Prognostic Value of Tumor Infiltrating Lymphocytes in the Vertical Growth Phase of Primary Cutaneous Melanoma

Claudio G. Clemente, M.D.[1]
Martin C. Mihm, Jr., M.D., F.A.C.P.[2]
Rosaria Bufalino[3]
Stefano Zurrida, M.D.[4]
Paola Collini, M.D.[1]
Natale Cascinelli, M.D.[4]

[1] Division of Anatomic Pathology and Cytopathology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy.

[2] Division of Dermatopathology, Albany Medical College, Albany, New York.

[3] WHO Melanoma Programme, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy.

[4] Division of General Surgery B, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Italy.

This study was partially supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) grant Clemente-1994/420.198.002.

Address for reprints: Claudio Clemente, M.D., Division of Anatomic Pathology and Cytopathology, Istituto Nazionale per lo Studio e la Cura dei Tumori, Milano, Via Venezian 1, 20133 Milano, Italy.

Stefano Zurrida present address is European Institute of Oncology, Milano, Italy.

Received May 9, 1995; revision received November 27, 1995; accepted November 27, 1995.

BACKGROUND. Primary cutaneous melanoma is often infiltrated by lymphocytes that provide the opportunity to study what may be the local immunologic reaction to the tumor and to correlate the presence of these lymphocytes with overall survival. In an attempt to delineate the histologic diagnostic criteria, to classify different categories of lymphocytic infiltrates, previously described by Elder et al. as brisk, nonbrisk, and absent, and to verify their prognostic significance, we reviewed 285 consecutive cases of primary cutaneous melanomas (American Joint Committee on Cancer Stage I and II).

METHODS. In addition to clinical variables (age, sex, and location of tumor) and the presence of tumor infiltrating lymphocytes in the vertical growth phase, the histopathologic attributes reviewed included mitotic rate, thickness, and regression. The results were derived from independent histopathologic review by two pathologists (C.G.C., M.C.M., Jr.) on separate occasions. A multivariate analysis of survival was performed with the Cox's regression model.

RESULTS. The 5- and 10-year survival rates for melanoma with a vertical growth phase and a brisk infiltrate were 77% and 55%, respectively. For tumors with a nonbrisk infiltrate, the 5- and 10-year survival rates were 53% and 45%, respectively, and for tumors with absent tumor infiltrating lymphocytes, the 5- and 10-year survival rates were 37% and 27%, respectively. Mitotic index, thickness, and tumor infiltrating lymphocytes were statistically (univariate analysis) significant prognostic factors ($P = 0.003$, 0.000001, 0.0003, respectively), whereas the presence or absence of regression is not. In the univariate statistical analysis, the sex of patients and site of melanoma also were statistically significant ($P = 0.00001$ and 0.002 respectively), whereas age ($P = 0.98$) was not statistically significant. The multivariate analysis of thickness, mitotic rate, and tumor infiltrating lymphocytes showed that thickness and presence of tumor infiltrating lymphocytes were significant and independent histologic prognostic factors. With regard to the clinical factors, sex retained its independent prognostic significance. The histologic characteristics of melanoma with vertical growth phase (brisk, nonbrisk, and absent) are exemplified.

CONCLUSIONS. We demonstrated that when categories of tumor infiltrating lymphocytes are strictly defined, they indeed have very strong predictive value for primary cutaneous melanomas with a vertical growth phase. This work confirms the work of Clark et al. and fully illustrates the brisk, nonbrisk, and absent categories of infiltration. Finally, a multivariate analysis comparing thickness, mitotic rate and presence of tumor infiltrating lymphocytes showed that only thickness and presence of tumor infiltrating lymphocytes are significant and independent positive histologic prognostic factors. ***Cancer* 1996; 77:1303–10.**
© 1996 American Cancer Society.

KEYWORDS: tumor infiltrating lymphocytes, TIL, melanoma, vertical growth phase, prognosis, survival.

TABLE 1
Distribution and Survival of 285 Patients with Primary (AJCC Stage) Cutaneous Melanoma I and II

Factors	No. of patients	%	5-year survival (%)	10-year survival (%)	*P* value
Age of patient					
≤ 19 yr	4	1.4	50	—	
20–44 yr	103	36.1	51	41	
45–64 yr	125	43.9	52	40	0.98
≥65 yr	53	18.6	46	37	
Sex					
Males	94	33	32	23	
Females	191	67	60	49	0.00001
Site of primary					
Head and neck	45	15.8	49	39	
Extremities	171	60	58	46	0.002
Trunk	69	24.2	36	26	
Mitotic rate					
0/mm^2	101	35.4	61	47	
≤ 6/mm^2	117	41.1	52	41	0.003
> 6/mm^2	67	23.5	31	27	
Tumor infiltrating lymphocytes					
Brisk	47	16.5	77	55	
Nonbrisk	133	46.7	53	45	0.0003
Absent	105	36.8	37	27	
Thickness					
≤ 2 mm	52	18.2	82	65	
2.1–3 mm	57	20	58	36	
3.1–4 mm	51	17.9	50	45	0.000001
> 4 mm	125	43.9	33	29	
Regression					
Present	37	13	57	42	ns
Absent	248	87	49	40	

ns: not significant; AJCC: American Joint Committee on Cancer.

Primary [American Joint Comittee on Cancer (AJCC) Stages I and II] cutaneous melanoma is nearly always associated with a chronic inflammatory infiltrate, composed predominantly of lymphocytes. This morphologic observation provides an opportunity not only to study what may be the local immunologic reaction to the tumor but also to seek any correlation between its presence and survival. The evidence of necrotic tumor cells, associated with a lymphocyte infiltrate, suggests that killing of melanoma cells by lymphocytes occurs. Therefore, one might expect to see a better prognosis for those tumors with a more prominent lymphocytic infiltrate. Nevertheless, the published results are contradictory.[1] Possible reasons for the discrepancies among the different studies are several. Some of the studies that found no prognostic effect appear not to have examined the topography of the lymphocytic component or whether infiltrative and noninfiltrative lymphoid cells were distinguished. According to Elder et al.[2] the lymphocytes, to be of prognostic importance, must penetrate the vertical growth phase and disrupt the melanoma cells. Another possible reason is the lack of a full understanding of the definitions of tumor infiltrating lymphocyte categories (brisk, nonbrisk, and absent) as proposed by Clark et al.[3] The present study was an attempt to define fully and to assess the prognostic significance of tumor infiltrating lymphocytes evaluated histologically in the vertical growth phase of cutaneous melanoma.

PATIENTS AND METHODS

Between 1967 and 1975, 777 cases of primary cutaneous melanoma were collected by the WHO Melanoma Study Group. From this number we selected only patients for whom adequate histology was available who had vertical growth phase disease and a minimum of 10 years of follow-up; 285 such cases formed the basis of our study. The patient were accrued from multiple centers involved in a clinical trial that included lymph node dissection; patients with clinically negative but histologically positive lymph nodes (N0+) were excluded from this study.

The slides from all patients were reviewed to record histopathologic characteristics possibly associated with survival: mitotic rate; presence of tumor infiltrating lymphocytes, categorized as brisk, nonbrisk, and absent;

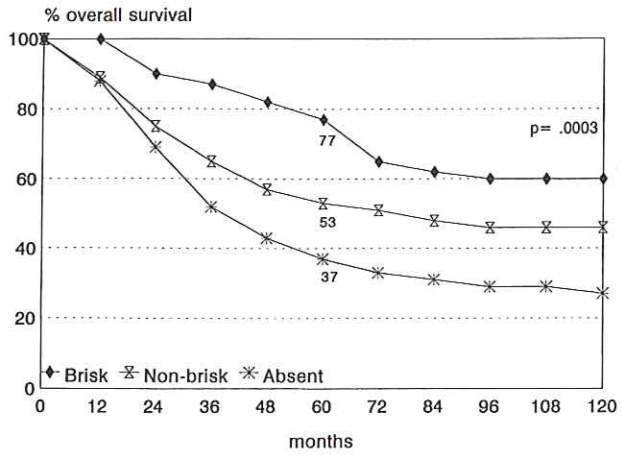

FIGURE 1. Presence of tumor infiltrating lymphocytes and overall survival in 285 Stage I and II (AJCC) cutaneous melanoma patients.

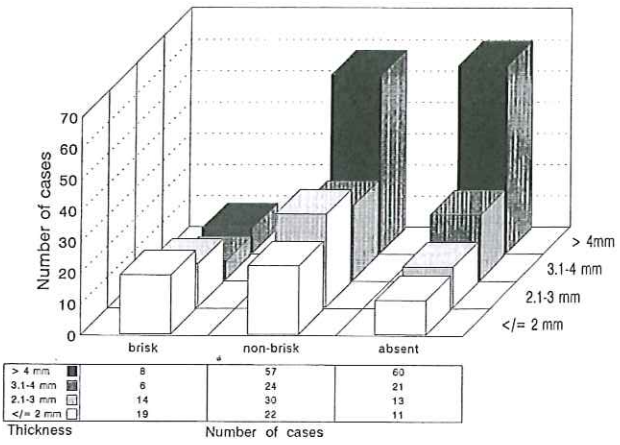

FIGURE 2. Distribution of 285 Stage I and II (AJCC) melanoma patients according to brisk, nonbrisk, and absent tumor infiltrating lymphocytes and thickness.

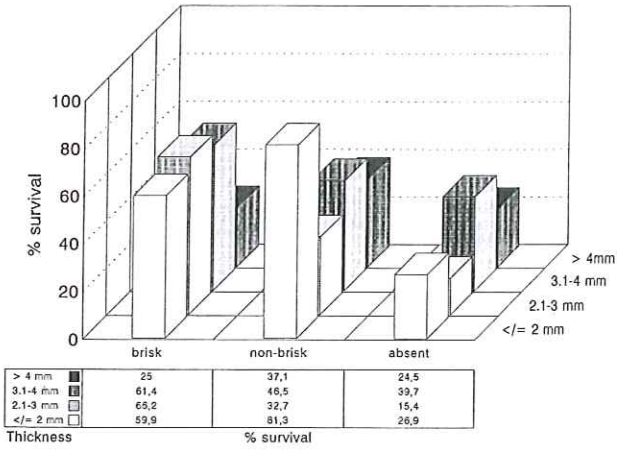

FIGURE 3. Ten-year survival of 285 Stage I and II (AJCC) cutaneous melanoma patients according to brisk, nonbrisk, and absent tumor infiltrating lymphocytes and thickness.

thickness in millimeters; and regression. The results derive from independent histopathologic review by two different pathologists (C.G.C. and M.C.M.) on separate occasions. Diagnostic differences between the two pathologists' assessments were resolved by consensus. The histologic review was performed without knowledge of outcome.

Vertical growth phase was determined by the histologic parameters described by Clark et al.[3,4] The cutaneous melanomas in horizontal growth phase, ten in situ and four with evidence of papillary dermis invasion, were not further analyzed. The mitotic rate was evaluated according to the method reported by Schmoeckel and Braun-Falco,[5] and the patients were subdivided into three groups (0 mitoses/mm^2, between 0.1 and 6 mitoses/mm^2 and, more than 6 mitoses/mm^2 with a microscopic field diameter of 0.63 mm, ×40).

Tumor infiltrating lymphocytes were classified, according to Elder et al.[2] and Clark et al.,[3] into three categories: brisk, nonbrisk, and absent. According to the criteria reported by Elder et al. and Clark et al. and used in our review, tumor infiltrating lymphocytes in cutaneous melanoma are defined as follows: 1) "brisk" (see Fig. 4), if the lymphocytes were present throughout the substance of the vertical growth phase (diffuse) or were present and infiltrating across the entire base of the vertical growth phase (peripheral); 2) "nonbrisk" (see Fig. 5), if the lymphocytes were in one or more foci of the vertical growth phase, either dispersed throughout ("diffuse") or situated focally in the periphery, so-called peripheral lymphocytes (for ease of understanding and communication we have suggested the terms diffuse and peripheral to facilitate description of patterns that have the same prognostic significance.); or 3) "absent" (see Fig. 6), if there were no lymphocytes or if the lymphocyte were actually present but did not infiltrate the melanoma (totally absent). In cutaneous melanomas with evidence of multiple foci of vertical growth phase, the lymphocytic infiltrate was considered to be brisk if all the neoplastic foci presented lymphocytic infiltration and to be absent (focally absent) if only one focus was free of lymphocytes (Fig. 6). In the case of evidence of regression, the same rules were applied.

Thickness was evaluated according to the method of Breslow[6] from the upper portion to the depth of the tumor at its thickest part. The strata of thickness were those reported by van der Esch et al.[7]

Histologic regression is characterized by absence of melanoma cells in a focal region of horizontal growth

TABLE 2
Multivariate Analysis of 285 Cutaneous Melanoma Patients

Factors (adjusted by the others)	Beta coefficient	SE	Zeta	R Risk	*P* value	DF
Thickness					0.0002	3
≤ 2 mm	−1.05517	0.274838	−3.83925	0.348133		
2.1–3 mm	−0.344256	0.211556	−1.62726	0.708747		
3.1–4 mm	−0.519943	0.228895	−2.27154	0.594554		
> 4 mm	—	—	—	1		
Sex					0.0003	1
Males	0.592205	0.162313	3.64853	1.80797		
Females	—	—	—	1		
Tumor infiltrating lymphocytes					0.04	2
Brisk	−0.566288	0.269315	−2.10269	0.567629		
Nonbrisk	−0.331143	0.171211	−1.93413	0.718102		
Absent	—	—	—	1		

SE: standard error; R risk: relative risk; DF: degree of freedom.

phase adjacent to vertical growth phase, widening of papillary dermis with increased collagen fibers parallel to the surface, lymphocytes infiltrating diffusely, sprinkling of melanophages in papillary dermis, and effacement of rete ridge pattern, as described by Elder et al. and referred to by Kang et al.[8]

Statistical Analysis

The association among factors was tested using the chi-square method. Survival rates were calculated using the actuarial life tables approach, breaking up the entire series into main subgroups according to all possible prognostic factors. The strength of association of each factor with survival was tested resorting to the log rank test.[9–12]

The multifactorial analysis of survival was performed with the Cox regression model.[13] The relative role of each factor in affecting the prognosis was assessed by the step-down procedure for variable selection at the 5% significant level.

RESULTS

In Table 1 the distribution of patients according to the single factors (age, sex, site of primary, mitotic rate, presence of tumor infiltrating lymphocytes, thickness, and regression) and the corresponding 5- and 10-year survivals is reported. For each group of factors the *P* value are calculated. The differences in 5- and 10-year survivals only for regression and age are not statistically significant; therefore, for the subsequent statistical analysis, regression and age were discharged.

Figure 1 shows the overall survival trend for 285 patients according to brisk, nonbrisk, and absent categories. The distribution of brisk, nonbrisk, and absent cases and the thickness in 285 melanomas is shown in Figure 2, and for the same groups the 10-year survival is reported in Figure 3.

Mitotic rate, thickness, and presence of tumor infiltrating lymphocytes were compared for their prognostic strength using multivariate analyses. Thickness was highly predictive after adjusting by mitotic rate and presence of tumor infiltrating lymphocytes ($P = 0.0006$), and also presence of tumor infiltrating lymphocytes, adjusted by thickness and mitotic rate, retains its statistical value ($P = 0.072$). The *P* value was not significant for mitotic rate after adjusting by thickness and presence of tumor infiltrating lymphocytes ($P = 0.76$), and a close correlation between mitotic index and thickness ($P < 0.000001$) was also demonstrated. The site of origin of the primary tumor, adjusted by the other prognostic factors, was not an independent prognostic factor; its *P* value in the multifactorial analysis was not significant ($P = 0.19$).

The next step was to compare thickness, presence of tumor infiltrating lymphocytes, and sex (Table 2). Thickness adjusted by presence of tumor infiltrating lymphocytes shows a highly significant *P* value ($P = 0.0001$), but also presence of tumor infiltrating lymphocytes corrected by thickness retains its statistical significance ($P = 0.03$). Considering the thickness of primary tumors as a continuous variable in a multifactorial regression model (the Cox model) together with sex, site of primary, mitotic index, and presence of tumor infiltrating lymphocytes, it maintains its prognostic significance when adjusted by the others. At the same time, the variables sex and presence of tumor infiltrating lymphocyte show a significant prognostic impact on survival.

With regard to thickness of the lesions, 33% of lesions in our series were greater than 4.0 mm in diameter. We selected a group of 69 patients with maximum lesion di-

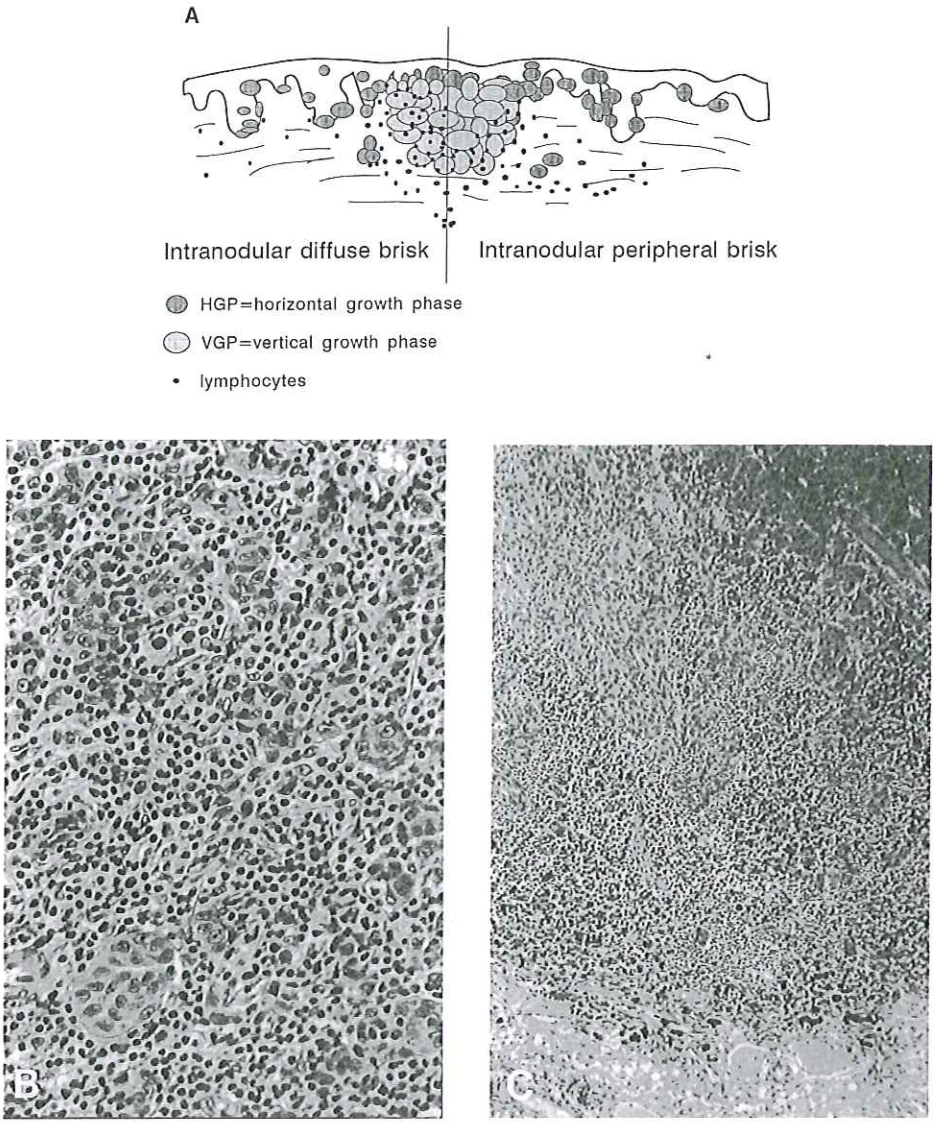

FIGURE 4. A–C: "Brisk" tumor infiltrating lymphocytes. B: The entire vertical growth phase is infiltrated by lymphocytes diffusely interposed between the melanoma cells. C: The lymphocytic infiltrate is predominantly localized through the lower one-third of the vertical growth phase (Original magnifications ×312 in B, ×100 in C).

ameter less than 6.0 mm and tested them for tumor infiltrating lymphocytes, 59% of which were less than 2.0 mm thick. The patients showed 100% survival with brisk infiltrates, 77% with nonbrisk infiltrates, and 45% with absent infiltrates. Of the clinical factors analyzed, including age, sex, and site of primary tumor, we found that prognosis was better in females.

DISCUSSION

The Clark et al.[3] model of tumor progression in cutaneous melanoma recognizes horizontal growth phase and vertical growth phase as having different biologic significance. In the horizontal growth phase, or nontumorigenic phase, the cutaneous melanoma lacks metastatic competence; in the vertical growth phase, the cutaneous melanoma acquires the ability to metastasize. However, metastatic potential is modulated by several factors, the most important of which is the thickness of the tumor. Clark et al.[3] tested 23 different attributes of vertical growth phase cutaneous melanomas and found that six of them were independent predictors of survival. Similarly to the case in that study and other studies, we found the prognosis to be better in females. One of the histologic parameters of Clark et al. was presence of tumor infiltrating lymphocytes, categorized as brisk, nonbrisk, and absent. A correlation between tumor infiltrating lymphocyte presence

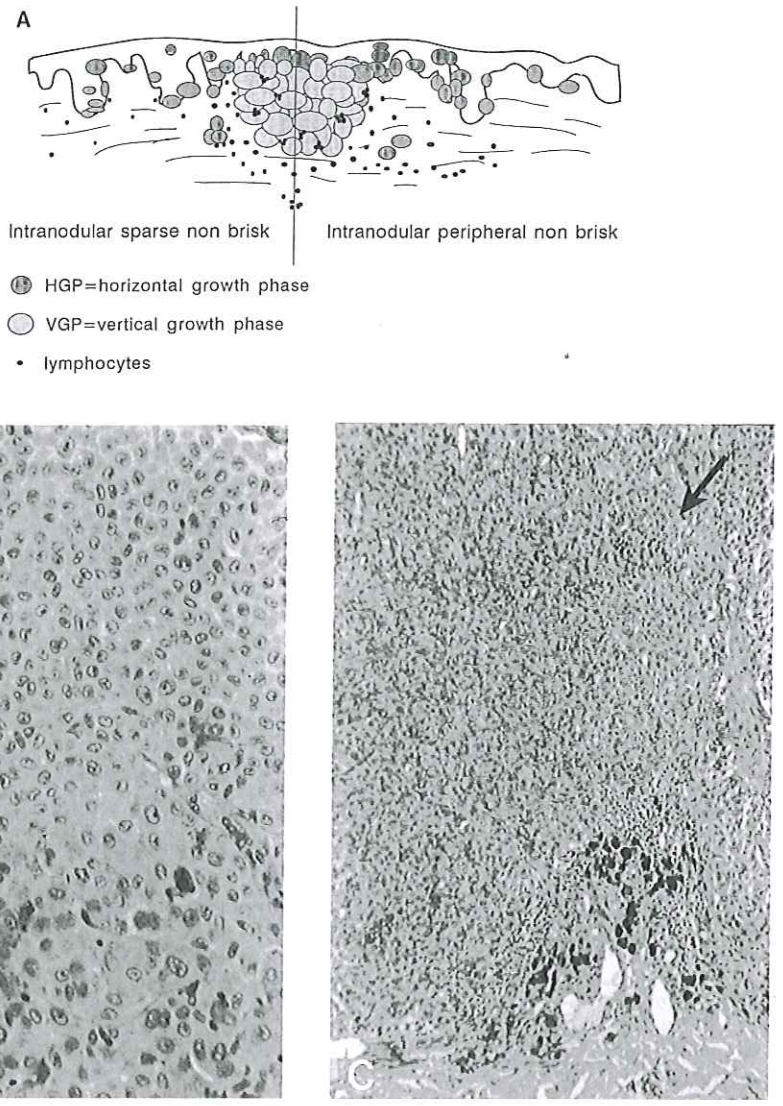

FIGURE 5. A–C: "Nonbrisk" tumor infiltrating lymphocytes. B: Sparse and isolated lymphocytes between the melanoma cells have to be accurately identified. C: One peripheral focus of lymphocytes (arrow) is present in the vertical growth phase (Original magnifications ×500 in B, ×100 in C).

and prognosis has been also reported for bladder,[14] kidney,[15] and breast[16] carcinoma.

In an attempt to verify the discriminant competence of presence of tumor infiltrating lymphocytes as an independent histopathologic prognostic parameter, we have evaluated 285 consecutive cases of vertical growth phase cutaneous melanomas. The presence of lymphocytes outside the vertical growth phase, either below or lateral to it, or in the context of the adjacent horizontal growth phase, has been found by others to be without prognostic significance[2] and was not tested here. The only lymphocytic component with a prognostic significance is that present within the vertical growth phase.[3] When an infiltrate of lymphocytes is present within the vertical growth phase, it may show different patterns.

In brisk cases, the lymphocytes infiltrate predominantly the lower portion of the vertical growth phase (Fig. 4), virtually all along the base of the vertical growth phase. However, at times they may infiltrate through only the lower one-third or even, vertically, one-half of the vertical growth phase. Occasionally, the entire vertical growth phase is infiltrated by the lymphoid cells. In the upper part of the vertical growth phase, the lymphocytic infiltration around melanoma cells must be distinguished from reactive inflammatory infiltrates secondary to superficial cutaneous ulceration. To qualify as brisk, the

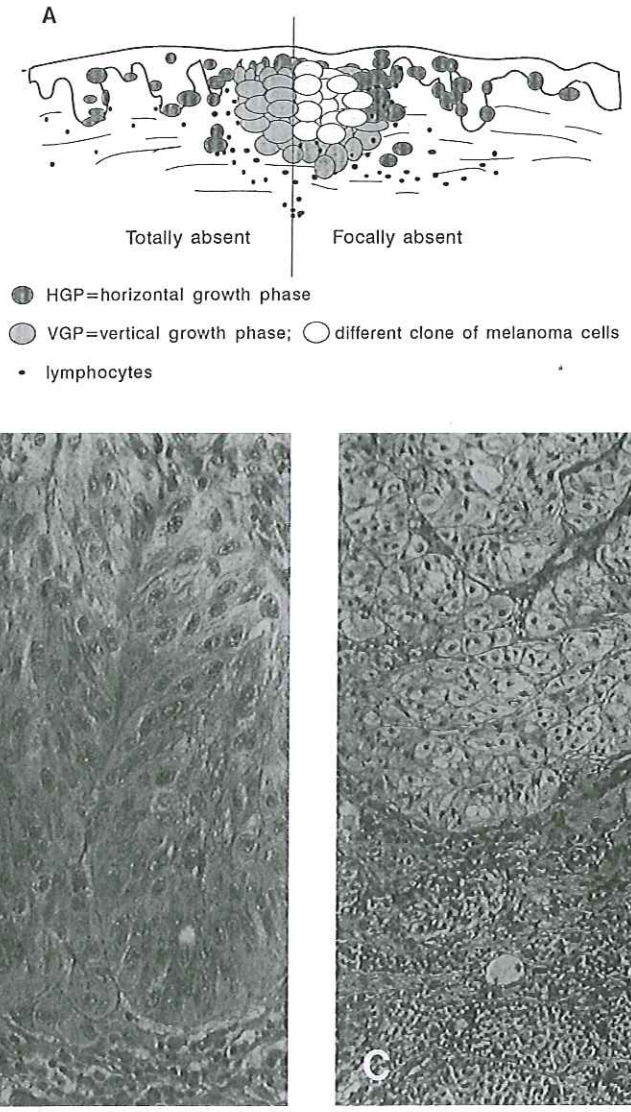

FIGURE 6. A–C: "Absent" tumor infiltrating lymphocytes. B: No lymphocytes are present in the vertical growth phase. C: Two different and distinct clones of melanoma cells: one (upper part) without lymphocytes between the melanoma cells and the other (lower part) with scattered lymphocytes. Both patterns of absent tumor infiltrating lymphocytes (B and C) have the same prognostic implications (Original magnifications ×312 in B, ×200 in C).

lymphocytes must be diffusely interposed between tumor cells, surrounding and disrupting them, with evidence of scattered individual tumor cell necrosis.

Nonbrisk infiltrates are composed of one or more foci of infiltrating lymphocytes that may occupy one-half to two-third of the vertical growth phase base (Fig. 5). This pattern must be very carefully assessed to evaluate small and occasionally rare foci of lymphocytes, away from vessels, between the proliferating melanoma cells, particularly in the center of the nodule.

Differential diagnosis between brisk and nonbrisk histologic patterns depends on quantitative estimation of lymphocytes and their location in the vertical growth phase and does not usually present difficulties. In nonbrisk cases, lymphocytes are scarce, occurring singly or in small groups; in brisk cases, they are more numerous and more diffuse and often occur in bands. For ease of understanding and communication, we have suggested the terms diffuse and peripheral to facilitate description of patterns that have the same prognostic significance.

The term absent of course refers to those cutaneous melanomas in which no lymphocytes are present or in which lymphocytes are not associated with any part of the vertical growth phase (Fig. 6). It also applies to cutaneous

melanomas in which lymphocytes, no matter how dense, are arrayed as a band around the vertical growth phase, without infiltration through the tumor cells. Furthermore, multifocal aggregates of lymphocytes around vessels within the vertical growth phase, but without any infiltration away from the perivascular site, are also defined as absent.

When several foci, often cytologically different, of vertical growth phase are present, each must be carefully examined. If there are two separate nodules of vertical growth phase, one infiltrated, the other not, we consider the pattern absent, but if, for example, there is only a nodule of vertical growth phase, one-half infiltrated by sparse lymphocytes and the other one-half without infiltration, the lesion is characterized as nonbrisk. Generally, the diagnosis gives precedence to absent rather than brisk or nonbrisk.

The results of our review are quite similar to those published by Clark et al.[3] in terms of distribution of tumor infiltrating lymphocytes, mitotic rate, thickness, and 5- and 10-year survivals. In the present series, the main difference is the differing prognostic significance of regression, but roughly equal opposing results have been reported,[1] and the histopathologic diagnostic parameters and the biologic significance of regression have to be reconsidered and verified on large controlled series.

Our results also differ from those of Clark et al. in that 33.9% of cutaneous melanomas were larger than 4.0 mm in our series as opposed to 13.6% larger than 3.60 mm in the Clark et al. series. Of great importance is the finding of highly significant prognostic differences among brisk, nonbrisk, and absent in a series of melanomas enriched for thinner cases. We reviewed 69 melanomas with a maximal diameter less than 6.0 mm., 59 (85.5%) of which were less than 2.0 mm thick. The difference in 7-year survival, in cases in which infiltrating lymphocytes were brisk (100%), nonbrisk (77%) and absent (45%) implies that the presence of tumor infiltrating lymphocytes is an important prognostic factor independent of size and thickness of the lesion.

The highly significant relationship between presence of tumor infiltrating lymphocytes and survival suggests that this lymphocyte-tumor cell interaction is a direct reflection of the host's ability to respond to the tumor cells by a cell-mediated mechanism. Further studies are essential to elucidate the biologic mechanism explaining this morphologic prognostic pattern.

REFERENCES

1. Cook MG. The significance of inflammation and regression in melanoma. *Virchows Arch A Pathol Anat* 1992;420:113–5.
2. Elder DE, Guerry D IV, Van Horn M, Hurwitz S, Zehngebot L, Goldman LI, et al. The role of lymph node dissection for clinical stage I malignant melanoma of intermediate thickness (1.51–3.99 mm). *Cancer* 1985;56:413–8.
3. Clark WH Jr, Elder DE, Guerry DP IV, Braitmen LE, Trock BJ, Schultz D, et al. Model predicting survival in stage I melanoma based on tumor progression. *J Natl Cancer Inst* 1989;81:1893–904.
4. Clark WH Jr, Elder DE, Van Horn M. The biologic forms of malignant melanoma. *Hum Pathol* 1986;17:443–50.
5. Schmoeckel C, Braun-Falco O. Prognostic index in malignant melanoma. *Arch Dermatol* 1978;144:871–3.
6. Breslow A. Thickness, cross-sectional area and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* 1970;172:902–8.
7. van der Esch EP, Cascinelli N, Preda F, Morabito A, Bufalino R. Stage I melanoma of the skin: evaluation of prognosis according to histologic characteristics. *Cancer* 1981;48:1668–73.
8. Kang S, Serri F, Sober AJ. Regressing melanoma and depigmentation. In: Cascinelli N, Santinami M, Veronesi U, editors. Cutaneous melanomas: biology and management. Milan: Masson, 1990;159–66.
9. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. *J Am Stat Assoc* 1958;53:457–81.
10. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719–48.
11. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient: part I—Introduction and design. *Br J Cancer* 1976;34:585–612.
12. Peto R, Pike MC Armitage P, Breslow NE, Cox DR, Howard SV, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient: part II—Analysis and examples. *Br J Cancer* 1977;35:1–39.
13. Cox DR. Regression models and life tables (with discussion). *J R Stat Soc B* 1972;34:187–220.
14. Lipponen PK, Eskelinen MJ, Jauhiainen K, Harju E, Terho R. Tumor infiltrating lymphocytes as an independent prognostic factor in transitional cell bladder cancer. *Eur J Cancer* 1993;29A:69–75.
15. Balch CM, Riley LB, Bao YJ, Salmeron MA, Platsoucas CD, von Eschenbach A, et al. Patterns of human tumor-infiltrating lymphocytes in 120 human cancers. *Arch Surg* 1990;125:200–5.
16. Rilke F, Colnaghi MI, Cascinelli N, Andreola S, Baldini MT, Bufalino R, et al. Prognostic significance of HER-2/NEU expression in breast cancer and its relationship to other prognostic factors. *Int J Cancer* 1991;49:44–9.