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Acquired Pigmented Macules in Human Piebald Lesions. Ultrastructure of Melanocytes in Hypomelanotic Skin

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Two cases of piebaldism are reported. The first patient was a 9-month-old girl with inborn hypopigmented areas on the frontal region of the scalp and both knees. There were no melanocytes in the lesions. In the second case, we observed the patient from 2 months of age for a period of 9 years. Many hyperpigmented spots appeared on the hypomelanotic areas on the frontal region of the scalp, abdomen and both knees. Electronmicroscopic examinations of the hypomelanotic skin disclosed an area with regularly distributed melanocytes as well as an area with no melanocytes. Most of the melanosomes were ellipsoidal and lamellar. They were in stage II to III, which signified delayed pigmentation. Hyperpigmented spots were slightly enlarged following PUVA treatment.

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Piebaldism is a rare, autosomal dominant inherited disorder, characterized by inborn hypopigmented skin and hair. The lesions are usually found on the forehead, ventral aspect of the trunk, and the extremities. Accepted knowledge of this disease is that white macules and hair do not change throughout the life time and that there are no melanocytes in the affected skin (1). Herein, we report 2 cases of piebaldism, in one of which we found the spontaneous appearance of hyperpigmented spots and the presence of well-distributed melanocytes in piebald skin.

CASE REPORTS

Case 1

A 9-month-old girl was brought to the dermatological department of Osaka City University Hospital with a problem of white skin and hair on the frontal region of the scalp and on both knees. In the hypopigmented area there were hyperpigmented spots. No macroscopic changes of the hypomelanotic skin were seen during next 3 years. An electronmicroscopic study failed to detect any melanocytes in the hypomelanotic skin.

Case 2

A full-term female baby was noted at birth to have depigmented lesions on the forehead, abdomen and knees. Six months later, in the absence of therapy, pigmented macules appeared on the knees (Fig. 1). Over the next few years, the pigmented macules gradually increased in size and number (Fig. 2). Light brownish macules also appeared on the depigmented lesions on the abdomen and forehead.

An electronmicroscopic study of hypopigmented skin in the left knee disclosed regularly distributed melanocytes (seven melanocytes out of 50 basal keratinocytes). Most of the melanosomes were in stage II to III (Fig. 3), and a small number of stage IV melanosomes were recognized in some of the dendrites. They were ellipsoidal in shape and had regular lamellar structures, but a few were irregular in shape and had irregular melanization. Several melanosomes were found disintegrating in a limiting membrane in one of the dendrites

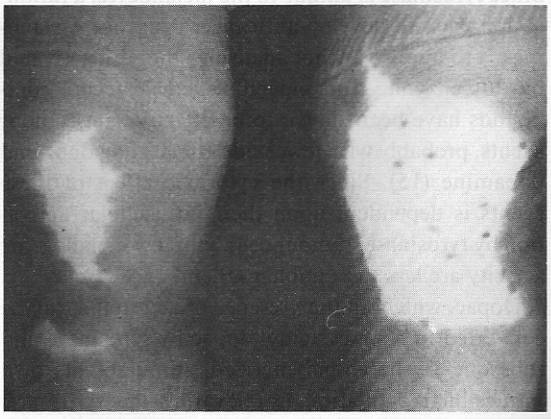

Fig. 1. Case 2 (6 months old). Pigmented macules appeared on the leukoderma, there being no such spots at birth.

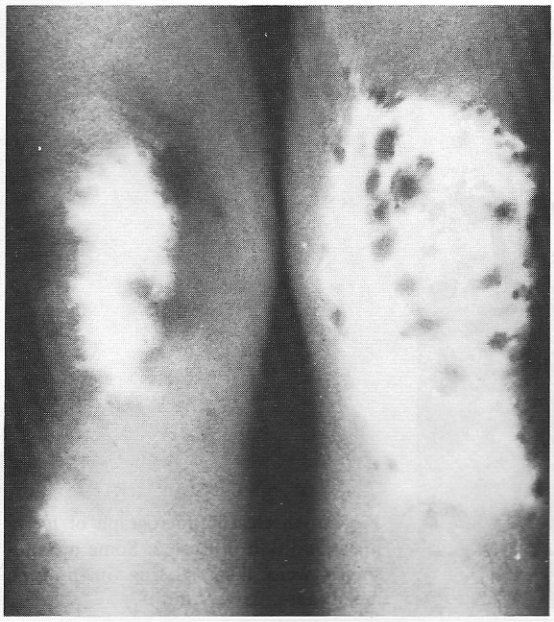

Fig. 2. Case 2 (9 years old). Pigmented spots increased in size and number, which reduced the size of the hypomelanotic area and made the margins irregular.

(Fig. 4). No melanosomes were noted in keratinocytes. In the hyperpigmented skin, there were also regularly distributed melanocytes, most of whose melanosomes were irregular in shape.

The presence of melanocytes in the leukoderma encouraged us to conduct topical administration of 8-methoxypsoralen with UV-A irradiation (PUVA therapy), which slightly reduced the size of the hypopigmented area. After the PUVA therapy, another skin sample from the hypopigmented skin was examined with an electron microscope. However, there were no melanocytes in the skin sample.

DISCUSSION

It has been generally believed that the hypopigmented area in piebaldism does not change throughout life (1). However, there are several reports of the contraction of the piebald skin and/or of the appearance of pigmented spots, especially on sun-exposed areas. All of these reporters had observed their patients for a period of years, from infancy or early childhood. Pearson examined a 4-year-old patient until 8 years of age (2). Davis & Verdol observed a 4-day-old baby girl for 4 years, compared a picture of her father at the age of 7 with a picture at age 27, and found contraction and expansion of the white skin in both cases (3). The white forelock of a 4-month-old female baby, reported by Hori, became obscured within several months (4). Ishii et al. examined 2 cases: a 16-month-old child for 2 years and a baby at birth for 4 years. They reported that hyperpigmented spots appeared on the piebald skin of both patients (5).

In the present study, we followed the second case for 9 years, from 2 months after birth, and have clearly demonstrated acquired pigmented macules. There are more reports of Japanese patients with

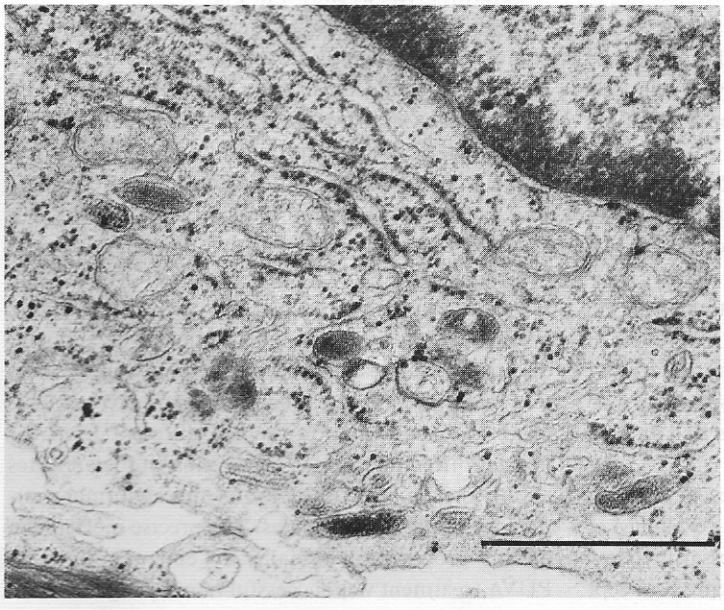

Fig. 3. An electronmicrograph of a melanocyte in hypomelanotic skin of Case 2. Most of the melanosomes were ellipsoidal in shape and in stage II to III, and have inner lamellar structures. Bar = 1 μm.

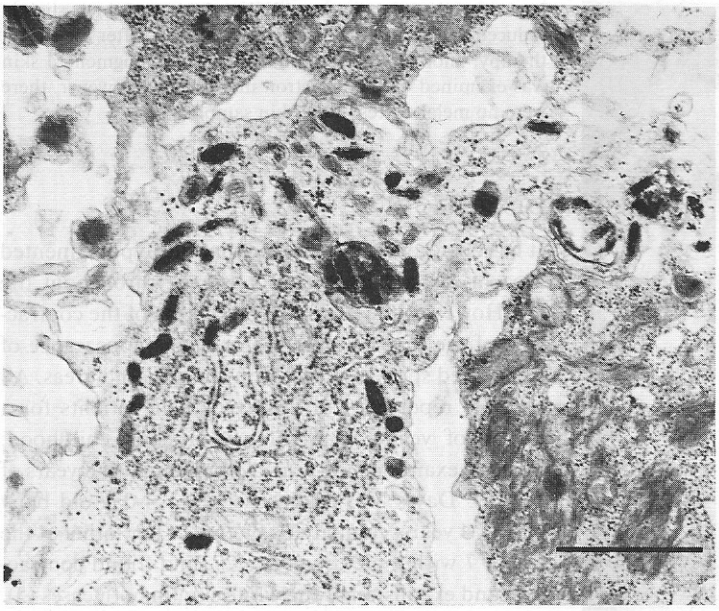

Fig. 4. An electronmicrograph of hypomelanotic skin in Case 2. Some melanosomes were disintegrating in limiting membranes. Bar = 1 μm.

piebaldism than of Caucasians. The lesions in the latter are probably not so distinct and can easily be overlooked, especially in infancy. It is therefore possible that the appearance of the hyperpigmented spots on piebald skin is more common with long and careful observation.

It is generally agreed that piebald skin is devoid of melanocytes, with only one exception, which contained only a single melanocyte (6). Although many electronmicroscopic studies have so far failed to detect melanocytes in piebald skin (7–10), there are three reports in the Japanese literature of the presence of DOPA-positive melanocytes in hypomelanotic skin or a hair bulb (4, 11, 12). Hayashibe & Mishima have recently reported an almost regular distribution of melanocytes in 3 piebald patients, by employing the split DOPA reaction technique and immunohistochemical study with monoclonal antibody (A4F11), which reacts to melanocytes (13).

In the first case in the present study, there were no melanocytes in an area of piebald skin. However, in the second case, even though there was an area of regularly distributed melanocytes, we would like to stress that in the same subject there was also an area devoid of melanocytes.

There is increasing evidence of the presence of melanocytes in piebald lesions. However, the existence of the subtypes in piebaldism does not necessarily follow (14). It seems to be explained by the accepted concept of the pathogenesis of this disorder; failure of melanoblasts either to migrate into the skin or to differentiate into melanocytes. Melanocytes may be present in hypomelanotic skin, but they are still immature and are not active enough to produce visible melanin. The grade of their maturation or differentiation seems to differ from one melanocyte to another. One of the steps that immature melanocytes must overcome may be the disturbed ability of transferring melanosomes to keratinocytes, as shown in Fig. 4.

Although Jimbow et al. suggested that spherical granular melanosomes are characteristic in hypomelanotic lesions of this disorder (6), we have found, in the second case, that typical ellipsoidal lamellar melanosomes were dominant and that there were a few irregularly shaped non-lamellar melanosomes. There were no characteristic morphological features of melanocytes or melanosomes in this patient. Many abnormal (non-ellipsoidal lamellar) melanosomes were recognized in melanocytes in a hyperpigmented spot. We believe that these spots, stimulated by sunlight, occurred as the result of accelerated melanin production of the melanocytes that had been inactive. This process of pigmentation seemed different from the normal differentiation or maturation of melanocytes and/or melanoblasts, because of the abnormal morphology of the melanosomes. The precise mechanism of this process still remains an enigma. Although PUVA treatment was successful in reducing the white

macule, we do not consider PUVA to be a useful treatment, as its effectiveness was minimal.

The facts, in most reported cases, that there were no melanocytes, as in the first case in this study, and that even though there was an area with regularly distributed melanocytes in the second case, there was also an area with no melanocytes in the same lesion, suggest that the primary defect in this disorder is the absence of melanocytes in the white macule. However, melanocytes may be present and regularly distributed in a small area of a white macule of piebaldism.

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Plasmin-like Proteinase Associated with High Molecular Weight Complexes in Blister Fluid of Bullous Pemphigoid

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Blister fluid from tense bullae of 10 patients with bullous pemphigoid was investigated using a radial caseinolysis assay and zymography. Proteolytic activity, varying from 4.7 to 10 μg/ml, was found in 3 out of 10 patients, by using the caseinolysis assay. Zymography revealed that a major part of this caseinolytic activity co-migrated with plasmin standard. In addition, in the zymography, proteolytically active high molecular weight complexes were seen. This characteristic pattern was seen in the zymography of both positive and negative samples in the caseinolysis assay. These high molecular weight complexes were not seen in the zymography of the blister fluid of 2 patients with epidermolysis bullosa or in the suction blister fluid of 3 healthy control patients. These findings suggest that plasmin is generated at some phase of the blister formation, being possibly involved in the pathomechanism of bullous pemphigoid. ***Key words: Proteolysis; Zymography; Dermo-epidermal separation***

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Several studies (1, 2) have suggested that the blister formation in pemphigoid involves binding of the antibodies to the bullous pemphigoid antigen in the basement membrane which initiates complement activation. This leads to production of several inflammatory mediators and migration of inflammatory cells which release inflammatory mediators and proteolytic enzymes. The ultimate result is a subepidermal blister. This proposed model contrasts with theory of blister formation in pemphigus vulgaris, which