

significance of the complement deposits demonstrated in the areas of amyloid fibrils of cutaneous lesions.

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## Lymphocytotoxic Autoantibodies in Pemphigus and Systemic Lupus erythematosus

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**Abstract.** Lymphocytotoxic autoantibody (LCTA) was studied in 9 patients with active pemphigus and 19 patients with active systemic lupus erythematosus (SLE). All patients with SLE showed high titres of LCTA but only one patient with pemphigus had LCTA. In this paper we discuss the immunological significance of LCTA and the difference in the incidence of LCTA between pemphigus and SLE. In addition we present the clinical features of the case with LCTA-positive pemphigus and suggests that the presence of LCTA is due to the common viral infection.

**Key words:** Lymphocytotoxic autoantibody; Pemphigus; Systemic Lupus erythematosus

In 1970 Terasaki et al. (10) reported the presence of lymphocytotoxic autoantibody (LCTA) in the

serum of patients with systemic lupus erythematosus (SLE). Stastny & Ziff (9) demonstrated that LCTA was correlated with the degree of clinical activity and suggested that LCTA might offer an explanation for the lymphopenia so commonly found in SLE patients. T cell specificity of LCTA in SLE was demonstrated (5) and Koike et al. (4) reported that LCTA had the analogy of natural thymocytotoxic autoantibodies appearing in New Zealand mice (8) and suggested, furthermore, that LCTA in patients with SLE is responsible for the selective loss of certain functional T cell subsets including suppressor T cells which are closely related to autoantibody production and play a significant role in the pathogenesis of SLE. Disturbance of suppressor T cells was also reported in pemphigus (2) and we therefore made a preliminary study of LCTA in pemphigus in order to elucidate the etiological analogy between SLE and pemphigus.

## MATERIALS AND METHODS

### Patients and sera

Sera from patients with SLE who satisfied the diagnostic criteria of the American Rheumatism Association for SLE (1) were collected. Sera from 9 patients with pemphigus, whose diagnosis was established by typical clinical features, histological and immunopathological findings and the presence of circulating IgG or polyvalent Ig antibodies against intercellular substances, were also collected. The numbers of patients with pemphigus vulgaris and foliaceus were 7 and 2, respectively. These patients were all in the active stage. All sera obtained were stored at  $-80^{\circ}\text{C}$  until use.

### Cells

Peripheral blood lymphocytes (PBL) were isolated from 5 normal volunteers with differing HLA by Ficoll-Paque (Pharmacia, Sweden) gradient.

### Cytotoxic test

The test for lymphocytotoxic autoantibody was performed by the modified method of Shirai and Mellors (8). Briefly, a mixture of 0.025 ml of PBL suspension ( $6 \times 10^5$ /ml) in RPMI medium supplemented with 2% fetal calf serum and 0.05 ml patient serum in serial dilutions was incubated at  $4^{\circ}\text{C}$  for 30 minutes. Then the mixture was incubated again with 0.05 ml rabbit serum at concentration of 1:2 as a complement source. This rabbit serum was previously selected individually for low cytotoxicity for human PBL. The trypan blue dye exclusion method was employed to determine the dead cells and alive cells. Any serum that killed more than 20% of the cells in the test was graded positive, because the sera taken from 20 normal controls killed less than 20% of the cells. The cytotoxic titre of the serum tested was tabulated as the reciprocal of the serum dilution that produced more than

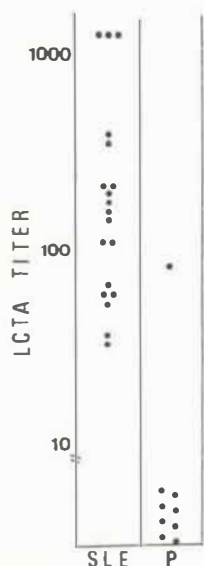


Fig. 1. Lymphocytotoxic autoantibody in patients with pemphigus and systemic lupus erythematosus. *p.*, pemphigus; *SLE*, systemic lupus erythematosus.

20% dead cells. Test serum without complement and complement without test serum were always employed as negative controls. To examine the isotype of LCTA, patient's serum was treated with 2-Mercaptoethanol (2ME). 0.2 M 2ME was mixed with an equal volume of patient serum and incubated for 15 minutes at 37°C followed by 4 hr at room temperature, and then dialysed in running tap water for 2 hr, followed by phosphate-buffered saline solution, pH 7.6, overnight. Following this treatment, the activity was lost in all samples. These results suggested that LCTA was IgM antibody. In order to test whether other lymphocytotoxic sera such as those directed against HLA antigens could possibly influence the reactivity, a comparison of reactivity of the test sample against five HLA-different PBL was made. No difference in cytotoxic titre was noted.

## RESULTS

As shown in Fig. 1, LCTA was found in all patients with active SLE but in only one out of 9 patients with active pemphigus. The clinical features of a case of LCTA-positive pemphigus are as follows. The patient, a 64-year-old man, developed erythematous and bullous lesions on the cheek, chest and back regions in September, 1978. Scaly erythematous lesions developed on the face. Mucosal lesions were absent throughout the course. No abnormalities in the chest, abdomen or cardiovascular system were found. Immunofluorescent technique showed

IgG deposits in the intercellular substance of epidermis by direct method and also by circulating antibody against intercellular substances at a titre of 1:40.

The patient was treated with oral corticosteroids and the lesions gradually improved. Routine blood examination disclosed no abnormalities and lymphopenia was not found during the course. He denied any past history of blood transfusion. Anti-nuclear antibodies were not demonstrated when using chicken erythrocytes, *Clithidial luciliae*, and lip and liver of guinea pig as substrates. Although his clinical features were similar to those of pemphigus erythematosus, histological findings revealed the suprabasal bulla with acantholysis. The diagnosis in this case was pemphigus vulgaris.

## DISCUSSION

It is generally accepted that all patients with pemphigus have autoantibodies in the sera which are directed against intercellular areas of stratified squamous epithelia. The mechanisms of the production of such autoantibodies are not well known. Recently Hashimoto et al. (2) studied the ConA-induced suppressor activity of peripheral blood lymphocytes in patients with pemphigus and showed a significantly low activity of suppressor cells, compared with healthy individuals. Of special interest is whether or not LCTA-mediated dysfunction of suppressor cells is present in pemphigus. However, our present study showed the incidence of LCTA in patients with pemphigus to be very low. Hosokawa et al. (3) also reported a similar low incidence of LCTA. This accumulating evidence indicates that the mechanisms of disturbance of suppressor T cells in pemphigus, if present, are different from those in SLE.

LCTA is demonstrated in infectious diseases, autoimmune diseases, malignancies and so on. Of these diseases, infectious diseases are the most common. Mottironi & Terasaki (7) reported the association of LCTA with diseases of viral infection such as measles and rubella. Mayer et al. (6) also demonstrated the presence of LCTA in mumps, influenza and herpes virus infection. Bearing in mind their investigations, the presence of LCTA in the present case of pemphigus may be a consequence of a common viral infection, by chance, due to the absence of anti-nuclear antibody, lymphopenia and malignant diseases during the course.

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## Leukoderma syphiliticum: Ultrastructural Observations on Melanocyte Function

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**Abstract.** A case of genital leukoderma syphiliticum was analysed submicroscopically. No treponema pallidum organisms could be detected intra- or extracellularly in the epidermis or in the dermis. The melanocytes were only slightly reduced in number and had mostly normal outlines. The melanogenesis was impaired and small melanosomes with decreased deposition of melanin were mostly produced at the expense of normal melanin granules. A partial block in the melanin transfer mechanism seems to be in evidence. As no direct destructive action of spirochetes on the melanocytes is observed, an indirect effect is assumed, e.g. by a tyrosinase inhibitor.

**Key words:** Lues; Melanosomes; Pigmentation

Leukoderma has been known since ancient times as a classical syphilitic stigma. Nowadays this sign is infrequently observed and occasional reports concern uncommon localizations (6). Leukoderma syphiliticum (LS) develops during the resolution of a roseol or papular syphilis and often persists throughout life. The occurrence of treponema pallidum in other, secondary lesions such as roseol, papules, and condyloma lata has long been well known. The exact localization in the various lesions of the spirochetes intra- and extracellularly has recently been classified by electron microscopic investigations (4, 8, 9). However, in the cases of LS, it is not known if treponema pallidum produces its effect upon the melanocytes via a destructive action or by an inhibitory effect. To clarify this, electron microscopic analyses were performed in one case of LS. This case was the first one in a large material of syphilitic patients observed for many years and is furthermore unusual in its localization.

## MATERIAL

The patient was a 24-year-old male homosexual who presented asymptomatic macular hypopigmentations of penis and scrotum (Fig. 1a, b). He was in good health and a routine serology check-up one year ago proved negative. Anamnestic data indicated that primary sclerosis preceded the pigment changes by 2 months.