Immunoperoxidase Localization of Lymphocyte Subsets in the Host Response to Melanoma and Nevi

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ABSTRACT

Using an avidin:biotin immunoperoxidase system with monoclonal antibodies to lymphocyte subsets, we have investigated the host response to malignant melanoma and melanocytic nevi in frozen sections. Eight primary melanomas, eight metastases, three dysplastic nevi, and two dermal nevi were studied with antibodies T11, T4, T8, and B1. Sections were read in a semiquantitative manner by two observers. Virtually all lymphocytes in these lesions were T-cells (T11 positive). In all primary melanomas, in the majority of metastases, and in all dysplastic nevi, both T4- and T8-positive cells were present. In two of eight metastases, tumor cells stained with T4, and in one case, melanoma cells stained with B1 antibody. The host response to melanoma involves primarily T-cells and includes both the helperinducer (T4) and suppressor:cytotoxic (T8) subsets.

INTRODUCTION

The lymphocytic infiltrate and the common phenomenon of partial regression in melanoma (5) suggest that there is a host response to the tumor. Functional subsets of lymphocytes in tissue sections can now be identified by surface antigens using monoclonal antibodies (2, 4, 10, 11, 15-17, 24).

We have applied an avidin:biotin immunoperoxidase system to analyze the host response to melanoma and other pigmented lesions. Frozen sections permit morphological identification of the antigen-positive cells.

MATERIALS AND METHODS

Tissue obtained fresh from 19 patients seen by the Pigmented Lesion Group at the Hospital of the University of Pennsylvania was frozen in liquid nitrogen and stored at −70° until used. Cryostat sections (5 μm) were cut and allowed to dry overnight. Sections were then fixed in acetone for 10 min and allowed to dry. After a 3-min wash in PBS at pH 7.4, the primary antibody was applied in a 1:50 PBS dilution for 15 min. Dilutions were selected in checkerboard titrations to allow for crisp staining of reactive cells against minimal interstitial background. Primary murine monoclonal antibodies T11, T4, T8, and B1 were obtained from Coulter Corporation, Hialeah, Fla. T11 is a pan-T-cell marker (24); T4 labels the helper:inducer T-cell subset (16); T8 stains the suppressor:cytotoxic T-cell (16); and B1 is a B-cell marker (1). After another 3-min wash in PBS, biotinylated antibody (Vector Laboratories, Burlingame, Calif.) was applied in a 1:75 dilution for 15 min. Sections were then rinsed in PBS. Next, the avidin:biotinylated peroxidase complex (Vector Laboratories) was applied for 15 min.

After another PBS wash, AEC (Aldrich Chemical Company, Milwaukee, Wis.) (20 mg AEC in 5 ml N,N-dimethylformamide:95 ml 0.05 M acetate buffer, pH 5.2, with 0.03% hydrogen peroxide) was applied for 10 min. AEC was used because the red pigment contrasts well with melanin. Sections were then rinsed in tap water, counterstained in hematoxylin (45 sec), and mounted in Elvanol (E. I. Dupont Company, Wilmington, Del.).

All incubations were at room temperature. Negative control slides had PBS substituted for the primary antibody. In addition, positive controls utilized the same antibodies on frozen sections of lymph nodes. Sections incubated in B1 antibody were essentially negative controls as well. Positive results consisted of red staining of cells. For lymphocytes, this was circumferential as is consistent with the known surface membrane location of these antigens.

Sections were examined independently by 2 observers using routine light microscopy. The following features were evaluated semiquantitatively: density of the lymphocytic response, distinguishing infiltrative and noninfiltrative areas as well as areas of partial regression; proportion and intensity of lymphocyte staining with T11, T4, T8, and B1 antibodies in these 3 areas of the lesion; proportion and intensity of tumor cell staining with the above panel of antibodies; and staining of other cell types, such as macrophages and Langerhans cells. Infiltrative host response was defined as the permeation of lymphocytes among tumor cells. The noninfiltrative host response was defined as lymphocytes beneath or around the tumor but not permeating between tumor cells. Partial regression represents an area within a tumor characterized by fibroplasia, melanophages, and a lymphocytic infiltrate (5).

The material studied included 8 primary melanomas. All of these were of the superficial spreading type (5), and 5 of them included areas of partial regression in the frozen section block. In addition, we studied 8 metastatic lesions (2 cutaneous, 4 nodal or s.c., and 2 visceral), 3 dysplastic nevi, and 2 common, acquired i.d. nevi (6).

RESULTS

The semiquantitative analysis was generally reproducible between the 2 observers, but these data are not reported in detail here. As we accumulate more cases, we anticipate that formal analysis using quantitative point counting methodology will be required to demonstrate statistically significant differences among subsets of melanoma cases, should such differences exist. In this present material, some conclusions were readily apparent to both observers at the qualitative level. These observations form the subject matter of this report (Table 1).

PBS controls were, for the most part, negative. One case showed granular cytoplasmic staining of sweat duct epithelium in PBS and all antibody stains. This was presumably nonspecific due to endogenous peroxidase. Sweat gland and duct staining was seen in a few other control and experimental sections. A
few control sections showed occasional cytoplasmic staining of small round apparently lymphoid cells. Membrane staining was not observed. The tissue sections stained with antibody also serve as internal negative controls, since all sections included a population of negative lymphocytes. In particular, the B1 antibody stained only a small proportion of lymphocytes in every case studied. Lymph node-positive controls showed the patterns characteristic of the particular antibody (1, 16).

All 8 primary melanomas demonstrated a prominent lymphocyte response. These lymphocytes were virtually all T11 positive (Figs. 1 and 2). Only rare cells stained with B1.

Both T4- and T8-positive lymphocytes were present in all cases in the noninfiltrating component of the host response. In 3 of 8 cases, these lymphocytes were predominantly T4 positive. In the other 5 cases, the proportion of T4- and T8-positive lymphocytes was approximately equal. Among the infiltrating lymphocytes, T8-positive cells clearly predominated in 2 cases, whereas in the remaining cases, the proportion was again approximately equal.

In areas of regression, T4-positive cells clearly predominated in 2 of 5 cases. In one case, T8-positive cells predominated. In the other cases, T4- and T8-positive cells were approximately equally represented.

In all metastatic lesions, a lymphocytic response was noted, although it was usually sparse. One dermal metastasis had a brisk lymphocytic response. Virtually all lymphocytes stained with T11, while B1 stained only rare cells. The majority of cases contained both T4- and T8-positive lymphocytes. However, one case had lymphocytes stained with T4 and not T8; one case had cells stained with T8 and not T4.

An additional finding was that the T4 antibody stained melanoma cells in 2 of the metastatic lesions and that B1 antibody stained one of these 2 lesions. The staining of individual cells was diffuse and cytoplasmic in contrast to the surface staining that was observed in positive lymphocytes (Fig. 3). The proportion of positive tumor cells ranged from 10 to 50%. No cell types other than tumor cells in frozen sections of skin reacted with T4 antibody. The tumor cells were negative for T11 and T8. A PBS control was also negative. All other metastases and all primary lesions were negative for tumor staining with T11, T4, T8, and B1 antibodies. Necrotic areas of tumors, however, appeared diffusely positive with all antibodies but negative with PBS control. This is consistent with nonspecific sticking of the antibody to necrotic tissue.

The dysplastic nevi demonstrated a mild host response consisting of T11-positive lymphocytes with rare B1-positive cells. The T4-positive cells tended to predominate over T8, although both types were present in all cases. The i.d. nevi contained rare T4-positive lymphocytes as well as rare T4- and T8-positive cells. No B1-positive cells were identified.

**DISCUSSION**

Using monoclonal antibodies and an immunoperoxidase system, we have demonstrated in situ the phenotype of lymphocytes responding to malignant melanoma and nevi. In accord with other in situ studies (3, 9, 18, 19), our investigation finds that virtually all the lymphocytes responding to melanoma are T-cells. The host response to dysplastic nevi is similar, although less brisk. With nuclear counterstaining, the relationship of the T-cells to lesional cells can be appreciated. Some of the individual T-cells can clearly be seen to infiltrate among melanoma cells or dysplastic nevus cells. The band-like accumulation of noninfiltrating lymphocytes in the dermis beneath primary melanomas and dysplastic nevi also stains intensely with T11 antibody. T11-positive cells also comprise nearly all the lymphocytes in areas of regression.

B1 antibody recognizes an antigen specific for B-cells at various stages of differentiation. This marker is lost when the antigen-stimulated B-cell acquires cytoplasmic IgG, and it is not present on plasma cells (1). Thus, a role for mature B-cells in the host response is not ruled out by our observations. Indeed, plasma cells are observed in the host response to some melanomas (5). In this material, however, there were few responding lymphocytes that did not stain for the T11 antigen.

This predominance of T-cells has been noted in the host response to other solid tumors using other techniques (3, 9, 20, 21). This consistent preponderance of T-cells in lymphocytic responses to tumors emphasizes the apparent importance of cell-mediated immunity to solid tumors.

Using monoclonal antibodies to T-cell subsets, we further investigated the nature of the lymphocyte response. In all cases of primary melanoma and 6 of 8 metastases, both T4- and T8-positive lymphocytes are present. It has been postulated that T4-positive cells are necessary for the production of lymphokines...
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in response to antigen (12), for the induction of precursor cytotoxic T-cells to become cytotoxic (7), and for the generation of suppressor T-cells (28). Recently, it has been demonstrated that, with the appropriate target cells, a subset of T4-positive lymphocytes may be cytotoxic as well (13). Furthermore, within the T4-positive population, cells with suppressor function have been identified (22). The T8-positive lymphocytes include cells with both suppressor and cytotoxic functions (16).

In view of these multiple interactions between T4- and T8-positive cells, it is not surprising that both populations are usually present in the host response to melanoma. Perhaps, a preponderance of one or the other type represents a benefit or detriment to the host and thus might correlate with prognosis. We plan to evaluate this possibility with a larger series of patients followed by the Pigmented Lesion Group. Functional studies can further elucidate the host response, particularly the important question of whether T8-positive cells are functioning in a cytotoxic or a suppressor role. However, those few studies that have attempted to evaluate the functional status of lymphocytes eluted from solid tumors have produced contradictory results. In one study, tumor-infiltrating lymphocytes from some lesions had cytotoxic activity (26), whereas, in other studies, tumors did not contain infiltrating cytotoxic cells (23, 25). A recent functional study of infiltrating lymphocytes in melanoma did demonstrate tumor-specific and tumor-nonspecific cytotoxic T-cells. The roles of natural killer cells (8) and monocytes also remain to be considered. We have preliminary data, not presented here in detail, from a few cases to suggest that the natural killer cell as identified by monoclonal antibody B73.1 (14) comprises only a very small proportion of tumor-associated lymphocytes. A monocyte marker (anti-human monocyte 0.2; Bethesda Research Laboratories, Gaithersburg, Md.) similarly was present on only a small number of mononuclear cells in addition to the obvious pigment-laden melanophages that are present in the majority of melanomas.

An unexpected finding was the staining of melanoma cells with T4 antibody (2 cases) and with B1 antibody (one case). This staining was cytoplasmic and diffuse in contrast to the surface staining of lymphocytes with these antibodies. A PBS control and sections stained with other antibodies (T11 and T8) were negative for melanoma cell staining. The significance of this finding is unclear.

In summary, the lymphocytic response to malignant melanoma and dysplastic nevi consists primarily of T-cells of both the helper:inducer and suppressor:cytotoxic phenotypes. In situ identification of cell surface antigens in conjunction with functional studies will permit further elucidation of the host response to melanoma, to melanoma precursors, and to other solid tumors as well.

REFERENCES


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Fig. 3. a, metastatic melanoma, control section stained as in Fig. 1a. Note large pleomorphic melanoma cells, some containing dusty melanin pigment (arrow). × 270.
b, same lesion stained with T4 antibody. Scattered tumor cells react positively. Red AEC pigment is readily distinguished from brown-green melanin in original sections. No lymphocytes are present in this field. × 270.

Fig. 1. a, control section of malignant melanoma, sectioned in cryostat and stained with PBS, biotinylated anti-mouse antibody, avidin:biotinylated peroxidase complex, and AEC. Note epidermis at top, tumor in center, and lymphocytic response at base. × 68. b, adjacent section of lesion shown in a, stained in the same manner but with addition of primary antibody to T11. Note dark staining of small lymphocytes. Scattered lymphocytes (center) are infiltrative among tumor cells; dense response at base of tumor is noninfiltrative. Epidermis (top) and tumor cells are negative (granular layer of epidermis is blue in original section). × 68.

Fig. 2. a, high-power view of base of control section (see also Fig. 1a). Note large tumor cells with unstained infiltrative and noninfiltrative lymphocytes. × 270. b, base of section shown in Fig. 1b. Note membrane staining of small lymphocytes with T11 antibody. Red AEC dye is even more prominent in color image, and it is readily differentiated from brown-green melanin pigment. × 270.
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