CAM 5.2 Positive Cells in the Epidermis of Nevus Sebaceus

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Background: In the course of the study of keratin expression in the epidermis of nevus sebaceus, several cells in the epidermis of nevus sebaceus were positively stained with CAM 5.2 antibody, which is known to be specific for the lower molecular weight cytokeratin and used as a marker of Merkel cell.

Objective: This study was intended to verify that CAM 5.2 positive cells found in the epidermis of nevus sebaceus are Merkel cells and to understand the meaning of CAM 5.2 positive cells in the epidermis of nevus sebaceus.

Methods: The immunohistochemical stainings with CAM 5.2 and antibody to epithelial membrane antigen (EMA) performed on specimens of normal skin, epidermal nevus, nevus sebaceus and some appendage tumors. In order to confirm the nature of CAM 5.2 positive cells, the distribution of those were compared to that of Merkel cells and double labeling with CAM 5.2 and neurofilament was performed.

Results: CAM 5.2 positive cells were also found in trichilemmoma developed associated with nevus sebaceus and the epidermis of normal palmoplantar skin. CAM 5.2 positive cells were also stained with antibody to EMA on serial sections cut from the same tissue blocks. The association of CAM 5.2 positive cell and nerve fiber was also demonstrated.

Conclusion: CAM 5.2 positive cells are seemed to be Merkel cells and their presence in the covering epidermis of nevus sebaceus suggests to the epidermis of nevus sebaceus may not be nevoid proliferation of epidermal keratinocytes. (Ann Dermatol 5:(1) 5–8, 1993)

Key Words: CAM 5.2, Merkel cell, Nevus sebaceus

Nevus sebaceus shows various morphological and histological changes with the increase of the age of lesion, eventually the lesion develops large numbers of sebaceous glands and papillomatous hyperplasia of the covering epidermis[1]. In addition to those changes, various types of appendage tumors can develop secondarily. About the later phenomenon, those tumors are known to have some differences from their counterparts which are developed primarily and are not thought to be true neoplasms, but hamartomas of primary epithelial germ cells[2]. For the papillomatous hyperplasia of the covering epidermis, it may be different from nevoid proliferation of epidermal keratinocytes like epidermal nevus also. Epidermal proliferation in the epidemis of nevus sebaceus often contains glycogen and which suggested that the covering epidermis of nevus might not consist of epidermal keratinocytes, but of outer root sheath cells[3]. In the course of the study of keratin expression in the epidermis of nevus sebaceus, we found several CAM 5.2 positive cells in the epidermis of nevus sebaceus. CAM 5.2 is the murine monoclonal antibody for the lower

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molecular weight cytokeratin and known to be a useful marker for Merker cell[4-8]. We thought these cells might be Merkel cells and have examined the presence of CAM 5.2 positive cells in the epidermis of nevus sebaceus, epidermal nevus, and some appendage tumors. Immunohistochemical staining with antibody to epithelial membrane antigen (EMA), another possible marker of Merkel cell has also been performed.

MATERIALS AND METHODS

Tissues. Thirty two cases of nevus sebaceus, 3 cases of epidermal appendage tumors which had developed from nevus sebaceus (1 trichilemmoma, 1 tumor of follicular infundibulm, 1 sebaceous epithelioma), and 5 proliferating trichilemmal tumors were collected for the files of Seoul National University Hospital, department of pathology. For the control, 5 specimens of epidermal nevus and 20 specimens of normal skin from various sites were also included. The details of specimens included are listed in table 1. All tissues had been fixed in formalin and then paraffin embedded according to conventional procedures.

Immunohistochemical study. The immunoperoxidase staining was carried out on sections cut from the same tissue blocks employing avidin-biotin-peroxidase complex (ABC) technique[9]. Monoclonal murine antibody. CAM 5.2 (Becton-Dickinson) and polyclonal rabbit antibody to EMA (Dako) were used. To confirm the specificity of CAM 5.2 to Merkel cells, double labeling with CAM 5.2 and antibody to neurofilament (Dako) was performed. When staining two antigens simultaneously, we used only ABC technique. CAM 5.2 was applied first and after washing with phosphate-buffered saline, second antibody, anti-neurofilament was applied. For color reaction, 3-3'-diaminobenzidine tetrahydrochloride was used with Mayer's hematoxylin counterstain.

Table 1. Profiles of specimens studied

Nevus sebaceus	: 32
Epidermal nevus	: 5
Normal skin	
Scalp	: 5
Palm & sole	: 5
Others	: 10
Appendage tumors	
Trichilemmoma*	: 1
Tumor of follicular infundibulum*	: 1
Sebaceous epithelioma*	: 1
Proliferating trichilemmal tumor	: 5

* developed associated with nevus sebaceus

RESULTS

Normal skin. There were no CAM 5.2 or anti-EMA positive cells in the epidermis of normal skin from various sites other than palmoplantar skin. In adult palmoplantar skin, several CAM 5.2 positive cells were found at the basal layer of the epidermis. In adult scalp skin, CAM 5.2 positive cells were located at the outer root sheath of hair follicle from the level of isthmus to follicular opening (Fig. 1). Double labeling with CAM 5.2 and anti-neurofilament antibody demonstrated that CAM 5.2 positive cells were associated with nerve fibers (Fig. 2). In all specimens, staining cells with CAM 5.2 and anti-EMA were generally correlated. The staining pattern of CAM 5.2 was cytoplasmic, wherease that of anti-EMA was membranous.

Epidermal nevus. There were no CAM 5.2 or anti-EMA positive cells in tissue specimens obtained from epidermal nevus.

Nevus sebaceus. In some lesions of nevus se baceus, several CAM 5.2 positive cells were found in the basal layer of the epidermis, follicular infundibulum, and basal layer of immature hair follicles connected with the epidermis (Fig. 3A, B). These cells appeared more frequently in the lesions of nevus sebaceus from the patients older than 14 years of age. They were found in 35% of all 32 nevus sebaceus lesions and in 50% of 22 nevus sebaceus lesion from the patients older than 14 years of age.

Appendage tumors. At the margins of trichilemmoma (Fig. 4) and tumor of follicular infundibulum developed associated with nevus sebaceus, many basal cells were stained with CAM 5.2 but those of sebaceous epithelioma developed associated with nevus sebaceus and proliferating trichilemmal tumors were not.

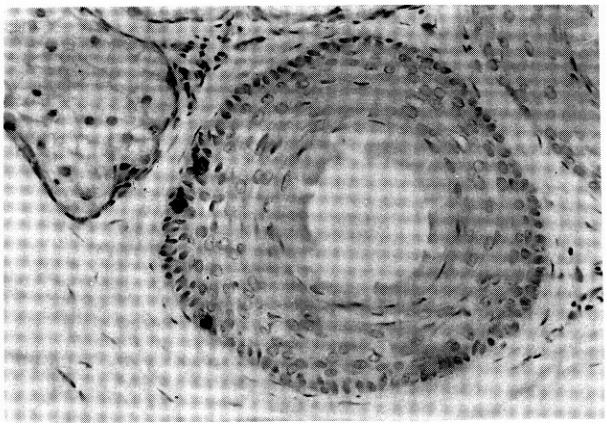

Fig. 1. Immunoperoxidase staining of transverse section of normal scalp skin. CAM 5.2 positive cells are located in the outer root sheath of isthmic portion of hair follicle (×200).

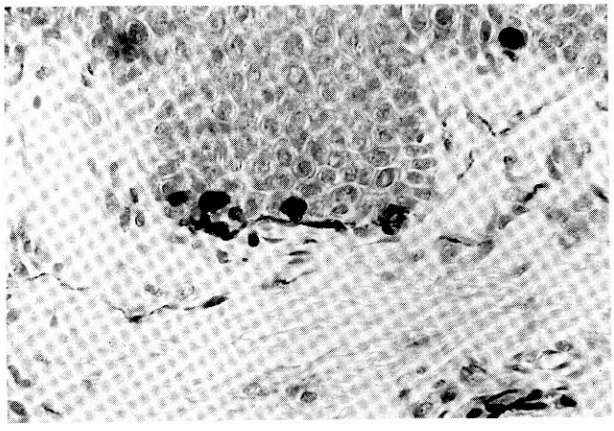

Fig. 2. Double labeling with CAM 5.2 and anti-neurofilament antibody of normal plantar skin. Fine nerve fibers are in contact with CAM 5.2 positive cells (×100).

DISCUSSION

Morioka[3] has shown that the epidermis of nevus sebaceus often contains glycogen positive cells. In that report, he suggested that by about 38% the covering epidermis of the lesion of nevus sebaceus consisted not of epidermal keratinocytes but of out root sheath cells. He also insisted that more than half of nevus sebaceus lesions with epidermal hyperplasia were essentially due to trichilemmoma composed of clear cells with rich coarse glycogen granules. In order to prove his hypothesis, the authors have tried immunohistochemical staining of the lesion of nevus sebaceus with various kinds of antibodies. In the course of the study of keratin expression in the epidermis of nevus, we found several CAM 5.2 positive cells in the epidermis of nevus sebaceus. CAM 5.2 is a murine monoclonal antibody which recognizes lower molecular weight cytokeratin proteins within secretory epithelia[4].

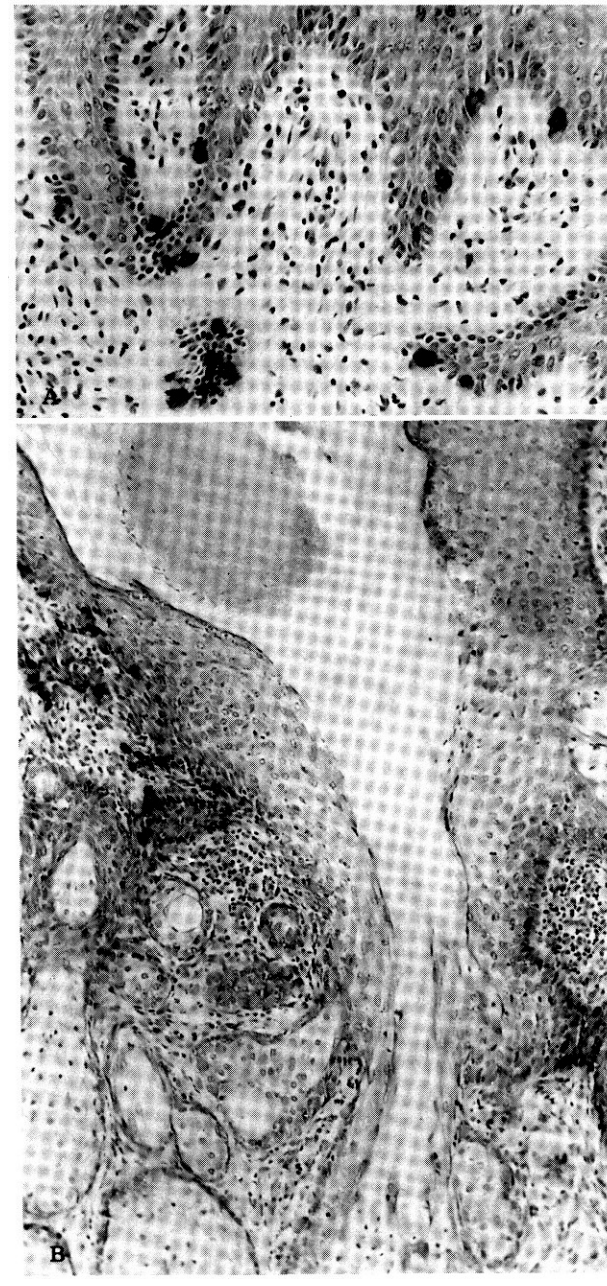

Fig. 3. Immunoperoxidase staining of nevus sebaceus with CAM 5.2. A) Several basal cells are positively stained with CAM 5.2 (×200). B) CAM 5.2 positive cells are crowded in the follicular infundibulm and immature hair follicles connected with the epidermis (×100).

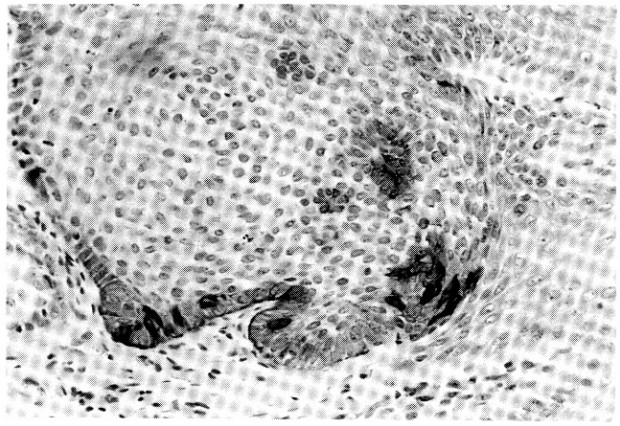

Fig. 4. In trichilemmoma occurred in the lesion of nevus sebaceus, CAM 5.2 positive cells are found at the margin of tumor nests (×200)

Merkel cell has the unique cytokeratin polypeptides nos. 8, 18 and 19 which are typical of diverse simple epithelia of the human body not present in epidermal keratinocytes[5]. Several antibodies specific for cytokeratins of Merkel cell have been used for the study of distribution of Merkel cells during embryogeneis[6,7]. Narisawa et al[8] demonstrated that CAM 5.2 positive epidermal cells are indeed Merkel cells by identifying Merkel cell granules and CAM 5.2-positive keratin filaments in such cells using the immunogold technique at the ultrastructural level. So we thought that CAM 5.2 positive epidermal cell in nevus sebaceus might be Merkel cell. In order to confirm that those CAM 5.2 positive epidermal cells were really Merkel cells, we performed immunohistochemical staining with antibody to EMA, another marker of Merkel cell not only in the lesion of nevus sebaceus but in the skin of palm and sole, and the scalp. In some lesions of nevus sebaceus, the epidermis of palmoplantar skin, and folliullar epithelium of the scalp, CAM 5.2 positive cells were also stained with antibody to EMA on serial sections cut from the same tissue blocks. Double labeling with CAM 5.2 and anti-neurofilament antibody demonstrated that CAM 5.2 positive cells were associated with nerve fibers. From these results, we regard CAM 5.2 positive epidermal cells in the lesion of nevus sebaceus as Merkel cells. The significance of Merkel cell in the epidermis of nevus sebaceus is uncertain. In mammals, Merkel cells are found mostly in the outer root sheath of hair follicles and the epidermal touch corpuscles. We also found Merkel cells in the isthmic portion of hair follicles and the epidermis of palmoplantar skin. In our study, another interesting finding was demonstrated in trichilemmoma and tumor of follicular infundibulum which were developed associated with nevus sebaceus. Many Merkel cells were found at the periphery of two tumors which are known to differentiate to outer root sheath cells. The presence of Merkel cells in the epidermis of nevus sebaceus and outer root sheath cells derived tumors might provide a clue to the nature of the epidermal change of nevus sebaceus lesion from the patients after childhood. At least, our result suggests that the covering epidermis of nevus sebaceus should be biologically different from that of epidermal nevus.

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