Natural Killer Cells in the Host Response to Melanoma

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ABSTRACT

Using an immunoperoxidase technique, we have investigated natural killer (NK) cells in the host response to malignant melanomas and melanocytic nevi in frozen sections. Eight primary melanomas, 12 metastatic melanomas, and 31 dysplastic nevi were studied. NK cells were identified phenotypically using an antibody, B73.1, against an Fc receptor present only on NK cells and neutrophils. Rare NK cells were identified in three of 31 dysplastic nevi and in one of eight melanomas. In contrast, significant numbers of NK cells were identified in ten of 12 metastases.

INTRODUCTION

NK3 cells are thought to be important in the host defense against tumors, including melanomas (1). The NK cell is able to kill tumor cells spontaneously, but without prior sensitization. The cell is postulated to be involved in immune surveillance and in the prevention of metastases. There is circumstantial evidence to support a role for NK cells in the response to melanoma. Hersey et al. (2) found that melanoma patients with high NK activity in the peripheral blood were significantly less likely to have recurrent local disease or metastases than patients with low NK activity. The NK cell can now be identified phenotypically by reactivity with a mouse monoclonal antibody, B73.1. This antibody is directed against an Fc receptor which is present only on NK cells and neutrophils (1).

In our study of the host response to melanoma, we have used this antibody to identify phenotypic NK cells for dysplastic nevi as well as primary and metastatic melanomas. Previously, we have reported the in situ identification of T-cells and T-cell subsets in these lesions (3).

MATERIALS AND METHODS

Fresh tissue was obtained from patients at the Pigmented Lesion Clinic at the Hospital of the University of Pennsylvania. Specimens consisted of 8 primary melanomas, 12 metastatic melanomas, and 31 dysplastic nevi, which were all confirmed histologically by previously described criteria (4). Tissue was frozen in liquid nitrogen and stored at —70°C. Frozen sections were acetone fixed and stained using the peroxidase-antiperoxidase immunoperoxidase kit obtained from Ortho Diagnostics (Raritan, NJ). Primary mouse monoclonal antibodies used were B73.1 (kindly provided by Dr. G. Trinchieri, Wistar Institute) and OKT11 (Ortho Diagnostics), or 13-17, an antibody against DR antigen (5), as technical controls. Sections were incubated with the primary antibody for 30 min at room temperature, rinsed in phosphate-buffered saline (pH 7.4), and incubated with secondary rabbit anti-mouse antibodies, followed by phosphate-buffered saline wash and the peroxidase-antiperoxidase reagent. Finally, sections were incubated with 2-aminochrome carbozole with 0.03% hydrogen peroxide, counterstained with hematoxylin, and mounted with Gelvatol (Monsanto, St. Louis, MO). For negative control sections, the primary antibody was omitted. For positive controls, cytospin preparations of peripheral blood mononuclear cells were frozen at —70°C and then stained with the frozen sections.

RESULTS

Dysplastic Nevi and Primary Melanomas. Sections of dysplastic nevi and primary malignant melanomas always contained a lymphocytic infiltrate of variable intensity, as previously reported (3). Rare NK cells were identified in 3 of 31 dysplastic nevi and in 1 of 8 primary melanomas. In contrast, numerous T11- and/or DR-positive lymphocytes comprised nearly all of the dermal infiltrate. In positive control slides, approximately 10% of peripheral blood lymphocytes were B73.1 positive. These positive cells were relatively large, with evident cytoplasmic granules. In negative control sections, only endogenous peroxidase activity was noted in rare neutrophils.

Metastatic Melanoma. Sections of metastatic melanomas contained, in general, only sparse T11- and/or DR-positive lymphocytes. However, significant numbers of B73.1-positive cells were identified in 10 of 12 metastases (Fig. 1). In these lesions, B73.1-positive cells were intimately admixed with tumor cells and were mononuclear with a lymphoid appearance but with prominent cytoplasmic granules. These cells did not have the polyclonal pattern of granulocytes; neutrophils were not observed in the tumor on alternative sections stained with hematoxylin-eosin. In a lymph node metastasis, the B73.1-positive cells were seen within the tumor with virtually no positivity in the rest of the nodal tissue. While most of the metastases contained at least a few characteristic lymphocytes, these were seen, in general, at the periphery of the tumor masses. By contrast, the B73.1-positive cells located within the tumor masses were intimately admixed with the tumor cells, and they were difficult or impossible to distinguish from fibroblasts, endothelial cells, and/or macrophages in sections not stained with the specific antibody (Fig. 1). In some lesions, the positive cells are quite numerous; overall, ratios of responders to tumor cells ranged from 1 of 20 to 1 of 3.

DISCUSSION

An unexpected finding, seen clearly in only 1 of the 12 metastatic melanomas, was reactivity of the tumor cells themselves with the B73.1 antibody. In some other cases, it was difficult to rule out the possibility that a few tumor cells were stained. However, the majority of reactive cells had small nuclei and granular cytoplasm, quite different from the tumor cells, and consistent with known NK cell morphology. Our observation that NK cells are not prominent at the site of primary tumors or precursor lesions is in accord with functional studies which found virtually no NK activity among infiltrating lymphocytes (6) in various tumors. A few functional studies have dealt specifically with melanomas. NK cell activity was not found in a primary melanoma studied by Klein et al. (7) or in metastatic melanomas studied by Burns et al. (7) (cases) (8) and Vose et al. (1 case) (9).

Herberman has argued that, if NK cells are important in defense against primary tumor growth, then they are likely to
NK CELLS IN MELANOMA

Fig. 1. Frozen section of a metastatic melanoma demonstrating scattered B73.1-positive cells. Tumor cells are not apparent in this photograph due to lack of counterstain. This section was not counterstained in order to highlight B73.1-positive cells for illustrative purposes. × 400. Inset, higher power photograph demonstrating staining pattern of B73.1-positive cell. × 1000.

act at an early stage before clinically detectable malignancy arises (10). However, cells of the NK phenotype were virtually absent in dysplastic nevi, a potential melanoma precursor and a marker of risk (4, 11) for melanoma. While it is possible that NK cells in situ might not have typical surface markers, the evidence favors the hypothesis that few, if any, NK cells are present at the site of primary melanomas or dysplastic nevi, despite the fact that a lymphocytic infiltrate is regularly seen in these lesions (4). Similarly, in a study of several solid tumors other than melanomas, Pizzolo et al. (12) found virtually no phenotypic NK cells infiltrating solid tumors. If indeed NK cells are important in tumor defense, they presumably exert their influence at a site other than the primary tumor mass.

NK cells may be more important in the peripheral blood, where they circulate in significant numbers (1), or at sites of metastases. In a functional study, Eremin detected NK cell activity in nontumorous lymph nodes draining breast and colon cancers, but not in the primary tumors (13). Recently, in a flow cytometric analysis, Morton et al. reported increased numbers of phenotypic NK cells (Leu 7 to 11+) in axillary lymph nodes from patients with Stage II breast cancer (nodal metastases present) compared to nodes from those with Stage I disease (negative nodes) (14). In the present study, we have identified striking numbers of phenotypic NK cells within metastatic melanomas. Thus, perhaps NK cells have a role in control of hematogenous dissemination and growth of metastases. More functional studies are now needed to examine NK cell activity within metastatic lesions.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Georgio Trinchieri for his helpful suggestions.

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