

Factor XIIIa-positive dermal dendritic cells and HLA-DR expression in radial versus vertical growth-phase melanomas

Fifty nevomelanocytic lesions, including 10 typical compound nevi and 20 radial and 20 vertical growth-phase melanomas, were evaluated for factor XIIIa and HLA-DR (LN3) expression within dermal dendritic cells (DDCs) or dermal dendrocytes to determine if DDCs proliferate and/or participate as possible antigen-presenting cells in the local tissue response to benign and malignant nevomelanocytic lesions. There was no statistical difference in factor XIIIa staining of DDCs between nevi and radial or vertical growth-phase melanomas, suggesting that DDCs do not significantly proliferate in nevomelanocytic lesions. However, studies to determine proliferation rate, apoptosis, and influences of local mediators on cell growth and/or recruitment were not done. HLA-DR staining by DDCs was significantly increased ($p < 0.001$) in both melanoma groups when compared to compound nevi, but did not significantly differ between radial and vertical growth-phase melanomas. The intensity of HLA-DR expression appeared to correlate with the presence or absence of lymphocytic inflammation; HLA-DR intensity was judged greater in melanomas characterized by a brisk and infiltrative lymphocytic host response. We propose that DDCs may participate in the dermal immune response to invasive melanomas, probably as antigen-presenting cells to skin-associated lymphocytes.

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Dermal dendritic cells (DDCs) or dermal dendrocytes are normal resident cells of the reticular dermal interstitium, occurring predominantly perivascularly and within the papillary dermis. They are characterized by their highly dendritic morphology (1). Although the origin of the DDC remains a topic of debate, the preponderance of evidence from immunohistochemistry and immunofluorescence studies supports their derivation from bone marrow progenitor cells (2, 3).

Most DDCs express cytoplasmic factor XIIIa, a protransglutaminase involved in hemostasis (4-6). In addition to their putative role in wound healing, DDCs are capable of phagocytosis of hemosiderin, melanin, and exogenous pigments, such as are present in tattoos (1, 4, 7).

DDCs proliferate in a variety of inflammatory and neoplastic processes. In inflammatory dermatoses, such as psoriasis and atopic eczema, an increase in DDCs associated with a lymphocytic infiltrate was previously reported (3). A proliferation of factor XIIIa-positive DDCs is the sine qua non of the dermatofibroma, and is also seen in fibrous papules of the face (8, 9). Moreover, DDCs are readily identified at the periphery of Kaposi's sarcomas (10). Denton et al. evaluated the distribution of factor XIIIa-positive DDCs in distinguishing nodular melanomas from Spitz nevi (11). They demonstrated that DDCs were either diffusely distributed within the tumor or primarily localized to the periphery, and that the pattern of distribution could not be used to distinguish between these two entities.

**Douglas R. Fullen and
John T. Headington**

Department of Pathology, University of Michigan
Hospitals, Ann Arbor, Michigan, USA

Douglas R. Fullen, M.D., Department of Pathology,
Cornell University Weill Medical Center, 1300 York
Avenue, F-309, New York, NY 10021, USA.
Tel: 212-746-6434, Fax: 212-746-8570

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A proposed antigen-presenting role for DDCs is based on their proliferation and enhanced expression of HLA-DR, a class II, nonconstitutively expressed major histocompatibility antigen, in intra-dermal delayed hypersensitivity reactions (1) and as potent stimulators of allogeneic mixed lymphocyte reactions *in vitro* (12). Although HLA-DR expression in melanomas has been extensively studied, the primary focus has been on antigen expression in melanoma cells (13–19). A few groups have evaluated HLA-DR expression by dendritic cells (Langerhans cells) residing within the dermis (20, 21); however, to our knowledge, differences in HLA-DR expression by factor XIIIa-positive DDCs in radial versus vertical growth-phase melanomas has not been done.

In this study, we semiquantitatively evaluated factor XIIIa expression by DDCs in benign and malignant nevocellular lesions to determine if there was a hyperplasia of DDCs and, if so, whether there specifically was a difference between radial and vertical growth-phase melanomas. Moreover, we assessed HLA-DR expression by DDCs to delineate a possible immunologic role as antigen-presenting cells in the progression of malignant melanomas.

Material and methods

Case selection

Fifty cases, 20 radial and 20 vertical growth-phase melanomas and 10 typical compound nevi, were obtained through the Melanoma Clinic registry and the SNOMED retrieval program of the Pathology Data Systems of the University of Michigan Medical Center. Cases were excluded if they were re-excisions of a previously diagnosed melanoma or compound nevus.

Radial were compared to vertical growth-phase melanomas for sex, age, body site, presence or absence of regression, and sun exposure. There were more males than females in the radial growth-phase group (14 vs. 6), as opposed to slightly more females than males in the vertical growth-phase group (11 vs. 9). The mean age was not appreciably different between the two groups (radial, 56 years; vertical, 61 years). Melanomas were separated into sites as follows: head and neck (radial, 4; vertical, 5), trunk (radial, 12; vertical, 8), extremities (radial, 3; vertical, 7), and site not provided (radial, 1). Presence of regression did not differ between the two groups (radial, 5; vertical, 4). In cases with regression, it was either focal (radial, 1; vertical, 2), or diffuse (radial, 4; vertical, 2). The degree of sun exposure was determined based on the degree of elastosis seen on the original hematoxylin and eosin stained slides. There was no sig-

nificant difference between radial or vertical growth-phase melanomas with respect to presence (radial, 14; vertical, 15), or degree of elastosis.

Immunoperoxidase methods

Four- μ m sections of formalin-fixed, paraffin-embedded tissue were prepared on plus slides (Baxter Scientific). In the vast majority of cases, sequential slides were prepared at approximately 4- μ m intervals and consecutively stained with the primary antibodies in question.

Single labeling

The slides were deparaffinized in xylene and ethanol. Endogenous peroxidases were blocked with hydrogen peroxide. Pretreatment with protease was done for factor XIIIa and CD1a stains, and microwave for CD34 (HPCA-1/2) stains; no pretreatment was done for HLA-DR (LN3). The tissues were stained with primary antibody followed by a biotinylated, polyspecific secondary antibody and an avidin-biotin horseradish peroxidase amplification system using an automated (Ventana) method. Each case was stained with a 1:800 dilution of rabbit polyclonal antibody to factor XIIIa (Becton-Dickinson), an undiluted mouse monoclonal antibody to HLA-DR (BioGenex), an undiluted mouse monoclonal antibody to CD1a (Immunotech), and a 1:10 dilution of mouse monoclonal antibody to CD34 (Becton-Dickinson). The peroxidase was visualized with 3',3'-diaminobenzidine (DAB). All slides were counterstained with Mayer's hematoxylin. Each antibody was applied to all 50 cases in a single run to avoid interbatch variability.

Double labeling

Six cases, three radial and three vertical growth-phase melanomas, were double labeled with HLA-DR vs. factor XIIIa, HLA-DR vs. CD34, and CD34 vs. factor XIIIa. The slides were deparaffinized as above. Endogenous peroxidases were blocked with hydrogen peroxide. Microwave pretreatment was performed for optimal antigen retrieval of HLA-DR, factor XIIIa, and CD34, as determined by single stain control runs using different forms of pretreatment, i.e. no pretreatment, enzyme pretreatment, or microwave pretreatment. The tissues were stained with the first primary antibody (HLA-DR for HLA-DR vs. factor XIIIa or HLA-DR vs. CD34, or CD34 for CD34 vs. factor XIIIa) at the same titers as above, followed by a biotinylated, polyspecific (anti-mouse and anti-rabbit) secondary antibody and an avidin-biotin horseradish

Table 1. Results of factor XIIIa staining of DDCs.

Diagnosis	Intensity of staining			
	0	1+	2+	3+
Compound nevi	6	4	0	0
Radial growth-phase melanomas	11	9	0	0
Vertical growth-phase melanomas	8	9	3	0

Table 2. Results of HLA-DR staining of DDCs.

Diagnosis	Intensity of staining			
	0	1+	2+	3+
Compound nevi	8	2	0	0
Radial growth-phase melanomas	3	10	7	0
Vertical growth-phase melanomas	3	11	6	0

peroxidase amplification system by an automated (Ventana) method. The peroxidase reaction product was detected using DAB. The slides were then subjected to the second primary antibody (factor XIIIa or CD34 for HLA-DR, or factor XIIIa for CD34) followed by a biotinylated, polyspecific (anti-mouse and anti-rabbit) secondary antibody and an avidin-biotin alkaline phosphatase amplification system also using an automated (Ventana) system. The alkaline phosphatase reaction product was detected using fast red. All slides were counterstained with Mayer's hematoxylin.

Interpretation of stains

Factor XIIIa, HLA-DR, CD-34, and CD1a slides were interpreted for staining intensity and scored semiquantitatively on a scale of 0-3+. The scoring system for staining intensity was as follows: 0 (none) = rare to no cells staining; 1+ (slight) = low density, patchy staining; 2+ (moderate) = moderate density, focal or confluent paralesional staining; and 3+ (marked) = high density with broad, confluent paralesional staining. Care was taken to exclude melanin-containing macrophages (melanophages) as a source of false positive staining. Moreover, distinguishing lymphocyte from DDC staining with HLA-DR was important in avoiding a false positive interpretation; this was readily achieved on morphologic grounds.

Statistical analysis

Chi-square analysis was performed using Sigma Stat 2.0 software (Jandel Scientific, San Raphael, Ca) run on a Compaq Desk pro 590 PC. Statistical significance was assigned at the $p < 0.05$ level.

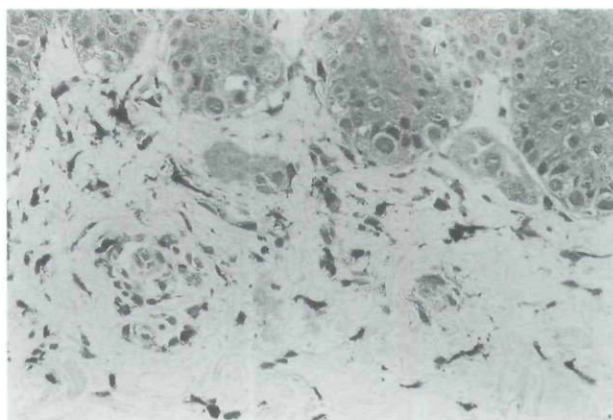


Fig. 1. Slightly increased (1+) factor XIIIa-positive staining of DDCs in a case of radial growth-phase melanoma (Immunoperoxidase, 115 \times).

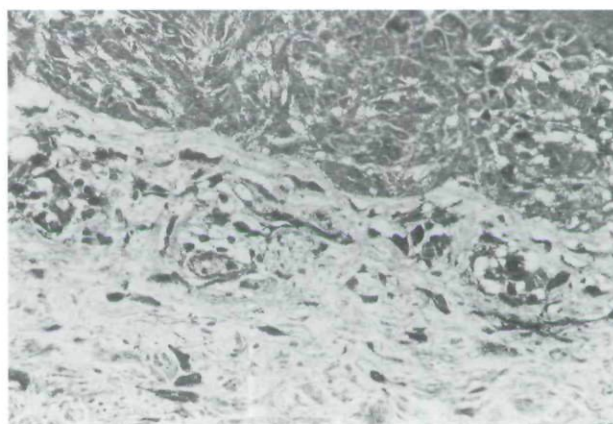


Fig. 2. Slight (1+) HLA-DR (LN3) staining of DDCs in a case of radial growth-phase melanoma (Immunoperoxidase, 115 \times).

Results

Factor XIIIa staining

Four of 10 (40%) compound nevi, 9 of 20 (45%) radial growth-phase melanomas, and 12 of 20 (60%) vertical growth-phase melanomas showed an increase in factor XIIIa staining of DDCs (Table 1). The majority of cases, with the exception of 3 vertical growth-phase melanomas, had only slight (1+) increased staining (Fig. 1). When compared to compound nevi, there was no statistical difference in factor XIIIa staining for radial ($p=1.00$) or vertical ($p=0.44$) growth-phase melanomas.

HLA-DR staining

Two of 10 (20%) compound nevi and 17 each of 20 (85%) radial and vertical growth-phase

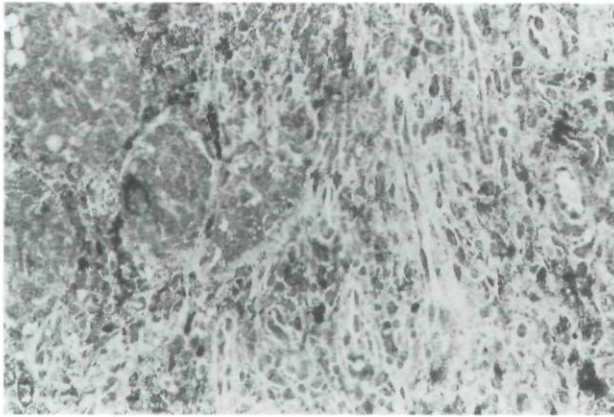


Fig. 3. Moderate (2+) HLA-DR staining of DDCs in a case of vertical growth-phase melanoma. Note the association of positive staining DDCs with the brisk inflammatory lymphocytic infiltrate (Immunoperoxidase, 115 \times).

melanomas showed HLA-DR expression by DDCs (Table 2). The majority of cases showed slight HLA-DR staining (Fig. 2). However, in 7 of 17 (41%) radial and 6 of 17 (35%) vertical growth-phase melanomas, the intensity of staining was judged to be moderate (2+) (Fig. 3). HLA-DR expression was judged to be similar in both melanoma groups, but was significantly increased in both melanoma groups when compared to compound nevi ($p < 0.001$). Of particular interest, HLA-DR expression by DDCs appeared to correlate with the presence or absence of a lymphocytic infiltrate in both radial and vertical growth-phase melanomas. Furthermore, the intensity of staining was greater in the presence of a brisk and infiltrative lymphocytic host response, whereas the intensity of staining was less or absent in a nonbrisk and non-infiltrative or absent lymphocytic host response (Fig. 3).

CD1a staining

Two of 10 (20%) compound nevi, 3 of 20 (15%) radial growth-phase melanomas, and 5 of 20 (25%) vertical growth-phase melanomas demonstrated slight (1+) increased CD1a staining of cells in the superficial dermis (Table 3). There was good internal control staining of CD1a-positive Langerhans cells within the epidermis. The Langerhans cells within the dermis tended to cluster as a few cells around blood vessels in association with a lymphocytic infiltrate of variable intensity. However, most lesions demonstrating a lymphocytic host response, including both radial and vertical growth-phase melanomas, did not show cells staining for CD1a within the dermis. Based on the few cases with CD1a-positive cells in the dermis and the small number of cells that were tightly distributed

around blood vessels in a very focal pattern, it is apparent that these cells did not significantly contribute to the HLA-DR expression seen in DDCs.

CD34 staining

Seven of 10 (70%) and 2 of 10 (20%) compound nevi showed slight (1+) and moderate (2+) CD34 staining, respectively. Eight of 20 (40%), 6 of 20 (30%), and 3 of 20 (15%) radial growth-phase melanomas showed slight, moderate, and marked CD34 staining, respectively. No vertical growth-phase melanomas had marked staining; however, 5 of 20 (25%) had slight and 5 of 20 (25%) had moderate staining (Table 4). DDCs that stained with CD34 had morphologic features indistinguishable from factor XIIIa-positive DDCs, although those CD34-positive DDCs within the interstitium tended to be more evenly distributed from upper to deep dermis. When increased staining was observed, it was usually seen within DDCs along the base or periphery of the lesion. CD34-positive DDCs were located both perivascularly and within the dermal interstitium. As expected, CD34 also stained endothelial cells, as well as dendritic cells within the connective tissue surrounding eccrine coils. Since the vascularity varied, in some cases significantly, from case to case, no attempt was made to factor out the contribution of vascular staining to the total score, as reproducibility would be difficult to achieve.

Double labeling

In 4 of 6 melanoma cases (radial, 2; vertical, 2) on which double labeling for HLA-DR vs. factor XIIIa was performed, frequent factor XIIIa-positive DDCs coexpressing HLA-DR were seen (Fig. 4). There did not appear to be a difference between radial and vertical growth-phase melanomas in number of DDCs staining with both factor XIIIa and HLA-DR. However, there were other cells, mostly lymphocytes but occasional factor XIIIa-negative DDCs, expressing only HLA-DR. Occasional factor XIIIa-positive DDCs did not show HLA-DR coexpression. Two cases showed strong HLA-DR staining but very weak and patchy factor XIIIa staining; these cases were considered unreliable for interpretation. It is possible that antigen retrieval for factor XIIIa by microwaving was suboptimal on these two cases.

When CD34 and factor XIIIa were applied in our double labeling technique, DDCs were readily identified with restricted staining for either CD34 or factor XIIIa in 3 of 6 cases (Fig. 5). No DDCs coexpressing both epitopes were seen. In 3 of 6 cases, CD34 stained DDCs well; however, factor XIIIa

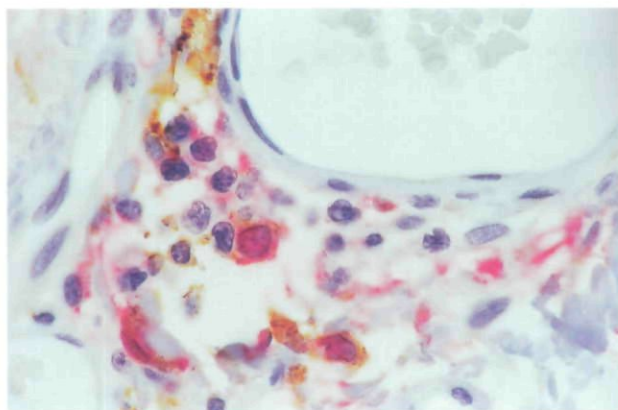


Fig. 4. Perivascular factor XIIIa-positive (red) DDCs demonstrating coexpression of HLA-DR (brown) by double labeling (Immunoperoxidase, 825 \times).

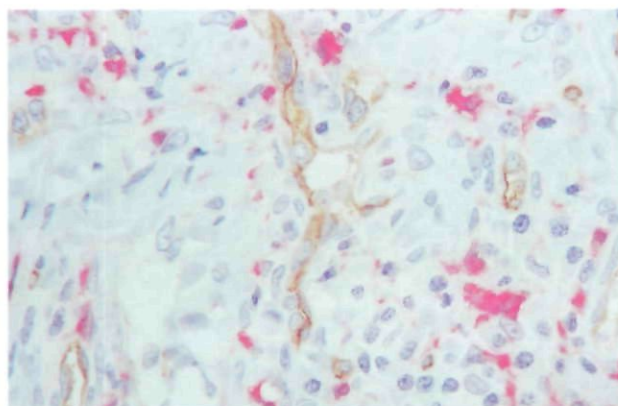


Fig. 5. Restricted staining of CD34 (brown) within endothelial cells and occasional hematopoietic cells, and factor XIIIa (red) within DDCs (Immunoperoxidase, 528 \times).

staining was weak and focal. Therefore, as with a few cases of HLA-DR vs. factor XIIIa, these cases were considered unreliable for interpretation.

CD34 was stained against HLA-DR to see if any CD34-positive DDCs may coexpress HLA-DR, possibly accounting for the occasional DDCs expressing HLA-DR that were factor XIIIa-negative. Five of 6 cases showed strong staining for both HLA-DR and CD34. No CD34 DDCs were observed to coexpress HLA-DR. In one case, HLA-DR staining was strong but CD34 staining was absent. Therefore, this case was considered unreliable for interpretation.

Discussion

In contrast to fibroproliferative lesions, such as dermatofibromas or fibrous papules of the face, there was no significant hyperplasia of DDCs, as determined by cytoplasmic factor XIIIa labeling, in the nevomelanocytic lesions we evaluated. Our

Table 3. Results of CD1a staining of cells within the dermis.

Diagnosis	Intensity of staining			
	0	1+	2+	3+
Compound nevi	8	2	0	0
Radial growth-phase melanomas	17	3	0	0
Vertical growth-phase melanomas	15	5	0	0

Table 4. Results of CD34 staining of DDCs.

Diagnosis	Intensity of staining			
	0	1+	2+	3+
Compound nevi	1	7	2	0
Radial growth-phase melanomas	3	8	6	3
Vertical growth-phase melanomas	10	5	5	0

postulate that DDCs would be increased above background in invasive melanomas when compared to a benign nevomelanocytic control group was not confirmed in this study. Moreover, if DDCs increased in invasive melanomas, either due to proliferation or recruitment, we hypothesized that the number of DDCs would be either unchanged or, more likely, reduced in vertical growth-phase melanomas when compared to radial growth-phase melanomas. We found no difference in the number of factor XIIIa-positive DDCs between vertical and radial growth-phase melanomas, as determined semiquantitatively.

Radial and vertical growth-phase melanomas are associated with a significantly increased expression of HLA-DR by DDCs in single antibody immunoperoxidase studies, suggesting that DDCs may have an antigen-presenting role in the dermal immune response to invasive melanomas. This is perhaps not surprising, since HLA-DR expression by DDCs in intradermal delayed hypersensitivity reactions has been described (1). In order to confirm that factor XIIIa-positive cells may coexpress HLA-DR, we subjected a small subset of radial and vertical growth-phase melanomas to an immunoperoxidase double labeling technique. We found that some factor XIIIa-positive DDCs coexpress HLA-DR (cells with cytoplasmic staining for both antigens), thereby confirming our impression from single antibody studies. We acknowledge the remote possibility of cross reactivity in our double labeling experiment, since the secondary antibody used against both primary antibodies was polyspecific. However, based on the pattern of DDCs staining for HLA-DR and factor XIIIa, i.e. coexpression as well as cells with restricted staining with both antibodies, purely restricted staining within DDCs for either CD34 or factor XIIIa, and cells with either CD34 or HLA-DR expression, we are confident that all of the binding sites on the

initial polyspecific secondary antibody were adequately blocked by the avidin-biotin amplification system such that no sites were available for cross reactivity.

In most radial and vertical growth-phase melanomas, HLA-DR expressing DDCs were found in association with the host lymphocytic inflammatory response and, more importantly, appeared to be associated with a brisk and infiltrative lymphocytic response as described by Elder and coworkers (22, 23). This finding may further support a putative antigen-presenting role by DDCs to tumor infiltrating lymphocytes.

The DDC remains an under studied cell in the dermal immune response to the wide range of inflammatory and neoplastic processes that involve the skin. Additional studies of HLA-DR-expressing DDCs in melanomas with a brisk and infiltrative lymphocytic host response compared to melanomas with a brisk and noninfiltrative, nonbrisk, or absent lymphocytic host response should be done.

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