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CLINICAL INVESTIGATION

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Vitiligo in Vogt-Koyanagi-Harada disease: immunohistological analysis of inflammatory site

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Abstract ● Background: Vogt-Koyanagi-Harada disease (VKH) consists of uveitis with associated non-ocular symptoms, such as vitiligo or central nervous system disorders. A cell-mediated immune disorder is believed to play an important role in VKH; however, the skin lesion has not been well studied. Therefore, for this study, we examined the skin of a 56-year-old Japanese man with Vogt-Koyanagi-Harada disease (VKH). ● Methods: Skin biopsy specimens from the patient's vitiligo were obtained 1 month after the onset of ocular symptoms. Immunohistochemical analysis of the specimens study was performed using the following monoclonal antibodies: anti-T cell, anti-B cell, anti-major histocompatibility complex class II (HLA-DR), anti-T helper/inducer (CD4), and anti-T suppressor/cytotoxic lymphocytes (CD8). ● Results: Histopathologic analysis revealed mononuclear cell infiltration of the slightly edematous dermis, especially surrounding the hair follicles and sweat glands. Melanin-laden cells in the epidermis were partially lost. The infiltrating mononuclear cells consisted primarily of T lymphocytes with a smaller number of B lymphocytes. Most showed expression of HLA-DR. CD4-positive lymphocytes were dominant over CD8-positive cells (3:1). ● Conclusions: The results indicate that vitiligo of VKH is infiltrated by mostly activated helper/inducer lymphocytes and that cell-mediated immunity plays an important role in the pathogenesis of the dermal lesions of VKH as well.

Introduction

Vogt-Koyanagi-Harada disease (VKH) is characterized by uveitis with associated extraocular symptoms, such as skin lesions and central nervous system disorders [1, 4, 5]. VKH has a unique etiology and clinical symptoms; its pathogenesis, however, is not fully understood [1, 4, 5]. A viral origin for VKH has been proposed but not demonstrated [6]. Recently much attention has been focused on the possibility that VKH is an immunologic disorder and that the pathogenesis and progression of this disease involve a cell-mediated immune process [1, 3–10]. We previously reported that activated T lymphocytes are the predominant cell types in choroidal inflammation of VKH patients and that choroidal melanocytes express major histocompatibility complex class II (HLA-DR), indicating that cell-mediated immunity plays an important role in the pathogenesis of the ocular lesions of VKH [4, 5, 7, 8].

There are few studies of the skin lesions of VKH. Lymphocytic infiltration has been found in the dermis and epidermis of VKH by histological study, and recently Ariga et al. reported the presence of T lymphocytes in the corneoscleral lesion and skin of a VKH patient [7, 9]. However, the cellular element has not been studied in detail [1]. To elucidate the more detailed mechanism of the pathophysiology of skin lesions in this disease, we

studied the vitiligo of one patient with VKH. We applied an immunohistochemical analysis focusing primarily on a subset of lymphocytes which infiltrated the skin lesion and on the expression of major histocompatibility complex class II antigen.

Case report

The patient was a 56-year-old Japanese man who was seen at Kyushu University on 21 May 1990, complaining of bilateral visual disturbance. Visual acuity at the first admission was 20/20 OD and 20/40 OS. Slit-lamp examination disclosed inflammatory cells in the bilateral anterior chamber. Optic disc edema and exudative retinal detachment were noted bilaterally (Fig. 1). Cerebrospinal fluid (CSF) examination revealed pleocytosis. Audiograms revealed sensorineural hearing loss. VKH was diagnosed and treated with corticosteroids (dexamethasone 5 mg/day) administered systemically and locally beginning on 25 May. On 28 May, vitiligo with surrounding red-colored lesions appeared on his left arm, both feet, his back and the sides of his neck, with associated pruritus (Fig. 2).

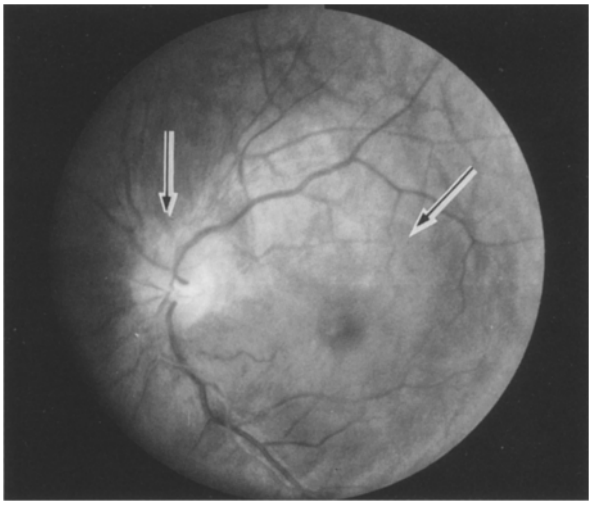

Fig. 1 Fundus of the left eye at the first admission (1 week after onset). Optic disc edema and exudative retinal detachment are observed (*arrows*)

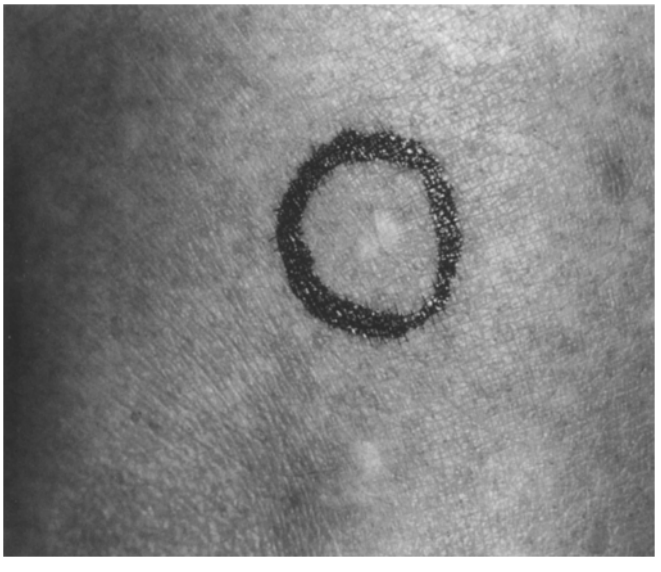

Fig. 2 Vitiligo on the left forearm at biopsy (1 month after onset)

Materials and methods

The procedures used in this study were conducted according to the ethical standards laid down in the 1964 Declaration of Helsinki. The patient was fully informed and he consented to be included in this study. When the skin biopsy was performed (1 month after the onset of ocular symptoms), dexamethasone (total 50 mg) had already been administered. Although mild inflammatory signs were present in the anterior chamber of both eyes, exudative retinal detachment had subsided and visual acuity had improved to 20/20 OD and 20/20 OS. The specimen of vitiligo from the left forearm was collected on 1June. It was divided into two pieces, one of which was frozen and the other fixed with neutral formalin using the method previously described [5, 7, 8]. The paraffin-embedded sections were stained with hematoxylin and eosin.

Paraffin-embedded sections and frozen sections were collected on lysine-coated glass slides. Immnohistochemical study was performed using the avidin-biotin peroxidase complex (ABC) method and the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. The two methods are equally sensitive. Specimens prepared with the same staining procedure were examined to quantify the CD4/CD8 ratio. The immunohistochemical study was performed by the methods described previously [5–7]. The following monoclonal antibodies and their dilution were used and were incubated for 2 h at room temperature: anti-T cell (UCHL-1, 1/100, Dakopatts, Glostrup, Denmark), anti-B cell (MB-1, 1/200, Kyowa, Tokyo), anti-HLA-DR (CR3/43, 1/25, Dakopatts), anti-T helper/inducer (CD4, Leu 3a+3b, Becton Dickinson, Franklin Lake, NJ), and anti-T suppressor/cytotoxic lymphocytes (CD8, Leu 2a, Becton Dickinson). Polyclonal antibodies for S-100 protein (rabbit 1/100, Dakopatts) were also used to differentiate Langerhans cell in the epidermis.The sections were evaluated by masked observers. Control normal skin was obtained at autopsy from a 50-year-old Japanese man who had died of lung cancer with no dermal diseases.

Results

The dermis was slightly edematous, but the epidermis did not show any edematous alteration. Lymphocytic infiltration was seen around the sweat glands, hair follicles, and vessels in the dermis (Fig. 3a). In lower parts of the epidermis, melanin granules were decreased in number and there was infiltration of mononuclear cells (Fig. 3b,c). Since most of the melanin-containing cells were present in the basal layer of the epidermis, this might reflect the accumulation of lymphocytes around the melanocytes. However, the direct contact between lymphocytes and melanocytes were not identified by the present microscopic study. Most of the lymphocytes found around the sweat glands and hair follicles were positive for anti-T cell antibody (Fig. 4a), while B

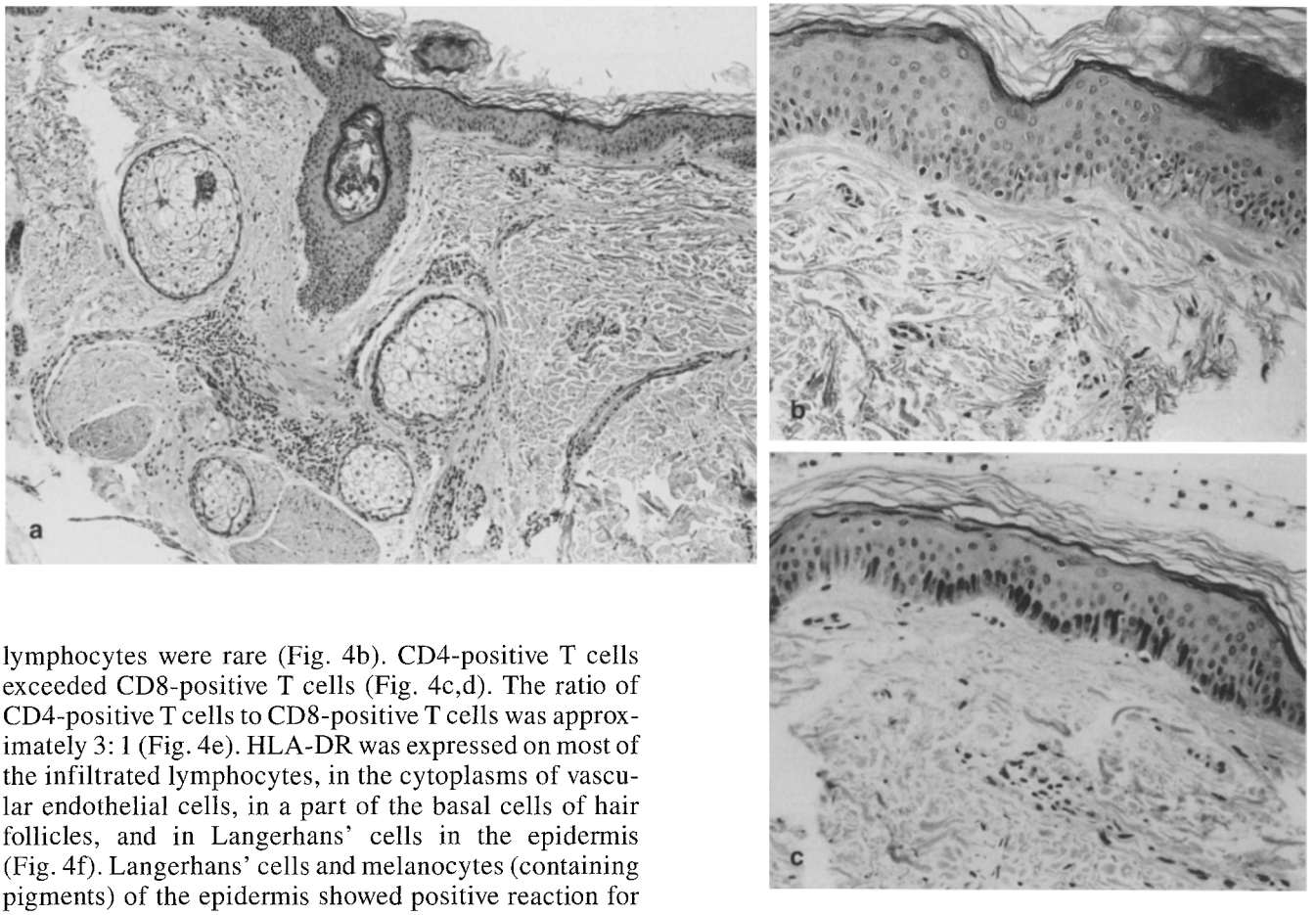

Fig. 3a–c Light microscopy picture of skin. **a** Infiltration of mononuclear cells is seen around the gland, hair follicles and vessels in the dermis. **b** Central part of the vitiligo. **c** Peripheral part of the specimen. In the lower part of the dermis, lymphocytic infiltration is noticed and pigment granules were partially lost (hematoxylin and eosin; **a** ×130, **b,c** ×330)

lymphocytes were rare (Fig. 4b). CD4-positive T cells exceeded CD8-positive T cells (Fig. 4c,d). The ratio of CD4-positive T cells to CD8-positive T cells was approximately 3: 1 (Fig. 4e). HLA-DR was expressed on most of the infiltrated lymphocytes, in the cytoplasms of vascular endothelial cells, in a part of the basal cells of hair follicles, and in Langerhans' cells in the epidermis (Fig. 4f). Langerhans' cells and melanocytes (containing pigments) of the epidermis showed positive reaction for S-100 protein antibody, but infiltrating lymphocytes in the epidermis did not show positive reaction. Apparent positive staining of HLA-DR antibody was not found on the surface of melanocytes in the skin. In control specimens, few cells showed positive reaction for anti-T cell, -B cell, -CD4, and -CD8 antibody. Langerhans cells and a few scattered cells in the skin reacted to HLA-DR antibody.

Discussion

This patient's VKH was diagnosed on the basis of cellular infiltration in the anterior chamber, exudative retinal detachment and pleocytosis in CSF. The patient's skin lesions appeared on his limbs, back, and the sides of his neck during the acute stage and the recovery stage of ocular symptoms. Partial loss of melanin-containing cells at the basal layer with associated lymphocytic infiltration was found, consistent with the previous histologic findings of VKH skin lesions [9]. Therefore, we diagnosed this patient's skin lesions as associated with VKH.

Melanocytes have been shown to be primary targets of inflammation in VKH [3–10]. In this case, lymphocytic infiltration was observed primarily around the sweat glands, the hair follicles and the upper part of the dermis, especially around the vessels, but the loss or degeneration of the melanocytes and the cell infiltration around these structures were not so prominent as that of choroidal melanocytes in our previous study [5, 7, 8]. This may be due to the fact that the present specimen was collected soon (1 month) after the onset of disease, while in the previous study, the specimen was collected 3 years after disease onset. Also the present case was at a more acute stage than that in the previous study, and may have been at too early a stage to show the severe loss of melanocytes. Another possible reason for the discrepancy is the difference in the amount of melanin or melanocytes contained in the skin and the choroid, which may result in different inflammatory reactions in the skin and in the eye.

Immunohistochemical study of this case reveals that a large proportion of the infiltrated lymphocytes are CD4-

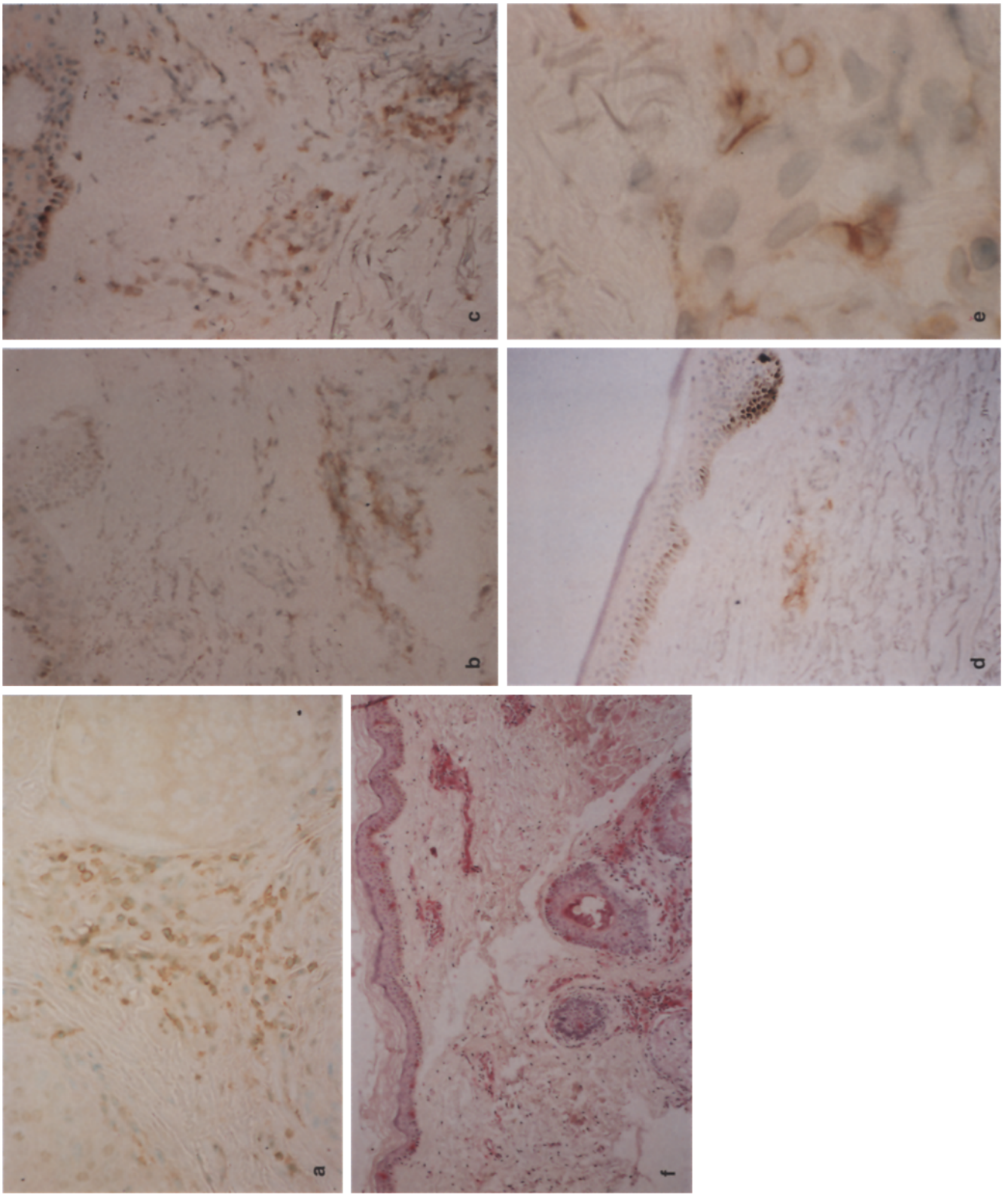
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Fig. 4a–f Immunohistochemical staining of the skin of a VKH patient. **a** Anti-T cell antibody (ABC method). Most of the lymphocytes infiltrating around the hair follicles and sweat glands are positive for anti-T cell antibody (*brown*). (×200). **b** Anti-B cell antibody (ABC method). B lymphocytes are rarely seen (*brown*) (×50). **c** Anti-CD4 antibody (helper/inducer T cells, ABC method). Most of the infiltrated lymphocytes in the dermis are positive for anti-CD4 antibody (*brown*) (×200). **d** Anti-CD8 antibody (suppressor/cytotoxic T cells, ABC method) (×200). **e** Anti-CD8 antibody (suppressor/cytotoxic T cells, ABC method). In higher magnification, ratio of CD4/CD8 was 3:1 (×600). **f** Anti-HLA-DR antibody (APAAP method). HLA-DR is expressed in most infiltrated lymphocytes, vascular endothelial cells, some basal cells of hair follicles, and Langerhans' cells in the epidermis (*red*) (×40)

positive helper/inducer T cells and that CD8-positive suppressor/cytotoxic T cells are fewer in number (ratio, 3:1). Most of the infiltrated cells are HLA-DR-positive T cells. HLA-DR is expressed on antigen-presenting cells, activated T cells or on the B cells [2]. Therefore, most of the infiltrated lymphocytes in the skin lesions in this case are activated T helper/inducer lymphocytes. In our previous study, choroidal melanocytes expressed HLA-DR and the more direct role of choroidal melanocytes in the disease progression was suggested [5, 7, 8]. In the specimen analyzed for this study, HLA-DR was apparently not expressed on the melanocytes of the skin. The primary lesions of VKH disease are in the eye, and ocular symptoms are usually noticed earlier and are more severe than the lesions of the skin [4, 6]. Therefore, it is possible that choroidal melanocytes and skin melanocytes might act differently in the progression of VKH disease. Our previous study was done on eyes at a clinically quiescent stage [5, 8], while this patient was at the early stage, just after the onset. The expression of HLA-DR might be altered in each disease stage, which could affect the results of the present study.

Chan et al. [3] reported that the predominant infiltrating cells in the choroid of VKH are T lymphocytes and that the ratio of helper/inducer to suppressor/cytotoxic cells was 3:2. Our previous reports also showed predominance of the T lymphocyte in the choroid and retina and a ratio of T helper/inducer and T suppressor/cytotoxic cells of 2:1 [5, 8]. The present findings with skin lesions of VKH disease were consistent with those of ocular lesions. The presence of T lymphocytes in a tissue does not necessarily imply that an immune response occurs and progresses. It is therefore important to clarify whether infiltrating lymphocytes were activated or not. In this study, HLA-DR was strongly expressed on the infiltrating T cells in the skin, indicating that infiltrating T lymphocytes are active and that a cell-mediated immune response may play an intimate and important role in the progression of skin lesion of VKH. These findings will contribute to a better understanding of the actual disease mechanism and to finding a better treatment for VKH disease.

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