. Each locus acting alone distinctly conferred a partial resistant phenotype. *Pct*^{r1} and *Pct*^{r2} did not appear to prevent the formation of clonal foci but did appear to limit the ability of the plasma cells in foci to acquire greater autonomy; thus, these genes affect tumor progression.

INTRODUCTION

PCTs[2] induced by the i.p. injection of paraffin oils (1) develop in the chronic inflammatory tissue that forms on peritoneal surfaces in response to the oils (2, 3). The earliest morphological evidence of a developing PCT is a focal plasma cell proliferation (2, 3). The end stage PCT development in the primary host is a massive overgrowth of the peritoneal oil granuloma tissue by PCT cells and the shedding of PCT cells into the peritoneal space. Microscopic plasma cell proliferative lesions (foci) may appear in the oil granuloma months before the mice have clinical evidence of a PCT. When a group of mice are sampled at various time points after 100 days post pristane, individual mice will be found to be in different stages of PCT development; this reflects the different rates of progression to neoplasia in this susceptible strain. The number of foci in histological sections of the entire intestinal mesenteries can be used to estimate the stage of development and progression of plasmacytomagenesis.

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[2] The abbreviations used are: PCT, plasmacytoma; Chr, chromosome; OG, oil granuloma; H & E, hematoxylin and eosin; PBS, physiologically buffered saline; PCR, polymerase chain reaction.

PCTs are induced in high frequency in BALB/cAn mice, whereas most other strains with the exception of NZB are resistant to this type of tumor induction (4–6). Using DBA/2 as a model resistant strain and BALB/cAn as a susceptible one, we have previously shown that (BALB/c × DBA/2)F1 ($CD2F_1$) hybrids are almost completely resistant to PCT development and that only 11% of first generation backcross progeny from either (DBA/2N × BALB/cAn)F1 × BALB/cAn or BALB/cAn × (BALB/cAn × DBA/2)F1 mice were susceptible (7, 15).

In previous work, we used the incidence of PCTs between 300 and 360 days to determine the PCT susceptibility/resistance phenotype and have shown that a major determinant of susceptibility to PCT formation resides in the distal portion of mouse Chr 4 (15). In the present study, we have used a focus enumeration method in order to accelerate typing of the S/R phenotype and have concentrated on the effects of resistance genes in DBA/2 mice.

Since $CD2F_1$ mice were resistant, BALB/c.DBA/2 congenic strains that carry different segments of the DBA/2 genome were constructed and tested to determine the incidence of PCTs at day 300 and/or the percentage of mice with 6 or more foci between days 150 and 260. In an earlier study, C.D2-Fv-$1^{n/n}$ (Chr 4) mice derived by introgressively backcrossing the *Fv-1*^{n} allele from DBA/2 onto the BALB/c background for 10 generations (N10) were found to have an intermediate but not completely resistant phenotype (7). These studies have been extended by developing new C.D2-Chr 4 congenics (C.D2-MIA and C.D2-TF3). Furthermore, we backcrossed C.D2-MIA to BALB/cAnPt and selected a series of recombinant strains (the C.D2-Lgm-1 and C.D2-Pnd series). The congenic strains were made homozygous at selected loci prior to testing. Studies involving these C.D2-Chr 4 congenics identified at least two separate regions of Chr 4 involved in resistance to tumor formation.

MATERIALS AND METHODS

Mice. BALB/cAnPt (originally obtained from H. B. Andervont in 1964), DBA/2NPt, $CD2F_1$, and C. D2 congenics mice were reared under conventional conditions in our mouse colony maintained by Hazleton Laboratories, Rockville, MD under National Cancer Institute contract NO1-CB-21075. The mice received i.p. injecions of 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Aldrich Chemical Co., Milwaukee, WI) on Days 0, 60, and 120 (8, 1). The experiments reported here were carried out from 1984–1993. During this period, the mouse colony, although maintained in the same facility, has become more increasingly isolated from other animal care personnel and mice in the building. In the late 1970s and early 1980s, the incidence of PCTs in BALB/c mice was 60% or higher (1), whereas in the 1990s, the incidences have varied from 28 to 50% at 300 days. Every 3 to 4 generations, a single pair of pedigreed mice was used to found a population for production breeding. Many of the mice used in the 1991–1993 experiments originated from a single pair of mice in 1989. This production colony, initiated in 1989, was selected for increased litter size, but the progeny gave a marked reduction in plasmacytoma incidence (28–50% as compared with 60% in earlier years) over a period of 300 days.

Harvesting Mesenteric Oil Granulomatous Tissue. Foci appear to develop almost exclusively in the OG and connective tissues of the intestinal mesenteries. Two alternative methods were used for removing the mesenteric tissue for histological studies. In the first method, beginning at the ileocecal junction and extending to the duodenum, segments of the small intestine

approximately 1–1.5 cm long with attached mesentery and including the mesenteric lymph node when present were dissected and fixed. The proximal sector contained the duodenum, some of the pancreas, and part of the mesentery of the ascending colon. The omentum, pancreas, spleen, and duodenum were taken in a single block. The descending colon and its mesentery containing some retroperitoneal tissues were also included. In the second method, the gut was separated from its serosal attachment, leaving the serosa and mesenteries. The intestinal mesentery was cut into 5–20 fragments before or after fixation. Additional separate sections were taken of the omentum and duodenal and colonic mesenteries.

After fixation in Fekete's modification of Tellyesniczky's fixative (70% ethanol:formalin:glacial acetic acid in 20:2:1 parts) and transfer to 70% ethanol, the tissues were trimmed to remove roughly one-half of the gut tissue opposite the mesenteric attachment site. The gut, with the extended sheet of mesentery, was placed in a flat plane during paraffin embedding, and four μm sections were stained with H & E. The preparation of the H & E slides was carried out at American Histo Labs (Gaithersburg, MD). The number of foci per mouse was made by counting the foci in single H & E slides. In the 62 cases that were used for immunoperoxidase staining, additional sections were cut; only a few contained foci not identified in the original sections.

Plasmacytoma Diagnosis by Peritoneal Smears. Peritoneal fluid was aspirated by paracentesis with a sterile 25-gauge needle, allowing the peritoneal fluid to flow into the sleeve. The fluid was diluted with tissue culture medium and cytofuged. The slides were stained with Wright-Giemsa stain. Ten or more intensely staining plasma cells per slide were indicative of a PCT. When there were only 10–20 cells per slide, a second confirmatory diagnosis was obtained. When 50 or more plasma cells were found, the mouse was considered to have an advanced PCT.

Immunoperoxidase Stains. Immunoperoxidase stains were made using avidin-biotin peroxidase complexes as described by Hsu *et al.* (9) and Thor *et al.* (10). The paraffin was removed by xylene, and the sections were then hydrated in ethanol, treated with 99.7% methanol plus 0.3% H_2O_2, and washed in PBS without calcium or magnesium. Each section was treated for 15 min in 0.1% bovine serum albumin that contained 10% normal serum of the species from which the second antibody was derived. The section was then overlayed with the primary heterologous antibody to the specific Ig class for 60 min at room temperature. The slide was washed three times in PBS and treated with the second biotin conjugated antibody that had been raised in a different heterologous species; then the primary antibody was added. Following three washes in PBS, the section was treated with commercial avidin-biotin peroxidase (Vectastain) for 30 min, followed by three washes in PBS, and then overlayed with a solution of 6.0 mg/ml of 3,3′-diaminobenzidine-tetrahydrochloride dehydrate in 0.1% bovine serum albumin. The slides were washed in PBS and counterstained with hematoxylin and lithium chloride.

Congenic Strains. The BALB/cAnPt.DBA/2N (C.D2) congenic strains were constructed by consecutive introgressive backcrosses in which DBA/2 chromatin was introduced onto the BALB/cAnPt background. The C.D2-MIA congenic mice were coselected for the DBA/2 alleles of either *Mtv-13* or *D4Lgm1* and *Pnd* for 10 consecutive backcrosses. N10 mice positive for all three markers were mated to each other to produce homozygotes. The C.D2-MIA N10 strain carries a segment of DBA/2 chromatin that represents approximately 44% of the entire chromosome. The homozygous line was backcrossed to BALB/cAnPt and several $D4Lgm1^{C/D}Pnd^{C/C}$ and $D4Lgm1^{C/C}Pnd^{C/D}$ recombinants were identified. These recombinants were used to derive two separate series of congenic strains referred to as the C.D2-Lgm and C.D2-Pnd series. The C.D2-TF3 strain was found to carry the *Mtv-13* locus of DBA/2 origin on Chr 4 during the development of another congenic line. C.D2-Fv-$1^{n/n}$ N19F11–13 were derived as described previously (7). Four non-Chr 4 congenics used in this study were C.D2-Idh1-Pep-3, $N_{20}F_9$ (Chr 1); C.D2-Qa2, $N_{14}F_{2-3}$ (Chr 17); CD-Es-Hba, $N_{10}F_{30}$ (Chr 11); and C.D2-Lyt-1, N_9F_9 (Chr 19).

Marker Typing and Molecular Analyses of Congenic Strains. DNA isolation (kidney), restriction enzyme digestion, agarose gel electrophoresis, and Southern blot analyses were performed as described (12). D4Rck microclones were amplified and labeled by PCR as described previously (13). PCR conditions for the *D4Mit* simple sequence repeats were a modification of those described previously (14). Many of the probes and restriction fragment variants for the loci examined among the various congenic strains have been described (11, 15–21). Probes for markers not previously described were a 1.9-kilobase *Pst*I fragment of pIL 11301 for *Il-135*, a 190-base pair Sau3a fragment of pDai for *Dai*,[3] a 1.8-kilobase *Eco*RI fragment of pK14 for *Kras-2*,[3] and a 1-kilobase *Hin*dIII/Pst fragment of pUC1.OHP for *Evi-1* (22). Isozyme analyses for *Idh-1, Pep-3, Akp-1, Pgm-1, Gus, Hba,* and *Es-3* were performed by Animal Genetic Systems (College Park, MD). The Fv-1 types for each of the strains were determined by the method previously described (7).

As a quality control measure, DNAs from the C.D2 congenic strains C.D2-Idh1-Pep3 N20F11–13; C.D2-Qa2 N14F2–3; C.D2-TF3 N12F3–6; C.D2-Fv-1 N19F10–14; and C.D2-MIA N10F7 were screened for BALB/c-DBA/2 allelomorphic differences on each autosome and for the X chromosome to check for the presence of unselected passenger segments from the DBA/2 donor strain. Fifty-two markers were used in this screen: for Chr 1, *Idh-1, Pep-3, Mtv-7,* and *Akp-1*; for Chr 2, *Neb, Lym23,* and *Il-1,a*; for Chr 3, *Dai, Evi-1,* and *Egf*; for Chr 4, *Mtv-14, Mtv-13, D4Rp1,* and *D4Lgm1,Pnd*; for Chr 5, *Pgm-1* and *B-Gus*; for Chr 6, *Kras-2*; for Chr 7, *Mtv1,c*; for Chr 8, *D8Mit14*; for Chr9, *d,D9Mit9* and *Cck*; for Chr 10, *Myb* and *Gli*; for Chr 11, *Hba, Sparc, U1a,* and *Es-3*; for Chr 12, *D12Mit4, Mtv-9,* and *Igh-C*; for Chr 13, *D13Mit9, Tcrg,* and *Il-9*; for Chr 14, *D14Mit5, Mtv-11, and Tcra*; for Chr 15, *Rpl30, Tgn, Ly-6,* and *Gdc-1*; for Chr 16, *D16Mit4*; for Chr 17, *Qa2* and *D17Mit41*; for Chr 18, *Ii*; for Chr 19, *Ly1, Fce1b,* and *D19Mit16*; and for Chr X, *DXNds3*. Each of these congenic strains were found to carry BALB/c alleles for all nonselected markers. The allelic composition of the C.D2-Idh1-Pep3 strain was discussed previously (11), and the C.D2-Qa2 congenic strain carries the DBA/2 allele of *Qa2*.

RESULTS

Plasma Cell Foci

Single plasma cells can be found scattered throughout the OG, and in some parts of the OG, plasma cells are seen in clusters. However, there are also much larger aggregates of plasma cells referred to here as foci. Foci are defined as a collection of 50 or more hyperchromatic plasma cells in a field width of approximately 0.4–2.0 mm^2. Some foci are much larger and can contain as many as several thousand cells. Morphologically, a focus may be a compact or diffuse aggregate of plasma cells. In diffuse foci, the cells invade OGs or other loose connective tissues. The mean (crude) mitotic index of cells in foci ranges from 0 to 3% with a mean of 0.74% (Fig. 1) based on the presence of mitotic figures. Foci are rarely observed before Day 50 post pristane administration but make their appearence in increasing numbers thereafter.

The clonal relationships of plasma cells in foci were examined by immunoperoxidase staining with antisera to the light and heavy chain immunoglobulin classes in 62 BALB/c mice exhibiting 4 or more foci by immunoperoxidase staining with antisera to the light and heavy chain immunoglobulin classes. All of the cells in a focus expressed the same light and heavy chain isotypes (Table 1). IgA was the most common heavy chain isotype expressed in isolated plasma cells and in foci; 60 of the 62 mice had one or more IgA foci, and only 12 mice had IgG staining foci (Table 1). The predominant light chain class was kappa, and only 6 mice had lambda staining foci. Twenty-one mice had one or more foci that stained for isotypes other than IgAκ; 11 of these mice had foci expressing only light chains. In four of the mice, foci that expressed lambda light chains were found (Table 1). Expression of one of the IgG subclasses was also relatively rare. Because of the rarity of lambda and IgG-staining foci, it appears that those mice with foci producing these rare isotypes represent examples of multiple foci that were almost certainly clonally related. The presence of clonally related foci in different noncontiguous sites in the OG of these mice further indicated that cells from one focus could leave that site and migrate to another peritoneal site. Single, isolated plasma cells were also found throughout this tissue, and the most common heavy chain class expressed in these cells was IgAκ.

[3] K. Huppi, personal communication.

In 20 mice, there were two to four different kinds of Ig-secreting foci. These are summarized in Table 1. In 11 mice, one of the Ig-secreting types produced only light chains, a common variant in plasmacytomas (23). IgA and IgG subclass-producing foci were found in 12 mice. Among the 62 mice, there were 15 biclonal, 3 triclonal, and 2 tetraclonal examples (Table 1). Often there was a preponderance of one clonotype and a single representative of any additional clones.

Table 1 *Immunotyping of foci*

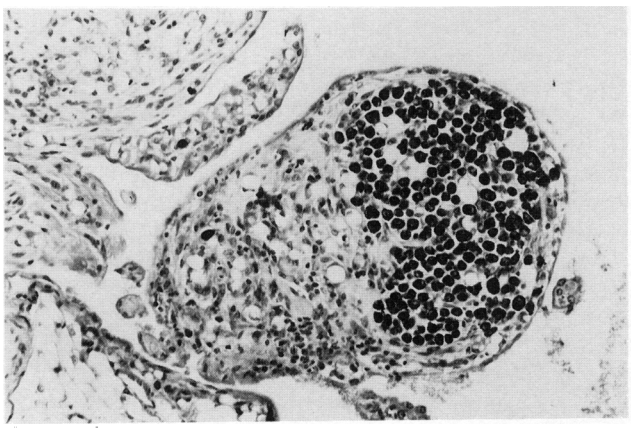
No. of mice	Foci						
	IgA		IgG		L[a] chain only		
	No.	Type	No.	Type	No.	Type	Not typed
30	4–20	Aκ	0		0		0
11	2–20	Aκ	0		0		1
1*[b]	0		0		14	κ	0
1*	20	Aκ	0		6	κ	0
1*	2	Aκ	0		20	κ	0
1*	1	Aκ	0		4	κ	0
1*	9	Aκ	0		6	κ	0
1*	0		0		4	κ + 24	
1*	1	Aκ + 6 Aλ	0		1	κ	0
1*	7	Aκ + 15 Aλ	0		2	κ	0
1*	10	Aκ	0		7	λ	0
1*	3	Aκ	1	G2aκ	1	κ + 1λ	0
1*	3	Aκ	14	G1κ	1	κ +1λ	0
1*	2	Aκ	1	G2aκ	3		0
1*	12	Aκ	8	G2bκ	0		0
1*	20	Aκ	8	G2bκ	0		1
1*	1	Aκ	16	G2bκ	0		0
1*	2	Aκ	13	G2aκ	0		0
1*	1	Aκ	1	Gκ	0		0
1*	19	Aκ	1	Gκ	0		0
1*	2	Aκ	1	Gκ	0		0
1*	8	Aκ	1	G2bκ	0		0
1*	7	Aκ	1	G2bκ	0		0
Total no. of mice							
62	60		12		12		

[a] L, light.
[b] * = 20 (bi, tri, or tetraclonal).

Quantitative Studies of Focus Formation

To determine whether the numbers of plasma cell foci in histological sections of mesenteric OG (assessed between 100 and 260 days post pristane) could be used as an earlier indicator of PCT susceptibility and resistance, the numbers of foci and the incidence of tumorigenesis were compared among resistant and susceptible strains of mice. Because the number of foci per mouse within a strain is variable, ranging from mice with no foci to those with over 20 foci, groups of 20 mice were used for determining PCT susceptibility and resistance. Depending upon the number of foci found, the mice were placed into three categories: no foci, 1–5 foci, and 6 or more foci (6+). This latter category also included mice with 20 or more foci, *i.e.,* fully developed plasmacytomas. Mice with 6+ foci are in the process of progressing to the plasmacytoma stage, a process that proceeds at different rates in each mouse. Foci studies were terminated between Days 130 to 260.

Inbred Strains of Mice (1984–1985). A summary of focus development in different primary strains of mice which had been typed previously for PCT formation by ascites smears over the course of 103–230 days is shown in Table 2. Less than 5% of DBA/2, $CD2F_1$, C57BL/6, and BALB/cJ mice (all shown previously to be resistant to plasmacytomagenesis; Refs. 4–7) developed 6 or more foci at 150 days, whereas 57% of BALB/cAn mice developed 6 or more foci. None of the DBA/2 mice and only a few $CD2F_1$ hybrids developed 6 or more foci. In general, a similar amount of OG tissue was found in BALB/cAn, DBA/2N, and $CD2F_1$ mice. There were some histological differences in OG tissue that will be described separately.[4] The results show that multiple focus development is a strain-specific phenotype of BALB/cAn mice. The low incidence of $CD2F_1$ mice with six or more foci indicated the dominance of the DBA/2 resistant phenotype.



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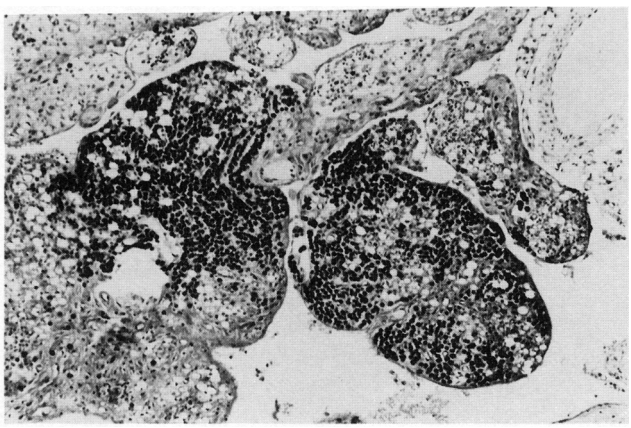

B

Fig. 1. Photomicrographs of foci. *A.* Single, small compact focus (darkly stained cells on the *right*) in a polyp-containing OG from a BALB/cAn mouse which developed a total of 14 foci at 127 days after first injection of pristane. The tissue section was stained with anti-IgA and hematoxylin (× 25). *B.* Two foci (darkly stained cells) in 2 contiguous, polyp-like structures in the OG from a BALB/c mouse that developed 12 foci at 131 days post-pristane. The tissue section was stained with anti-IgA and hematoxylin (× 12.5).

C.D2-Chr 4 Congenic Strains (1990–1993) between Days 125 and 260. The percentage of mice with 1–5 and 6+ foci from individual experiments taken at different days is shown graphically in Fig. 2. The percentages obtained with mice (BALB/cAn, 28–47%; C.D2-Idh1-Pep3, 61%; and C.D2-Qa2, 60%) which had been shown previously to be susceptible were compared with those in the C.D2-Chr4 strains (Table 3). As shown, the 6+ values were nonoverlapping (Fig. 2*B*) with the two groups, whereas the percentages obtained for 1–5 foci (Fig. 2*A*) were overlapping. This finding indicates that both groups are able to form foci but that cells in mice with only 1–5 foci have less tendency to develop autonomous growth.

Five and 8% of mice in the strains C.D2-Fv-$1^{n/n}$ and C.D2-Pnd-7, respectively, had 6+ foci between 125 and 220 days post pristane (Table 3). These mice carry a short segment of DBA/2 chromatin at the distal end of Chr 4 from *Tnfr-1* to *D4Smh6b* (Fig. 3); the smallest region of overlap extends for roughly 6 cM from *Pnd* to *D4Mit42*. Ten to 13% of mice in the 4 strains C.D2-TF3, C.D2-Lgm-1A, C.D2-Lgm-1B, and C.D2-Lgm-1C had 6+ foci between Days 125 and 260 (Table 3; Fig. 2*B*). C.D2-Lgm-1H was tested at 260 days and found to be resistant (Table 4). These 5 strains carry segments of DBA/2 chromatin in the region of Chr 4 from *Ifa* to *D4Rck41*, a distance of roughly 10 cM (Fig. 3). As shown in Fig. 3, the mid and distal segments of DBA/2 chromatin from Chr 4 were discontinuous among the C.D2-Chr 4 congenics. The susceptibility/resistance phenotype of these strains was considered to be resistant or partially resistant. These

[4] M. Potter and E. B. Mushinski, unpublished observations.

Table 2 *Summary of focus data*

Strain	Year of 1st pristane injection	Days	Total no. exps.[a]	Total no. mice	% of Mice with			% of 6+ mice w/PCTs	PCT phenotype
					0 foci	1–5 foci	6+ foci		
BALB/cAn	1984–1985	103–186	7	159	13.9	29.5	56.6	24.0	S
C57BL/6	1984	150	1	23	70.0	26.0	4.3	0.0	R
BALB/cJ	1985	150	1	25	76.0	20.0	4.0	4.0	R
DBA/2	1984–1985	150–230	3	72	90.2	9.7	0.0	0.0	R
CDF1	1984–1985	150–230	3	72	75.0	22.0	2.7	0.6	R

[a] exps., experiments; w/PCTs, with plasmacytomas; S, susceptibility; R, resistance.

results indicate there must be at least two genes on Chr 4 that determine partial resistance to PCT development. The C.D2-MIA congenic mice, which contained DBA/2N chromatin from the entire mid-distal region of Chr 4 from *Ifa* to *D4Smh6b*, also developed a low incidence (10%) of mice with 6+ foci (Table 3; Fig. 3).

C.D2-Congenic Strains at Day 300. In some experiments, groups of mice were followed for 300 days by periodic peritoneal smears. At that time, the surviving mice were autopsied, and mesenteric tissues examined (Table 4). The incidence of plasmacytomas at Day 300 was given as the sum of the cases detected by ascites smears and the cases diagnosed from tissue sections; this value is listed under incidence in Table 4. The incidence of PCTs at 300 days was low in C.D2-MIA, C.D2-Fv-1, and C.D2-Lgm-1H and high in BALB/c and the non-chromosome 4 congenics C.D2-Idh1-Pep3, C.D2-Es3-Hba, C.D2-Qa2, and C.D2-Lyt1. A 22% PCT incidence was obtained with C.D2-TF3 which was due to the late development of PCTs in this strain.

BALB/cAnPt Mice (1984–85 *versus* 1990–1993). The incidence of foci in our BALB/cAnPt colony of mice showed a progressive change; beginning in 1991, the incidence of mice with 6 or more foci at Days 130–150 declined from 47 to 28% (Table 3). Initially, we attributed this difference to the increased isolation of our mouse colony from exposures to other mice and improvements in the cage-washing practices. We had shown that specific pathogen-free BALB/cAn mice receiving injections of pristane have a reduced incidence of plasmacytomas compared to conventionally reared BALB/cAn mice (25). However, the susceptible C.D2 congenics C.D2-Idh1-Pep3 and C.D2-Qa2 that were pristane treated during the same period developed high incidences of mice with 6+ foci (Table 3), suggesting the possibility of a genetic rather than an environmental explanation. The "1991–1992" BALB/cAn mice were derived from a single pair of BALB/cAn mice. As such, this raised the possibility that a mutation conferring resistance to plasmacytoma formation had occurred in our BALB/cAnPt colony through genetic drift.

DISCUSSION

To accelerate assessment of genetically determined susceptibility or resistance to plasmacytoma induction, we have counted the number of foci in tissue sections of the mesenteric OG during PCT development. Foci are defined here as colonies of plasma cells that take up stain more avidly than normal cells and which exhibit varying degrees of atypia. Foci develop well before PCT cells appear in the ascites. The interval between the time a focus develops and a PCT can be diagnosed by the presence of PCT cells in the ascites is not accurately established and is variable. Results of immunoperoxidase staining indicate that cells in a focus are clonal (*i.e.*, all of the cells make the same isotypic immunoglobulin light and heavy chains). A single mouse, however, may carry more than one clone. The predominant immunoglobulin class expressed in foci is IgAκ. IgAκ foci were found in over 95% of the mice and are the only type of focus in 80% of the mice. At present, we have not subtyped the IgAκ foci for V-region specific markers which may reveal further evidence of oligoclonality of foci. The cells in some foci appear to acquire the ability to spread to other locations in the OG and form new satellite foci. A mouse with 6 or more (6+) foci was considered to have cells that were

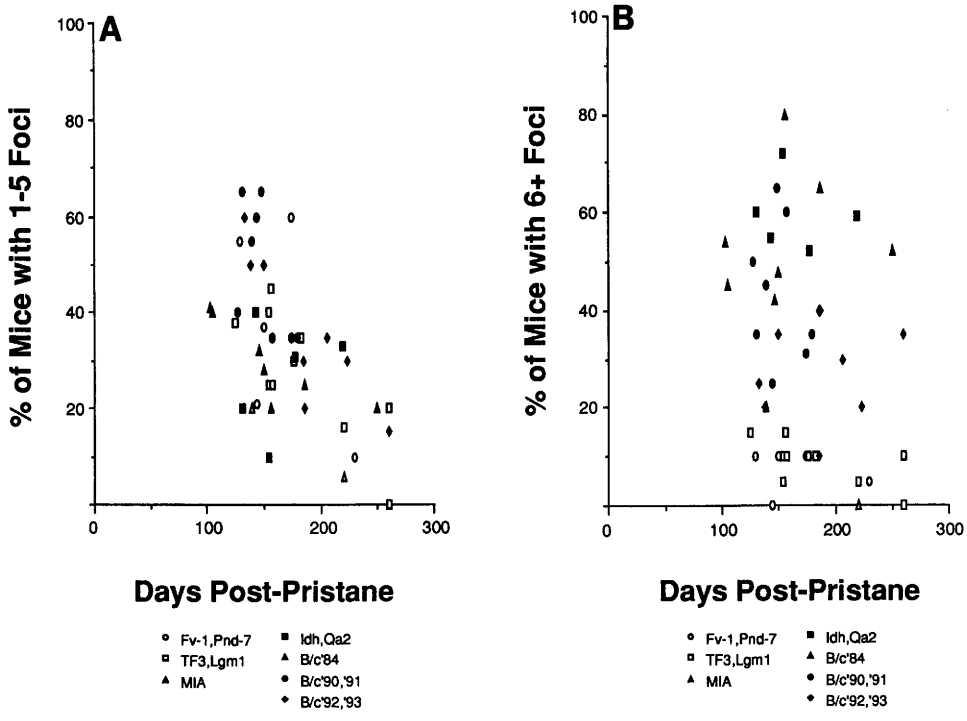

Fig. 2. The percentage of mice with 1–5 foci (*A*) and 6+ foci (*B*) in the survey of intestinal mesenteric tissues. *Closed symbols,* the various partially susceptible strains BALB/cAn; C.D2-Idh1-Pep3; and C.D2-Qa2. *Open symbols,* the C.D2-Chr 4 resistant strains. *Points,* the percentage for a single group of mice. The average number of mice per group was 20 (range, 18–25) from the experiments summarized in Tables 2 and 3. The data points were plotted according to the day post pristane when the sections were taken. As shown, there was a gradual decline in the number of mice in the 1–5 foci categories (*A*) with time. The C.D2-Chr 4 congenics had fewer mice with 6+ foci than in the susceptible strain (*B*).

Table 3 *Summary of focus data for C.D2-Chr 4 congenic strains and controls*

Strain	Year of 1st pristane injection	Days	Total no. exps.[a]	Total no. mice	% of Mice with			% of 6+ mice w/PCTs	PCT phenotype
					0 foci	1–5 foci	6+ foci		
BALB/cAn	1990	131–157	4	80	3.7	48.7	47.0	13.7	S
BALB/cAn[b]	1991	144–175	4	79	25.3	45.5	29.1	7.6	S
BALB/cAn[b]	1992	150–260	3	60	40.0	31.6	28.3	16.6	S
BALB/cAn(B)[b]	1992–1993	185–186	2	40	35.0	25.0	40.0	25.0	S
C.D2-Idh1-Pep3	1991–1993	143–219	4	95	11.6	27.4	61.0	37.7	S
C.D2 Qa2	1991	131	1	20	20.0	20.0	60.0	35.0	S
C.D2-MIA	1990–1992	139–220	2	39	74.3	15.4	10.3	0.0	PR
C.D2-TF3	1990–1992	125–220	3	58	65.5	24.2	10.3	5.1	PR
C.D2-Fv-1*n*/*n*	1990–1992	125–220	4	78	57.5	34.5	7.7	2.5	PR
C.D2-Lgm-1A	1991	154–182	2	40	52.5	37.5	10.5	2.5	PR
C.D2-Lgm-1B	1992	154–260	2	40	80.0	12.5	7.5	2.5	PR
C.D2-Lgm-1C	1992	156–176	2	40	50.0	37.5	12.5	5.0	PR
C.D2-Pnd-7	1991	144–174	2	39	53.6	41.0	5.1	5.1	PR

[a] exps., experiments; w/PCTs, with plasmacytomas; S, susceptibility; PR, partial resistance.
[b] See description in text.

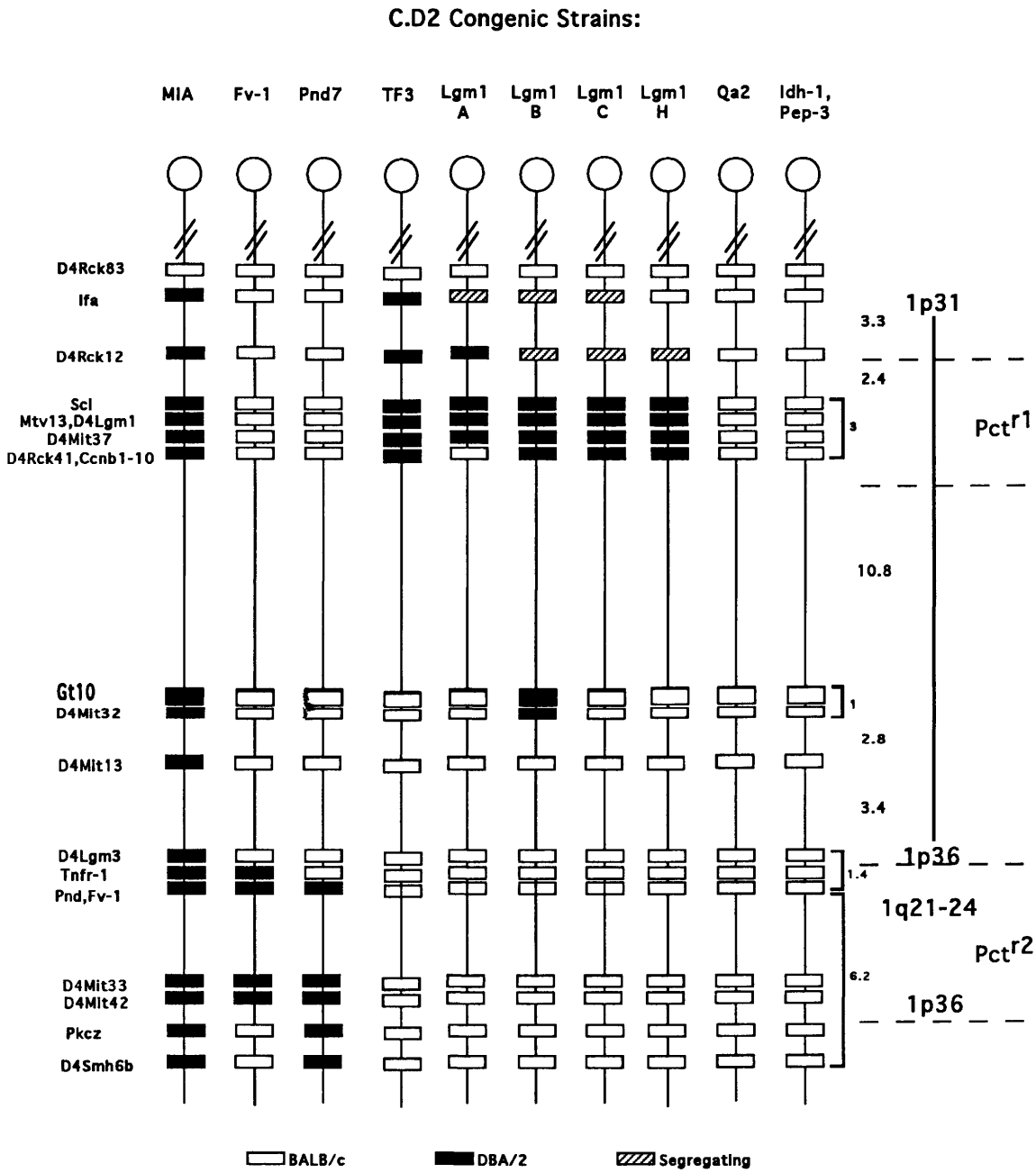

Fig. 3. Genotypes of allelomorphic genes in the distal half of Chr 4 for the BALB/c.DBA/2 congenic strains described in the text. *Open rectangles,* the BALB/cAnPt allele; *darkened rectangles,* the DBA/2N allele. *Hatched rectangles,* genes that were still segregating when the mice were tested for foci. Recombinational distances between loci and the locations of loci are indicated on the *right* and were originally determined by Mock *et al.* (15) in an intraspecific backcross of BALB/c and CD2F$_1$. Probable locations of *Pct*^{r1} and *Pct*^{r2} are indicated on the *right*. Regions of linkage homology with human Chr 1 are also denoted on the *right*.

Table 4 *Incidence of PCTs*

Strain (Chr)	Date 1st pristane	Total no. mice	Autopsied at Day 300				No. PCTs diagnosed by smear	Incidence of PCTs[a] %	PCT phenotype
			No.	%	Day	No. PCTs			
BALB/c	06/04/90	83	30		300	6	37	52	S
	05/03/91	21	17		300	1	5	28	S[b]
	11/05/91	40	21		300	0	12	30	S[b]
C.D2-Qa2 (Chr 17)	06/04/91	84	10		335	0	46	54	S[c]
C.D2-Idh1-Pep3 (Chr 1)	11/05/91	40			296		29	72	S[c]
C.D2-Es-Hba (Chr 11)	05/10/93	39			206		22	56	S[c]
C.D2-Lyt1 (Chr 19)	05/10/93	47			206		28	57	S[c]
C.D2-MIA (Chr 4)	09/11/90	80	65		300	1	2	4	R
C.D2-Fv-1 (Chr 4)	09/11/90	68	48		300	1	6	10	PR
C.D2-TF3 (Chr 4)	07/06/90	63	37		300	2	13	22	PR[d]
C.D2-Lgm-1H (Chr 4)	04/07/92	20	20		260[e]	0	0	0	R

[a] Incidence of PCTs = number of PCTs diagnosed at autopsy plus the PCTs diagnosed by smear.
[b] Reflects declining incidence in BALB/cAnPt (see text).
[c] Early onset of PCTs, very similar to results obtained with BALB/cAnPt in 1984–1985.
[d] C.D2-TF-3 developed PCTs late.
[e] Autopsied at Day 260.

capable of spreading around the mesenteric OG and eventually overgrowing the peritoneal surfaces.

Although focus enumeration is not a precise quantitative method, it is a useful index to determine progression in plasma cell tumor development. High percentages of mice in PCT susceptible strains are found to have 6+ foci between Days 100 to 260 post pristane. We used two types of control strains in this study. The first was our conventionally raised BALB/cAnPt strain. For many years, this particular subline of the BALB/cAn family was highly susceptible to PCT development (1). The studies performed from 1983 to 1991 reflect this hypersusceptibility. Sometime around 1991, however, the incidence of PCTs following the routine three injections of 0.5 ml pristane began to decline. All of the BALB/cAnPt mice in this period can be traced to a single breeding pair. Four non-Chr-4 C.D2 congenic strains were used as susceptible controls (Table 4). These congenic strains were derived during the same period and have a high degree of susceptibility. In a previous survey of various BALB/c sublines imported into our colony from other laboratories that were carried out in 1985, we found five available sublines (*e.g.*, BALB/c Wt, BALB/c ORNL, and BALB/c Arg) to be highly susceptible to PCT induction (26). One exceptional strain, however, was the BALB/cJ subline which developed a very low incidence of plasmacytomas (24). In subsequent unpublished studies, we found that BALB/c Wm, which was separated from the MacDowell stock in 1931, also to be susceptible.[5] This evidence suggests that the genetic predisposition associated with PCT susceptibility was established many decades ago. In view of the experiences reported here, it is important to continuously monitor the phenotype of an inbred strain when multiple genes participate in forming the susceptible genotype.

The development of multiple plasmacytic foci is highly strain dependent. BALB/cAn develop multiple foci whereas DBA/2, C57BL/6, and BALB/cJ do not. We found that BALB/cAn.DBA/2 congenic mice, carrying segments of the distal end of Chr 4 from DBA/2, developed a greatly reduced number of multiple foci, indicating that specific genes in DBA/2 mice are able to suppress the formation of foci. These effects thus far have been assessed in homozygotes, *i.e.*, congenic mice carrying both alleles derived from the DBA/2 parent. The C.D2-Chr 4 congenics can be divided into two groups, one that has a mid-distal segment of Chr 4 from the DBA/2 donor (C.D2-TF3, C.D2-Lgm-1A, C.D2-Lgm-1B, C.D2-Lgm-1C, and C.D2-Lgm-1H strains) and a second group with telomeric segments of Chr 4 (C.D2-Fv-$1^{n/n}$ and C.D2-Pnd-7). The mid-distal and telomeric segments of chromatin are nonoverlapping. This indicates the presence of at least two independent DBA/2 genes that reduce PCT incidence and suppress the development of multiple foci. Mock *et al.*(15) have recently shown that BALB/cAnPt plasmacytoma susceptibility genes (Pct^s) reside on Chr 4 in the same regions as the proposed Pct^r genes. This suggests that the phenotypes of PCT-susceptible and -resistant strains are due to different alleles of *Pct* genes. We are currently surveying other C.D2 congenic strains harboring regions of DBA/2 chromatin from other chromosomes for their susceptibility/resistance phenotype. The most remarkable finding in the present study is the strong effects of the Chr 4 DBA/2 genes, Pct^{r1} and Pct^{r2}, in producing a partially resistant phenotype in response to pristane induction of plasmacytomagenesis.

While there were striking differences in the percentage of mice with 6+ foci in susceptible and resistant strains, the percentages of mice with 1–5 foci were overlapping (Fig. 2*A*), indicating partially resistant mice could form foci. The major action of the DBA/2 genes, then, appears to impede the development of multiple foci rather than inhibit focus formation.

The mechanism of action of Pct^r genes is not known. These genes do not appear to be associated with obvious immunological disorders such as immunodeficiency or autoimmunity in normal BALB/c mice. *Pct* susceptibility/resistance genes may be regulated and expressed differently depending upon the physiological, developmental, or pathological state of the B-cell. Over 90% of pristane-induced plasmacytomas develop characteristic chromosomal translocations, t(12;15) and t(6;15), that deregulate the transcription of the c-*myc* protooncogene (see Ref. 27 for review). *Pct* susceptibility/resistance genes could exert their biological action at different stages of PCT development. This may include the step leading to the formation of a focus cell or in focus cells themselves through an enhanced ability to proliferate and spread to new locations in the peritoneum. It is likely that plasma cells in foci have one of the c-*myc* deregulating chromosomal translocations. Using PCR primers, we have recently found evidence of illegitimately recombined c-*myc*-Sα genes 30 days after the injection of pristane (28). Although very few foci have ever been found at this early time point,[5] it is possible that B-cells with translocations are proliferating in OG tissue even before foci can be detected in histological sections. We are currently studying the OG tissue in C.D2-Fv-1 and C.D2-MIA mice; preliminary studies have identified illegitimately recombined c-*myc*-Sα sequences in C.D2-Fv-1 and C.D2-MIA mice,[6] indicating that these events can take place in mice that are partially resistant to PCT development. Thus, it seems likely that the Chr 4 Pct^r genes identified here are acting in cells that have undergone c-*myc* deregulation. An important characteristic of the Pct^r genes described here is their independent action. Either Pct^{r1} or Pct^{r2}

[5] M. Potter, unpublished observations.

[6] S. Janz, J. Müller, G. Jones, and M. Potter, unpublished results.

alone appears to reverse the susceptibility phenotype. This suggests that PCT suseptibility in the BALB/cAn mouse is dependent upon the cooperative action of several allelomorphic genes.

Jonasson *et al.* (29) in 1977 described a gene located in the vicinity of *Pct*^{r1} on Chr 4 that suppressed the malignant phenotype in hybrids of normal and tumor cells. If this gene is *Pct*^{r1}, it suggests that the BALB/c mouse carries a variant that does not suppress the neoplastic phenotype and may explain the tumorigenicity of plasmacytoma-normal lymphocyte hybrids.

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