Growth and Regression of Cutaneous Melanomas in Sinclair Miniature Swine

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Previous studies have demonstrated pathologic similarities between human melanoma and the spontaneous melanoma in the Sinclair miniature swine (SMS) with respect to cutaneous histologic features and patterns of metastasis. The current biopsy series, correlating growth curves and histopathologic features of cutaneous melanomas, was undertaken for documentation of the histologic events associated with the successful regression of melanoma in the SMS. Cutaneous growth and regression was characterized by a series of cellular events that eventually led to depigmentation and scar formation. Mononuclear inflammatory infiltrates, seen in over half of the 104 biopsies, showed several temporal and topographic distribution patterns, similar to that described in human melanoma. Histopathologic observations in the SMS confirm clinical observations that the host can, with consistent effectiveness, react with the tumors to modify their biologic aggressiveness. Although regression is associated with lymphocytic and macrophage infiltration, the exact role of the immune response in the regression of the cutaneous melanoma remains to be elucidated. (Am J Pathol 1982, 109:259–269)

SINCLAIR MINIATURE swine (SMS) provide a useful model of malignant melanoma, because they have a high incidence of spontaneously occurring cutaneous malignant melanomas that show nearly 100% incidence of spontaneous cutaneous regression.[1]

Although human malignant melanomas frequently undergo partial cutaneous regression, the host is generally not successful in diminishing the tumor burden. Therefore, a thorough understanding of the biologic processes responsible for the *successful* regression of cutaneous malignant melanoma in SMS should provide important insights into host tumor mechanisms that can be manipulated in favor of the host in human melanoma.

We undertook this study to correlate cutaneous histopathologic features with tumor volume measurements over time and to describe the morphologic sequence of tumor growth and regression.

Materials and Methods

Fifty-five black tumors from 23 SMS (15 males and 8 females) were selected for sequential punch biopsies

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and tumor volume correlations. "Tumor" is used here in the classical gross sense to mean "swelling."

These swine were derived from melanoma female × melanoma male matings. The swine were examined for the presence of melanocytic lesions within 72 hours after birth and, in general, at 2-week intervals thereafter. Cutaneous tumor volume was calculated as the volume of a cylinder.

One hundred four biopsies were carried out. The tissue was processed for light-microscopic examination as previously described.[2] The slides were coded and interpreted by one of us (R.W.O.) without knowledge of the time course or tumor volume measurements. For the purpose of relating tumor volume measurements over time to the changes in numbers of neoplastic cells, we have arbitrarily assigned the sum of the infiltrating tumor cells plus pigment-laden macrophages (PLMs) as 100%. We are, of course, well aware that other cellular and noncellular elements contribute to tumor volume. The ratio of infiltrating neoplastic cells to PLM was estimated by the scanning of multiple microscopic fields. Lymphocytic and plasma cell infiltrates were graded in a manner analogous to that of McGovern et al,[3] and the number of mitotic figures per 10 hpf was counted.

Results

Nineteen tumors were present at birth (congenital) as flat black spots. All swine developed raised black tumors, which, according to biopsy results, were invasive melanomas (Clark's Level IV or V). Six of the tumors were present at birth (congenital) as raised black nodules with an average tumor volume of 1841 cu mm (range 300–5426 cu mm). All of these proved to be Level V melanomas. Thirty tumors arose spontaneously an averaged of 29 days after birth. Twenty-three were Level IV–V, while single early biopsies on 7 other tumors showed Clark's Level I–III lesions.

Tumors developing from congenital raised and flat lesions reached their maximum tumor volume (MTV) an average of 28 and 56 days, respectively, after birth. In contrast, tumors developing from normal skin reached their MTV an average of 100 days after birth (Table 1, Figure 1).

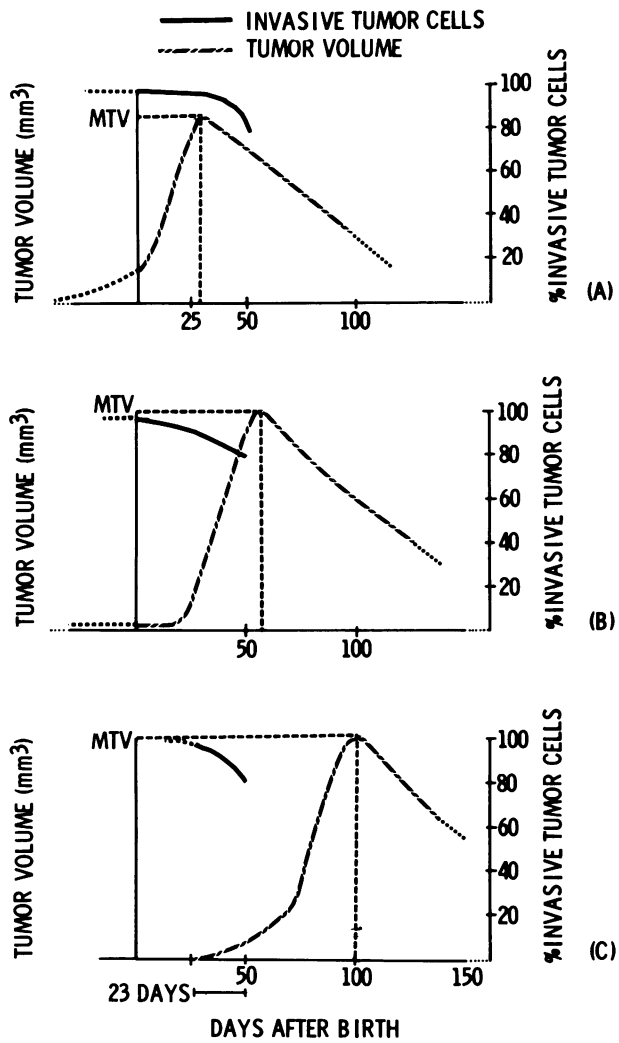


Figure 1—Graphic representations of characteristic pathobiologic features of tumors developing from congenital raised lesions (**A**), congenital flat lesions (**B**), and normal skin (**C**).

Table 1—Timetable to Reach MTV* and TC† Regression as a Function of the Origin of the Cutaneous Melanoma

Tumors developing from	Average time (days) after birth: To reach MTV	For ratio TC/PLM‡ to decrease
Congenital raised lesions	28 days	49 days
Congenital flat lesions	56 days	53 days
Normal skin	100 days	48 days§

* MTV = maximum tumor volume.
† TC = tumor cell.
‡ PLM = pigment-laden macrophage.
§ Note: 23 days from the first appearance of the tumor.

Decreasing numbers of neoplastic cells relative to PLMs (tumor regression) occurred approximately at the same time *from birth* regardless of whether tumors developed from normal skin (mean, 48 days from birth) or from preexisting congenital lesions (mean, 50 days from birth for congenital flat and raised). However, when numbers of tumor cells were evaluated from the time that each tumor first appeared, tumor regression was found to occur sooner in noncongenital lesions (mean, 23 days) than in congenital lesions (mean, 50 days) (Table 1 and Figure 1). Relative decreases in neoplastic cells with concomitant in-

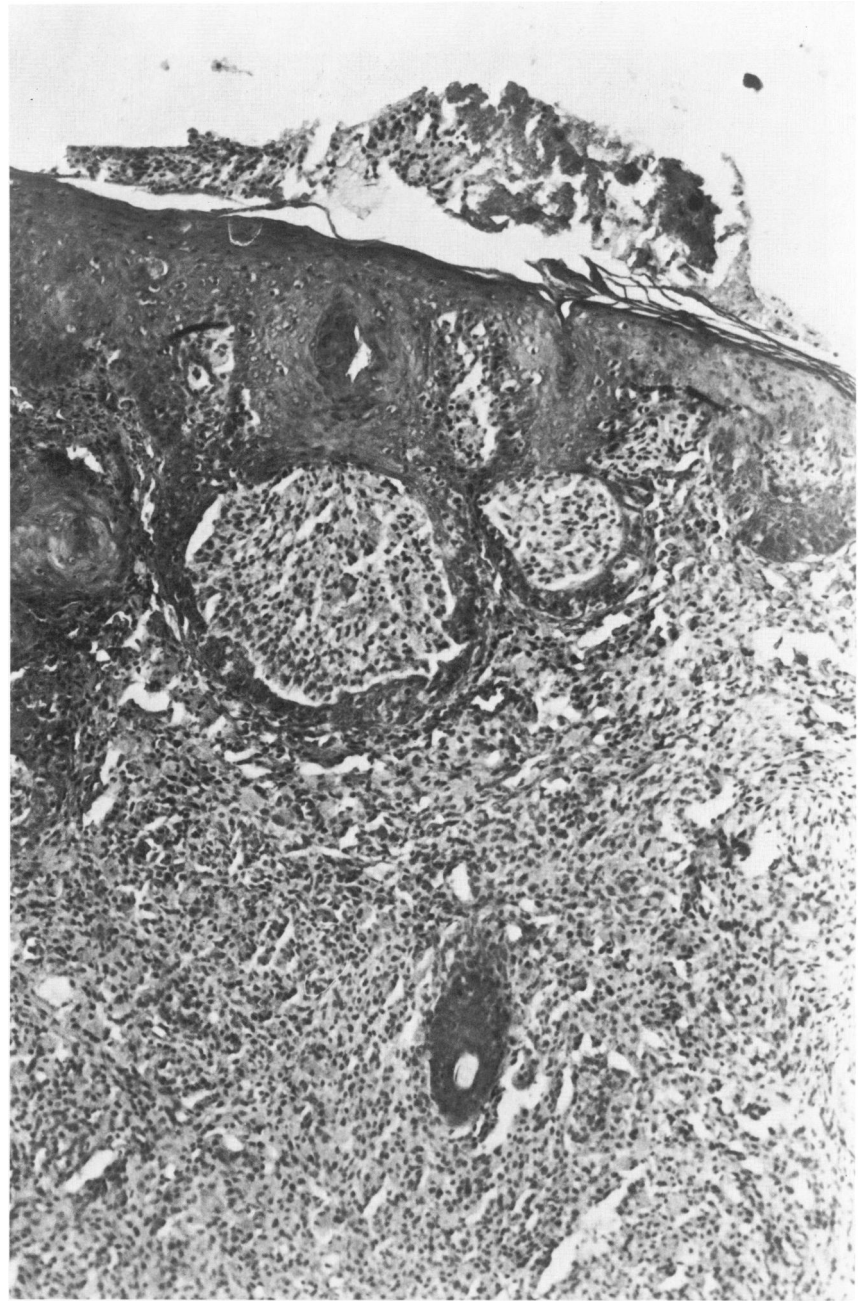


Figure 2— Melanin-bleached H&E-stained sections, demonstrating both epidermal and dermal invasion by this heavily pigmented melanoma. (×25)

creases in PLMs occurred before the maximum tumor volume was reached in 14 of 17 (82%) noncongenital tumors and 8 of 16 (50%) tumors arising from congenital flat black spots, but in only 1 of 6 (17%) tumors developing from congenital raised lesions (Figure 1).

Histologic Evolution of Cutaneous Melanoma

The histology of the neoplastic component, previously described in detail,[2] is that of a superficial spreading malignant melanoma having both intraepidermal and deeply invasive growth of heavily melanized melanocytes (Figure 2). In general, the invasive tumor cells grow in cohesive nests and have elongated nuclei with occasional prominent nucleoli (Figure 3). The cytoplasm is obscured generally by abundant fine melanin pigment, in contrast to the more coarsely granular melanin-filled cytoplasm of the PLMs. Pigmented dendritic cytoplasmic extensions are prominent (Figures 3 and 5D). Intralesional transformation with respect to cell shape and melanin content was seen frequently, analogous to that seen in human melanomas. Twelve tumors from 8 swine contained con-

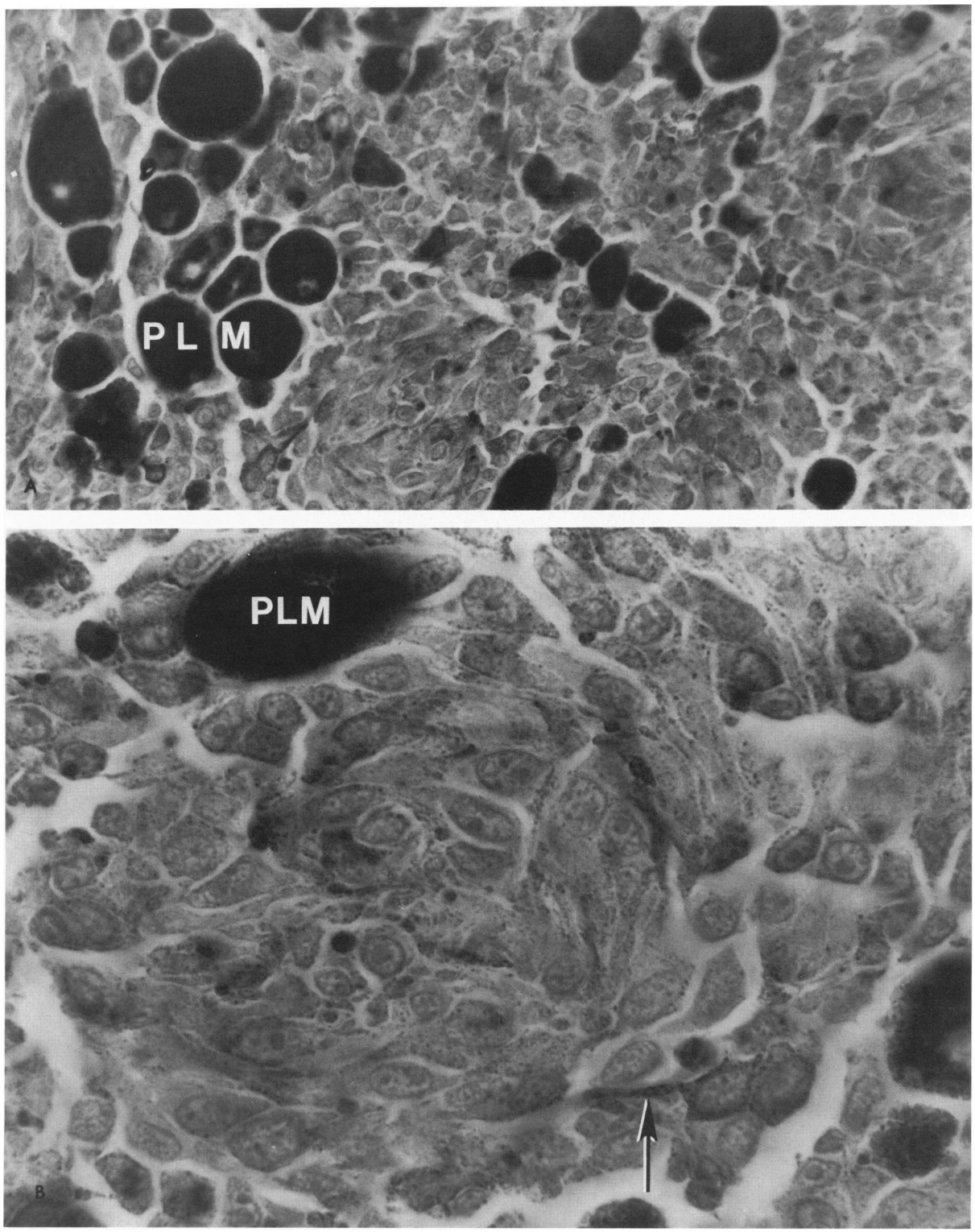


Figure 3 A and B — H&E-stained sections of a deeply invasive melanoma. The tumor cells are growing in cohesive nests and contain relatively scarce amounts of finely distributed melanin pigment. The tumor cells also show large singularly prominent nucleoli. Dendritic cytoplasmic extensions are highlighted by fine melanin pigment (*arrow*). The PLMs are larger than the tumor cells, with abundant cytoplasm filled by coarsely granular melanin pigment. (**A**, ×100; **B**, ×250)

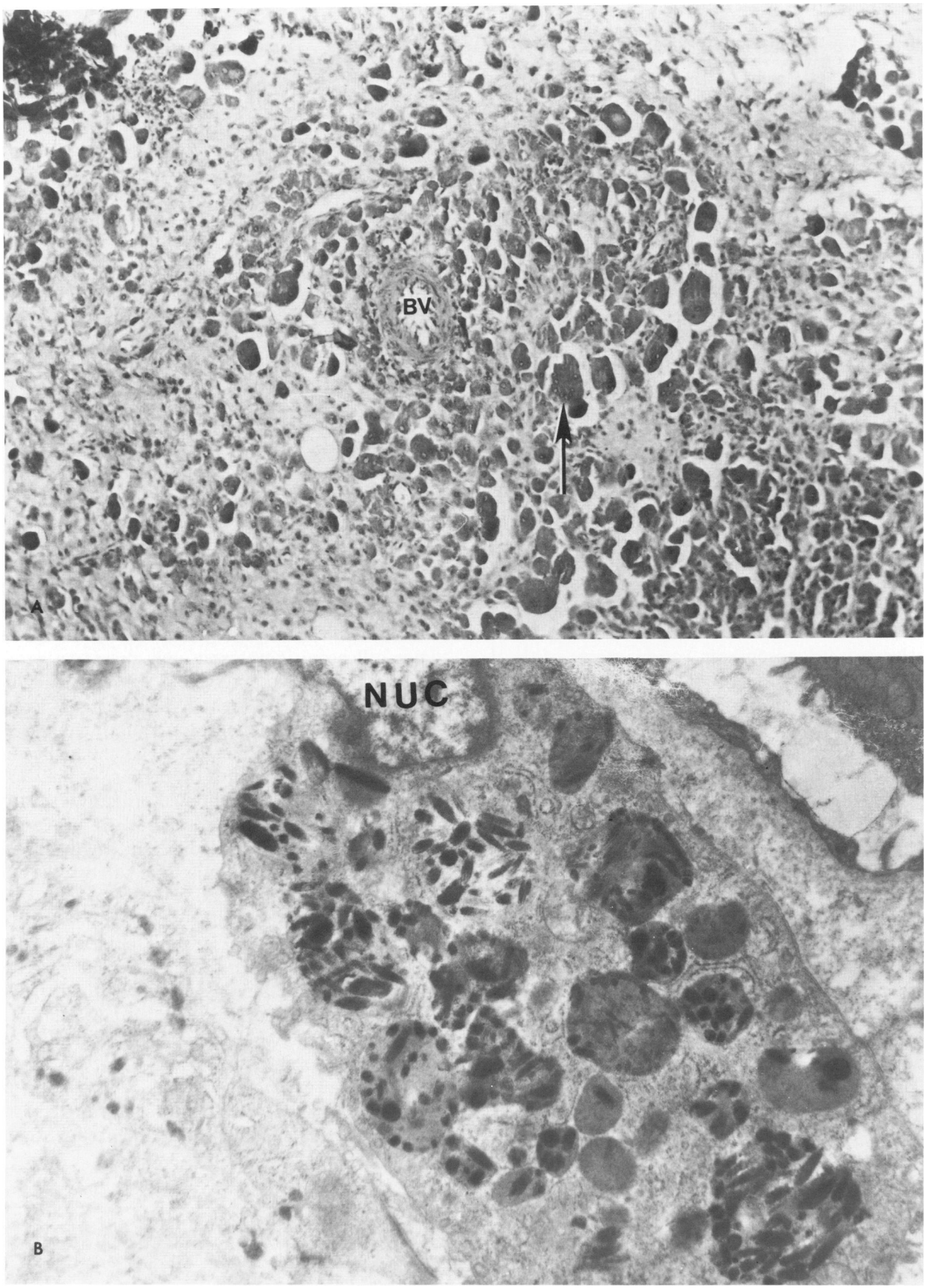


Figure 4 A and B—A melanin-bleached H&E-stained section of an aggregate of PLMs from a regressed tumor. Many of the macrophages have multiple nuclei (*arrows*). The abundant cytoplasm is filled with coarsely granular melanin. The electron micrograph below reveals lysosomal packets of melanin granules in various stages of formation. *Nuc* = nucleus; *BV* = blood vessel. (**A**, ×25)

Table 2—Mononuclear Inflammation (MI)

	Total number	Number with MI	% with MI
Swine	23	19	82.6
Tumors	55	31	56.4
Biopsies	104	42	40.4

spicuous areas of epithelioid tumor cells. Amelanotic nests of tumor cells were seen in five tumors, three of which also showed a change in tumor cell shape, from spindle to epithelioid.

In general, mitotic figuires were extremely hard to find. Between 1 and 4 mitotic figures per 10 hpf were seen, however, in 8 tumors from 6 swine. No difference in tumor growth or regression was seen in those tumors exhibiting mitotic counts between 1 and 4 per 10 hpf.

The PLM is a larger cell than the neoplastic melanocyte, with a much smaller nuclear/cytoplasmic ratio due to its abundant cytoplasm. Coarsely granular melanin fills the cytoplasm, frequently pushing the nucleus to an eccentric position (Figures 3 and 4). Electron-microscopic examination reveals that the coarsely granular nature of the melanin pigment is due to the presence of membrane-bound lysosomal packets of melanosomes in various stages of development (Figure 4). Occasional multinucleate forms are present. Melanin bleach stains reveal that some of the nuclei have little heterochromatin with prominent nucleoli, while most the nuclei are darkly staining without nucleoli.

Thirty-one of the 55 tumors studied had mononuclear inflammatory infiltrates (Table 2). Ten tumors demonstrated infiltrates on more than one biopsy. The infiltrates showed several temporal and topographic distribution patterns.

Inflammatory infiltrates were present before the MTV was reached in 38% of the tumors showing mononuclear inflammation; most of these tumors were noncongenital (11 of 16 tumors, or 69%), (Table 3). Inflammatory infiltrates were seen in 50% of the biopsies after the maximum tumor volume was reached (Table 3). Early in the evolution of the tumor (Clark's Levels I and II), moderate numbers of lymphocytes were found around papillary blood vessels, often in close proximity to infiltrating tumor cells

Table 3—Temporal Distribution of Mononuclear Inflammation (MI) in 42 Biopsies

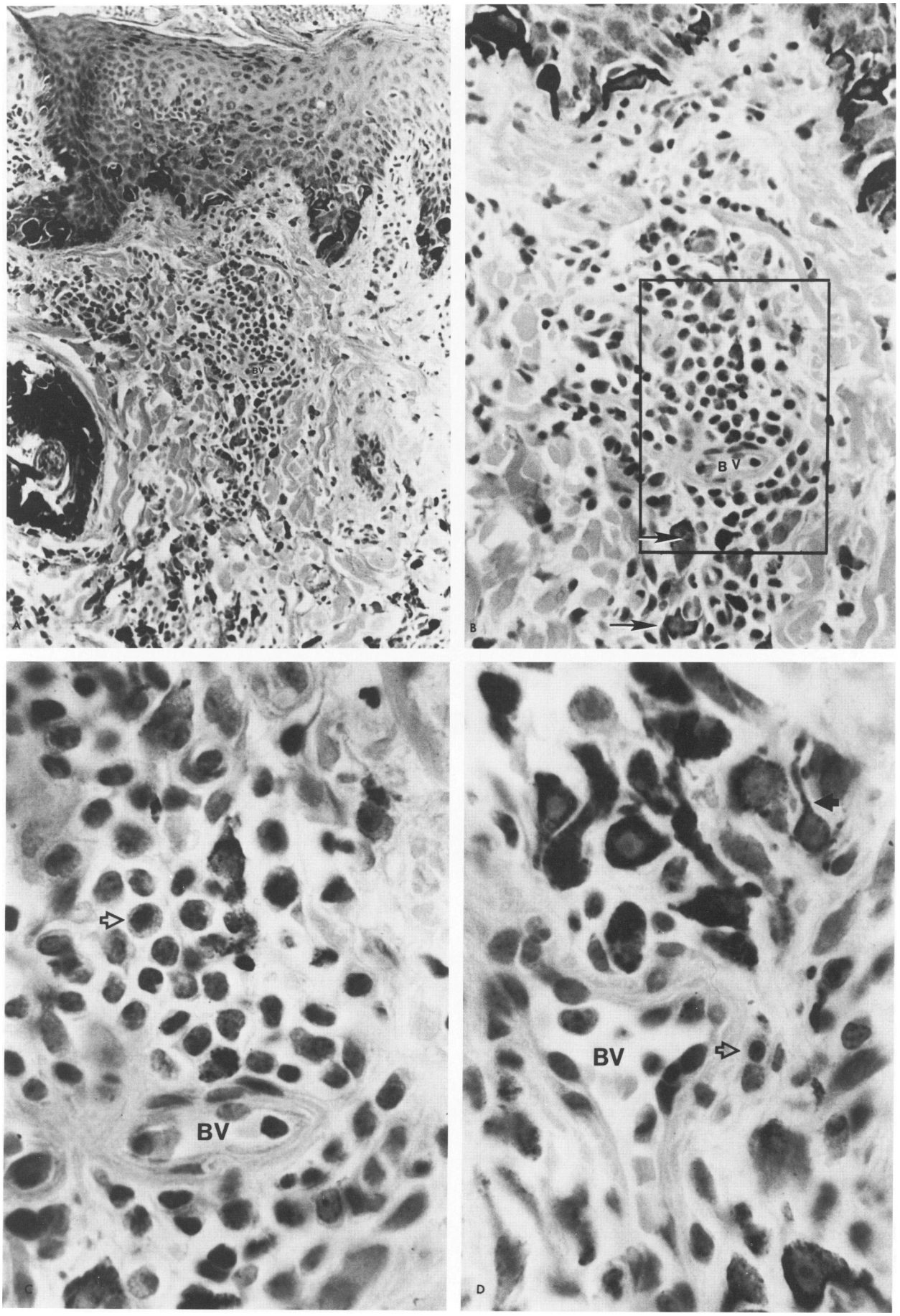
	Number	% (n = 42)	Average time (days) before/after MTV* that MI seen
Before MTV	16	38	60
Tumors developing from congenital flat lesions	5		37
Tumors developing from congenital raised lesions	0		NA†
Tumors developing from normal skin	11		70
At MTV	5	12	0
Congenital flat	1		
Congenital raised	0		
Noncongenital	4		
After MTV	21	50	61
Congenital flat	10		38
Congenital raised	3		95
Noncongenital	8		77
Total		100	

* MTV = maximum tumor volume.
† NA = not applicable.

(Figure 5). Small numbers of mast cells were frequently apparent. Occasionally this lymphocytic infiltrate extended into the epidermis (5A), but actual tumor cell necrosis was seen only once. Apoptotic bodies have not been observed. A dense bandlike infiltrate has not been seen in these early invasive (Clark's Levels I–III) lesions. Later, the mononuclear host response became unapparent as the malignant cells progressively infiltrated the dermis. At this time, the tumor volume was composed primarily of varying numbers of malignant cells and PLMs. Occasional focal collections of medium and small lymphocytes could be seen within nests of tumor cells and PLMs (Figure 6). Again, no evidence of individual tumor cell necrosis has been seen.

Late in this dynamic regressive process (Figures 7–9), the histologic picture progressively gave way to an absence of neoplastic melanocytes in the epidermis, with the epidermal region replaced by acanthotic nonpigmented epithelium. The papillary dermis below now contained dilated small blood vessels surrounded by an edematous fibrous connective tissue (delicate fibroplasia) containing small numbers of mononuclear inflammatory cells (Figure 8A). Some

Figure 5A—An H&E-stained section of a biopsy specimen of noncongenital melanoma taken approximately 22 days after the lesion was first noted. This sample, taken from the periphery of a tumor with a volume of 3165 cu mm, demonstrates neoplastic melanocytes with long dendritic processes within the basilar epidermis associated with a prominent papillary mononuclear inflammatory infiltrate. Lymphocytic exocytosis is also seen. (*BV* = blood vessel) (×25) **B**—Higher magnification showing the papillary perivascular mononuclear inflammatory infiltrate among scattered tumor cells (*arrows*). (*BV* = blood vessel) (H&E, ×50) **C**—Higher magnification of the area within the rectangle in **C**, demonstrating the variably sized lymphocytes as well as a mast cell (*open arrow*). (*BV* = blood vessel) (H&E, ×100) **D**—From an adjacent field, a group of invasive tumor cells demonstrates melanin-highlighted dendritic processes (*arrow*). Nucleoli can be seen in the tumor nuclei. The mononuclear inflammatory infiltrate consists primarily of lymphocytes. A mast cell is also seen (*open arrow*). (*BV* = blood vessel) (H&E, ×100) (With a photographic reduction of 28%)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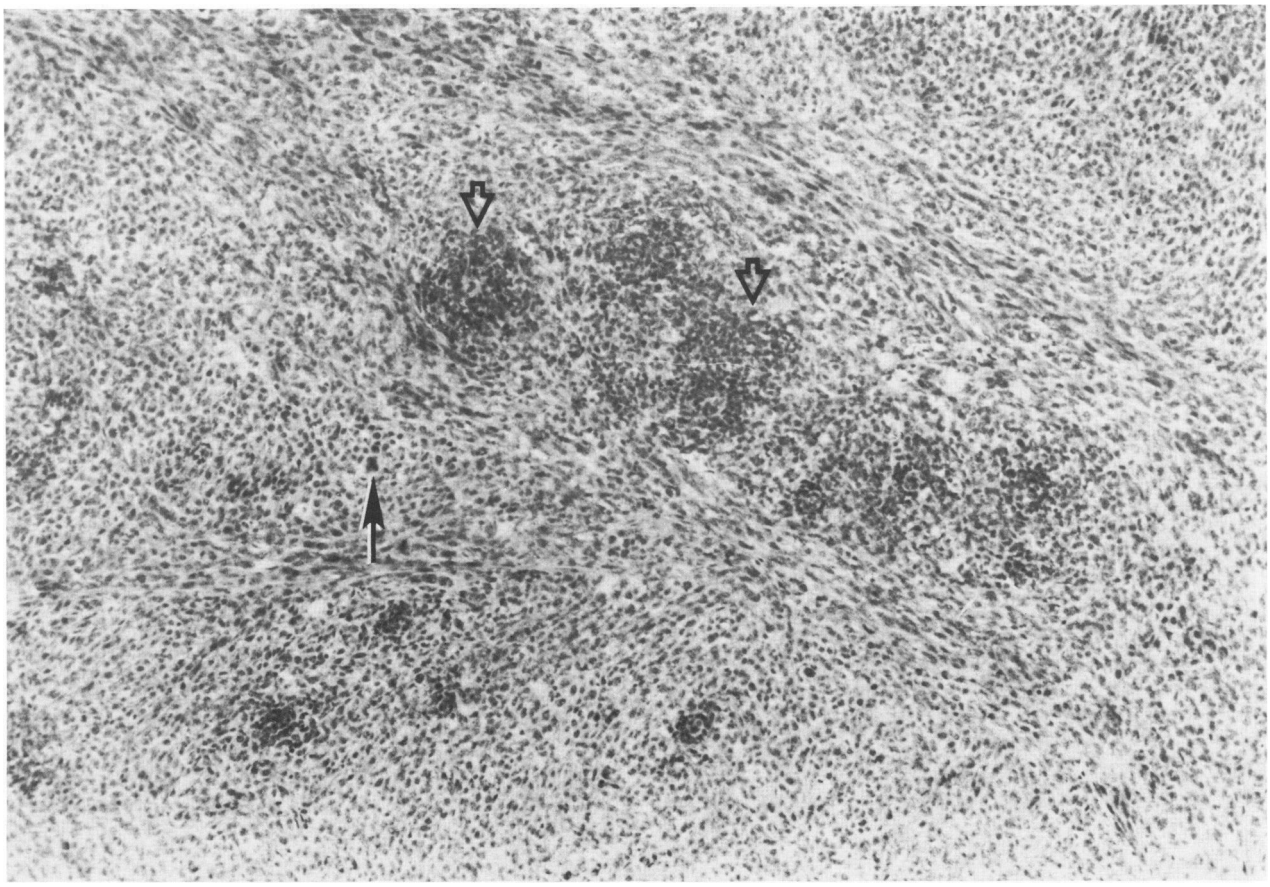

Figure 6 – Bleached H&E-stained section demonstrates interlacing fasicles of deeply invasive spindle-shaped tumor cells with focal nests of medium and small lymphocytes (*open arrow*). A large mitotic figure is shown (*closed arrow*). (×25)

cases have shown a lymphocytic exocytosis into the overlying epithelium (Figure 8B). Adjacent epithelium may still contain neoplastic melanocytes. Frequently, below the remaining epidermal neoplastic cells, there is a mild papillary dermal lymphocytic infiltrate. The grossly apparent pigmented nodules (Figure 7) were now composed primarily of sheets of PLMs frequently centered around a dilated blood vessel (Figure 9). These large sheets of PLMs were separated by densely sclerotic acellular connective tissue. Occasional deep dermal nests of neoplastic cells were still found. In general, a minimal lymphocytic infiltrate associated with small numbers of plasma cells could be seen among these remaining tumor cells.

Discussion

The evolutionary changes leading to depigmentation and complete tumor regression no doubt take place in a continuum. We have segregated the changes artificially in relation to the growth curve and the frequency with which biopsies were done on the lesions. This study has revealed the following.

1) Tumors that develop from normal skin are more likely (82%) to show a decreasing ratio of tumor cells to PLMs before maximum tumor volume is reached than tumors developing from congenital flat black spots (50%) or tumors developing from congenital raised lesions (17%). In fact, the increasing slope approaching maximum tumor volume appears to be the period of time when tumor cells are decreasing and PLMs are increasing, particularly for tumors developing from normal skin and congenital flat lesions (Figure 1).

2) Mononuclear inflammatory infiltrates were seen in over half of the biopsies, further suggesting they have a role in controlling the biologic processes of this tumor. Furthermore, tumors developing from normal skin appear more likely to show early postnatal inflammatory infiltrates (before MTV is reached) than tumors arising from congenital flat or raised le-

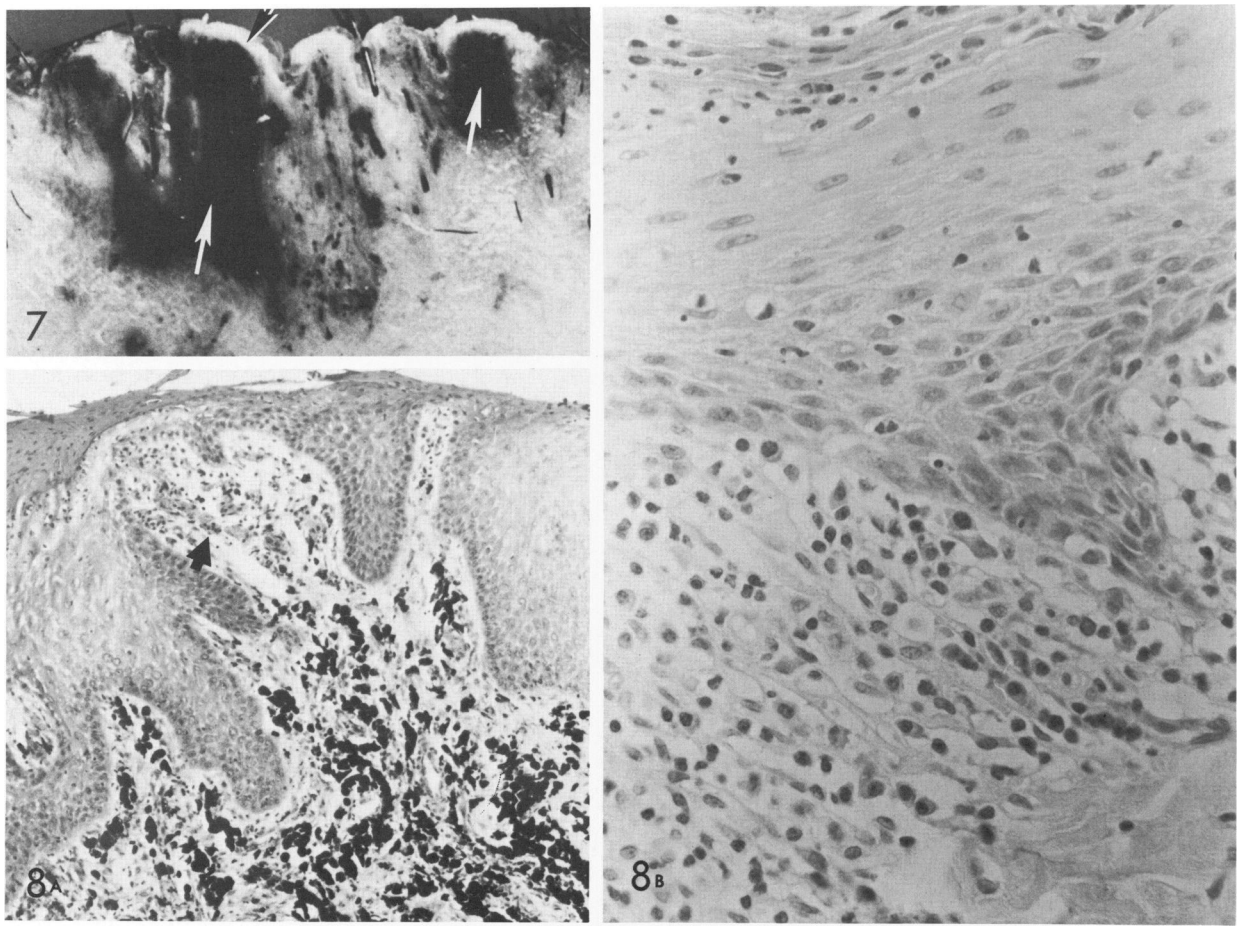

Figure 7—A gross cross-section of a tumor undergoing late regression. The white, thickened epidermis (*arrow*) shows acanthotic squamous cells with an absence of melanocytes (see 8A). The black dermal nodules (*white arrows*) consist predominantly of PLM (see figure 9) with only rare foci of remaining tumor cells. **Figure 8A**—This section demonstrates late regression and is characterized by acanthotic epithelium lacking melanocytes. There is telangectasia of the papillary blood vessels (*arrow*), below which are PLMs. (H&E, ×25) **B**—Lymphocytic exocytosis is seen within the acanthotic epithelium of this late-regressing melanoma. (H&E, ×100) (With a photographic reduction of 26%)

sions. It is possible that a comparative early wave of mononuclear inflammation occurs in the prenatal period.

We have obtained evidence both *in vivo* and *in vitro* that a host immune response plays a role in the spontaneous regression of Sinclair swine melanoma.[1,2,4-7] We have demonstrated that leukocytes from SMS specifically recognize and react *in vitro* with autologous and allogeneic swine melanoma extracts[6] and cultured allogeneic swine melanoma cells.[6] In addition, the leukocyte reactivity against allogeneic swine melanoma cells was found to correlate with spontaneous regression of swine melanoma.[7] In a study currently under way, swine demonstrating increased leukocyte reactivity prior to a decrease in tumor volume histologically show that increases in tumor volume were a result of host inflammatory cells, particularly PLMs, infiltrating the tumors. Thus, rather than representing neoplastic cell growth, increases in tumor volume may actually be a sign of *in vivo* tumor regression. No doubt, previous gross classification schemes, where the swine were classified as progressors or regressors according to changes in measured tumor volume, were misleading and resulted from the fallacious assumption that neoplastic cells were necessarily responsible for the changes in the size of the tumor (swelling).

Histologic observations in the SMS confirm clinical observations that the host can, with consistent effectiveness, react with the tumors to modify its biologic aggressiveness. Histologic observations in the Sinclair swine, as well as in human melanoma,[8]

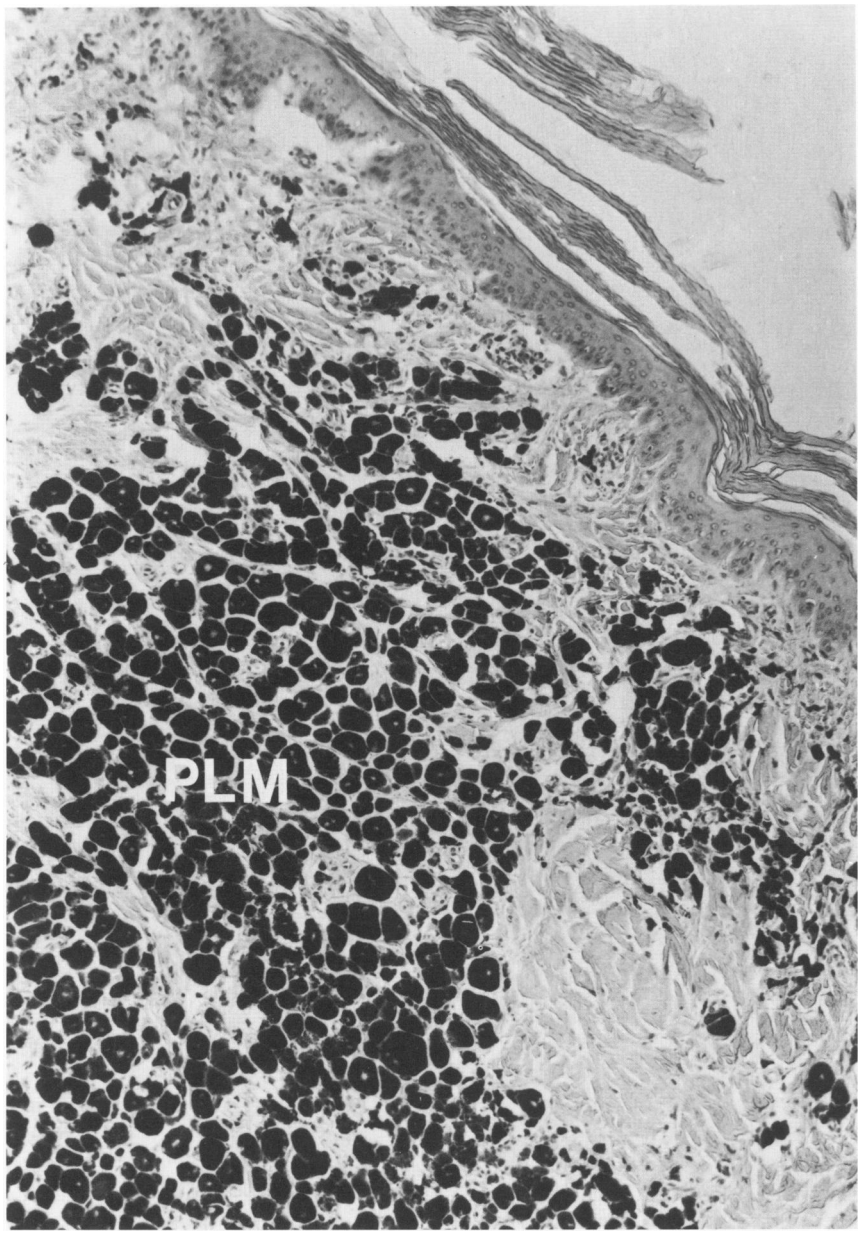

Figure 9—The dermal black nodules, as seen grossly in the late-regressed lesion pictured in Figure 7, consist almost entirely of PLMs, as seen here. (H&E, ×25)

partly relate the regressive phenomena to lymphocytic and macrophage infiltration.

We must be aware that the control of neoplastic growth may involve not only destruction of neoplastic cells (by the immune system) but also modulation of the neoplastic process by growth regulators that may induce maturation or transformation to a nonaggressive cell type. Such regulators have been described in *in vitro* systems, including melanoma tissue.[9,10] Such types of regulatory mechanisms are intriguing because of the possibility that we have erroneously called the large cell with coarsely granular packages of melanin a macrophage, when, in fact, it may be a tumor cell whose aggressive qualities have been redirected.

The exact role of the immune response in tumor regression in the SMS remains to be elucidated. Studies are currently under way to correlate *in vitro* immune parameters with growth curves and histopathologic features. This model continues to provide a clinically relevant system for the study of the pathogenesis of melanoma growth and regression.

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Acknowledgments

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