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CONTENTS

Development of Skin Measurement Instruments

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Abstract: The observation of skin changes has long been conducted with an emphasis on the visually identifiable and palpable lesions. However, because of the limitation of sensory evaluation, efforts have been made during the last 30 years toward the introduction of various types of instrumental measurement. Skin conditions that have conventionally been categorized as normal can now be classified into various types on the basis of numerical values. Achievements from such studies are widely utilized not only for the treatment of diseases, but also in the field of cosmetics. Of particular importance is the measurement of the barrier function of the stratum corneum in the outermost layer of the skin, as well as its water content. Such measurement enables us to perform numerical assessment of skin irritation and abnormal cornification. It also facilitates the quantitative evaluation of the action of topical drugs in softening and smoothing the skin surface. Skin color, surface topography, and stiffness can also be evaluated numerically. The magnified observation of the skin assists the differential diagnosis of malignant tumors. Recent developments are enabling us to perform *in-situ* non-invasive observation of the internal structures of the skin, eliminating the need for invasive biopsy and histopathological studies to some extent.

Key words: Barrier function; Biophysical measurement; Hydration state; Instrumental measurement; Skin; Stratum corneum

Introduction

Gross observation has been and is an essential part of the examination of skin in our routine practice. Descriptive dermatology has a long history, and experienced dermatologists are usually reputed for their capability in identifying subtle changes in the patient's skin. Typically, the presence of a skin rash is obvious. A dermatologist makes a diagnosis by observing the characteristic morphology of the skin lesion, applies appropriate topical drugs, and confirms the disappearance of macroscopic lesions. The whole process of treatment

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is performed with the naked eye. The use of instruments has traditionally been limited to the use of a magnifier by presbyopic physicians and the use of a microscope for mycological examination.

However, the field of dermatology is not limited to overt pathologic changes, but also shares a number of elements with cosmetic field. Even the skin of healthy individuals shows subtle variations in tone and the degree of softness, which are very difficult to describe verbally. Needless to say, the characteristics of diseased skin are much more diverse.

When we measure various characteristics of the skin using appropriate instruments, we find that the normal skin shows wide variations that are difficult to discern with the naked eye. It is now clear that there are even "invisible dermatoses," which are conditions detected only by the use of instruments.[1)] What we see with the naked eye is not everything.

Reflecting this situation, there has been a movement toward the introduction of measurement instruments in dermatology and the development of skin biometry in the past quarter of a century. Specialized academic societies have been established and dedicated scientific journals have come into publication. Nowadays, more and more instruments are being used in our daily practice.

Measurement of Skin Surface Properties

The outermost layer of the skin consists of the stratum corneum, which functions as a barrier preventing the free passage of substances and maintains the suppleness of the skin. The stratum corneum is composed of corneocytes, which are dead epidermal keratinocytes commonly called scurf. The spaces among these cells are filled with intercellular lipids arranged in a special lamellar structure. The stratum corneum as a whole is a biologically produced membranous structure less than 20μm thick. The reason for the presence of the stratum corneum all over the human body surface is the basic need to retain water in the body living in a dry environment and warding off the invasion of microbes and other harmful substances.

As compared with the healthy skin, diseased skin looks whitish and is rough and dry to the touch. The stratum corneum of the skin showing such pathological appearance lacks sufficient barrier functions. Because it loses water to dry air and becomes hard and brittle, cracks develop on the skin surface as a result of body movement. This leads to the formation of hard, thick lumps of horny tissues called scales. From the standpoint of maintaining a beautiful, healthy skin, the moisture content of the stratum corneum is one of the major factors affecting the properties of both healthy and diseased skin, and this tissue is considered to be one of the most important targets of instrumentation in dermatology. In addition, this measurement is important to evaluate the effectiveness of topical drugs and skin care cosmetics.

1. Measurement of the barrier function of stratum corneum

Recently, it has become possible to examine *in situ* the barrier function of the stratum corneum using a non-invasive method called transepidermal water loss (TEWL) measurement. This method uses an electric hygrometer to measure the amount of water being lost slowly from the body through the stratum corneum.

Because the measurement is affected by perspiration, it is performed at an ambient temperature of 22°C or less and under conditions that cause no sweating. The skin with inflammatory conditions forming scales consistently shows high values of TEWL reflecting the loss of barrier function. A study using this measurement has revealed a loss of barrier function in the skin damaged by detergents, as well as in atopic xerosis (areas of skin in atopic dermatitis patients that are simply dry but have

no visible lesions).[2] The situation differs in the xerosis of elderly people. While inflammatory skin lesions involve the formation of pathological stratum corneum and appearance of scales as a result of enhanced epidermal metabolism, the xerosis of elderly people is characterized by the presence of intact barrier function, and the dryness results from the lack of moisture supply from inside after moisture is lost to the dry air of winter. Despite the similar dryness in clinical appearance, the latter is characterized by the thick accumulation of the stratum corneum and reduced TEWL.[3]

On the other hand, fresh scars, hypertrophic scars, and keloids with inflammation and fibrous tissue proliferation in deep layers of the dermis show a reduction of barrier function, even if no scales are observed. This is also the case with the topical applications of retinoic acid for photo-aging in the exposed skin areas of elderly patients and the oral use of retinoids for dyskeratotic dermatoses.[4] Naturally, normal skin in different parts of the body shows different values of TEWL. The skin of the face and the external genitalia present high values of TEWL approximating those in skin lesions in other locations.[5]

2. Measurement of hydration state of stratum corneum

Normal stratum corneum binds water and performs the important hydration (moisture retention) function for maintaining the softness and smoothness of the skin and allowing the unrestricted movement of the skin. A loss of this function may result in dryness, rough skin, and even the scales and cracks that frequently accompany skin diseases. Thus, the hydration state of the stratum corneum is a very important measure both for cosmetic scientists, who attempt to design fundamental cosmetics that would enhance the water content of a normal stratum corneum, and for dermatologists, who treat skin diseases by the use of topical drugs increasing the water content of an abnormal stratum corneum.

In 1980, the authors[6] reported that we could perform *in-vivo* measurement of the hydration state of the stratum corneum surface instantly and non-invasively by the measurement of the high-frequency conductance or electric capacitance. Since then, many types of instruments based on this principle have been developed and used widely for diagnosis and the development of moisturizing agents and other topical drugs.

Along with the measurement of the barrier function of the stratum corneum, that of the hydration state of the stratum corneum provides the basis for the functional analysis of the stratum corneum. This measurement also should be performed at an ambient temperature of 22°C or less and a relative humidity of about 50% to avoid the influence of perspiration.

Among the parts of the body, the hydration value tends to be high in the face and the upper parts of the body, where sebum secretion is high, and low in the lower limbs.[5] Elderly people who show a loss of hydration function of the stratum corneum often suffer from dryness in the lower limbs in winter, and this may lead to senile xerosis characterized by itchiness due to surface cracking.[3] Normal individuals without skin rashes also show lower barrier function and higher TEWL of the stratum corneum in winter than in summer, when the same individuals are examined under the same conditions.[7] This means that the skin in winter is more prone to dermatitis and aggravation of conditions such as atopic dermatitis.

The water content of the stratum corneum decreases when there are scales or crusts and increases promptly after the application of moisturizing agents. The oral use of retinoid for abnormal cornification and the topical use of retinoic acid for acne and photo-aging increase the softness of the skin within 1 or 2 weeks.[4] The afore-mentioned keloids and fresh scars also show similar changes associated with the increase in the water content of the stratum corneum.

3. Measurement of skin surface topography

The most widely used method for detailed observation of skin surface topography seems to be the replica (microrelief) method. The surface topography of the skin is transferred to a soft nitrocellulose or silicone rubber material, transferred further from it to a resin or other appropriate material, and then observed under the light or electron microscope. Attempts have been made to visualize the surface topography in 2-dimensional representation and to achieve 3-dimensional reconstruction.

At present, specimens are usually observed under oblique light and the resulting shadows are read into an image analyzer connected to a computer, which calculates the area of detected shadows. For example, in Paget's disease and mycosis fungoides, a type of T lymphoma, we can accurately define the borderline between the margin and normal tissues based on the difference in the form of minute wrinkles.[8)]

Measurement of Skin Color

The measurement of the redness, whiteness, and pigmentation of the skin has been showing extremely remarkable progress. One of the most notable developments is Minolta Chroma Meter®, which has the capability to analyze skin tone based on hue, lightness, and chroma. With the convenience for routine use, it is fairly close to the ideal of instruments of this type. The facial skin of Japanese people shows a decrease in lightness (L*) and an increase in yellowness (b*) with the advancement of age.

Magnified Observation of Skin Surface

The observation of the skin surface under a magnification of about 20× using a portable optical device called a dermatoscope facilitates the evaluation of superficial tumors, pigment lesions, and blood vessel network. It improved the accuracy of clinical diagnosis of seborrheic keratosis, solar lentigo, pigmented mole, basal cell carcinoma, and malignant melanoma, which often present difficulties in differential diagnosis.[9)]

Non-Invasive *In-Vivo* Observation of Microscopic Skin Structures

While histopathological examination of the skin can be performed by simple procedures, it has a problem of scars remaining on the skin. The use of instruments enables us to conduct *in-situ* observation of microscopic changes in skin tissues. The construction of the skin can be examined by the use of high-frequency ultrasound. The numerical data of the hypertrophy and atrophy of the skin and the tumor location may be useful in assessing the extent of lesions or the effectiveness of treatment.

With respect to non-invasive observation of smaller structures, confocal laser microscopes are useful to examine the shallower layers of the skin, i.e., the epidermis and the papillary layer of the dermis. This method can provide information on the nature and thickness of corneocytes, nature of epidermal cells, and hemodynamics in capillary vessels.[10)] However, in view of the fact that we have been accustomed to the observation of artifacts in HE stained histopathological specimens, the value of this method in the diagnosis of diseases has yet to be established.

Identification of Substances in Skin Tissues

Microdialysis is a method in which physiological saline is injected via a thick injection needle inserted intradermally or subcutaneously while the tissue fluid is recovered via another needle to analyze and identify substances occurring in it. This method enables us to analyze various substances in the tissue fluid sampled in real time and to study the roles and metabolism of these substances in normal and pathological skin.[11)]

Measurement of Viscoelasticity and Stiffness of Skin

Cutometers are commercially available devices that comprehensively measure the mechanical and physical properties of the skin, such as extensibility and viscoelasticity. These instruments apply suction to the skin and measure its displacement and time to recovery. Because changes in the skin detected by this method reflect not only the hydration state of the stratum corneum, but also the thickness of epidermis, the extent of fibrous changes, the state of the dermal matrix, etc., it is necessary to understand the characteristics of each device and select appropriate models to suit the purpose of the study.

We recently developed a method of measuring skin stiffness with robotic tactile sensors and analyze it into superficial stiffness due to drying of the stratum corneum and deep stiffness reflecting fibrotic changes in the dermis.[12)]

Conclusion

Subjective observation that can be recognized only by the observer himself lacks reproducibility and reliability. The instrumental measurement of the living skin has been developed based on the idea of analytical science to understand skin conditions through analyzing instrumental measurement data. The application of biometry technology to the skin has enabled us to grasp numerically the properties of the skin that cannot be evaluated by the human senses. This approach has become essential to the understanding of not only the changes caused by skin diseases, but also the characteristics of normal skin reflecting age, sex, and anatomical location. With respect to normal individuals, we need to pay particular attention to the skin of elderly people, who represent an increasingly large part of our society. It is hoped that this approach will facilitate the measurement of the age-related changes in the skin and the scientific evaluation of the efficacy of treatment for aged skin.

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Animal Models of Atopic Dermatitis

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Abstract: Atopic dermatitis (AD) is a chronic, recurrent eczematous skin disease with itching. Many AD patients have a family history of asthma, allergic rhinitis, and a tendency to IgE overproduction. AD is recognized as a form of persistent antigen-specific dermatitis with a Th2 cytokine profile. Various antigens induce AD, but uniformity of their clinical manifestations suggest the involvement of a common non-antigen-specific pathway. We have generated a keratinocyte-specific caspase-1 transgenic mouse (KCASP1Tg) using the keratin 14 promoter. KCASP1Tg over-secreted IL-1alpha/beta and IL-18 from the skin and developed persistent itching dermatitis with marked serum IgE and histamine elevation at age 8 weeks in specific pathogen-free conditions. We also produced a keratin 14-driven mature IL-18 transgenic mouse (KIL-18Tg). KIL-18Tg developed similar AD-like skin changes with IgE elevation at 24 weeks. KCASP1Tg crossed with stat6-/- developed skin manifestations at age 8 weeks without IgE elevation; however, KCASP1Tg crossed with IL-18-/- did not develop dermatitis. KCASP1Tg crossed with IL-1alpha/beta-/- developed dermatitis at age 24 weeks. Thus, epidermal IL-18 secretion induces AD-like dermatitis, and epidermal IL-1s enhances AD-like changes. Both KCASP1Tg and KIL-18Tg showed Th2-type cytokine profiles and satisfied the human AD criteria. These mice models indicated the involvement of an antigen-independent innate-type pathway in addition to the antigen-specific acquired-type pathway in AD. These models responded to various therapeutic agents for AD, and are ideal, potent tools for the development of gene therapy and other new therapies for AD.

Key words: Atopic dermatitis; Interleukin 18 (IL-18); Mast cell; Mouse; Caspase

Introduction

According to the definition by the Japanese Dermatological Association (JDA), atopic dermatitis (AD) is "a chronic and recurrent eczematous skin disease with itching; many AD

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patients have a family history of atopic predisposition (bronchial asthma, AD or allergic rhinitis) and a tendency to IgE overproduction."[1] Understanding AD pathology is very difficult because it involves immediate type allergic reactions despite its basically eczematous reaction.

The clinical efficacy of topical steroids has been remarkable, but the uncritical use of corticosteroids has resulted in frequent adverse effects. A flood of incorrect information that ensued created mistrust of corticosteroids therapy, which continues to hinder the proper treatment of AD. In this situation, animal models of AD are essential for answering questions about AD and developing new therapies. The mouse is the most suitable species for this purpose since it is a mammal, the reproduction cycle is short, the immunological background of this species has been established, and diagnostic reagents are available.

Several AD mouse models have been used for years. Mutant mouse models include the Nc/Nga mouse,[2] which is constantly infested by mites on the skin, and the NOA (Naruto Research Institute Otsuka Atrichia) mouse.[3] Other models have been produced by repeated sensitization with antigens.[4] The production of a sensitization model takes a long time, and can be achieved only in a few strains of mice such as BALB/c. These mice have a different genetic background from commonly used genetically manipulated mice based on C57BL/6 mice, and this causes difficulty in cross breeding analysis. The genetic background of Nc/Nga mice has not been well characterized, and these mice do not develop dermatitis under specific pathogen-free (SPF) conditions because dermatitis in this model depends on the presence of mite infestation.[2] Thus, it is difficult to use these mice to develop therapeutic drugs.

The NOA mouse, a hairless strain created by mutation, is considered an AD model because the mouse develops ulcerating skin lesions mainly on the trunk, shows scratching behavior, and presents elevated serum IgE levels. This mouse has been found to show an elevated platelet factor 4 and eotaxin, an eosinophil chemotactic factor, in the skin.[3] However, the genetic background of this mouse has not been established. While backcrossing with Th2-dominant BALB/c mice results in the development of symptoms, backcrossing with Th1-dominant mice such as C57BL/6 results in low occurrence of dermatitis. Because of these problems, it has been necessary to develop new genetically modified AD mouse models.

Clinically, AD has been regarded as a persistant antigen-specific eczematous reaction to environmental antigens such as food antigens and mites. The pathogenesis of AD is believed to involve Langerhans cells and activated T cells. The overproduction of IgE antibodies in AD has led researchers to pay attention to the roles of B cells, mast cells, and basophils, and AD has been considered a Th2-type disease involving cytokines such as IL-4, IL-10, IL-13, and IL-5. Although various antigens induce AD, the uniformity of their clinical manifestations suggests the presence of a non-antigen specific pathway in the pathogenesis of AD.

The authors produced a mouse model that showed continuous secretion of proinflammatory cytokines in the skin,[5] which developed AD-like dermatitis under SPF conditions.[6] We identified IL-18 as a cytokine involved in AD pathogenesis,[7] and proposed a new concept of AD.[8]

Skin-Specific Caspase 1 Transgenic Mouse (KCASP1Tg)

Inflammation is believed to involve proinflammatory cytokines including IL-1α, IL-1β, tumor necrosis factor (TNF) α, and IL-18. These substances are produced in the cell as inactive precursors and released from the cell after enzymatic activation. The IL-1β-converting enzyme that activates IL-1β was later found to be coded by a member of a gene family related to apoptosis. This enzyme, named caspase, is a cysteine protease that mediates proteolysis at

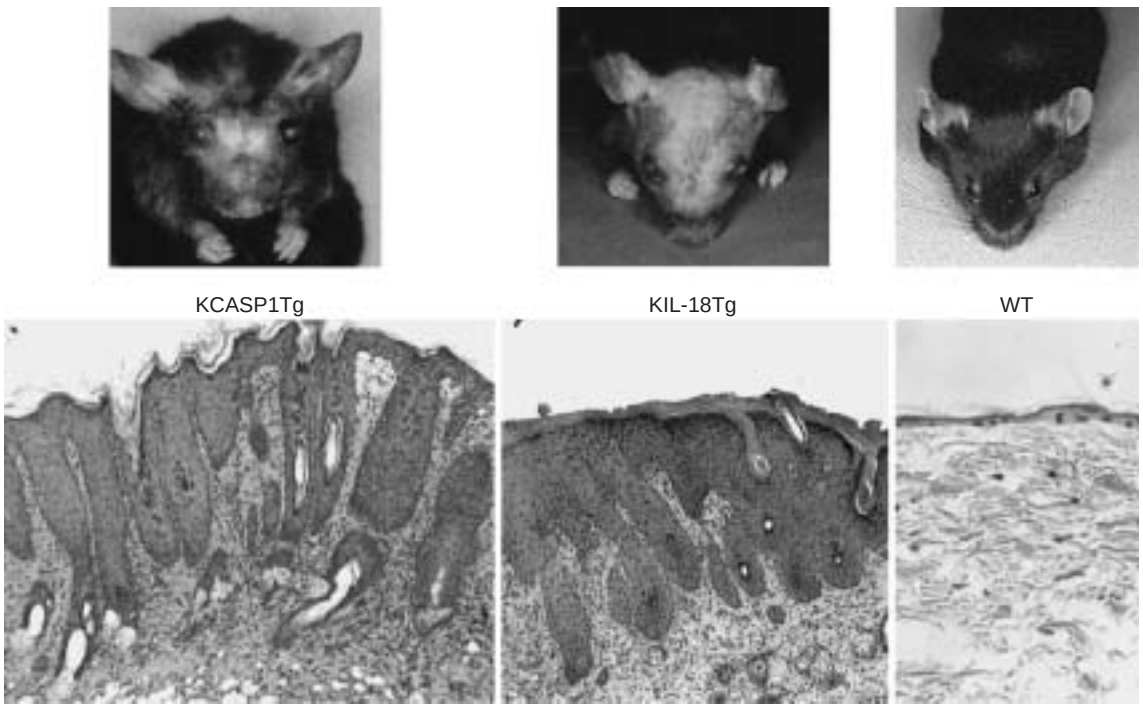

Fig. 1 Clinical and histopathological findings in KCASP1Tg and KIL-18Tg

an aspartate site.

Monocytes produce caspase 1, which promotes the activation and secretion of IL-1β and IL-18. While epidermal keratinocytes produce pro-IL-1β and IL-18, they do not secrete these cytokines because they normally lack caspase 1 activity.[9] We attached a keratin14 promoter to human caspase 1 DNA segments and introduced the DNA into fertilized eggs so that caspase 1 would be expressed only in the epidermal basal cells.[5]

Clinical Symptoms in Model Mice

About 8 weeks after birth, the mice started to show dermatitis around the eyes and neck. The lesions extended to the ears and legs and finally became generalized. The dermatitis started as erosive erythematous patches and moist crusted areas. After repeated re-epithelization and inflammation, the lesions progressed to chronic lichenified plaques (Fig. 1). Almost all the mice developed cataract, as is the case with AD.

The frequency of scratching behavior increased remarkably with the onset of the skin lesions. In week 10 and after, the frequency of scratching per 10 minutes was 10 times as high as that in normal mice (Fig. 2b).

Histopathological Changes in Model Mice

The skin before the onset of skin symptoms showed no histopathological changes. After onset, it presented remarkable epidermotropic cell infiltration, papillomatous proliferation and thickening of the epidermis, partial epidermal defects, crusts, and parakeratosis. These changes resembled those observed in the acute stage of human atopic dermatitis (Fig. 1). The dermis showed an increase in the number of infiltrating CD4+ T cells and a remarkable increase in toluidine blue-positive mast cells, similar to the findings in AD[6] (Fig. 2c).

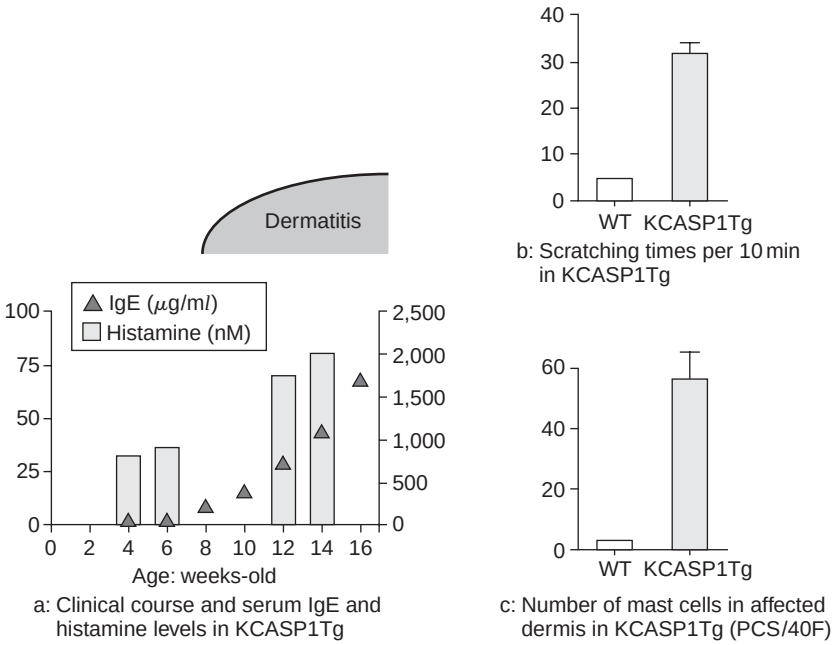

Fig. 2 Immunological profiles of KCASP1Tg

Serological Changes in Model Mice

Serum histamine levels correlate with the severity of AD in humans. The serum histamine levels in the model mice started to increase before the onset of symptoms, and it increased according to the extension of the skin lesions (Fig. 2a). Serum histamine levels corresponded with an increase in mast cells in the skin. Serum IgE levels were as low as that of wild-type mice. It increased by the time of symptom onset, and showed remarkable elevation to 50μg/m*l* or more, correlating with the severity of the skin rash (Fig. 2a). IgG1 increased remarkably, however, increases in IgG2 and IgM were limited, suggesting the allergic nature of the observed changes.

Cytokine Production and Immunological Profile of Model Mice

The sera of the model mice were found to contain high levels of active IL-1β and IL-18. The expression of cytokine mRNAs in the affected skin showed an elevation of IL-4 and IL-5, which are not detected in normal mice, and IFN-γ was not detected. The increase in IL-4 production was confirmed with an enzyme-linked immunosorbent assay (ELISA) for the culture condition medium of the lesions.

In addition, we measured the cytokine production in splenic cells after stimulation with anti-CD3 antibodies. We detected elevation of IL-3, IL-4, and IL-5, as well as the suppression of IFN-γ, indicating a systemic Th2 shift of cytokines. These mice showed an elevation of CD40L expression, implying the promotion of IgE production from B cells.

Appropriateness as a Model of Atopic Dermatitis

Because the definition of atopic dermatitis is based on clinical findings in humans, it is somewhat controversial whether the same criteria can be applied to mice. The definition by the Japanese Dermatological Association, i.e., recurrence, itching eczema, family history, and elevated serum IgE levels, applies to our mouse model.[1)]

Our model satisfies all the essential elements of the internationally accepted diagnostic criteria by Hanifin-Rajka,[10] i.e., (1) itching, (2) typical distribution of skin rash, (3) chronic recurrent dermatitis, and (4) family history. At least three of these major criteria are required. Our model satisfies 11 of 22 minor elements, including xeroderma, elevated serum IgE, early onset, dermatitis of the hands and feet, cheilitis, conjunctivitis, cataract, facial erythema, lesions around the eyes, and aggravation due to environmental changes. We could not detect environmental antigen specific IgE in their sera.

As discussed above, these mice are qualified as an animal model of AD.

Onset Mechanism of AD Studied in Model Mice

There has been a long debate as to whether the onset of AD involves type I or type IV allergy. However, it has been accepted that the distinction between type I and type IV does not have much significance in this context. The role of IgE and the mechanism for IgE elevation are nevertheless important issues.

KCASP1Tg mice produce IgE without antigen-specific reactions, and the skin of these mice secretes IL-1 and IL-18. IL-18 was originally discovered as a factor inducing IFN-γ in hepatitis, and thus had been considered a Th1 cytokine. This assumption contradicts the symptoms seen in these mice.[5] To answer this question, we injected recombinant IL-18 into wild-type mice, and observed the elevation of IgE. The results indicated that IL-18 is a Th2 cytokine and suggest the possibility that Th2 induction in these mice may be mediated by IL-18.[7]

On the other hand, the class switching and production of IgE essentially requires signal transduction via the stat6 system locating the downstream of IL-4. We therefore crossed KCASP1Tg with stat6 knockout mice. Because this resulted in the complete suppression of IgE production, we concluded that the IgE induction by IL-18 is mediated by stat6. On the other hand, we crossed our mice with IL-18 knockout mice to see whether IgE production in KCASP1Tg depends on IL-18. This experiment showed that the IgE level in KCASP1Tg IL-18-/- mice is as low as one-tenth of the reference IgE level. These mice possessed IL-4, suggesting that there are minor IgE induction pathways other than those involving IL-18.

While the stat6-dependence of IgE production was confirmed, this study produced the surprising results that the stat6-/-KCASP1Tg mice lacking IgE developed similar dermatitis with the same timing as the KCASP1Tg mice. Furthermore, the IL-18-/-KCASP1Tg mice did not develop dermatitis.

The above findings indicate that IgE is not essential to the development of dermatitis, and IL-18 plays a key role.

IL-18 Transgenic Mice

Next, it was necessary to examine the *in vivo* function of epidermal IL-18. We produced epidermis-specific mature IL-18Tg (KIL-18Tg) for this purpose. While KCASP1Tg developed symptoms within 8 weeks, KIL-18Tg took 6 months before the onset of symptoms.[8] These mice developed lichenification of the skin from the early stage of the skin rash (Fig. 1).

Because KCASP1Tg mice also secrete IL-1β, we crossed the model mice with IL-1α/β knockout mice to examine the effect of IL-1β. While the IL-1s-/-KCASP1Tg mice presented a similar phenotype to the KCASP1Tg mice, they resembled KIL-18Tg mice in that 6 months was required before the onset of symptoms. These observations suggest that IL-18 is essential for the onset of skin rash and that IL-1s acts as an onset booster.

Suggestions from AD Model Studies and Future Prospects

The model mice described above introduced a new concept of AD. High IgE levels and

RAST (radioallergosorbent test) scores have been emphasized in the clinical management of AD. These indices sometimes cause excessive dietary restriction and limitation on bedding and clothing, and these may be exploited as an opportunity for home reform businesses. In addition to the conventional antigen-specific mechanism, it is possible that epidermal damage due to scratching promotes the release of epidermal proinflammatory cytokines (in particular, IL-18), that activates mast cells, and results in aggravation of AD.

We proposed a new concept that the part of AD caused by environmental antigen-specific eczema reaction as "acquired-type AD" and the part consisting of non-antigen specific dermatitis seen in these mice as "innate-type AD."[8] In Europe, the term "extrinsic AD" was proposed to describe AD showing antigen-specific IgE antibodies and the term "intrinsic AD" for AD lacking these antibodies, and the latter has been reported to be increasing recently.[11] In our daily practice, about 10 to 20% of AD cases do not have high serum IgE levels. It is likely that these cases are affected by epidermal injury due to scratching caused by psychological factors or ichthyosis.

Therapeutically, KCASP1Tg mice responded well to topical steroids and steroid injections. These mice showed disappearance of erosions and remarkable improvement in dermatitis. The effectiveness of the topical use of anti-allergic drugs and immunosuppressive agents was also confirmed. Because KCASP1Tg mice are tested in SPF conditions, they are expected to be ideal tools for the development of new drugs and the evaluation of existing drugs.

The past pococurante use of corticosteroids and the flood of incorrect information about adverse effects once caused much confusion about the treatment of AD. Owing to the efforts of the JDA to promote correct information, we have overcome most of the misconceptions. The development of gene therapy and other new future therapies will require animal models. These two types of model mouse discussed here are expected to open new possibilities in the development of new AD therapies.

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The Molecular Basis of Keratinizing Disorders

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Abstract: Technological advances in molecular biology have brought about a number of major scientific achievements in the field of normal and abnormal epidermal differentiation. This article reviews the molecular defects that cause many types of keratinizing disorders, and highlights the roles of affected genes encoding a variety of epidermal proteins.

Key words: Keratinizing disorders; Pathogenic gene; Mutation

Introduction

Keratinizing disorders are conditions in which qualitative or quantitative abnormality based on a certain genetic background affects the pathway of epidermal differentiation from the birth of basal cells in the epidermis to their exfoliation as cornified cells at the skin surface. This paper reviews biological aspects of pathogenic genes encoding cytoskeletal proteins (keratins), desmosomal proteins (desmoplakin, desmoglein, plakoglobin, and plakophilin), gap junction proteins (connexins), cornified envelope protein (loricrin), and certain enzymes/enzyme inhibitors

Keratinizing Disorders and Pathogenic Genes (Table 1)

1. Enzymes and their inhibitors

(1) Lipoxygenase

Nonbullous ichthyosiform erythroderma is caused by the mutation of the *lipoxygenase-3* (ALOXE3) or the *12R-lipoxygenase* (ALOX12B) gene.[1] ALOXE3 and ALOX12B are both enzymes involved in the metabolism of arachidonic acid. The loss of activity of these enzymes results in a shortage of essential fatty acids and compromises the hydration ability of the horny layer.

(2) Transglutaminase 1 (TGase 1)

Lamellar ichthyosis, a condition resembling nonbullous ichthyosiform erythroderma, is caused by the mutation of the *TGase 1* gene.[2] TGase 1 catalyzes the bridging of proteins at the γ-carboxyl groups in glutamine residues and at the lysine residues. Through these reactions, TGase 1 bridges the components of the cornified cell envelope, which is a structure that lines the cell membrane, and plays an important role in maintaining the construction of cornified cells. In this disease, the activity of

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Table 1 Molecular Basis of Keratinizing Disorders

Pathogenic genes	Keratinizing disorders
Keratins	
K1	palmoplantar keratoderma Unna-Thost
	ichthyoisis hystrix Curth-Macklin
K1/K10	bullous congenital ichthyosiform erythroderma
	annular epidermolytic ichthyosis
K2e	bullous ichthyosis of Siemens
K16	focal palmoplantar keratoderma
K6a/K16	pachyonychia congenital, type 1
K6b/K17	pachyonychia congenital, type 2
K9	palmoplantar keratoderma Vorner
hHa1, hHb1, hHb6	monilethrix
Desmosomal proteins	
desmoglein 1	striate palmoplantar keratoderma
desmoplakin	striate palmoplantar keratoderma
plakoglobin	Naxos disease
plakophilin	skin fragility syndrome
Gap junction proteins	
Connexin 31	Erythrokeratoderma variabilis
Connexin 30	Clouston syndrome
Connexin 26	Vohwinkel's syndrome with deafness
	keratitis-ichthyosis-deafness syndrome
Cornified envelope protein	
loricrin	Vohwinkel's syndrome with ichthyosis
	progressive symmetric erythrokeratoderma
Enzymes and their inhibitors	
lipoxygenase	non-bullous congenital ichthyosiform erythroderma
transglutaminase 1	lamellar ichthyosis
SERCA 2	Darier disease
3beta-hydroxysteroid dehydrogenase	CHILD syndrome
cathepsin C	Papillon-Lefevre syndrome
steroid sulfatase	X-linked ichthyosis
fatty aldehyde dehydrogenase	Sjögren-Larsson syndrome
phytanoyl-CoA hydroxylase	Refsum disease
SPINK5	Netherton syndrome
Others	
SLURP-1	palmoplantar keratoderma Mal de Meleda
dyskerin	X-linked recessive dyskeratosis congenita
telomerase RNA	autosomal dominant dyskeratosis congenital
Unknown	
18q21.3	harlequin fetus
17q25	palmoplantar keratoderma Howel-Evans

TGase 1 is reduced, and the ability to bridge the cornified cell envelope precursor proteins is insufficient. As a result, the ceramide that normally binds to the cornified cell envelope components is accumulated in intercellular spaces, and the decomposition of desmosomes by protease is inhibited.

(3) Sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA)

Darrier's disease is caused by the mutation

of the sarco/endoplasmic reticulum Ca^{2+}-ATPase type 2 isoform (*SERCA2*) gene.[3] SERCA2 belongs to the large family of P-type cation pumps that couple ATP hydrolysis with cation transport across membranes. SERCA pumps specifically maintain low cytosolic Ca^{2+} concentrations by actively transporting Ca^{2+} from the cytosol into the sarco/endoplasmic reticulum lumen. In this disease, the level of SERCA2b that has a normal pump function is reduced by half, and this results in the impairment of Ca^{2+}-mediated signal transduction and cell adhesion.

(4) 3beta-hydroxysteroid dehydrogenase

CHILD (congenital hemidysplasia with ichthyosiform erythroderma and limb defects) syndrome is caused by the mutation of the *3beta-hydroxysteroid dehydrogenase* gene.[4] This enzyme functions in the pathway for cholesterol biosynthesis. Since cholesterol is important in embryonic development controlled by the hedgehog signaling pathway, this led to the notion that a cholesterol precursor without the potential to form esters might impair signaling through hedgehog proteins and thus explain the dysplasias observed in CHILD syndrome.

(5) Cathepsin C

Papillon-Lefèvre syndrome is caused by the mutation of the *cathepsin C* gene.[5] Cathepsin C is a cysteine protease of the papain family and is a dipeptidyl aminopeptidase capable of removing dipeptides from amino terminus of proteins. The Cathepsin C knockout mice show insufficiency in the cytotoxic activity of CTL cells and NK cells through activation impairment of serine proteases called granzymes A and B. In this disease, the immune response to infections becomes abnormal via the same mechanism seen in the knockout mice and characteristically induces severe periodontitis.

(6) Steroid sulfatase (STS)

X-linked ichthyosis vulgaris is caused by the mutation of the *STS* gene.[6] STS is an enzyme hydrolyzing the sulfate group at the sterol ring 3β position in steroid hormones and cholesterols. In this disease, the degradation of cholesterol sulfate, the substrate for this enzyme, to cholesterol, does not take place, and cholesterol sulfate accumulates in the spaces between cornified cells. As a result, cholesterol sulfate inhibits the activity of chymotrypsin-type serine proteases and hinders the decomposition of desmosome components.

(7) Fatty aldehyde dehydrogenase (FALDH)

Sjögren-Larsson syndrome is caused by the mutation of the *FALDH* gene.[7] FALDH is a component of the fatty alcohol: NAD oxidoreductase enzyme complex that catalyzes the sequential oxidation of fatty alcohol to aldehyde and fatty acid. In this disease, FALDH deficiency causes the accumulation of fatty alcohol and wax esters in the intercellular membrane lamella, which may disrupt the epidermal water barrier.

(8) Phytanoyl-CoA hydroxylase (PAHX)

Refsum syndrome is caused by the mutation of the *PAHX* gene.[8] PAHX is involved in the α-oxidation of phytanic acid. In this disease, PAHX deficiency leads to the accumulation of phytanic acid followed by replacement of essential fatty acid with phytanic acid in the lipid moieties of various tissues.

(9) LEKTI (lymphoepithelial Kazal-type-related inhibitor)

Netherton syndrome is caused by the mutation of the *SPINK5* (serine protease inhibitor, Kazal-type 5) gene, which codes LEKTI, a serine protease inhibitor.[9] LEKTI is involved in T-cell differentiation in thymic epithelium as well as anti-inflammatory and bactericidal activities in mucosal epithelium. Defective LEKTI expression may cause unbalanced Th2 immune response with markedly elevated IgE levels, increased susceptibility to infection and impaired desquamation of cornified cells.

2. Adhesion Molecules

(1) Desmosome components

Striate palmoplantar keratoderma, Naxos disease, and skin fragility syndrome are caused by mutations of the *desmoplakin* gene or the *desmoglein 1* gene, the *plakoglobin* gene, and

the *plakophilin* gene, respectively.[10–13] Desmosomes are intracellular adhesion apparatuses occurring in epithelial cells and myocardial cells. The protein component of a desmosome is composed of a group of transmembrane glycoproteins and a group of attachment plaque proteins binding to them on the cytoplasm side. Desmoglein 1 belongs to the former group, and desmoplakin, plakoglobin, and plakophylin belong to the latter. Mutations in these desmosome components are considered to cause abnormalities in cell adhesion, but little is known about their relation to palmoplantar keratosis, the common clinical manifestation of the above-mentioned diseases.

(2) Connexins

Erythrokeratoderma variabilis, Clouston syndrome, Vohwinkel's syndrome with deafness and KID (keratitis-ichthyosis-deafness) syndrome are caused by mutations of the *connexin 31* gene, the *connexin 30* gene and the *connexin 26* gene, respectively.[14–17]

Connexins are the main protein components of the gap junction. Six connexin molecules assembled in a ring form a doughnut-shaped structure. The tunnel at the center of this structure opens and closes to adjust the transportation of small molecules between adjacent cells, which play an important role in intercellular interaction. Mutant connexins are likely to be incapable not only of forming the normal gap junction but also of playing a crucial role in epithelial homeostasis and differentiation.

3. Cornified Cell Envelope Proteins

(1) Loricrin

Both Vohwinkel syndrome with ichthyosis and progressive erythrokeratodermia are caused by the mutation of the *loricrin* gene.[18,19] Loricrin deposits on the 15-nm-thick layer of the cytoplasmic surface of the cell periphery (cornified cell envelope). Frameshift mutations in the loricrin gene produce amino acid sequences that contain motifs resembling nuclear localization signals. As a result, the mutant loricrin moves into the nucleus being associated with normal loricrin, and the formation of the cornified cell envelope is impaired.

4. Cytoskeletal Proteins (Table 1)

(1) Keratins

Various diseases collectively called keratin diseases are caused by the mutation of various *keratin* genes.[20] Keratins are an extremely diversified group of proteins that play an important role in cell shape maintenance. In keratin diseases, the ability of keratins to maintain cell shape is weakened, and cells are easily destroyed by even slight external force.

5. Others

(1) Dyskerin and telomerase RNA

X-linked recessive dyskeratosis congenita and autosomal dominant dyskeratosis congenita are caused by mutations of the *dyskerin* gene and *telomerase RNA* gene, respectively.[21,22] These components are suspected to play a role in the activity adjustment of telomerase, which governs the life span of cells. However, their functional significance in these diseases is unknown.

(2) SLURP-1 (secreted Ly6/uPAR-related protein 1)

Mal de Meleda type of palmoplantar keratosis is caused by the mutation of the *SLURP-1* gene.[23] SLURP-1 belongs to the leukocyte antigen-6 (Ly6)/urokinase-type plasminogen activator (uPAR) protein family, and the structure of SLURP-1 suggests its relationship with snake and frog toxins. SLURP-1 receptors are assumed to play an important role in signal transduction, cell growth, cell adhesion, etc., but their functional significance in this disease is unknown.

6. Diseases Being Studied for Identification of Pathogenic Genes

The genes for harlequin fetus and palmoplantar keratoderma Howel-Evans map to chromosomes 18q21.3 and 17q25, respectively.[24,25]

Conclusion

These findings of genes responsible for keratinizing disorders are expected to provide valuable information in the elucidation of pathogenic mechanisms and the search for new treatment modalities.

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Updates on Autoimmune Skin Bullous Diseases

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Abstract: Autoimmune skin bullous diseases are a group of diseases induced by autoantibodies against adhesion molecules in the skin. The understanding of these diseases has progressed greatly through studies combining cell and molecular biological investigation of cell adhesion. A technique for producing recombinant pemphigus antigen proteins became available, and the development of the ELISA method facilitated serodiagnosis and monitoring of disease activity. Furthermore, a mouse model of pemphigus was developed through a new methodology using autoantigen knockout mice produced by gene manipulation. The pemphigus model mice produce the IgG antibodies against desmoglein3 (Dsg3) for over 6 months. This model is useful for the study of the mechanisms for antibody production and immune tolerance to peripheral antigens, as well as the evaluation of various immunosuppression therapies. In addition, monoclonal antibodies against Dsg3 were isolated from the pemphigus model mouse, and the studies using these antibodies expanded our knowledge of the molecular basis for blister formation. It is hoped that the study on autoimmune diseases of the skin will lead to elucidation of the pathophysiological mechanisms of autoimmune diseases and the development of treatment with minimal side effects.

Key words: Pemphigus; Bullous pemphigoid; ELISA; Model mouse

Introduction

Autoimmune skin bullous diseases are a group of diseases induced by autoantibodies against adhesion molecules in the skin. Typical examples of these diseases are pemphigus, which impairs adhesion between epidermal cells, and bullous pemphigoid, which impairs adhesion in the basement membrane area. The understanding of autoimmune bullous dermatoses has progressed greatly through studies combining cell and molecular biological investigation of cell adhesion. Based on the findings from these studies, an ELISA (enzyme-linked immunosorbent assay) method using recombinant antigen proteins was developed as a

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diagnostic tool for clinical application. Furthermore, a mouse model of pemphigus was developed through a new methodology using autoantigen knockout mice produced by gene manipulation.

Focusing on these topics, this article reviews the updates on autoimmune skin bullous diseases.

Pemphigus and Bullous Pemphigoid

Pemphigus is divided into two major forms, pemphigus vulgaris (PV) and pemphigus foliaceus (PF). PV is further classified into mucosal dominant type mainly affecting the oral mucosa and mucocutaneous type forming blisters and erosion not only in the oral mucosa but also in the skin. PF does not affect mucous membranes, but forms erosion and scaly erythema only in the skin.

Patients with pemphigus produce IgG autoantibodies against desmoglein (Dsg), a cadherin-type cell-cell adhesion molecule that plays important roles in the adhesion between epidermal cells. Dsg1 and Dsg3 are mainly expressed in stratified squamous epithelium including the skin and mucous membranes. Patients with mucosal dominant type of PV show anti-Dsg3 antibodies alone, while those with mucocutaneous type of PV show both anti-Dsg1 and anti-Dsg3 IgG autoantibodies. Patients with PF produce anti-Dsg1 antibodies alone. An indirect immunofluorescence (IF) assay using normal human skin as the substrate has demonstrated that the IgG autoantibodies in pemphigus react only with the cell surfaces of keratinocytes.

Bullous pemphigoid (BP) is an autoimmune disease in which the target antigens are the 230 kD BP antigen (BP230, BPAG1) and 180 kD BP antigen (BP180, BPAG2) found in hemidesmosomes, which are adhesion structures connecting the epidermal basal cells and the basement membrane. BP is prone to develop in aged persons. Typical clinical symptoms of BP include tense blisters on itchy urticarial erythema. Histopathologically, BP shows subepidermal blisters.

In addition to typical BP that produces skin rashes all over the body, there are various clinical subtypes such as localized BP (the type showing skin rashes in limited anatomical areas such as anterior tibial area, head, face, or neck); vesicular BP (the type showing only small vesicles); nodular BP (the type showing skin rashes resembling nodular purigo); and vegetative BP (the type showing papillomatous growth and rising of the intertriginous areas). In any type, an IF assay using healthy human skin as the substrate demonstrates that the IgG autoantibodies in pemphigoid react with the basement membrane zone.

Serodiagnosis with ELISA Using Recombinant Antigen Proteins

ELISA can be applied to any antigen, provided that highly purified antigen has been obtained. A major advantage of this assay is the ability to measure antibody titers, because the assay quantifies the reactivity of autoantibodies by means of a color reaction, and the result is read on a spectrophotometer. Inter-assay and inter-facility comparisons of assay results can be achieved by the use of the ELISA score (index value) calculated from the comparison with the reactivity of a standard specimen.

As a tool for serodiagnosis of pemphigus, an ELISA method using recombinant Dsg1 and Dsg3 as coated antigens has been developed and put into clinical use.[1–4] The recombinant proteins used in this method are produced in baculovirus expression system using cultured insect cells, and have been confirmed to have proper 3-dimensional structures. Serodiagnosis is made as described below, based on the combination of ELISA result using Dsg1 and Dsg3 (Fig. 1).[8]

If the tested serum is positive for Dsg3 and negative for Dsg1, the diagnosis is mucosal dominant type of PV. If the serum is positive

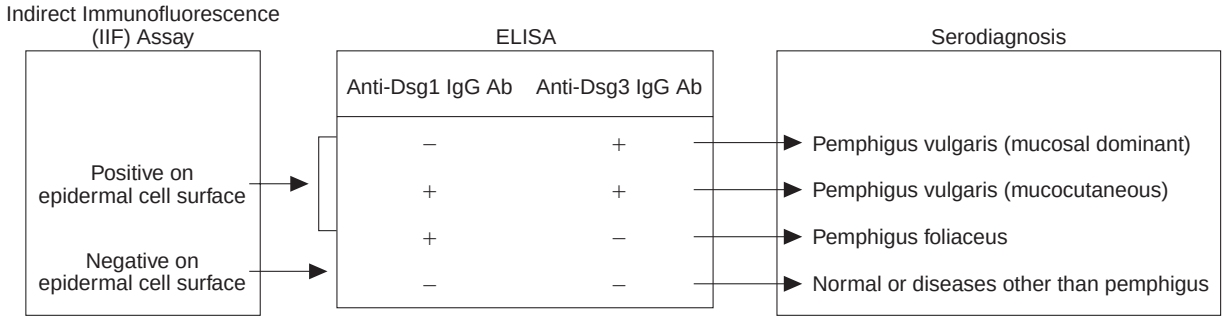

Fig. 1 Serodiagnosis of pemphigus

The serodiagnosis of pemphigus should preferably be conducted by a combination of indirect immunofluorescence (IIF) assay and ELISA. A serodiagnosis of pemphigus is made, if the reactivity of IgG to epidermal cell surfaces by IIF and the presence of IgG antibodies against Dsg1 or Dsg3 are confirmed by ELISA. Differentiation of mucosal dominant type of PV, mucocutaneous type of PV, and PF is made by the combination of the presence or absence of anti-Dsg1 and anti-Dsg3 antibodies.
(Modified from Amagai, M.: *Newest Dermatology Series Vol. 6*, Nakayama Shoten, 2002; pp. 21–26.)

for both Dsg3 and Dsg1, the diagnosis is mucocutaneous type of PV. If the serum is negative for Dsg3 and positive for Dsg1, the diagnosis is PF. It should be noted that serodiagnosis is not the final diagnosis. Final diagnosis should be made based on the clinical as well as histopathological findings.

For serodiagnosis of bullous pemphigoid, an ELISA method using recombinant protein of NC16a domain, where the main epitope of BP180 is located, was developed.[6,7)]

Because the ELISA method quantitatively measures antibody titers, it is useful for monitoring disease progression.[1,2,8)] ELISA scores can be used as a guide in the determination of a steroid tapering schedule. In plasmapheresis, clearance rate can be calculated from the ELISA score of the serum before treatment and that of the waste fluid, and this provides an index for objective evaluation of the antibody removal rate. In patients showing repeated remissions and aggravations, an increase in the ELISA score sometimes precedes an aggravation and aids early treatment.

The measurement in ELISA is based on an enzyme reaction. When the patient has a very high antibody titer, this means that the serum dilution factor must be increased more than usual 100 fold and the result of ELISA must be converted to the true antigen titer.[8)]

The Dsg1 and Dsg3 ELISA has been covered by the health insurance system and the results of basic studies have been widely utilized in daily clinical practice.

A Novel Mouse Model for Pemphigus

Why are autoantibodies attacking the self components formed in autoimmune diseases? We have not clearly answered this question as yet even after the turn of the century. The treatment for autoimmune diseases is mainly based on steroids, immunosuppressants, and plasmapheresis. These therapies suppress the immune system in general, and thus have the problem of causing severe side effects. The study of the mechanisms for autoantibody production and the development of new antigen-specific immunosuppressive therapies will require animal models simulating diseases in humans.

Conventionally, mouse models of autoimmune diseases have been produced by forced immune reactions using repeated immunization of various wild-type mice with antigen proteins in various immune adjuvants. While several models have been produced by this method, the success of model preparation strongly depends on empirical factors, because

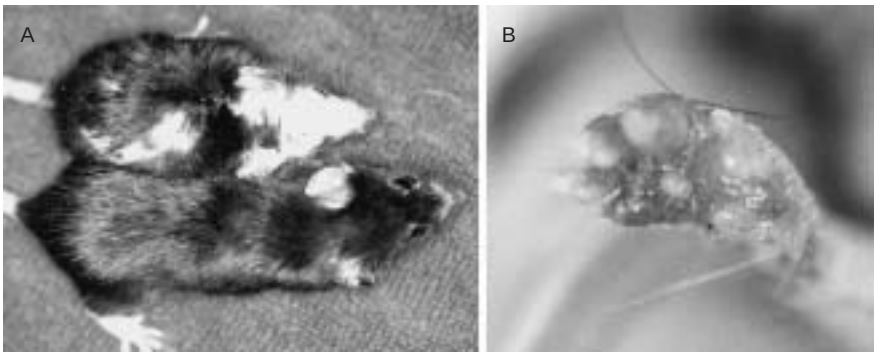

Fig. 2 Pemphigus model mouse
Pemphigus model mice present feeding impairment due to extensive erosion in the oral cavity and body weight loss (A: the pemphigus model mouse above is smaller than the normal control mouse below). Some pemphigus model mice develop crusted erosions in areas constantly receiving external force, such as the soles (B).

immune responses vary depending on mouse strains and the type of adjuvants. The autoimmune response of these model mice is often transient, and few achieve a persistent autoimmune state similar to actual diseases.

In addition, organ-specific autoimmune diseases in humans usually involve antigen-specific disruption of autoimmune tolerance, and other immune functions remain in normal conditions. However, the conventional method using forced immunity induces the activation of the immune system in general, and often results in great differences from actual diseases, such as the manifestation of systemic inflammatory reactions.

A new method for producing mouse models of organ-specific autoimmune diseases has been invented recently.[9] In autoantigen knockout mice, the immune system in the process of development does not encounter an autoantigen that is missing in such mice. Therefore, the lymphocytes that are reactive to this autoantigen are not removed or inactivated in these mice, and the immune tolerance to the knocked out gene product is not established.

Utilizing this fact, the splenocytes (T and B cells) of immunized autoantigen knockout mice are transferred into mice that express the autoantigen. To prevent the rejection of transplanted splenic cells, this procedure is conducted in a Rag2 knockout mouse, which is an immunodeficient mouse lacking mature T and B cells. The transferred T and B cells derived from the autoantigen knockout mouse encounter the autoantigen in the recipient mouse, and a persistent autoimmune response is expected to take place.

Based on this principle, a mouse model of pemphigus was produced using Dsg3 knockout mice[9] (Fig. 2). When Dsg3 knockout mice without immune tolerance to Dsg3 were immunized with rDsg3, this procedure easily induced antibodies that could bind to Dsg3 *in vivo*. Next, the splenocytes from the immunized Dsg3 knockout mice were adoptively transferred into Rag2 knockout mice expressing Dsg3. The production of the IgG antibodies against Dsg3 was detected in the blood of the recipient mice 4 to 7 days after transfer. The antibody titer reached a peak after 21 days, and persistent antibody production was observed for over 6 months. The antibodies did not show reactivity with Dsg1, and were specific to Dsg3.

Deposition of mouse IgG was observed in the cell membranes of Dsg3-expressing stratified squamous epithelium in the skin, oral mucosa, and esophagus of the recipient mice. In addition, impairment of cell adhesion was seen in the epidermis and mucous epithelium, as well as blister formation just above the basal

cell layer, which is a histopathological change characteristic of PV. Extensive erosions in the oral cavity caused inhibition of food intake, and body weight loss was observed from 7 days after the adoptive transfer, when antibody production became evident. Some recipient mice showed crusted erosions in areas usually scratched by the mice, such as the area around the nose.

Based on these findings, the mice produced here were considered a model showing characteristic clinical, histopathological, and immunological features of pemphigus. Among the types of pemphigus, these mice were considered a model of mucosal dominant type of PV, because they produced only anti-Dsg3 IgG antibodies and the main symptoms were observed in mucous membranes.

Isolation of Monoclonal Antibodies Inducing Pemphigus

Do all IgG autoantibodies that can bind to Dsg3 *in vivo* equally induce blisters, or do different antibodies have different pathogenic activity to induce blisters? The answer to this question is important for understanding the mechanism of blister formation in pemphigus and the reason why severity varies among cases. Taking advantage of the fact that the pemphigus model mice produced antibodies with pathogenic activity, we isolated several types of anti-Dsg3 IgG monoclonal antibodies and analyzed the relation between their pathogenic activity and the conformational epitopes recognized by the antibodies.[10] We obtained 9 monoclonal antibodies (AK mAb) that were reactive with Dsg3.

When the hybridoma cells of each AK mAb were inoculated into the intraperitoneal cavity of mice, only the hybridoma of AK23 mAb induced the phenotype of PV in mice. Next, we analyzed the conformational epitopes of these mAbs. Dsg1/Dsg3 chimeric molecules and point mutated molecules were used for this analysis. AK23 mAb was found to occur in V3, K7, P8, and D59 considered to comprise the adhesive interface formed between Dsg3 molecules. The other mAb types that did not induce blister formation were found to recognize functionally unimportant parts that are not directly related to the interaction between Dsg3 molecules.

These results indicated that not all autoantibodies recognizing Dsg3 have equal pathogenic activity, and autoantibodies recognizing different sites on the Dsg3 molecule have different degrees of pathogenicity. A possibility was suggested that the difference in severity might be explained by the different epitopes of autoantibodies in different cases.

In the future, the use of pemphigus model mice is expected to facilitate the elucidation of autoantibody production mechanisms and the development of new disease-specific therapies. In addition, the method using autoantigen knockout mice demonstrated in this study can be applied widely to other autoimmune diseases.

Conclusion

The pathogenetic mechanism of autoimmune diseases has not been clarified as yet. The dermatoses discussed in this paper are examples of those few diseases that can be explained in terms of molecular biology. It is hoped that the findings in dermatoses will open a path for true understanding of the pathophysiological mechanisms of autoimmune diseases.

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Latest Information on Alopecia

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Abstract: In most of mammals, hair plays a very important role and protects them against physical forces, defends them against parasites and UV rays, provides heat conservation, and acts as a sensory organ. During the process of evolution, human hair lost these essential life-supporting functions. Human hair is now viewed as an object of aesthetic interest. However, androgenetic alopecia (male pattered hair loss) has been a source of distress for many men since ancient times. Hair loss due to the side effect of anticancer agents and extensive alopecia areata often drastically impair the QOL of affected persons. Recent rapid progress in molecular biology has clarified the molecular mechanisms for hair generation and regeneration. The pathogenetic mechanism of androgenetic alopecia, reported to affect about one-third of all men in their 40's, and the nature of alopecia areata (as an autoimmune condition), have recently been clarified. New therapies based on these findings are expected to emerge.

Key words: Hair cycle; Male pattern alopecia; Alopecia areata

Introduction

The hair follicle is one of the smallest organs in the body. In many mammals, hair plays a very important role and protects the animal against physical forces, defends it against parasites and UV rays, provides heat conservation, and acts as a sensory organ. For human beings, hair has lost these essential life-supporting functions, and it is now viewed as an object of aesthetic interest that only serves to enhance physical attractiveness and to provide camouflage. However, unexpected hair loss causes serious psychological trauma in the affected persons, and it is sometimes described as a life-altering disease.

This paper reviews new topics on androgenetic alopecia and alopecia areata, which are classic types of hair loss.

Hair Cycle

Biologically, the hair follicle is a unique organ in the body where the process of tissue regeneration and involution is repeated throughout the lifespan. This process is called the hair cycle

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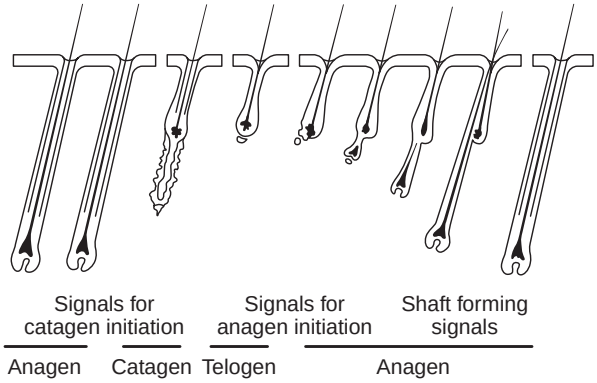

Fig. 1 Hair cycle

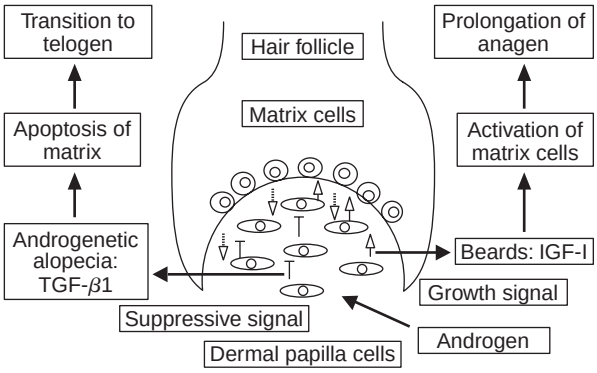

Fig. 2 Action mechanism of androgen in hair follicle

(from anagen to catagen to telogen) (Fig. 1). Because the regulation of the hair cycle involves many factors, various pathological conditions result in alopecia.[1)]

Effects of Androgen on Hair Cycles in Humans

Interestingly, most of the steroid receptor family affect hair cycles in humans, as exemplified by hypertrichosis due to glucocorticoids, alopecia due to thyroid hormone, retinoid, and vitamin D abnormalities, and hirsutism and male pattern alopecia due to androgen. While many of these abnormalities are caused by the action to epithelial cells of the hair follicle, the difference in androgen sensitivity is defined by dermal papilla cells, which are mesenchymal cells of hair follicles.

The dermal papilla is the only part of a hair follicle that has androgen receptors and type-II 5α-reductases (known isozymes of 5α-reductase), which are needed for the action of androgen in male sexual organs.[2)] Strong male phenotype expressions, such as beards and male pattern alopecia, require both androgen receptors and type-II 5α reductase. Because epithelial cells of hair follicle lack androgen receptors, hair growth seems to be controlled by the androgen-dependent release of signals from dermal papilla cells.

To find out the signals, we established an *in vitro* co-culture system using epithelial cells and dermal papilla cells from hair follicles. In the growth of sexual hair during puberty, we found that insulin-like growth factor I (IGF-I) is the hair growth factor released from beard dermal papilla cells (paracrine growth factor) in an androgen-dependent manner. In androgenetic alopecia in humans, we found that dermal papilla cells release transforming growth factor (TGF) β1 in an androgen-dependent manner. TGF-β1 suppressed the growth of keratinocytes in hair follicles and shortened the duration of anagen in male pattern alopecia (Fig. 2).[3,4)]

For the treatment of this common disease affecting 30% of Japanese males in their 40's, a 1% minoxidil solution was marketed in Japan several years ago, which generated great interest. Minoxidil was originally developed as a K channel opener to treat hypertension. Because this agent was found to cause the adverse effect of hypertrichosis, it is used as a topical medication for alopecia. Although the mode of action has not been clarified, minoxidil is suspected to promote hair growth because it activates prostaglandin endoperoxide synthase-1 in hair papilla cells and enhances PGE_2 synthesis.[5)]

In Western countries, a type-II 5α reductase inhibitor called finasteride is currently prescribed.[6)] A clinical trial of this agent is ongoing in Japan, it will probably be marketed in Japan before next year. While this agent causes

hypogonadism among a few percent of the males, oral administration of finasteride is contraindicated in females, because it causes feminization of male fetuses. We expect the development of new therapeutic agents targeted at TGF-β in the future.

Alopecia Areata

Alopecia areata is a common disease representing 2% to 5% of all new patients visiting dermatology departments. According to statistics in the U.S., the prevalence is about 2,000 per 100,000 persons, and the lifetime risk is reported to be 1.7%.[7] This disease is characterized by clearly defined patches of hair loss chiefly on the head. Alopecia areata is classified into 4 types: single, multiple, totalis, and universalis (i.e., total body hair loss). Usually, the disease appears as a single or a few coin-shaped patches of hair loss, but a total loss of hair on the head and body occurs in 7% of all cases. No differences between the sexes are observed. While this disorder develops in a wide range of ages, about 25% of all cases develop before the age of 15. About 20% of the patients have a family history of this disease. As noted above, alopecia areata is often regarded as a trivial problem, but it may cause serious psychological trauma in the affected persons. In the case of minors, it can be a cause of school truancy, and the patient may become the target of bullying by classmates. In adults, this disease can result in limited employment choices.

The pathogenesis of this disorder was explained by various theories in the past, including infection, nutritional disturbance, neurological (stress), and endocrine theories. Nowadays, it is considered an organ-specific autoimmune disease with a genetic background, because about 20% of patients are positive for antinuclear antibodies, thyroglobulin, and microsome antibodies. Human leukocyte antigen (HLA) DQB1*03 (DQ3) is commonly detected in this disease, and the presence of DQB1*1104 (DR11) and DQB1*0301 (DQ7) has been shown to cause extensive development and prolongation of the disease.[8]

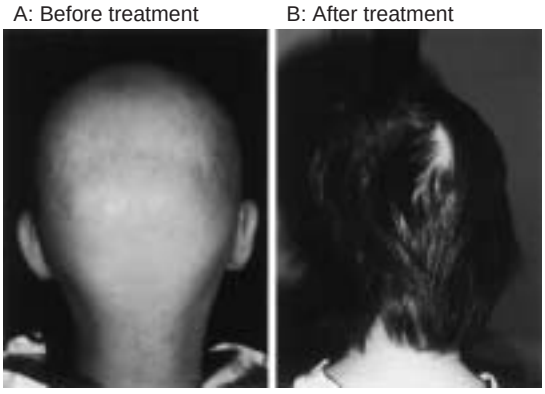

Fig. 3 Local immunotherapy for alopecia areata (universal)

In the affected area of the skin, CD4 lymphocyte infiltration is observed around the anagen hair roots, CD8 lymphocytes invade into hair roots, and matrix cells undergo apoptosis. However, no lymphocyte infiltration is seen in the vicinity of the hair bulge, where hair follicle stem cells are believed to be located. For this reason, permanent hair loss does not occur in alopecia areata. When an alopecic scalp skin from a patient is transplanted to a SCID mouse (an immunodeficient mouse), hair will regenerate. When T lymphocytes isolated from the affected skin are stimulated by a homogenate of hair follicles and introduced locally into the transplanted site, the disease is reproduced.[9] These results indicate that alopecia areata is an autoimmune disease in which T lymphocytes recognize and attack autoantigens in hair follicles.

Local immunotherapy is the most effective treatment for intractable multiple alopecia areata at present (Fig. 3). Though details of the mode of action have not been clarified, this therapy uses local application of hapten to induce allergic contact dermatitis aimed at stimulating hair generation. When the expression of cytokine mRNAs in locally infiltrating lymphocytes was compared before and after

local immunotherapy, the T_H1 type was seen before treatment, and an increase in IL-10 and a decrease in IL-1β were observed after treatment.[10)]

Systemic administration of steroids has long been criticized because the life prognosis of this disease does not warrant such treatment. However, this therapy is attracting new attention as understanding and recognition of this disease as an autoimmune condition and as a life-altering disease have grown. The efficacy of steroid pulse therapy has been reported in patients with multiple alopecia areata within one year of its onset.[11)]

Conclusion

Alopecia is often disregarded as a minor disease, but the distress of affected persons is serious. This article focuses on the mode of onset of male pattern alopecia and alopecia areata, which have been elucidated recently. The mechanism of the hair cycle is being clarified rapidly, since molecular biologists, in addition to dermatologists, have become interested in hair as an ideal tissue regeneration model.

Based on such findings, promising results have been reported from animal experiments to prevent chemotherapy induced alopecia. In addition, the study of the hair follicle, the smallest organ in the body, is expected to provide new information that may contribute to the progress of regenerative medicine of other organs.

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Recent Progress in Diagnosis and Treatment of Melanoma

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Abstract: New methods for the diagnosis and treatment of melanoma are outlined. Dermoscopy provides a simple and effective means of diagnosing melanoma, and it is expected to be used widely in primary care settings. The CGH method that detects abnormalities in the number of DNA copies in the genome is very useful where differential diagnosis between melanoma and benign pigmented lesions is difficult in histopathological study. The effectiveness of sentinel lymph node biopsy has been confirmed with respect to surgery. This method eliminates the need for lymph node dissection when the sentinel lymph node is negative for metastasis, and can improve the patient's QOL. The clinical use of leading-edge therapies for advanced cases, such as peptide vaccine and gene therapies based on achievements in immunological and molecular biological studies, is becoming a reality. A clinical study on gene therapy using liposomes containing the IFN-β gene has been approved by the Ministry of Health, Labor and Welfare, aiming at the first gene therapy for melanoma in Japan.

Key words: Dermoscopy; Sentinel lymph node biopsy; Peptide vaccine therapy; Gene therapy

Introduction

Melanoma is the malignancy of melanocytes (pigment cells) that produce the melanin pigment. Since the 1970s, the incidence of melanoma has doubled in Europe and the U.S., and this increase has become a serious social concern. Changes in lifestyle and the increase in UV radiation due to ozone depletion have been identified as causes that contribute to this increase.

On the other hand, the incidence of melanoma in Japan has been 1/10 to 1/20 of that in Europe and the U.S. While about 40% of melanoma cases in Japan used to have lesions in the plantar area, which is not associated with UV radiation, recent cases show an increase in lesions on the trunk and lower limbs that are exposed to sunlight. This trend is alarming.

Melanoma tends to undergo lymph node

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metastasis from the relatively early stages. The prognosis after progression is extremely poor since melanoma does not respond to most chemotherapeutic agents and has low sensitivity to radiation therapy. It is urgently necessary to develop methods for the accurate diagnosis of early-stage lesions and therapies for the advanced stages of melanoma.

This article explains the progress of diagnosis and treatment based on recent achievements in melanoma studies.

Progress in Diagnosis

1. Dermoscopy

Dermoscopy is a technique in which the affected skin is coated with a gel or another appropriate medium and observed with a dermatoscope or video microscope. A magnification of 10× is attained by the former, and 20× to 100× by the latter. Because the coating prevents the diffuse reflection of light and improves the transparency of the horny layer and the epidermis, this method is effective for the observation of skin down to the upper layer of the dermis. For this reason, dermoscopy is used for the diagnosis of pigmented skin lesions. Melanomas often develop in the plantar area, and about 1 in 10 people has melanocytic nevus in this area. It is important to establish differential diagnosis of these two types of lesion.

Characteristic features of plantar malanocytic nevus can be classified into 3 patterns as follows.[1)] (1) A parallel pattern along skin furrows: pigmentation occurs in parallel lines corresponding to skin furrows (Fig. 1). (2) Lattice pattern: a lattice is formed by a parallel pattern along skin furrows plus linear pigmentation perpendicular to it. (3) Filamentous pattern: linear pigmentation crosses skin furrows and skin ridges perpendicularly or obliquely.

In contrast with the parallel pattern along skin furrows seen in melanocytic nevus, early melanoma lesions characteristically show a parallel pattern along skin ridges, in which zonal or linear pigmentation occurs corresponding to skin ridges (Fig. 2). Other features of melanoma include: (1) black/dark brown pigmentation with marked color variegation, (2) abrupt termination of pigmentation in the lesion borders, and (3) irregular distribu-

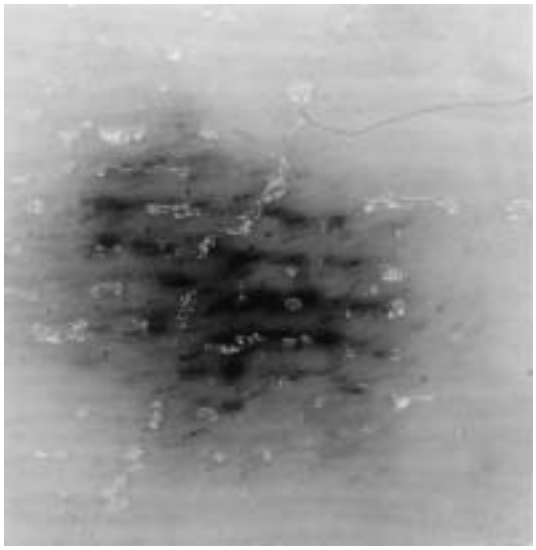

Fig. 1 Dermoscopic appearance of melanocytic nevus
Pigmentation is seen in parallel lines corresponding to skin furrows.

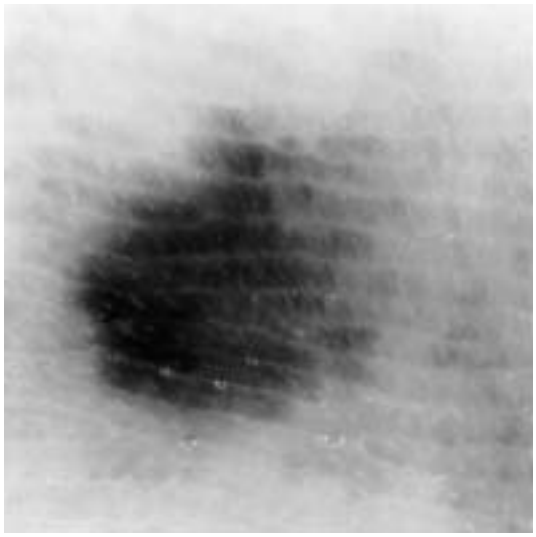

Fig. 2 Dermoscopic appearance of melanoma
Pigmentation is seen in bands corresponding to skin ridges.

tion of black spots of varying sizes in the lesion borders.

Because dermoscopy is a non-invasive and simple technique, it is useful for the diagnosis of pigmented lesions, particularly melanomas in the plantar area, which often present difficulty in clinical diagnosis.

2. Comparative genomic hybridization (CGH) method

Spitz nevus, a type of malanocytic nevus, is also called juvenile melanoma although it is a benign disease. It is important to differentiate this disease from melanoma, but differential diagnosis is difficult in many cases not only by clinical diagnosis but also by histopathological study. Some patients receive unnecessary treatment as a result of this difficulty, while some others do not receive the treatment needed, with tragic outcomes through relapse and metastasis. Hence, a method of differentiating these diseases has long been awaited. Recently, the CGH method is attracting a lot of attention.

CGH is a technique for analyzing alteration in the number of DNA copies in all chromosomes at a time. A DNA sample from cancer tissues and that from normal tissues are labeled with different fluorescent dyes. These are allowed to bind competitively to human metaphase chromosomes, and changes in the number of DNA copies are detected based on the relative intensity of the fluorescence.

In most cases of melanoma, this method detects alteration in the number of DNA copies in more than 1 chromosome. On the other hand, Spitz nevus either does not show alteration in the number of DNA copies or shows alteration only at a particular site (the short arm of chromosome 11). Because melanoma does not exhibit anomaly in the short arm of chromosome 11, this method can differentiate between the two diseases.[2] Although this method is currently used at research level, it will be used widely in the future, as assay can be performed in paraffin-embedded specimens.

Progress in Treatment

1. Sentinel lymph node biopsy

It is an important problem to decide whether or not prophylactic lymph node dissection should be performed when there is no swelling of the regional lymph nodes. In Japan, prophylactic lymph node dissection is usually performed when the thickness of the primary tumor is 3 mm or more or when ulceration is observed. However, metastasis actually occurs in 20 to 30% of cases with thicknesses of 1.5 to 4 mm, and micrometastasis is detected in a few percent of cases with a thickness of about 1 mm. If the presence or absence of micrometastasis to lymph nodes could be determined preoperatively, this information would greatly assist the decision on the necessity of lymph node dissection. A method invented for this purpose examines the sentinel lymph node, which is the first regional lymph node in the course of lymphatic migration of tumor cells from the primary lesion.[3]

In this method, 1 m*l* of a dye solution (1–2% patent blue) is administered into the skin near the primary lesion. When the dye has reached the sentinel lymph node about 15 min after injection, a skin incision is made. The lymph vessels and lymph node stained blue are confirmed visually and excised.[4] If the result of histopathological study is positive for metastasis, staged lymph node dissection is performed. If it is negative, no lymph node dissection is performed because the probability of metastasis to other lymph nodes is considered extremely low. A primary lesion located in the central part of the trunk presents difficulty in deciding the location of regional lymph nodes using the dye method as these may be inguinal or axillary lymph nodes on the right or left side. In addition, there may be more than one sentinel lymph nodes for a primary lesion. In these cases, the use of isotopes such as ^{99m}Tc-labeled tin colloid is effective for the preoperative identification of the accurate location and number of regional lymph nodes and sentinel

lymph nodes. A gamma probe can be used intraoperatively to identify sentinel lymph nodes through direct contact with lymph nodes.

Thus, it is desirable to combine preoperative or intraoperative isotope study and intraoperative dye study. The reliability of this combination is high, with a less than 1% occurrence of pseudo-negative results (i.e., the sentinel lymph nodes are negative and other lymph nodes are positive). This examination is essential in deciding whether prophylactic lymph node dissection should be conducted, and is expected to contribute to improvement in the patient's QOL.

2. Peptide vaccine therapy

Recently, many melanoma-associated peptides recognized by T cells have been identified. These are human leukocyte antigen (HLA)-restricted, and most of them are presented by HLA class I to cytotoxic T lymphocytes (CTLs). The clinical application of the hypodermal administration of these peptides with adjuvants has already been introduced in Europe and the U.S. A key to the success of this peptide therapy is the effective induction of CTLs. For this reason, attention is being directed to the use of dendritic cells, which are a type of antigen-presenting cell that plays the most important role in T cell activation.

Researchers at Geneva University induced dendritic cells from peripheral blood, allowed them to take up multiple melanoma-associated peptides with keyhole limpet hemocyanin (KLH), and directly injected the cells into lymph nodes.[5] As a result, tumor regression was observed in 5 of the 16 cases tested. This method has been tested repeatedly in various countries.

Because patients with melanoma in Japan and those in Europe and the U.S. have different HLA types, we need to select peptides that are suitable for Japanese patients. Under the leadership of the National Cancer Center, a clinical study using 5 types of peptide and dendritic cells has been conducted on patients with HLA A2 or A24. The results of this study are awaited with interest.

On the other hand, the effectiveness of peptide therapy and other immunotherapies aiming at CTL induction depends on the ability of CTLs to recognize the peptides presented by HLA class I in melanoma cells. However, in some cases of advanced melanoma, the tumor cells may lack tumor-associated peptides and HLA class I, and treatment can be ineffective despite success in CTL induction.[6] Overcoming this problem is a major challenge in the clinical use of T cell based immunotherapy.

3. Gene therapy

Like all cancers, genetic aberrations in melanoma are complicated. It is difficult to restore all causative genes by gene therapy. For this reason, the current focus is on immunogene therapy aiming to enhance immunity against cancer cells through gene transfer.

The authors conducted a study and the development of gene therapy for melanoma using the interferon (IFN) β gene embedded in positively charged multilayer liposomes, which were developed by Jun Yoshida at the Department of Neurosurgery, the Nagoya University School of Medicine. We have clarified the following facts:[7,8]

(1) The effectiveness of gene transfer in cultured human melanoma cells is about 10%.
(2) Melanoma cells with transferred IFN-β gene produce IFN-β.
(3) The produced IFN-β exhibits not only a growth inhibition effect but also a cytotoxic effect, and this eventually results in the complete extinction of melanoma cells. The administration of IFN-β in itself shows a growth inhibition effect, but does not show a cytotoxic effect. The IFN-β produced by melanoma cells after gene transfer exerts a stronger antitumor effect than IFN-β per se.
(4) Human melanoma cells transplanted into the hypodermis of nude mice die out after

30 to 60 days. A single local dose of liposomes containing the IFN-β gene after tumor formation results in the arrest of tumor growth for 30 days. Six repeated doses result in the complete disappearance of tumor after 40 days. The action mechanism involves the direct effect of IFN-β and the induction of apoptosis.

(5) In inbred mice, the effectiveness is also seen in tumors other than those in the administration site, and the induction of NK cells and CTLs is observed.

Liposomes containing the IFN-β gene have already been tested in a clinical trial on patients with glioma at Nagoya University, and the treatment was found to be effective without any adverse reactions. The clinical study of gene therapy for melanoma was approved by the Ministry of Health, Labor and Welfare in July 2003, and the first case of gene therapy for melanoma in Japan has just begun. (The clinical study of gene therapy is conducted in cooperation with the Department of Dermatology, Shinshu University; the Department of Neurosurgery, Nagoya University Postgraduate School; and the Department of Gene Therapy, Nagoya University Postgraduate School.)

Conclusion

This paper outlines new methods for the diagnosis and treatment of melanoma. Dermoscopy, with the advantage of simplicity of procedure, is expected to be used widely in primary care settings and to facilitate the early detection of patients with melanoma. With respect to surgery, the routine use of sentinel lymph node biopsy is expected to improve the patient's QOL. The clinical use of leading-edge therapies for advanced cases, such as peptide therapies and gene therapies based on achievements in immunological and molecular biological studies, is becoming a reality. It is hoped that the results of basic study will be increasingly applied to clinical treatment.

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Treatment of Postherpetic Neuralgia

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Abstract: Herpes zoster, a commonly seen condition in daily medical practice, is reported to occur in 10–20% of the population at some time during the lifespan. Chronic, intractable postherpetic neuralgia, a sequela of herpes zoster, presents a clinical challenge. In recent years, effective antiviral agents that can be used in the outpatient setting have been developed for the treatment of herpes zoster and have achieved good clinical efficacy. However, in the absence of any clear, decisive treatment for postherpetic neuralgia, a variety of therapies have been elaborated for use in clinical practice. This paper outlines the treatment of postherpetic neuralgia and introduces therapeutic iontophoresis, which we have been using with success in the clinical setting. The prevention and prediction of postherpetic neuralgia is also discussed.

Key words: Herpes zoster; Postherpetic neuralgia (PHN); Antiviral agents; Iontophoresis therapy

Introduction

Herpes zoster, a commonly occurring condition, is frequently encountered in the dermatology clinic and various other clinics. It is reported that the annual number of patients is 140–180 per 100,000 population and that 10–20% of the population suffers from this disease at some time during the lifespan. In Japan, approximately 500,000 people are affected by herpes zoster each year, and the total number of individuals affected is as high as 20 million.

Although herpes zoster is not life-threatening, it poses the clinical problems of severe neuralgia as a manifestation of the disease and chronic persistent postherpetic neuralgia (PHN), which follows the successful treatment of eruptions. PHN naturally does not occur in every patient with herpes zoster,[1)] although its incidence increases with age, particularly at about 60 years of age and above. The incidence of PHN is about 5% among patients with herpes zoster in their 60s, reaching about 10% among those in their 80s. In Japan, people aged 65 years or older already number 23 million, accounting for 18% of the total population.

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Table 1 Treatments of Postherpetic Neuralgia in Japan

Therapeutic modality		Dosage	Efficacy, adverse effects, characteristics, and others
Drug Therapy			
● Systemic therapy			
Nonsteroidal anti-inflammatory drugs		Usual oral dose. The dose is increased or decreased depending on symptoms. Suppositories are widely used.	Because the effectiveness of prolonged treatment is poor, care must be taken so as not to continue oral treatment for too long. Care must also be taken because these drugs cause various side effects when doses orally.
Antidepressants	Tricyclic	Clomipramine (25～75 mg/day)	Effective in 10 out of 12 cases, with side effects in 4
		Others including amitriptyline (30～150 mg/day) imipramine, and nortriptyline (10～30 mg/day)	
	Others	Carbamazepine (an antiepileptic agent)	Little efficacy, with side effects that pose problems
Extract of inflammatory rabbit skin inoculated with vaccinia virus		Neurotropin (__units/day divided into one morning and one evening dose)	Patients more than 6 months after onset of herpes zoster are amenable. Care must be taken not to continue therapy if there has been no response for 4 weeks.
Interferon		$4～50\times10^4$ units/kg/day	The incidence of PHN and the duration of neuralgia were reduced.
Chinese medicines (combined with nerve blocks)		Herbal extracts, Keisi-ka-zyutsubuto, 5 g, processed Japanese aconite daughter root powder, 1～5 g	70～80% improvement (in 1 case)
		Toki-sigyaku-ka-gosyuyu-shokyoto	Effective in 5 out of 12 cases
Antiarrhythmic drugs		Mexiletine hydrochloride	Alleviation in 10 out of 11 cases
Others		Antiviral agents (vidarabine, acyclovir, and others have been reported to be effective in preventing the development of PHN, but there is a tendency to rule out their efficacy for PHN itself), vitamin B_{12}, antiparkinson drugs (L-DOPA), immunoglobulin (intravenous infusion at high doses).	
● Topical therapy			
Nonsteroidal anti-inflammatory drugs	Aspirin	20 m*l* of a solution prepared by dissolving 50 g of aspirin in 1,000 m*l* of chloroform is applied topically 2～3 times weekly.	Alleviation in 5 out of 10 patients receiving 5～60 treatments
		2% aspirin ointment, ODT after application of 15 g	Alleviation in 5 cases. The effect lasted for 3～6 hours.
	Others	Indomethacin and others	Although this preparation is used widely because it is easy to apply, its efficacy is variable.
Capsaicin		0.025% capsaicin cream, 5 times daily	Effective in 12 out of 14 patients who had been treated for 4 weeks. Application causes a burning sensation.
		Capsaicin cataplasms. It is applied twice a day.	Symptomatic improvement achieved in 8 out of 10 cases. Treatment caused a burning sensation.
Local anesthetics		Xylocaine jelly	
		10% lidocaine cream (to be applied 3～5 times daily)	Alleviation in 5 of 10 patients receiving 5～60 treatments
		Lidocaine tape (containing 60% lidocaine)	Effective for 12 hours
Others		Nitrates (Isosorbide dinitrate is problematic because it causes headache.), topical anesthetics (Xylocaine jelly and others), and others	

Table 1 Treatments of Postherpetic Neuralgia in Japan *(continued)*

Therapeutic modality		Dosage	Efficacy, adverse effects, characteristics, and others
Physical Therapy			
Nerve blocks		The sympathetic, stellate, and somatic ganglions are blocked with local anesthetics 10～30 times, and if necessary, more than 100 times. As a rule, nerve blocks are administered at frequencies from daily to twice a week. In some cases, nerve blocks are administered by continuous infusion. Nerve blocks are administered in combination with other therapies such as epidural blocks and acupuncture in some cases.	Effective in 40～65% of PHN cases. With PHN lasting for more than 1 month, the efficacy decreases as the duration increases. In PHN lasting more than 1 year, it is almost ineffective. The younger the patient and the earlier the treatment, the more effective it is. It requires some skill.
Epidural blocks		Local anesthetic agents are used alone or in combination with steroids. A course consists of 10 blocks given twice a week or it is administered by continuous infusion.	It showed little effect in some studies, but produced improvement in more than 80% of patients treated in other studies. The longer PHN has lasted, the less effective it is.
Subarachnoid blocks		Injection of phenol or alcohol	Not adequately effective. The procedure is complicated. It may cause complications.
		Injection of 0.1～0.2 m*l* of 10% tetracaine solution	Effective in 11 out of 14 cases. Blood pressure was decreased in 2. Respiratory depression
Intravenous infusion		Infusion of 0.5% procaine	Effective, but not in all cases
Topical instillation		Injection into the painful site. Dibucaine; dibucaine and benzocain; camphor and sodium salicylate; triamcinolone and procaine; and others	The effect is transient.
Acupuncture		Anesthesia by acupuncture or with needles left inserted. Daily to once every three days for a total of about ten times	Anesthesia by acupuncture seems to be more effective. Efficacy rate: 36%. Effective in 96% if administered within 2 weeks after the onset. Skill is required. It is less painful for the patient.
Iontophoresis		A pad soaked with a solution of lidocaine and methyl predni-solone is applied to the skin. A weak electric current is applied through the pad so that the drugs penetrate into the skin. The electric current is applied for about 30 minutes. The treatment is administered at intervals of 2～6 weeks for a total of up to 5 times.	Pain was alleviated by ≥40% in 2/3 of the patients who received it 3.8 times on average. The procedure is not painful. The efficacy is independent of the duration of PHN. It is effective even if other forms of therapy are ineffective and in patients having underlying diseases. The procedure is simple.
Cryotherapy	Dry ice	After local anesthesia, a piece of dry ice is pressed onto the site.	Effective in 77% of the patients who received it 1～14 times (mean: 5.7 times). It causes frost-bite which gives rise to vesicles and pain.
	Liquid nitrogen	Apply liquid nitrogen with a cotton ball once or twice a week or once a day for 2 weeks, and then once or twice a week	Effective in 70～80% of patients treated 4～20 times.
Transepidermal nerve stimulation (TENS)		An active electrode attached directly to the skin is used to apply low frequency electric current (low frequency therapy). An implanted electrode is used to stimulate the spinal cord or the brain.	Effective in 78%. Transcutaneous nerve stimulation can be performed by the patients themselves and is useful as a home therapy for long-standing neuralgia.
Near infrared irradiation		Infrared light at a wavelength of 700～1,700 nm (mainly 970 nm) is irradiated for 30 minutes (temperature at the surface of the skin: 39°C).	Effective immediately after irradiation in 39 out of 64 patients, and effective in 12, 24 hours later, without side effects
Laser therapy		A GA-AI-As semiconductor laser is irradiated for about 10 minutes once a week for a total of 10～50 times. An Nd-YAG laser, a low reactive laser, and others are also used.	Effective in 50～90%
Others		Moxibustion (pain disappeared when it was repeated 8 times), surgery (interruption of the posterior root or sympathetic trunk, and others), skin excision (effective in some studies, but seldom satisfactory), radiofrequency thermocoagulation (may be effective in patients not responsive to other therapies), electroconvulsive therapy (pain reduced by an electric current of 110～115 V, applied for 5 seconds to the anterior temporal area under general anesthesia, 1～2 times weekly to a total of 6-12 treatments), and others	

(Source: Reference 5: *Dermatology Practice 10*, Bunkodo, 2000; pp. 110–114)

Thus, there is concern that the prevalence of herpes zoster and PHN will increase further.

In recent years, effective antiviral agents developed for the treatment of herpes zoster have been used in outpatient clinics with favorable clinical results.[2] However, no decisive treatment for PHN exists, necessitating various clinical elaborations for its treatment (Table 1). Various attempts to treat PHN are outlined below.

What Is PHN?

Postherpetic neuralgia is defined by the International Association for the Study of Pain as chronic pain following resolution of acute herpes zoster that is accompanied with skin degeneration in the affected dermatome.

Another view advocates that neuralgia following herpes zoster should be collectively considered postherpetic pain (PHP), in which PHN is only one constituent. This view regards PHN as "deafferentation pain due to nerve degeneration."[3] According to this theory, transition to PHN is presumed to occur about one month after the onset of herpes zoster and to persist thereafter. However, in many cases of herpes zoster, neuralgia as a form of PHP may be present for 2–3 months after the successful treatment of eruptions, and therefore, it is difficult to form a clear distinction between PHP and PHN.

Under these circumstances, PHN cases present an issue in evaluating the clinical efficacy of a particular treatment. Consultation among anesthesiologists and dermatologists in Japan has resulted in the recommendation that, when examining the efficacy of treatment for PHN, patients be examined at least 3 months after the onset of herpes zoster.[4]

Treatment of PHN

1. Current status and expected efficacy of anti-pain procedures

Surveys of anti-pain procedures used for PHN were carried out in Japan in 129 accredited facilities of anesthesiology and 259 accredited facilities of dermatology by the respective academic societies.[4] On the basis of these surveys, the current status and expected therapeutic efficacy of various anti-pain procedures for PHN were investigated and a report issued.

According to the report, therapies noted for their therapeutic efficacy and frequent clinical use include NSAIDs, psychotropics, and nerve block therapy. Therapies from which high efficacy was expected despite limited actual use included narcotic analgesics, steroids, laser therapy, iontophoresis, psychotherapy, and rehabilitation training.

However, no clear treatment has been established for PHN, although various procedures have been elaborated and employed.

2. Treatment policies for PHN

The basis of treatment for PHN consists of medical intervention and detailed instructions given to individual patients and their families.[5] Medical treatment alone often may be insufficient.

(1) Instructions for daily life

i) Patients should not be made anxious or given preconceived ideas about pain and PHN at the onset of herpes zoster.

ii) Patients should be instructed to return to normal daily activities after eruptions have been cured. In principle, there are no restrictions on daily life activities.

iii) Instructions in the creation of a pain-free environment should be given to patients and their families. Suggestions should be based on the patient's lifestyle, circumstances, personality, and relationships with family members.

(2) Medical treatment

i) Since no decisive treatment currently exists, the status of pain should be assessed objectively and treatment chosen according to the individual patient.

ii) A combination of several treatments may be necessary in some cases depending on

symptoms.

iii) The treatment chosen should be evaluated frequently to avoid its continued use merely because the patient complains of pain.

(3) Choice of medical treatment

Treatment should be chosen for each patient according to his or her symptoms and phase of illness. The goal of treatment should be to restore the patient's ability to carry out daily activities such as eating, sleeping, and so on. Antiviral agents are unlikely to have therapeutic efficacy for PHN.

i) Up to 3 months after the cure of eruptions

Although neuralgia as a form of PHP remains in many patients, the degree of its severity gradually decreases. Therefore, if there is no serious impediment to daily living, symptomatic treatment with NSAIDs and vitamin B preparations should constitute the core treatment. When there is severe pain, aggressive anti-pain procedures including physical therapies such as nerve block should be employed.

ii) Up to 6 months after the cure of eruptions

Drug treatment using NSAIDs, vitamin B preparations, or antidepressant drugs, and physical therapy including nerve block therapy, laser therapy, acupuncture, and iontophoresis therapy should be tried as monotherapy or combined therapy.

iii) More than 6 months after the cure of eruptions

Combined therapy including drug treatment and physical therapy should be employed, while exercising caution with regard to the possible adverse effects of prolonged use.

(4) Treatment of elderly patients

Elderly patients account for a considerable proportion of all patients with PHN. Particular attention to the following points is important in the treatment of this population.

i) Is it truly PHN?

It is possible that any pain in patients who have had herpes zoster may be wrongly attributed to PHN. Fracture pain, osteoarthritis, secondary muscle ache derived from pain-limited motion, and pain from other diseases such as cardiac disease may be reported as PHN by the patient.

ii) Psychological dependence

Patients with PHN tend to be isolated from social life, preoccupied with pain and the fear of pain, and psychologically dependent on others. It therefore is necessary for patients and their families to better understand the patient's response to pain and to reconsider the living environment.

iii) Assessment of pain

The assessment of pain in elderly patients can be difficult, often leading to difficulties in understanding symptoms. The physician should strive for objective assessment of the patient's pain, taking into account his/her speech and actions in the consultation room or reports from family members regarding the patient's daily life.

iv) Dependence on treatment

Elderly patients characteristically exhibit intense anxiety in regard to the cessation or alteration of treatment. The physician in charge should always try to assess the patient's pain objectively and make certain that the patient understands the need to continue, change, or terminate treatment.

(5) Iontophoresis therapy for PHN

Iontophoresis therapy is a method of topical drug delivery by which ionized drug in a solution is introduced into the body painlessly via the skin.

We have carried out iontophoresis therapy using lidocaine and methylprednisolone in the Department of Dermatology, Tokai University School of Medicine (Fig. 1), with favorable clinical results. Over two-thirds of more than 1,000 patients with PHN (mean duration of PHN, 30.6 months) showed 40–100% improvement in neuralgia after an average of 3.8 sessions of therapy.[5)]

This form of therapy is painless, and its efficacy is not affected by the duration of PHN. The treatment was effective in patients with

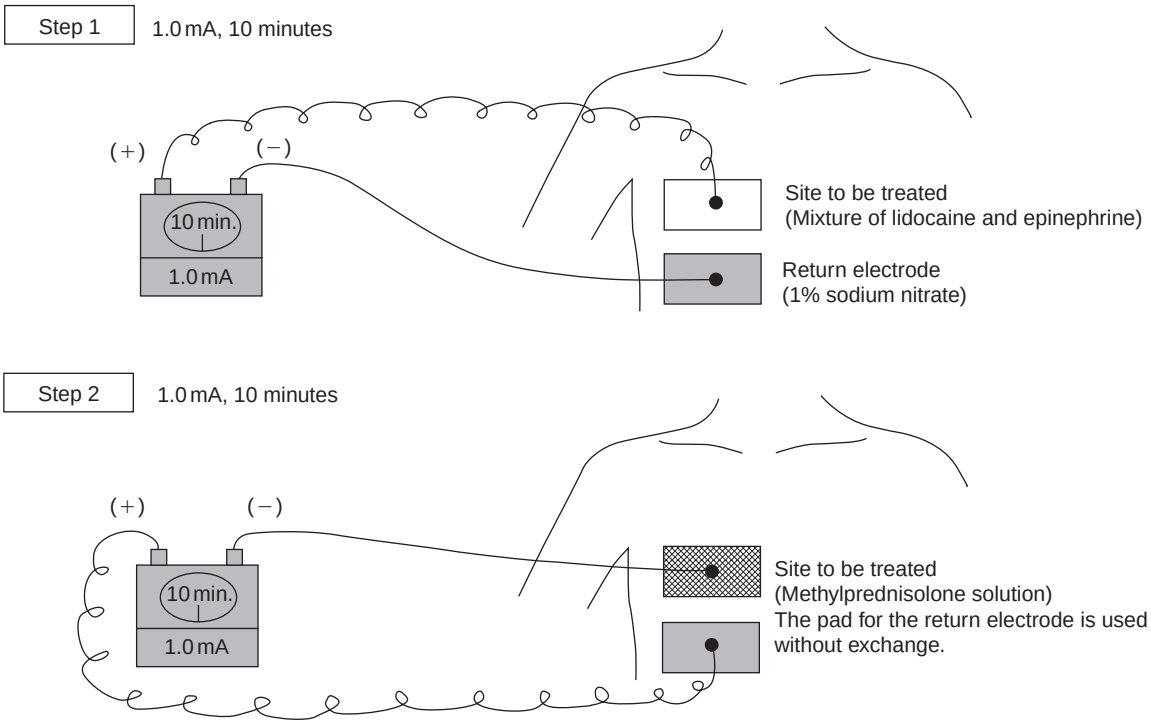

Fig. 1 Iontophoresis for postherpetic neuralgia
(Source: Reference 5: *Dermatology Practice 10*, Bunkodo, 2000; pp. 110–114.
For Information about the instrument, refer to BS Medical, Tel. +81-3-3299-6425.)

pain persisting for more than one year, those who did not respond to other treatments, and those who had underlying diseases such as malignant tumor, hypertension, or diabetes mellitus. Follow-up of patients for 1–5 years after the end of therapy confirmed a continuing therapeutic effect.[6)]

Therefore, iontophoresis therapy for PHN is a clinically useful therapeutic option. Many other therapies have been reported to be less effective in patients with neuralgia persisting for at least one year, indicating the usefulness of iontophoresis therapy for the treatment of PHN.

Is Prevention of PHN Possible?

Unfortunately, there is currently no absolute prophylaxis for PHN. However, since PHN occurs as a sequela to herpes zoster, the prevention of herpes zoster is useful.

1. Prevention of herpes zoster

Varicella vaccine is promising, and those who are of an age susceptible to herpes zoster, i.e., 50–55 years of age, should be inoculated with varicella vaccine to obtain booster immunity.[7)] Clinical trials of this procedure have been carried out in the US as well as Japan, with benefits reported.

2. Prevention of PHN in herpes zoster

Prevention of the occurrence of PHN is an important issue to be considered when a patient has already contracted herpes zoster.

(1) Antiviral drug therapy in the early phase of herpes zoster

Herpes zoster should be mitigated through early-phase antiviral drug therapy.[8)] Antiviral agents with excellent clinical efficacy have been developed, including Arasena A® ointment as topical therapy, Zovirax® and Barutorex® as oral preparations, and Arasena A® and

Table 2 Immunogenetic Analysis of VZV

Herpes zoster	Disease resistance: HLA-B*5101
PHN	Disease resistance: HLA-B*4001 Disease resistance: HLA haplotype (A*3303-B*4403-DRB1*1302)

Zovirax® as intravenous preparations. The main point of treatment is to use these antiviral agents in the early stage after onset. One report has documented a 50% decrease in the incidence of PHN after antiviral drug treatment for herpes zoster.

In dosage regimens of antiviral drug therapy, renal function is an important issue. Dose adjustment is necessary for elderly patients or those who have renal disease. Dosage regimens of intravenous formulations are described in detail in the manufacturer's instructions for use of the drug, and the treatment of patients should follow these instructions. When impaired renal function is present, the dose is determined according to serum creatinine clearance. In actual practice, serum creatinine clearance can be estimated from the serum creatinine level and the patient's body weight and age according to a simple formula.[2)]

It should be noted that the combined use of topical and oral antiviral drugs or topical and intravenous drip administration generally is not covered by health insurance in some areas of Japan (e.g., Kanagawa Prefecture).

(2) Proper topical therapy for skin lesions

Dermatologists should select an appropriate topical preparation for eruptions, with reference to the particular disease stage, and provide instructions as to its use.[2)]

(3) Aggressive treatment of neuralgia

Neuralgia should be treated as needed, in cooperation with an anesthesiologist.

(4) Instructions for daily life

For patients with herpes zoster, instructions for daily life that emphasize the importance of rest, recreation, and nutrition are necessary. In addition, patients should be instructed to return to their usual everyday life after eruptions have subsided. Rehabilitation training should also be considered in some cases, particularly those with limb lesions.

Prediction of Onset of Herpes Zoster and PHN

If PHN derives from nerve degeneration resulting from invasion of varicella-zoster virus (VZV), the body's immune response (sensitivity) to VZV may be involved in disease onset. If there were immunogenetic differences in patients affected by varicella, zoster, and PHN, and if such differences were clarified, the onset of disease might be predicted.

In this regard, we examined the HLA antigen gene region on the short arm of chromosome 6 for genetic control of the immune response to VZV.[9)] Results confirmed the involvement of HLA antigens in disease susceptibility and genes controlling resistance (Table 2). Therefore, if these diseases can be predicted, prevention of their onset may become possible by various means, including vaccination.

Conclusion

Antiviral agents for herpes zoster have been developed and are in widespread use in clinical practice, although the efficacy of these antiviral agents for PHN has been denied. However, methods of dealing with patients and the usage and place of antiviral agents in the actual clinical setting should be considered further, taking into account both the prediction and prevention of the onset of herpes zoster and PHN.

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