Reflectance-Mode Confocal Microscopy for the *In Vivo* Characterization of Pagetoid Melanocytosis in Melanomas and Nevi

Giovanni Pellacani,* Anna Maria Cesinaro,† and Stefania Seidenari*
*Department of Dermatology and †Department of Pathology, University of Modena and Reggio Emila, Italy, Modena, Italy

Pagetoid infiltration of the epidermis by melanocytes is a relevant criterion for the histologic diagnosis of melanoma, although sporadically observable in benign lesions. Since *in vivo* reflectance-mode confocal microscopy enables the visualization of superficial layers at cellular-level resolution, the different aspects and the diagnostic significance of epidermal alterations and pagetoid cell infiltration were investigated on 84 benign and malignant melanocytic lesions by confocal microscopy and compared with histopathology. The observation of a disarranged pattern in superficial layers appeared characteristic for malignant lesions. *In vivo* identification of pagetoid cells, clearly present in the majority of melanomas and in a few benign lesions, seemed useful for melanoma diagnosis. An excellent concordance between confocal microscopy and histopathology was achieved. Moreover, identification of some characteristic features by confocal microscopy, such as large and numerous closely arranged cells extended to the stratum corneum, was strongly correlated with malignancy. In conclusion, confocal microscopy enabled a very good identification of melanocytes spreading upward in a pagetoid fashion in melanocytic lesions. Thus, when pagetoid melanocytosis is observable by means of confocal microscopy, melanoma diagnosis should be considered, whereas it cannot be excluded in the absence of pagetoid cells, lacking in at least 10% of malignant lesions.

Key words: histopathology/melanoma/pagetoid infiltration/scanning laser microscopy/Spitz nevus
J Invest Dermatol 125:532–537, 2005

Pagetoid infiltration of the epidermis by single or grouped melanocytes is a relevant criterion orienting toward the histologic diagnosis of melanoma (McGovern, 1970; Urso *et al*, 1990; Fallowfield and Cook, 1992; Haupt and Stern, 1995). Since pagetoid melanocytosis may also occasionally be seen in benign melanocytic lesions, such as acquired nevi (Rivers *et al*, 1990; Urso *et al*, 1990), congenital nevi, Spitz nevi, and recurrent nevi (Haupt and Stern, 1995), it is suggestive, but not diagnostic, of melanoma.

The recent introduction of *in vivo* reflectance-mode confocal scanning laser microscopy (RCM) enabled the instant visualization of skin structures at a quasi-histopathologic resolution (Rajadhyaksha *et al*, 1995, 1999), representing a non-invasive approach for the *in vivo* study of physiologic and pathologic conditions of the skin. Melanin and melanosomes are strong sources of contrast, rendering melanocytic cells particularly evident by means of this technique (Rajadhyaksha *et al*, 1995; Busam *et al*, 2001). Some RCM features of melanocytic lesions have recently been identified (Langley *et al*, 2001; Busam *et al*, 2002; Tannous *et al*, 2002; Pellacani *et al*, 2004, 2005a, 2005b). Laser-light penetration limits the resolution to a maximum depth of approximately 200 μm, enabling only the visualization of superficial features. Studying four melanomas and two lentigo maligna-type melanomas, the honeycombed appearance of stratum spinosum and the presence of solitary bright cells within the epidermis spreading upward, suggesting a pagetoid fashion, were found by RCM (Langley *et al*, 2001). Moreover, the detection within the epidermis of large nucleated cells with a bright cytoplasm, sometimes presenting dendritic-like branches, was reported in a series of five melanomas (Busam *et al*, 2002) and in one case of *in situ* melanoma, lentigo maligna type (Tannous *et al*, 2002).

Since previous observations of pagetoid cells and disarrangement of epidermal structure by means of RCM seemed highly suggestive of malignancy, the aim of our study was to investigate systematically the different aspects and the diagnostic significance of epidermal alterations and pagetoid cell infiltration, as observed by means of RCM, in a large series of benign and malignant melanocytic lesions and to compare them with histopathology.

Abbreviations: OR, odds ratio; RCM, reflectance confocal microscopy

Results

RCM aspects of superficial layers The RCM aspect of stratum granulosum/spinosum is summarized in Table I. In the majority of cases, a honeycombed pattern is observed, especially in benign lesions (odds ratio (OR) = 0.30; 95% confidence interval (CI95%) 0.11–0.79). A cobblestone appearance is also frequently observed, in some cases, in combination with a honeycombed pattern, both in melanoma and nevi. The disarranged pattern is present in eight

Table I. Frequency of the aspects of granulosum/spinosum layers as observed by reflectance-mode confocal microscopy

	Melanoma (28)	Nevi (42)	Spitz (14)	TOT (84)	OR (CI95%)
Honeycombed	14# (50.0%)	31* (73.8%)	12 (85.7%)	57 (67.9%)	0.302 (0.115–0.795)
Cobblestone	12 (42.9%)	19 (45.2%)	4 (28.6%)	35 (41.7%)	NS
Disarranged	8# (28.6%)	0* (0%)	1 (7.1%)	9 (10.7%)	22.00 (2.586–187.161)

#Significant compared with benign lesions (acquired nevi + Spitz nevi).
*Significant compared with melanomas.
TOT, total; OR, odds ratio; CI95%, 95% confidence interval; NS, not significant.

out of 28 melanomas and in one Spitz nevus alone (OR = 22.00; CI95% 2.58–187.16).

RCM and histopathology of pagetoid cells The frequency and characteristics of pagetoid melanocytosis, as observed by means of RCM and histopathology, are reported in Table II. Pagetoid melanocytosis is observed in the great majority of melanomas but is also present in benign lesions. An excellent agreement between RCM and histopathologic evaluations is obtained (Cohen's $\kappa = 0.899$). Large cells predominate to small ones, especially with RCM compared with histopathology (71% *vs* 54.5%, respectively). By means of RCM, pagetoid cells are frequently roundish (Fig 1*a*), although sometimes dendritic cells are observable (Fig 1*b* and *c*). Large cells with long dendritic like branches are sporadically observed in superficial layers of both benign and malignant lesions, in association with roundish or small dendritic cells in all cases but one. A poor agreeement between histopathological and RCM evaluation is reported for cell pleomorphism (Fig 2) and cell aggregates, both aspects more frequently observed by means of RCM.

Whereas approximately 50% of melanomas are characterized by numerous pagetoid cells (Fig 3), in the majority of benign lesions, only a few pagetoid cells are observable. Widespread distribution of pagetoid cells is significantly correlated with malignancy. Pagetoid melanocytosis more frequently extends to the stratum corneum in melanomas compared with nevi. Fair-to-good agreement is obtained for confocal and histologic evaluation of cell density, cell distribution, and extension to the stratum corneum.

Discussion

Infiltration of the epidermis by melanocytes, also called pagetoid melanocytosis, is considered a relevant criterion for melanoma diagnosis by histopathologists (McGovern, 1970; Rivers *et al*, 1990; Urso *et al*, 1990; Fallowfield and Cook, 1992; Haupt and Stern, 1995). Since pagetoid melanocytosis can also be observed in benign lesions (Rivers *et al*, 1990; Urso *et al*, 1990; Haupt and Stern, 1995), it is only suggestive of melanoma diagnosis, and other criteria should be considered. In melanomas, pagetoid infiltration is usually extensive and diffuse, characterized by marked cellular atypia, whereas in benign lesions, it is generally focal rather than diffuse, without cellular atypia (Haupt and Stern, 1995).

RCM represents a new tool for *in vivo* observation of cytological and architectural aspects of physiological and pathological skin conditions, enabling skin structure visualization at a cellular-level resolution (Rajadhyaksha *et al*, 1995, 1999). The light source limits the depth of penetration to approximately 200–300 μm, permitting the characterization of superficially located structures and cells. Since cells rich in organelles and melanin appear highly contrasted, RCM was used for the characterization of benign and malignant melanocytic lesions (Busam *et al*, 2001, 2002; Langley *et al*, 2001; Tannous *et al*, 2002; Pellacani *et al*, 2004, 2005a, 2005b). In a series of 40 melanocytic lesions, comprising four melanomas and two lentigo maligna, marked alterations of the normal honeycombed appearance of the stratum spinosum and granulosum, resulting in a grainy picture with bright granular dust-like particles, together with individual bright cells, sometimes spreading upward in a pagetoid distribution, were reported in malignant lesions (Langley *et al*, 2001). Evaluating five cases of cutaneous melanomas by means of RCM, the presence of pagetoid cells seemed to be consistent for MM diagnosis (Busam *et al*, 2002). Moreover, detection of pagetoid infiltration appeared useful for distinguishing between a melanoma *in situ*, lentigo maligna type, from a lentigo maligna (Tannous *et al*, 2002). Recently, evaluating RCM aspects of globular Spitz nevi, we observed melanocytic cells with a pagetoid fashion in one of six cases (Pellacani *et al*, 2004).

In this study, we have evaluated the aspects of the superficial layers and the significance of the parameter "pagetoid infiltration" as observed by means of RCM for distinction between benign and malignant melanocytic lesions, systematically exploring 84 consecutive pigmented lesions excised in order to rule out a melanoma. Moreover, features of pagetoid cells as observed by RCM have been described and correlated with the corresponding histopathology. In our cases, epidermal disarrangement, considered consistent for melanoma diagnosis (Langley *et al*, 2001), was observed in less than 30% of melanomas and in one Spitz nevus. Moreover, the presence of honeycombed or cobblestone architectures in superficial layers, observable in almost all benign lesions and in over 70% of melanomas, did not appear relevant for diagnosis. On the other hand, *in vivo* identification of pagetoid cells, clearly present in the majority of melanomas and in six of 56 benign lesions, seemed useful for melanoma diagnosis. An excellent concordance between RCM and histopathology was obtained for the identification of pagetoid cells (Cohen's $\kappa = 0.899$), and the few discordant cases (three of 84) represented lesions in which pagetoid cells corresponded to occasionally scattered melanocytes in superficial layers, probably not included in histopathologic sections or not perceived during exploration of the lesions by means of

Table II. Presence and aspect of pagetoid cells as observed by means of reflectance-mode confocal microscopy and conventional histopathology

	Melanoma (28)		Nevi (42)		Spitz (14)		TOT (84)		OR (CI95%)[#]		
Pagetoid cells	**RCM**	**HIST**	**RCM**	**HIST**	**RCM**	**HIST**	**RCM**	**HIST**	**RCM**	**HIST**	**κ**
Presence	25[#] (89.3%)	24[#] (85.7%)	3* (7.1%)	4 (9.5%)	3 (21.4%)	5 (35.7%)	31 (36.9%)	33 (39.3%)	69.44 (16.01–301.03)	31.33 (8.74–112.3)	0.899
Aspects[a]											
(a) *Size*											
Small	7 (28.0%)	9 (37.5%)	1 (33.3%)	3 (75.0%)	1 (33.3%)	3 (60.0%)	9 (29.0%)	15 (45.5%)	9.00 (1.72–46.87)	3.94 (1.23–12.59)	0.577
Large	18 (72.0%)	15 (62.5%)	2 (66.7%)	1 (25.0%)	2 (66.7%)	2 (40.0%)	22 (71.0%)	18 (54.5%)	23.40 (6.52–83.94)	20.38 (5.12–81.03)	
Shape											
Roundish	25[#] (100%)	—	2 (66.7%)	—	2 (66.7%)	—	29 (93.5%)	—	108.33 (22.51–521.29)	—	—
Small dendritic	10 (40.0%)	—	1 (33.3%)	—	3 (100.0%)	—	14 (45.2%)	—	7.22 (2.01–25.90)	—	—
Large dendritic	5 (20.0%)	—	2 (66.7%)	—	1 (33.3%)	—	8 (25.8%)	—	NS	—	—
Pleomorphism	23 (92.0%)	12 (50.0%)	2 (66.7%)	1 (25.0%)	2 (66.7%)	1 (20.0%)	27 (87.1%)	14 (42.4%)	59.80 (14.69–243.32)	20.25 (4.09–100.05)	NS
(b) *Aggregates*	19[#] (76.0%)	13 (54.2%)	0* (0%)	0 (0%)	1* (33.3%)	2 (40.0%)	19 (61.3%)	15 (45.5%)	116.11 (13.78–977.84)	23.40 (4.74–115.30)	NS
(c) *Cell density*											
Slight	4 (16.0%)	5[#] (20.8%)	2 (66.7%)	3 (75.0%)	1 (33.3%)	3 (60.0%)	7 (22.6%)	11 (33.3%)	NS	NS	
Medium	7 (28.0%)	8 (33.4%)	1 (33.3%)	0 (0%)	1 (33.3%)	1 (20.0%)	9 (29.0%)	9 (27.3%)	9.00 (1.72–46.87)	22.00 (2.58–187.16)	0.559
Marked	14 (56.0%)	11 (45.8%)	0 (0%)	1 (25.0%)	1 (33.3%)	1 (20.0%)	15 (48.4%)	13 (39.4%)	55.00 (6.65–454.51)	17.47 (3.51–86.72)	
(d) *Cell distribution*											
Focal	7[#] (28.0%)	13 (54.2%)	3* (100%)	2 (50.0%)	2 (66.7%)	4 (80.0%)	12 (38.7%)	19 (57.6%)	NS	7.222 (2.34–22.27)	0.545
Widespread	18[#] (72.0%)	11 (45.8%)	0* (0%)	2 (50.0%)	1 (33.3%)	1 (20.0%)	19 (61.3%)	14 (42.4%)	99.00 (11.84–827.59)	11.431 (2.85–45.83)	
(e) *Extension to the stratum corneum*	19 (76.0%)	20[#] (83.3%)	2 (66.7%)	1* (25.0%)	1 (33.3%)	1* (20.0%)	22 (71.0%)	22 (66.7%)	37.29 (9.12–152.43)	67.50 (13.19–345.27)	0.489

[#]Significant compared with benign lesions (acquired nevi + Spitz nevi).
*Significant compared with melanomas.
[a]Percentage and statistics are calculated considering only lesions presenting pagetoid cells.
NS, not significant; RCM, reflectance-mode confocal microscopy; HIST, histopathology; TOT, total; OR, odds ratio; CI95%, 95% confidence interval.

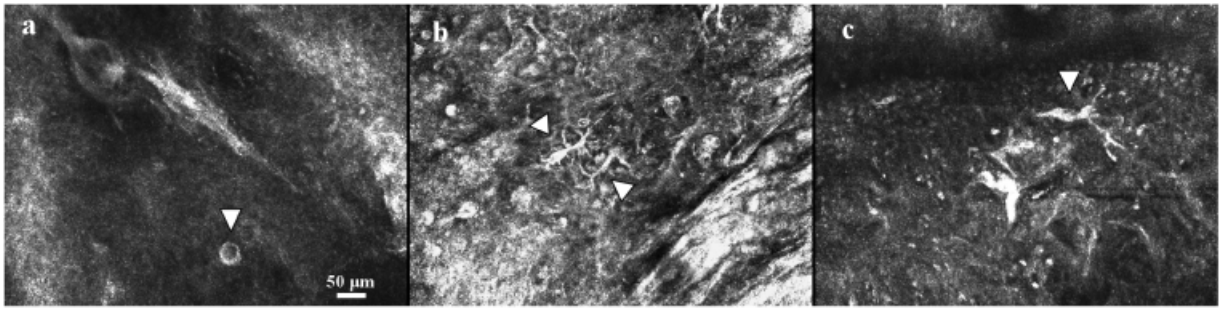

Figure 1
***In vivo* reflectance confocal microscopy aspects of pagetoid cells in melanocytic lesions.** (*a*) Roundish cell with bright cytoplasm and dark eccentric nucleus (*white arrowhead*); (*b*) small dendritic cells with short branches (*white arrowhead*); (*c*) large dendritic cells, with spindled body and long and thin dendritic-like branches.

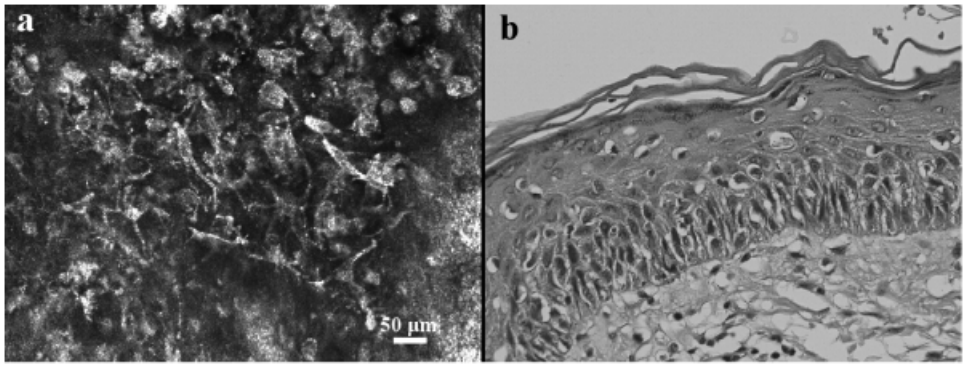

Figure 2
Pleomorphic pagetoid cells. (*a*) confocal microscopy: numerous irregularly shaped and sized cells with a bright cytoplasm and a dark nucleus in superficial layers; (*b*) corresponding histopathology.

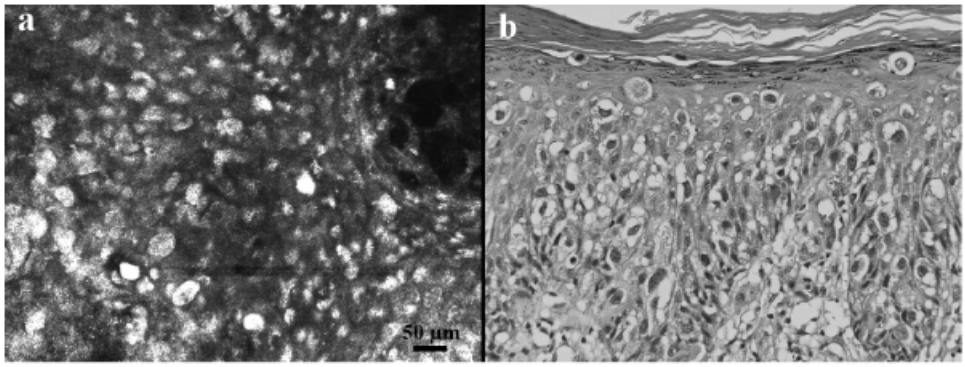

Figure 3
Pagetoid infiltration with marked density. (*a*) Confocal microscopy: numerous roundish bright cells in superficial layers; (*b*) corresponding histopathology.

RCM, or corresponded to non-melanocytic cells, such as Langerhans' cells.

According to previous histopathology studies (Urso *et al*, 1990; Haupt and Stern, 1995), the observation by RCM of some characteristic features, such as large and numerous closely arranged cells extended to the stratum corneum, was strongly correlated with malignancy (for OR, see Table II). The agreement between RCM and histopathology for some descriptors of pagetoid melanocytosis, such as cell density, cell distribution, and extension to the stratum corneum, was acceptable, in consideration of the limited field of view of RCM exploration (7.60 $\times$ 6.65 mm) and the difficulty in recognizing epidermal layers when a disarrangement of epidermis is present. On the other hand, the agreement for pleomorphism and cell aggregate identification was poor. Evaluation of pleomorphism by means of RCM was overestimated compared with histopathology (87% *vs* 42% of cases, respectively), since confocal evaluation was based only on differences in cell morphology and reflectivity, not easily quantifiable, whereas in histopathology, better standardized aspects, such as the difference in cell shape and size, nuclear pleomorphism, presence of small or conspicuous nucleoli, were considered. Moreover, by RCM, pagetoid cells were more frequently regarded as "aggregated," owing to the impossibility of distinguishing between true aggregates and close cells in lesions with marked pagetoid infiltration.

RCM enabled the evaluation of the morphology of pagetoid melanocytes, frequently appearing as roundish cells, sometimes together with squat cells with short branches. Furthermore, in a few cases, large spindled or triangular cells with long and thin dendritic-like branches were observed within the stratum corneum, in all cases except one in combination with roundish cells. No certain correlation

with histopathologic findings was identified for large spindled or triangular cells with long and thin dendritic-like branches, although the shape and the similarity with previous observations in eczematous lesions may suggest its correspondence with Langerhans' cells (Gonzalez *et al*, 1999).

In conclusion, RCM is a reliable tool for identification of pagetoid melanocytosis in melanocytic lesions, and the findings correlated strongly with routine histology. Thus, when pagetoid melanocytosis is observable by means of RCM, melanoma diagnosis should be considered, whereas it cannot be excluded in the absence of pagetoid cells, lacking in at least 10% of malignant lesions. Moreover, identification of some features, such as large and numerous cells extended to the startum corneum, seemed to be suggestive of a malignant lesion. Similar to routine histopathology, the diagnostic judgment in RCM should be based on the integration of all relevant cytological and architectural aspects. *In vivo*, RCM seems to represent a new era for skin oncology, enabling the high-resolution visualization of cells and structures located within the epidermis and superficial dermis, with a strong correlation with histopathology. The number of cases is the main source of the statistical instability, as indicated by the large OR confidence intervals, stressing the opportunity to implement systematic studies based on a larger population in order to assess the usefulness of confocal criteria, alone or in combination, for melanocytic lesion characterization and diagnostic interpretation.

Materials and Methods

Participants–subjects The study was conducted according to the Declaration of Helsinki Principles, and institutional approval and written informed patient consent were obtained. This study included a total of 14,190 images acquired by means of RCM referred to 84 consecutive melanocytic lesions from 83 subjects, 4791 of which referring to 28 melanomas, 9399 to 56 benign lesions (42 acquired nevi and 14 epithelioid and/or spindle cell nevi (Spitz nevi)), corresponding to an average of 176 images per lesion. Melanomas had a mean Breslow's thickness of 0.93 mm, ranging between 0 and 2.94 mm. In three cases they were *in situ*, 16 lesions corresponded to melanomas thinner than 1 mm (pathologic stage T1 according to American Joint Committee on Cancer classification (Balch *et al*, 2001)), four lesions were from 1.01 to 2.0 mm thick (pathologic stage T2), and five from 2.01 to 4.0 mm (pathologic stage T3). Nodular melanomas were not included in the study. Benign lesions corresponded to melanocytic nevi and Spitz nevi with equivocal aspects at clinical and dermoscopic inspection, and excision was performed in order to rule out a melanoma.

Protocol design and study evaluation Prior to biopsy, RCM images were acquired by means of a near-infrared reflectance confocal laser scanning microscope (Vivascope 1000, Lucid Inc, Henrietta, New York) (Rajadhyaksha *et al*, 1995), which uses an 830 nm laser beam with a maximum power of 35 mW. After acquiring the dermoscopic image, the 1 cm adapter ring was filled with water and the RCM arm with the 30 $\times$ water immersion objective lens (numerical aperture of 0.9) was placed onto it. High-resolution images have a spatial resolution of 0.5–1.0 μm in the lateral dimension and 4–5 μm in the axial dimension. Each image corresponds to a horizontal section at a selected depth with an effective 475 $\times$ 350 μm field of view, with a resolution of 640 $\times$ 480 pixels and 255 colors. An automated stepper was used to obtain a grid of 16 contiguous horizontal images at a selected depth, constructing a montage image with a 1.9 $\times$ 1.4 mm field of view ("block" image). A sequence of 30 "block" images was acquired for each lesion at the epidermal level in order to explore a 7.60 $\times$ 6.65 mm field of view per lesion (Pellacani *et al*, 2004). Sequences of confocal sections, beginning at the stratum corneum and ending inside the papillary dermis, were recorded at areas of interest.

All lesions were then excised positioning a silk suture at one pole of the specimen to make its orientation easier, and underwent histopathologic examination for diagnostic confirmation and pattern correlation.

***In vivo* RCM evaluation** Granulosum/spinosum layers were described, identifying three possible patterns: honeycombed pattern with regular appearance, constituted by large (10–20 μm) polygonal cells with dark nuclei and bright and thin cytoplasm; cobblestone pattern, consisting of small polygonal cells with refractive cytoplasm separated by a less refractive border; and the disarranged pattern, characterized by disarray of the normal architecture of the superficial layers with unevenly distributed bright granular particles and cells, irregular in shape and size.

In order to identify pagetoid cells, we selected three cases in which pagetoid cells were clearly present in histopathology. In the corresponding RCM images, we could clearly identify pagetoid cells as cells with a dark nucleus and bright cytoplasm and twice the size of keratinocytes, located in the superficial layers. Conversely, artifacts or blotches of melanin appeared as bright roundish structures without a nucleus. These criteria were applied to the other RCM images for pagetoid cell identification. Different features were evaluated as follows: (a) cell size, considering cells with the maximum diameter lower than 50 μm "small," and, if greater, "large"; cell shape, evaluated as "roundish" or "dendritic" (Fig 1); and cell pleomorphism (Fig 2); (b) presence of cells aggregated into nests; (c) cell density, regarded as "slight" when less than 10 cells per mm^2 were observed, "medium" for 11–20 cells per mm^2, and "marked" for more than 20 cells per mm^2 (Fig 3); (d) cell distribution, referred to as focal or widely diffused; and (e) extension to the stratum corneum, when cells were observable up to 20 μm below the surface.

Histopathology Lesions were excised with a 2 mm margin and stocked for histopathological analysis. After surgical excision, a silk suture was positioned on the specimen to clarify its orientation. The lesion was serially cut in slices about 3 mm thick, along the minor axis, and totally embedded in one or more blocks, depending on the size of the lesion. At least two sections for each block were then cut and scrutinized. According to Haupt and Stern (1995), pagetoid melanocytosis referred to the upward discontinuous extension of melanocytes into the superficial epidermis. Histological criteria for pagetoid melanocytosis included the clear presence of melanocytes in the suprabasilar epidermis, the discontinuous extension of melanocytes from the junctional component, and the melanocyte location in suprabasilar epidermis at a level above the basal epidermal layer overlying the most superficial papilla. In lesions where the epidermis was relatively flat and lacked well-defined dermal papillae, the melanocytes had to be separated from the basilar layer from at least one layer of keratinocytes. Cases displaying pagetoid melanocytosis were evaluated for the following features: (a) cytological features of pagetoid melanocytes (size, subdivided into small or large cells, and pleomorphism); (b) single cells versus nests; (c) amount of pagetoid cells ("slight density" corresponded to occasionally scattered melanocytes, "medium density" to some pagetoid cells, "marked density" to numerous closely arranged melanocytic cells); (d) distribution of pagetoid melanocytosis (focal distribution or widely diffused); and (e) extension to the stratum corneum.

Statistical analysis Statistical evaluation was carried out using the SPSS statistical package (release 10.0.6, 1999; SPSS Inc., Chicago, Illinois). Statistical analysis was performed for the patterns of superficial layers and for the presence of pagetoid cells, referring to all lesions examined, and for each pagetoid melanocytosis aspect, referring to lesions presenting pagetoid cells.

Absolute and relative frequencies of each confocal and histological criterion were calculated in melanomas, acquired nevi, and Spitz nevi. Significant differences between melanomas and benign lesions were evaluated by means of the χ^2 test of independence (Fisher's exact test was applied if any expected cell value in the 2×2 table was <5). For the estimate of the chance to represent a malignant lesion, the calculation of the OR and (CI95% was performed both on confocal and histopathologic data for the presence of pagetoid cells and for each cell aspect, referring to lesions presenting pagetoid cells. For the evaluation of the concordance between confocal and histopathologic feature identification, Cohen's κ index for agreement was calculated for each descriptor. κ values range between 1 and 0. A κ value of 1.0 indicates full agreement beyond chance, values greater than 0.70 are generally considered excellent, values less than 0.40 poor. and values between 0.40 and 0.70 fair to good. A p value less than 0.05 was considered significant.

This study was partially supported by a grant of the Fondazione Cassa di Risparmio di Modena (Italy).

DOI: 10.1111/j.0022-202X.2005.23813.x

Manuscript received January 26, 2005; revised March 17, 2005; accepted for publication March 29, 2005

Address correspondence to: Giovanni Pellacani, Department of Dermatology, University of Modena and Reggio Emilia, Via del Pozzo 71, 41100 Modena, Italy. Email: pellacani.giovanni@unimo.it

References

Balch CM, Buzaid AC, Soong SJ, *et al*: Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. J Clin Oncol 19:3635–3648, 2001

Busam KJ, Charles C, Lee G, Halpern AC: Morphological features of melanocytes, pigmented keratinocytes, and melanophages by *in vivo* confocal scanning laser microscopy. Mod Pathol 14:862–868, 2001

Busam KJ, Charles C, Lohmann CM, Marghoob A, Goldgeier M, Halpern AC: Detection of intraepidermal malignant melanoma *in vivo* by confocal scanning laser microscopy. Melanoma Res 12:349–355, 2002

Fallowfield ME, Cook MG: Pagetoid infiltration in primary cutaneous melanoma. Histopathology 20:417–420, 1992

Gonzalez S, Gonzalez E, White M, Rajadhyaksha M, Andersson RR.: Allergic contact dermatitis: Correlation of *in vivo* confocal imaging to routine histology. J Am Acad Dermatol 40:708–713, 1999

Haupt HM, Stern JB: Pagetoid melanocytosis. Histologic features in benign and malignant lesions. Am J Surg Pathol 19:792–797, 1995

Langley RGB, Rajadhyaksha M, Dwyer PJ, Sober AJ, Flotte TJ, Anderson RR: Confocal scanning laser microscopy of benign and malignant melanocytic skin lesions *in vivo*. J Am Acad Dermatol 45:365–376, 2001

McGovern VJ: The classification of melanoma and its relationship with prognosis. Pathology 2:85–98, 1970

Pellacani G, Cesinaro AM, Grana C, Seidenari S: *In-vivo* confocal scanning laser microscopy of pigmented Spitz nevi. Comparison of *in-vivo* confocal images with dermoscopy and routine histopathology. J Am Acad Dermatol 51:371–376, 2004

Pellacani G, Cesinaro AM, Longo C, Grana C, Seidenari S: Microscopic *in vivo* description of cellular architecture of dermoscopic pigment network in nevi and melanomas. Arch Dermatol 141:147–154, 2005

Pellacani G, Cesinaro AM, Seidenari S: *In vivo* assessment of melanocytic nests in nevi and melanomas by reflectance confocal microscopy. Mod Pathol 18:469–474, 2005

Rajadhyaksha M, Gonzalez S, Zavizlan JM, Anderson RR, Webb RH: *In vivo* confocal scanning laser microscopy of human skin II: Advances in instrument and comparison with histology. J Invest Dermatol 113:101–113, 1999

Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR: *In vivo* confocal scanning laser microscopy of human skin: Melanin provides strong contrast. J Invest Dermatol 104:946–952, 1995

Rivers JK, Cockerell CJ, McBride A, Kopf AW: Quantification of histological features of dysplastic nevi. Am J Dermatopathol 12:42–50, 1990

Tannous ZS, Mihm MC, Flotte TJ, Gonzalez S: *In vivo* examination of lentigo maligna and malignant melanoma *in situ*, lentigo maligna type by near-infrared reflectance confocal microscopy: Comparison of *in vivo* confocal images with histologic sections. J Am Acad Dermatol 46: 260–263, 2002

Urso C, Giannini A, Bartolini M, Bondi R: Histological analysis of intraepidermal proliferations of atypical melanocytes. Am J Dermatopathol 12:150–155, 1990