

## INHIBITION OF PLASMACYTOMA DEVELOPMENT IN BALB/c MICE BY INDOMETHACIN

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Plasmacytomas can be induced with high frequency in BALB/cAn  $\pi$  mice by the intraperitoneal injection of mineral oils (1), defined alkanes, e.g., pristane (2,6,10,14-tetramethylpentadecane) (2), or the implantation of plastic Lucite discs (3). Histological studies (4) of mineral oil- or pristane-induced plasmacytoma development have provided evidence that the tumors develop in the peritoneal inflammatory tissue (oil granuloma) that forms in response to the oil. The intraperitoneal transplantation of plasmacytomas derived from primary hosts to syngeneic hosts requires conditioning of the peritoneum by pristane (5, 6). This phase of plasmacytoma growth appears to depend upon factors supplied by the oil-induced inflammatory cells (6); however, this dependence is soon lost, since subsequent transfers grow independently. The close association of the developing plasmacytoma with the oil granulomatous process also suggests that inflammatory cells and their products play an important role in earlier steps in the evolution of plasmacytomas. Support for this notion comes from the data of Takakura et al. (7), who demonstrated that hydrocortisone administered chronically during the latent period of plasmacytoma development inhibited plasmacytomagenesis. Moreover, Cancro and Potter (6) found that hydrocortisone blocked the development of oil granuloma formation and greatly reduced the ability of the pristane-treated peritoneal tissues to support the growth of transplanted primary plasmacytomas. In the present study we have examined the effect of the nonsteroidal antiinflammatory drug (NSAID),<sup>1</sup> indomethacin, on plasmacytomagenesis. Indomethacin is not toxic and can be administered in the drinking water throughout the induction period (i.e., 300 d), thus providing a system for studying long-term effects of the drug. This paper will describe a dramatic inhibition of plasma cell tumor development in pristane-injected mice treated with indomethacin, and further demonstrate that indomethacin does not inhibit the formation of the oil granulomatous tissue during the latent period of plasmacytoma development (i.e., the first 120 d after the injection of pristane).

### Materials and Methods

BALB/cAn  $\pi$  female mice were used throughout. All of the mice were bred in our closed conventional colony, and the induction experiments were carried out in the same

<sup>1</sup> *Abbreviations used in this paper:* MPO, myeloperoxidase; NSAID, nonsteroidal antiinflammatory drug; PGE, -G, and -H, prostaglandins E, G, and H.

location. 40–60-d-old mice were given a single intraperitoneal injection of 0.5 or 1 ml of pristane (Aldrich Chemical Co., Milwaukee, WI) (8). Indomethacin (Sigma Chemical Co., St. Louis, MO) was put in solution at 10 g/ml in 100% ethyl alcohol and stored in the dark. The stock was diluted in tap water to a final concentration of 2, 10, or 20  $\mu\text{g}/\text{ml}$ . The drinking water was changed twice weekly. The mice were fed Purina Mouse Chow ad libitum. Controls were injected with pristane and given drinking water containing 0.2% ethyl alcohol.

Beginning at 120 d after the injection of pristane, the mice were examined every 14 d for the development of ascites. Ascitic fluid was obtained by paracentesis with a 25-gauge needle and the diagnosis of plasmacytoma was made from Wright's-stained slides. In experiment 2 (Table I), 20 of the mice in groups A and B alive at 300 d were autopsied and tissue sections were examined histologically for the presence of plasmacytomas.

Histological studies were also done on mice given 0.5 ml i.p. pristane in experiment 4. These mice were killed by cervical dislocation, and the tissues were fixed in Fekete's modified Tellyesniczky's fluid (70% ethyl alcohol/formalin/glacial acetic acid, 20:2:1), and stained with hematoxylin and eosin. Multiple sections were taken from mesenteric tissues. To obtain peritoneal exudates, mice that did not have visible ascites were injected intraperitoneally with 10 ml tissue culture medium containing 10% fetal calf serum. Cytofuge preparations were made in a cytospin centrifuge (Shandon Southern Instruments, Inc., Sewickley, PA).

## Results

*Effect of Indomethacin on the Development of Plasmacytomas and Arthritis.* Indomethacin given continuously in the drinking water at a concentration of 20  $\mu\text{g}/\text{ml}$  produced a dramatic reduction in plasmacytoma formation (Table I). Only 4 plasmacytomas developed in 180 mice (2.2%), as compared with 64 in 183 controls (34.9%) (Table I, groups A and B). During the time of maximal plasmacytoma development, i.e., from 150 to 240 d after the injection of pristane, most of the indomethacin-treated mice had a healthy, vigorous appearance and did not develop ascites. A few became severely emaciated and died, and the cause of death in these mice has not yet been determined. Mice treated with 10  $\mu\text{g}/\text{ml}$  indomethacin in the drinking water (Exp. 3, group C) also developed very few plasmacytomas, while 16% of the mice treated with 2  $\mu\text{g}/\text{ml}$  indomethacin developed ascites and plasmacytomas, indicating that this latter dose of indomethacin had only a mild inhibitory effect on plasmacytoma development.

When indomethacin (20  $\mu\text{g}/\text{ml}$ ) treatment was begun at 60 d after the injection of pristane, the incidence of plasmacytomas was also strikingly reduced. Only four plasmacytomas among 50 mice were observed (group I C, Table I). Delaying the beginning of indomethacin treatment until day 160 produced a reduction in plasmacytoma development to 22% (group I D, Table I).

A high percentage of BALB/c mice given intraperitoneal pristane developed arthritis after a latent period of 100 d, as observed in previous studies (9). The ankle joints were most frequently affected, and the process often appeared to be confined to these joints. Some of the mice, however, developed a severe debilitating arthritis that involved joints in both upper and lower extremities and which limited mobility. These mice often became emaciated. Indomethacin partially reduced the incidence of arthritis (Table I), but clearly did not prevent its development.

*Effect of Indomethacin on Transplantable Plasmacytomas.* When  $10^5$  to  $10^6$  plasmacytoma cells from a mouse with a primary plasmacytoma are transplanted

TABLE I  
*Effect of Indomethacin on the Induction of Plasmacytomas by the Intraperitoneal Injection of 1.0 ml Pristane*

Group	Concentration ( $\mu\text{g/ml}$ )	Indomethacin		Percent arthritis at day 200	Percent plasmacytomas at day:				Total No. mice
		Day begun	Day ended		150	200	250	300	
1 A	—	—	—	60	4	8	32	40	50
1 B	—	—	—	42	4	18	28	40	50
1 C	20	-7	300	22	0	0	0	0	50
1 D	20	+60	300	40	0	2	6	8	50
1 E	20	+160	300	36	6	8	12	22	50
2 A	—	—	—	50	8	22	30	38*	50
2 B	20	-7	300	18	0	0	2	5*	43
2 C	20, 10	-7, 60 <sup>‡</sup>	300	21	1	1	2	4	47
2 D	20	-7	120	26	0	4	5	10	50
3 A	—	—	300	42	2	2	22	30	50
3 B	20	-7	300	22	0	0	0	0	50
3 C	10	-7	300	12	0	0	2	7	50
3 D	2	-7	300	38	2	4	10	16	50
4 A <sup>§</sup>	—	—	—		0	21	38	56	34
4 B	20	-7	300		0	2	2	5	37

All mice received an intraperitoneal injection of pristane on day 0. Mice not receiving indomethacin were given diluent (0.2% ethyl alcohol) in the drinking water with the exception of group 2 B, which received tap water.

\* Remaining mice at day 300 (20 in each group) were autopsied and peritoneal tissues were examined histologically for plasmacytomas; eight plasmacytomas were found in 2 A and two in 2 B. These tumors were not included in the day 300 incidence.

<sup>‡</sup> Group 2 C received 20  $\mu\text{g/ml}$  indomethacin for the first 60 d, followed by 10  $\mu\text{g/ml}$  thereafter.

<sup>§</sup> Mice in groups 4 A and 4 B received a single injection of 0.5 ml pristane.

intraperitoneally to a normal syngeneic host, they usually do not grow progressively (5, 6). However, similar numbers of cells almost always grow progressively when transplanted to mice that have received an intraperitoneal injection of pristane several days before transplantation. This dependence on the pristane-conditioned peritoneal environment for growth is usually lost after a single transfer (5). In the present study, we tested the ability of indomethacin to inhibit the growth of four primary plasmacytomas in transplant recipients (Table II). The mice were conditioned with three different doses of pristane, 0.5, 0.2, or 0.1 ml. Indomethacin had no effect on the growth of the four tumors in conditioned mice. These results also indicate that indomethacin was not cytotoxic for plasmacytoma cells.

*Effect of Indomethacin on the Development of the Peritoneal Inflammatory Exudate.* The effects of indomethacin on the formation of the peritoneal exudate were studied in groups of mice on days 67, 116, 145, 170, and 180 after the intraperitoneal injection of 0.5 ml pristane. Preparations of the peritoneal exudate cells from each mouse were made using the cytofuge, which permitted the examination of very large numbers of well fixed and stained cells. The total

TABLE II  
Effect of Indomethacin on the Transplantation of Primary Plasmacytoma Cells

Tumor*	Control	Frequency of plasmacytoma occurrence in mice receiving:					
		Pristane only at dose (ml) pristane:			Pristane plus indomethacin <sup>‡</sup> at dose (ml) pristane		
		0.5	0.2	0.1	0.5	0.2	0.1
MyI 8-36	0/10 <sup>§</sup>	5/5	10/10	8/10	5/5	10/10	8/10
MyI 11-52	1/10	5/5	10/10	9/10	5/5	10/10	10/10
MyI 13-62	0/10	4/5	10/10	7/10	5/5	10/10	10/10
MyI 2-7	1/10	5/5	10/10	10/10	5/5	10/10	10/10

\* Each mouse received  $\sim 10^5$  viable primary plasmacytoma cells intraperitoneally. Pristane-treated mice received an intraperitoneal injection of pristane 8 d before receiving the tumor cells. Controls received no pristane.

<sup>‡</sup> Indomethacin treatment (20  $\mu\text{g}/\text{ml}$  in drinking water) was begun 7 d before pristane injection.

<sup>§</sup> Data consist of fractions of which the denominator is the total number of mice, and the numerator is the number of mice with progressively growing plasmacytomas.

TABLE III  
Free Cells in Peritoneal Cavity

Days after pristane injection	Free cells ( $\times 10^6$ )			
	Pristane (0.5 ml)		Pristane (0.5 ml) plus indomethacin (20 $\mu\text{g}/\text{ml}$ )**	
	Total mean (range)	Neutrophils mean (range)	Total mean (range)	Neutrophils mean (range)
67	31.7 (21-34)	18.6 (14-22)	20.7 (9-46)	9.4 (5.6-18.6)
116	19.9 (4-31)	11.6 (2.4-17.7)	11.7 (2.6-26.7)	5.8 (1-12.0)
145	17.6 (2-30)	6.4 (0.6-13.2)	11.9 (5.7-21.0)	5.1 (1.2-8.3)
170	22.2 (11-33)	4.5 (2.0-8.1)	13.7 (6-30)	4.9 (2.4-9.0)
180	31.3 (17-62)	3.7 (0.2-11.8)	8.6 (3.5-11)	2.2 (1.0-3.1)
	Alcohol-water		Indomethacin (20 $\mu\text{g}/\text{ml}$ )	
75	1.6 (1.2-1.9)	0 (0)	1.4 (0.9-2.4)	0 (0)

There were five mice in each group, except alcohol-water and indomethacin controls, which had only three mice, and the pristane-indomethacin day 116, which had only four mice. The mice in this experiment were groups C (pristane), group D (pristane and indomethacin), group E (alcohol and water), and group F (indomethacin) from Exp. 4 (see Table I).

free peritoneal cells in mice injected with 0.5 ml pristane were consistently higher than observed in nonpristane controls.

Three predominant cell types found in the exudates of pristane-injected mice were: (a) monocytic cells and macrophages, (b) neutrophils, and (c) lymphocytes. Because there were transitional stages with considerable morphological variation, particularly in the monocyte-macrophage series, it was often difficult to classify the cell types. Thus, the table indicates only the total numbers of polymorphonuclear leukocytes. Since neutrophils are not found or found only rarely in the peritoneal exudates of non-pristane-treated mice, their presence indicates an active inflammatory response. As seen in Table III, there was a modest reduction

in the total number of neutrophils in indomethacin-treated mice but this did not constitute a striking difference. Free plasmacytoma cells were found in four mice from the pristane-only group (Nos. 101, 118, 141, 144) and in one pristane-indomethacin-treated mouse (No. 137). The percentage of plasmacytoma cells ranged from 0.04 to 0.8% (Table IV).

*Histology of the Oil Granuloma.* Histological sections of peritoneal tissues from pristane- or pristane-indomethacin-treated mice that were obtained 30–100 d after pristane injection showed no remarkable differences in the morphology or quantity of oil granuloma development. A more detailed study of the oil granuloma was carried out on the groups of mice from which the peritoneal exudates were obtained (Table III). Tissue sections were obtained from five mice each on days 116, 145, 170, and 181 (Table IV). Two general differences in the oil granulomatous tissues from pristane- and pristane-indomethacin-treated mice were observed after day 116. First, there was less granulomatous tissue in many but not all of the mice treated with indomethacin, and, very often, the oil granuloma tissue in these mice showed less cellularity and inflammatory activity. It was difficult to quantitate these differences in mesenteric tissues, but some assessment could be made by comparing oil granuloma formation on the diaphragm. In most mice given only pristane the oil granulomatous process was extensive and appeared as large plaques of yellow, very hard, calcified tissue that

TABLE IV  
Summary of Histological Findings in the Mesenteric Oil Granuloma

Day	Pristane (0.5 ml)					Pristane (0.5 ml) plus indomethacin				
	Mouse No.	EMM*	Plasma-cytosis‡	PCT foci‡	Free PCT cells§	Mouse No.	EMM	Plasma-cytosis	PCT foci	Free PCT cells
116	101	+	+	+(m)¶	+(0.04%)	103	-	-	-	-
	102	+	+(m)	+(m)	-	104	-	-	-	-
	106	+	+	+(m)	-	105	-	-	-	-
	107	+	+	-	-	109	+	-	-	-
	108	-	+	-	-	110	-	-	+(2)	-
145	116	-	+(m)	+(3)¶	-	111	-	-	-	-
	117	+	-	+(5)	-	112	-	-	-	-
	118	-	-	+(m)	+(0.4%)	113	-	-	-	-
	119	-	+(m)	+(5)	-	114	-	+(2)	-	-
	120	-	-	+(1)	-	115	-	-	-	-
170	132	-	-	-	-	130	-	-	-	-
	131	-	-	+(2)	-	127	-	-	-	-
	133	+	-	+(5)	-	128	-	-	-	-
	134	-	+	+(3)	-	129	-	+	-	-
	135	+	-	+(m)	-	136	-	-	-	-
181	144	-	+	+(4)	+(0.29%)	146	-	+(1)	-	-
	143	-	+	+(m)	-	145	-	-	-	-
	142	-	+(m)	-	-	139	-	+(1)	-	-
	141	-	-	+(m)	+(0.8%)	138	-	+	-	-
	140	+	+	+(6)	-	137	-	-	+(m)	+(0.88%)
Totals				16/20					2/20	

\* Focus of extramedullary myelopoiesis that contains proliferating cells.

‡ m, multiple. Numbers in parentheses represent number of foci.

§ (-) No plasmacytoma cells were seen by scanning the cytofuge preparations that usually contain  $\sim 2.5 \times 10^5$  cells.

¶ Mouse 116 had a large infiltrate that replaced a polyp.

immobilized the diaphragm. The indomethacin-treated mice had less diaphragmatic infiltration and, in many of the mice, part of the diaphragm was virtually free of granulomatous tissue. These findings suggested that prolonged indomethacin treatment reduced the amount of granulomatous tissue by diminishing the inflammatory response to the oil and some of the associated complications, such as calcification and adhesion. This, however, was only observed in the tissues obtained after 116 d.

A second and more significant difference in oil granulomatous tissues was related to plasma cell proliferation. Surprisingly, in the mice studied between day 116 and day 180, foci of proliferating plasma cells that morphologically resembled plasmacytomas were found in 16 of 20 pristane-treated mice (Table IV) while only 2 of 20 pristane-indomethacin mice had microscopic plasmacytomas. These foci were multiple in eight of the mice (i.e., over five separate locations in the peritoneum were involved) and resembled typical primary plasmacytomas. In the other eight mice, five fewer foci were found (Table IV). These probably represent some of the earliest histological evidence of plasmacytoma development. Many of these foci did *not* appear to represent in situ developing tumors but rather appeared to be deposits of cells that had originated elsewhere and invaded the oil granuloma (Fig. 1). Two routes of invasion, consistent with histology, were seeding on mesothelial surfaces or entry through blood vessels. Much of the oil granuloma was organized into small polypoid structures that formed on the mesenteric peritoneal surfaces (Fig. 2). The morphology and development of these polyps will be described in a separate publication. Plasmacytoma infiltrates were found confined to single polyps, either as deposits on the surface or infiltrating the stalk of the polyp.

Other forms of plasma cells were found in the peritoneal tissues. In most mice of both pristane- and pristane-indomethacin-treated mice, it was easy to find plasma cells scattered throughout the oil granuloma. Also, there were focal collections of plasma cells with uniform round nuclei and abundant cytoplasm (plasmacytosis) (Fig. 3). Many of the foci were very small, containing as few as 10 cells, others were larger. Lymphocytes were frequently found in association with plasmacytotic foci. A few plasmacytotic foci contained an occasional atypical plasma cell, suggesting a potential relationship of the plasmacytotic focus to the plasmacytoma. However, more detailed histological studies will be required to establish such a relationship. Plasmacytosis appeared to be associated with active inflammation in the oil granuloma.

Plasmacytoma cells were found in only five of the mice by scanning cytofuge preparations (Table IV). Thus, in the majority, the only evidence of a developing plasmacytoma was the presence of a fixed focus of plasmacytoma cells. While this may appear to indicate that plasmacytoma development takes place in the peritoneal connective tissues, it is possible that some transformation steps occur in the peritoneal exudate population and that the foci represent seedings of these transformed cells on peritoneal surfaces.

In addition to plasmacytosis and plasmacytoma, foci of extramedullary myelopoiesis were found frequently in 8 of 20 mice given pristane alone, while only one was observed in the pristane-indomethacin-treated mice. These foci usually

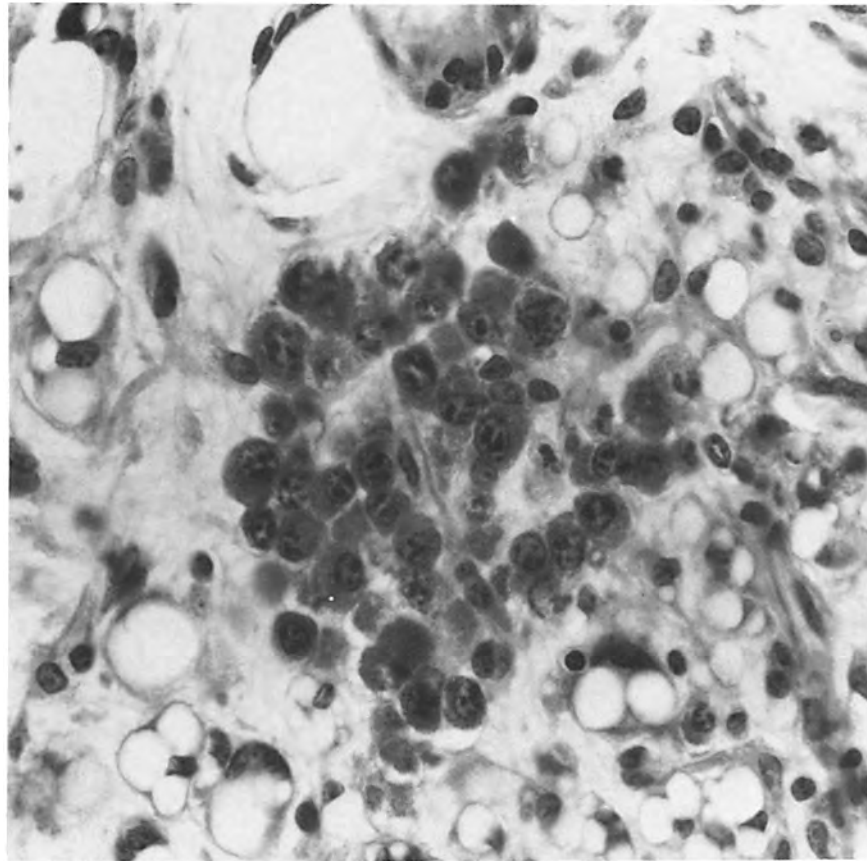


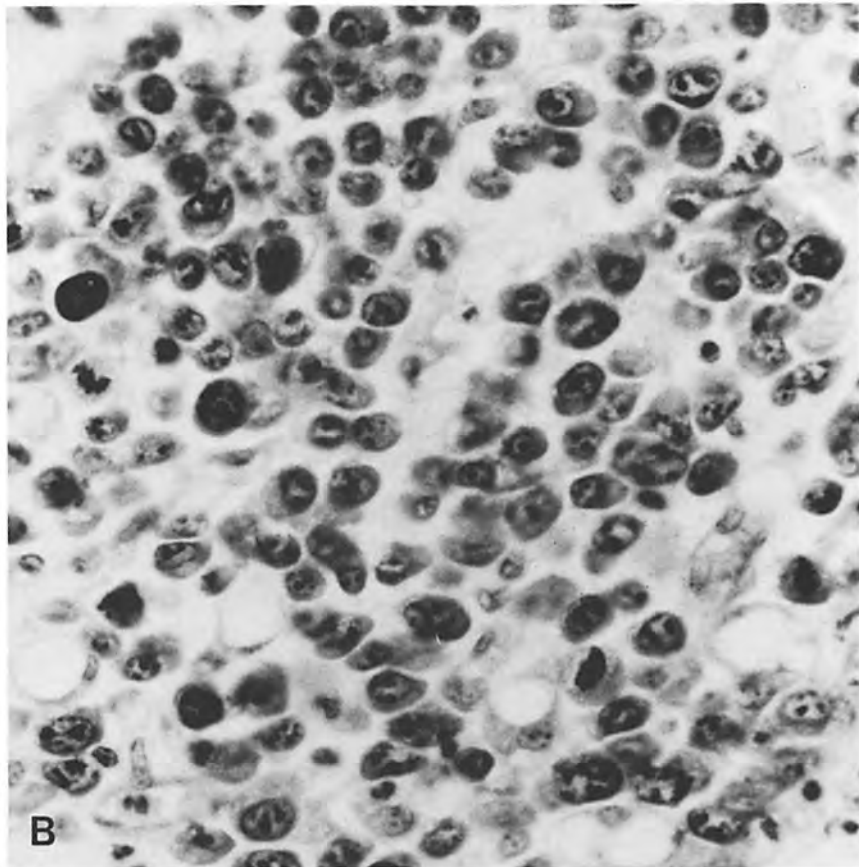
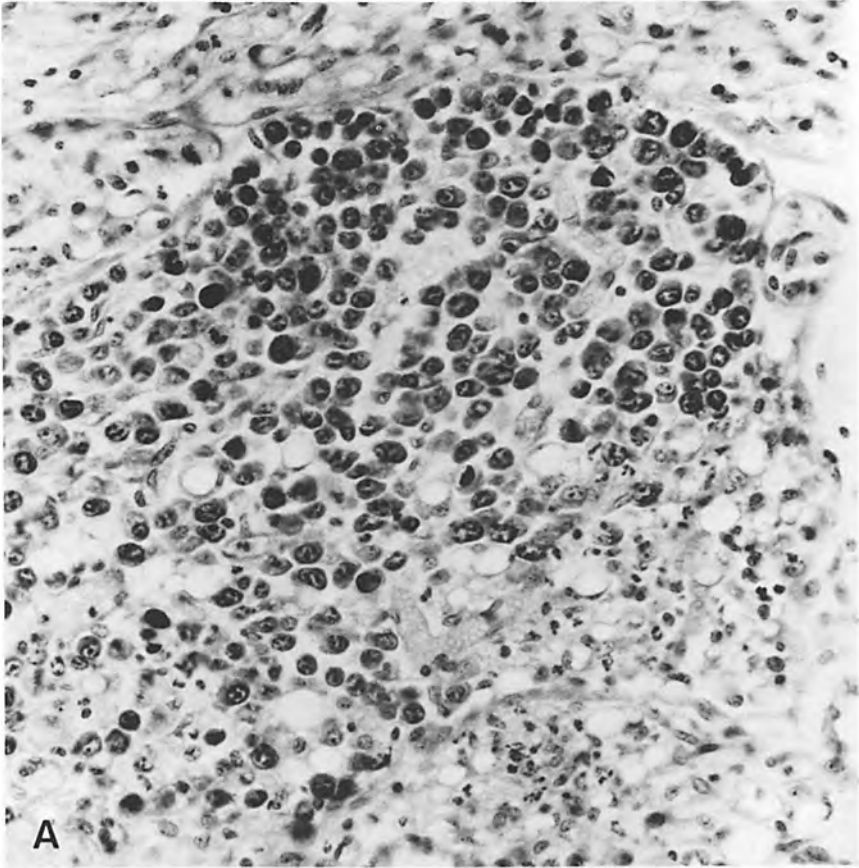
FIGURE 1. Deposit of atypical large, intensely staining plasma cells from pristane-only mouse 117 (Table IV) at day 145. Five small deposits were found in the mouse. The cluster shown in the figure was located near the base of a polyp. Hematoxylin and eosin.  $\times 134$ .

contained proliferating cells related to the myelomonocytic differentiation pathway.

A similar histological study was also performed on peritoneal tissues obtained from 20 mice in each of groups 2 A and 2 B (Table I) at day 300. These mice had not been diagnosed as having plasmacytomas by examination of the standard type of smears (see reference 8). In 8 of the 20 pristane-only mice, plasmacytomas were found, while two were found in the pristane-indomethacin-treated mice. The degree of infiltration of oil granulomatous tissue with inflammatory cells was much less in many of the mice in the pristane-indomethacin group, giving the oil granulomatous tissue a relatively acellular appearance. Furthermore, few

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FIGURE 2. Focus of atypical plasma cells that infiltrated a polyp from mouse 102 (Table IV) found at day 116. (A) Demarcated area occupied by the intensely staining atypical plasma cells.  $\times 67$ . The focus probably occupies a polyp that has become adhered to adjacent polyps. (B) Atypical nuclei. Hematoxylin and eosin.  $\times 134$ .





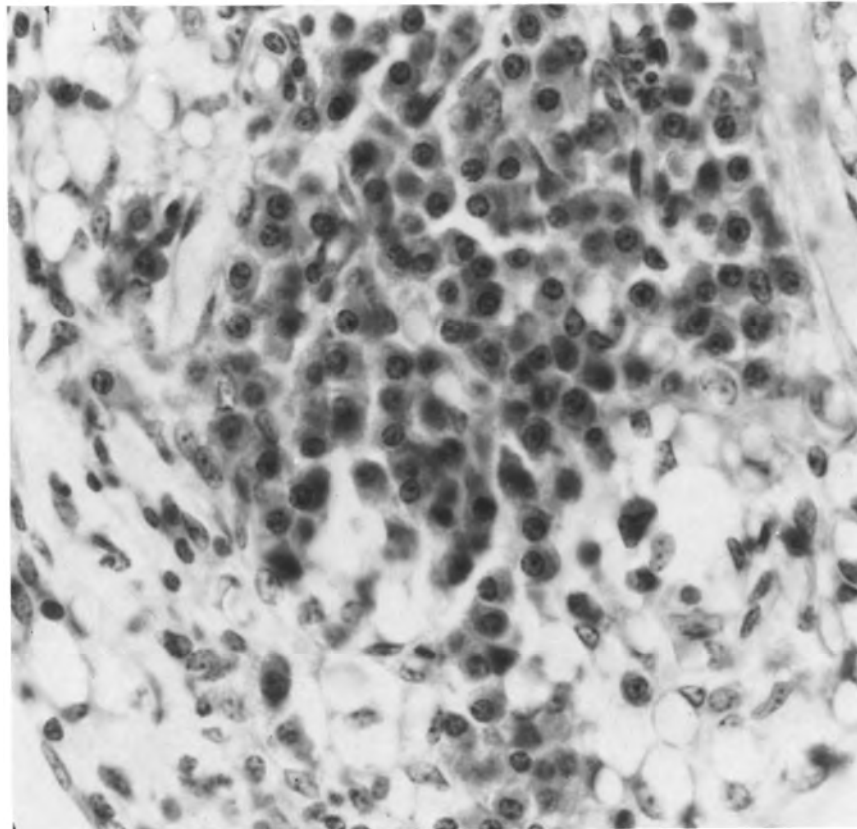


FIGURE 3. Oil granuloma showing a focus of plasmacytosis found at day 170 in pristane-only mouse 131 (Table IV). A cluster of plasma cells with uniform, relatively spherical nuclei and well-developed cytoplasm, surrounded by oil granulomas. Hematoxylin and eosin.  $\times 134$ .

plasma cells were seen. Histological studies of the early focal lesions will be described in a separate paper.

### Discussion

The chronic treatment of BALB/c mice with indomethacin during the latent period of the induction of plasmacytomas by pristane (minimum, 120 d) strikingly inhibited plasmacytoma formation. The inhibition of pristane-induced plasmacytoma development by indomethacin occurred despite the fact that the mice developed the characteristic oil granulomatous tissue, i.e., the microenvironment in which plasmacytomas develop. Indomethacin was effective when administered continuously or limited to just the first 120 d after the injection of pristane. Further, indomethacin treatment could be delayed until 60 d after the pristane injection and still exert a strong inhibitory effect. The effectiveness of the delayed treatment suggested that plasmacytoma development required the sustained maintenance of special conditions throughout the early 120 d period.

Histological studies revealed that during the first 116 d after the injection of 1 ml of pristane, the mice that were treated continuously with indomethacin developed oil granulomas and peritoneal exudates (Table III) that were often difficult to distinguish morphologically from those seen in mice given only 1 ml pristane. After 120 d, many of the indomethacin-treated mice appeared to have less deposition of oil granulomatous tissue, particularly on the diaphragm. The amount of oil granulomatous tissue in the mesentery has thus far been difficult to quantitate. After 116 d, many of the indomethacin-treated mice also had fewer inflammatory cells in the oil granuloma compared with those derived from mice treated only with pristane.

Probably the most striking and important histological difference in the oil granulomas of pristane- and pristane-indomethacin-treated mice was the number of foci of plasmacytoma cells in the oil granuloma. Between days 116 and 180, foci of developing plasmacytomas were found in 80% of the pristane mice but only 10% of indomethacin-pristane-treated mice. Plasmacytosis probably also occurred more frequently in the pristane mice but this was less easy to quantitate. Isolated plasma cells were scattered throughout oil granulomatous tissue in both groups of mice. It should be mentioned that a systematic study of the histogenesis of plasmacytomas has not yet been published. The findings in the present study are some of the first evidence that microscopic plasmacytomas precede the appearance of overt plasmacytomas, i.e., plasma cell tumors that have spread over the peritoneum and are associated with free tumor cells in the peritoneum.

The incidence of progressively growing plasmacytomas in BALB/c  $\pi$  mice given a single injection of 0.5 ml pristane has been reported to be 22% (8). Thus, the incidence of microscopic plasmacytomas observed in the pristane group between days 116 and 181 in this study far exceeds the number of clinical plasmacytomas expected at this dose or which were observed in group 4 A (Table I). Thus, not all of these foci of neoplastic plasma cell proliferation will progress to a stage where the tumor can be detected by finding free plasmacytoma cells in the peritoneal exudate.

The active oil granuloma also provided stimuli for the proliferation of cells in the myelomonocytic series. Frequent foci of extramedullary myelopoiesis were found in the pristane group and only one was found in indomethacin-treated mice. The cells in these foci were in the myelomonocytic lineage. This finding suggests that indomethacin treatment could inhibit the production of essential growth factors required by myelopoietic stem cells, and raised the possibility that growth factors for plasma cells may also be curtailed.

Biochemical and physiological actions of indomethacin have been extensively studied; its most specific and powerful action is as a competitive inhibitor of prostaglandin synthetase EC 1.14.99.1 (10). Indomethacin competes with arachidonic acid for one of the active sites on this enzyme. Prostaglandin synthetase has at least two active sites: a fatty acid cyclooxygenase site, which converts arachidonic acid to the endoperoxide/hydroperoxide intermediate PGG<sub>2</sub> in prostaglandin biosynthesis, and a peroxidase site that reduces PGG<sub>2</sub> to PGH<sub>2</sub> (11, 12). A second action of indomethacin is its ability to interact with certain oxygen radicals as a direct antioxidant or scavenger. Bodaness and Chan (13)

have shown that indomethacin can directly react with singlet molecular oxygen ( $^1\text{O}_2$ ) and scavenge this highly reactive radical. In other studies, Pekoe et al. (14) have found that indomethacin and other NSAID compounds can scavenge oxidants derived from the neutrophil myeloperoxidase (MPO)-catalyzed reaction of  $\text{H}_2\text{O}_2$  and  $\text{Cl}^-$  that generates  $\text{HClO}$ ,  $\text{OH}^-$ , and  $\text{OH}\cdot$ , presumably by some form of direct interaction with one or more of the oxidants.

Mineral oils or pristane injected into the peritoneal cavity are probably dispersed into small droplets in the peritoneal fluid and phagocytosed by neutrophils and macrophages. Leak (15) has described the rapid deposition of fibrins and breakdown of fibrin filaments after the intraperitoneal injection of Freund's adjuvants and endotoxin; this raises the possibility that oil or pristane droplets cause the outpouring of proteins that could potentially opsonize pristane droplets. Histological sections show a greater variety of configurations of macrophages and neutrophils around relatively large and variably sized oil drops. Moreover, the presence of a large reserve of oil droplets continuously stimulates the phagocytic system. There have been relatively few studies on the effects of oil injection on macrophage and neutrophil physiology, but it has been reported (16, 17) that oil-elicited peritoneal macrophages from both guinea pigs and mice are activated and can be stimulated by a variety of other nonphagocytic stimuli (e.g., phorbol esters) to produce superoxide anions.

It is likely that the phagocytosis of oil droplets and pristane by monocytes, macrophages, or neutrophils induces locally high concentrations of toxic and potentially mutagenic oxygen radicals. This would potentially occur in the peritoneal connective tissues (oil granuloma) or in the free space. Indomethacin then could reduce the generation of oxidants by: (a) blocking the formation of prostaglandin  $\text{G}_2$  ( $\text{PGG}_2$ ), (b) neutralizing oxygen radicals derived from MPO- $\text{H}_2\text{O}_2$ - $\text{Cl}^-$ , and (c) directly interacting with  $^1\text{O}_2$  and neutralize its effects. These actions would reduce the chance for exogenous oxygen radicals generated by phagocytes to attack B lymphocytes that are continuously entering the oil granuloma or that may be in transit in the peritoneal space.

It is tempting to speculate that oxygen radicals generated by continuous phagocytosis of oil might play a role in the formation of the nonrandom chromosomal translocations that have been found in >95% of plasmacytomas induced by mineral oils or pristane (see 18 and 19 for references). These translocations, rcpt12;15, rcpt6;15, and an interstitial deletion in chromosome 15, place the *c-myc* oncogene in a new context or disrupt its sequences. In both situations the *c-myc* gene is deregulated. The pathogenesis of these translocations are not understood but are thought to be facilitated by Ig gene rearrangement or by switching processes occurring on Ig gene-bearing chromosomes 12 and 6. The cause of chromosomal breaks is not known but could be enhanced nonspecifically by the availability of genotoxic substances. Oxygen radicals have been implicated in the formation of chromosomal aberrations in other systems including lymphocytes (20–23). It is not yet clear whether these oxygen radicals are generated endogenously in lymphocytes or are produced by nonlymphocytic accessory cells. Indomethacin, however, has been found to inhibit the development of chromosomal damage in at least one of these situations (21). Three ways

in which PG biosynthesis or prostaglandins may be implicated in tumor development are as immunosuppressors, as growth regulators, and as a source of intracellular oxidants.

*Immunosuppression.* Actively phagocytosing macrophages release metabolites of arachidonic acid (24–26). One of these, prostaglandin E<sub>2</sub>, (PGE<sub>2</sub>), has a number of important biological actions: (a) suppression of activation of macrophages (27); (b) inhibition of Ia antigen on macrophages (28); (c) inhibition of mitogen-stimulated proliferation of T suppressor cells (29, 30); and (d) inhibition of interleukin 2 (IL-2) production by human T lymphocytes (31). In regions of high local PGE<sub>2</sub> concentration, certain cell-mediated immune functions may be suppressed (for reviews, see 32–34). Immunosuppression may be a factor that permits developing plasmacytomas the chance to escape immunosurveillance. Some evidence to support this notion comes from Mandel and DeCosse (35), who showed that antithymocyte serum accelerates plasmacytomagenesis. Further, other types of antigenic tumors that produce large amounts of prostaglandins are thought to induce a local immunosuppression that can be relieved by indomethacin (41, 42).

Studies from other laboratories (36–39), have shown that transplantable plasmacytomas are potentially immunogenic to syngeneic hosts, and that they carry transplantation rejection antigens. We have repeatedly observed that primary plasmacytoma cells do not grow progressively when transplanted into the peritoneal cavities of normal BALB/c mice, yet these same cells grow progressively when introduced into peritoneal cavities conditioned by pristane. Further, we previously (40) provided some evidence that pristane priming has some immunosuppressive effects on established immunity. These results raised the possibility that pristane induces a microenvironment that suppresses tumor cell killing. We tested this hypothesis in the present study by transplanting primary plasmacytomas to pristane-conditioned mice and to pristane-conditioned, indomethacin-treated mice (Table II) and found that the “priming-dependent” plasmacytomas grew equally well in both pristane- and pristane-indomethacin-treated mice. We did not obtain evidence to support the notion that indomethacin relieves immunosuppression, although it could be argued that this finding was obtained with transplanted tumors and not with a developing neoplastic clone.

*Growth Regulation.* The effects of prostaglandins on B lymphocyte proliferation are less well studied, but the findings reveal a consistent picture. Using a soft agar colony-forming assay to measure murine splenic B cell proliferation, Kurland and Moore (43) showed that 10<sup>-10</sup> to 10<sup>-12</sup> M PGE<sub>1</sub> and PGE<sub>2</sub> inhibited B cell colony formation. In a separate study, indomethacin introduced into cultures of lymph node lymphocytes grown in the presence of macrophages increased the yield of B cell colonies in the presence of high macrophage/B cell ratios (44). Thus, indomethacin blocked the high exogenous production of PGE<sub>2</sub> from macrophages and permitted the B lymphocytes to proliferate and form colonies. Using human cells, Lydyard et al. (45) showed that PGE<sub>2</sub> promoted the development of pokeweed mitogen-stimulated plasma cell formation. PGE<sub>2</sub> also inhibited the proliferative response of *Staphylococcus aureus*-stimulated human B lymphocytes (46). These findings suggest that, if intraperitoneal pristane

stimulates production of locally high concentrations of prostaglandins, the effect on surrounding B lymphocytes would inhibit proliferation and promote differentiation.

Because indomethacin is a powerful inhibitor of prostaglandin synthetase, it is tempting to implicate the high local prostaglandin formation by oil-induced inflammatory cells in plasmacytoma development. As discussed above, the principal way in which high levels of prostaglandins could affect plasma cell tumor development is by an immunosuppressive action. If this were the case, the growth of primary plasmacytoma cells that depend upon the inflammatory microenvironment (and high prostaglandin levels) for growth should be inhibited by indomethacin. This was not found to be true. A second possible prostaglandin mechanism is stimulation of proliferation of B lymphocytes, but the opposite effect was observed in *in vitro* studies reported elsewhere (43–46). Thus, prostaglandins have not yet been implicated in plasmacytoma development through their ability to mediate cellular proliferation.

*Intracellular Oxidants.* Prostaglandin biosynthesis is a source of endogenous oxidants; in particular, the conversion of PGG<sub>2</sub> to PGH<sub>2</sub> by prostaglandin synthetase liberates an oxygen radical (47, 48). It has also been suggested (49) that lipid peroxides themselves may be biologically active. Tumor-promoting agents, such as the phorbol esters, activate protein kinase C and prostaglandin biosynthesis (50) and oxygen radicals (51). The promoting effects of phorbol esters in epidermal carcinogenesis in mice can be partially blocked by indomethacin (51). The promotion phase in epidermal carcinogenesis requires many weeks so there are temporal parallels between two-stage epidermal carcinogenesis and plasmacytomagenesis. Although the role of tumor promoters in tumorigenesis has not been settled, they seem to induce multiple genetic changes in the cells which are essential to achieving the neoplastic state. Since intracellular oxidant production has been associated with clastogenic effects in lymphocytes (21–23), it is possible that the nonrandom translocation in plasmacytomagenesis, rcpt12;15, could be induced by oxidants liberated from phagocytes and absorbed by B cells or be generated endogenously by a product of the chronic inflammation in the oil granuloma.

### Summary

Indomethacin given continuously in the drinking water (20 µg/ml) to BALB/cAn  $\pi$  mice during the latent period of pristane-induced plasmacytoma development dramatically reduced the plasmacytoma incidence from 34.9 to 2.2%. Additionally, indomethacin given from day 0 to 120 or begun as late as 60 d after a single injection of 1.0 ml pristane was also highly effective in reducing the development of plasmacytomas.

Indomethacin treatment did not prevent the formation of a peritoneal inflammatory exudate or peritoneal oil granulomatous tissue, although it had a mild inhibitory effect on the intensity of the cellular inflammation, particularly after extensive treatment of >100 d. Indomethacin treatment reduced the incidence of arthritis by 50%. A major effect of indomethacin treatment was a reduction

in the appearance of microscopic plasmacytomas that appear in the oil granuloma before plasmacytomas can be detected by routine sampling of the peritoneal exudate. Between days 116 and 181, 16 of 20 mice given 0.5 ml pristane were found to have foci of plasmacytoma cells, while only 2 of 20 indomethacin-treated mice had foci-containing plasmacytoma cells. The number of mice with microscopic foci in the pristane-treated group greatly exceeded the expected incidence of plasmacytomas (22%) at this dose of pristane. The growth of primary plasmacytomas in transplant that is dependent on the pristane-conditioned peritoneal environment was not inhibited by indomethacin treatment. The role of indomethacin in inhibiting plasmacytoma development was not established; two possibilities are that (a) it inhibits production of mutagenic and tissue destructive oxidants by inflammatory cells, and (b) it inhibits prostaglandin synthesis and intracellular production of oxidant biproducts.

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