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# Naturally Occurring T Lymphocytotoxic Antibody in Viral and Related Skin Diseases

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The naturally occurring T lymphocytotoxic antibodies in patients with viral and related skin diseases were investigated and compared with those of systemic lupus erythematosus (SLE). The incidences of T lymphocytotoxic antibodies in exanthema suspected of viral infection, infectious mononucleosis, rubella and pityriasis rosea were 28%, 44%, 8% and 28% respectively. Sera from patients with herpes zoster and erythema infectiosum did not show positivity. Incidence in SLE sera as positive control was 82%. The T lymphocytotoxic antibodies detected in skin diseases were similar in nature to those of SLE patients, but were transient and lower in titer than those of SLE. *Key words: T lymphocytotoxic antibody; Viral infection; Pityriasis rosea; SLE.* (Received September 16, 1983.)

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Naturally occurring T lymphocytotoxic antibody has been detected in sera of systemic lupus erythematosus (SLE) patients (1, 2). It was demonstrated that this antibody could be detected by its inhibiting ability in E-rosette formation by normal T lymphocytes (3).

Similar naturally occurring antibodies have been observed in a variety of diseases including autoimmune diseases (4), mycosis fungoides (5), viral infection (6, 7), transplantation (8) and following vaccination (9). However, there are few reports on association of naturally occurring T lymphocytotoxic antibodies with skin diseases (5, 10).

Based on these findings, we have investigated the occurrence of T lymphocytotoxic antibodies in patients with viral and related skin diseases and compared them with those of SLE.

## MATERIALS AND METHODS

### *Patients*

Sera were obtained from 25 patients with exanthema suspected of viral infection, 9 patients with infectious mononucleosis with exanthema, 50 patients with rubella, 18 patients with herpes zoster, 18 patients with pityriasis rosea, 9 patients with erythema infectiosum, 11 patients with SLE, and 28 healthy adults. All dermatological diseases were diagnosed by clinical features and laboratory data. All SLE patients fulfilled the American Rheumatism Association criteria for SLE (11). All sera were stored at −40°C until use.

### *Lymphocyte separation*

Peripheral blood lymphocytes (PBL) were obtained from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. PBL from the interface were washed 3 times in phosphate buffered saline (PBS) and resuspended in RPMI-1640 medium.

### *E-rosette inhibition test (ERIT)*

T lymphocytotoxic antibody was detected by the ability of sera to inhibit E-rosette formation of PBL and was evaluated by the slightly modified method of Koike et al. (3). A mixture of 25 μl of PBL

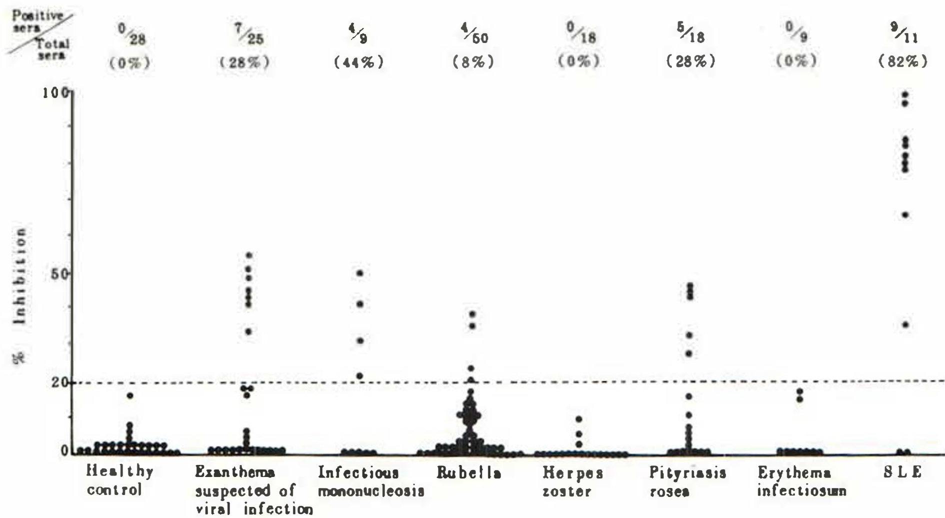


*Fig. 1.* Incidence of naturally occurring T lymphocytotoxic antibodies in sera from patients with viral and related skin diseases, and SLE. Each serum was diluted at 1:4.

suspension ($5\times10^6$ cells/ml) and 50 µl of serum at varying dilutions was incubated at 15°C for 30 min (first incubation). Next, 50 µl of rabbit complement were added. After incubation for 180 min (second incubation), the cells were then washed once in RPMI-1640 medium, and tested for sheep erythrocyte (E)-rosette formation as previously described by Mendes et al. (12). After overnight incubation at 4°C, the cells were gently resuspended, and E-rosette forming cells (RFC) were counted on prestained slide (Blutstan, Daiichi Pure Chemicals, Japan). More than 200 lymphocytes were counted, and the lymphocytes binding 3 SRBC or more were regarded as positive RFC. As controls, PBL were treated with serum alone or with the complement alone. Percentage of E-rosette inhibition was calculated as follows:

$$\%\,\text{inhibition} = \left(1.0 - \frac{\text{Serum treated }\%\,\text{RFC}}{\text{Untreated }\%\,\text{RFC}}\right) \times 100\ (\%)$$

Sera which showed more than 20% inhibition were considered to be positive.

### *2-Mercaptoethanol (2-ME) treatment of sera*

A mixture of equal volume of 0.2 M 2-ME and the serum was incubated for 15 min at 37°C followed by incubation for 4 hours at room temperature, and then dialysed with PBS overnight.

### *Membrane immunofluorescence*

A suspension of $5\times10^6$ PBL in 50 µl of serum was incubated for 60 min at 4°C or 15°C or 37°C. The cells were then washed twice with RPMI-1640 and resuspended in 50 µl of fluorescein-labeled goat antibodies to human IgM, G and A. After incubation for 60 min at 4°C, the cells were washed twice, resuspended in 50% glycerol with PBS (pH 8.0), and examined with a darkfield fluorescence microscope. More than 100 lymphocytes were counted. Percentage FITC-positive cells (%PC) were calculated as follows:

$\%\,\text{PC} = \%\,\text{PC in experiment} - \%\,\text{PC in control}.$

## RESULTS

### *Occurrence of natural antibodies to T cells in sera from various skin diseases*

By using E-rosette inhibition test (ERIT), we examined natural antibody activities in sera obtained from six skin diseases, SLE and healthy adults. As shown in Fig. 1, the

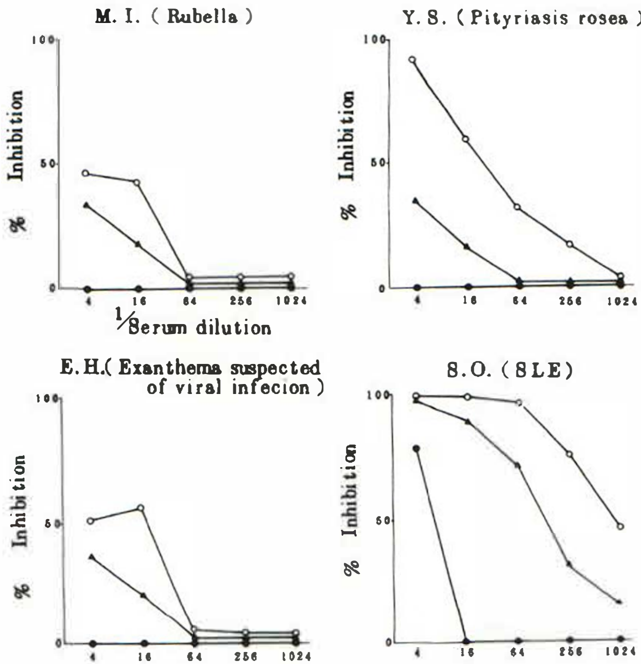


*Fig. 2.* Temperature dependency of naturally occurring T lymphocytotoxic antibodies in sera from viral and related skin diseases, and SLE. Serum dilution; 1 : 4. ▲—▲, 4°C; ○—○, 15°C: ●—●, 37°C.

frequencies of positive sera, which showed more than 20% inhibition, were 28%, 44% and 28% in exanthema suspected of viral infection, infectious mononucleosis and pityriasis rosea respectively. Four out of fifty rubella patients were positive (8%). There was no positive serum in herpes zoster and exanthema infectiosum. 82% of sera obtained from SLE patients were positive and most of them showed much higher inhibiting activity than those of other skin diseases. None of the sera from healthy adults showed positive activity.

Table I. *Effect of 2-mercaptoethanol treatment on naturally occurring T lymphocytotoxic antibodies in sera from patients with viral and related skin diseases and SLE*

| | 2-ME treatment | |
|---|---|---|
| Diagnosis | Before (%) | After (%) |
| Pityriasis rosea no. 1 | 46[a] | 0 |
| Pityriasis rosea no. 2 | 33 | 0 |
| Exanthema suspected of viral infection no. 1 | 54 | 0 |
| Exanthema suspected of viral infection no. 2 | 48 | 8 |
| Rubella | 39 | 4 |
| SLE no. 1 | >90 | 7 |
| SLE no. 2 | >90 | 0 |

[a] Percentage inhibition by ERIT. Serum dilution; 1 : 4.

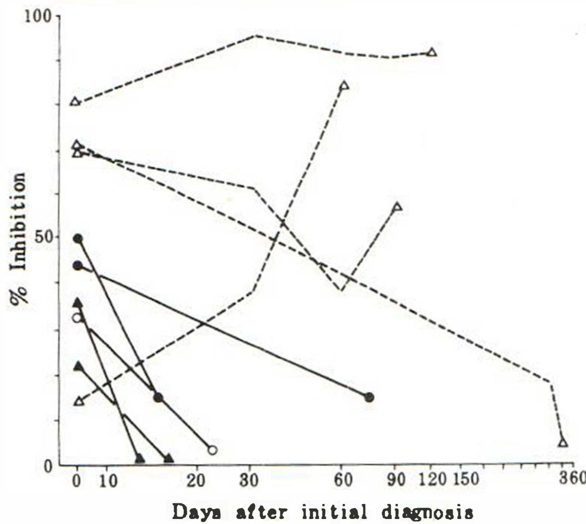


*Fig. 3.* Time course of naturally occurring T lymphocytotoxic antibodies in sera from viral and related skin diseases, and SLE. ●—●, exanthema suspected of viral infection; ○—○, pityriasis rosea; ▲—▲, rubella; △---△, SLE.

*Subclass of naturally occurring antibodies against T cells*

In order to characterize the naturally occurring T lymphocytotoxic antibodies in skin diseases, we performed the 2-mercaptoethanol treatment and the immunofluorescence test with representative sera in each disease group. All seven sera from the 4 disease groups markedly decreased the activities of E-rosette inhibition (Table I). Indirect immunofluorescence studies also demonstrated the presence of high binding activity of natural antibody in IgM subclass, low as IgG and negative as IgA (Table II).

*Comparison of temperature dependency of the antibody activity between skin diseases and SLE*

We next examined the temperature dependency of the antibody activity by using four representative sera from exanthema suspected of viral infection, pityriasis rosea, rubella and SLE. In this experiment, we performed the first incubation composed of PBL and serum to be tested, at various temperatures. The E-rosette inhibition activities of all sera tested were maximum at 15°C, intermediate at 4°C, and absent at 37°C (Fig. 2). This result indicates that the naturally occurring T lymphocytotoxic antibodies in skin diseases have a similar nature to those of SLE.

Table II. *Indirect immunofluorescence demonstrating antigen-binding activities of anti-lymphocyte antibodies in each Ig fraction and at indicated temperature*

| Serum from | 15°C | | 4°C | |
|---|---|---|---|---|
| | IgM (%) | IgG (%) | IgM (%) | IgG (%) |
| Pityriasis rosea | 28[a] | 13 | 32 | 11 |
| Exanthema suspected of viral infection | 13 | 5 | 20 | 11 |
| Rubella | 15 | 20 | 26 | 16 |
| SLE | 52 | 10 | 51 | 6 |

[a] Percentage FITC-positive cells (% PC). Serum dilution: 1:4.

*Time course of E-rosette inhibition activity*

We next examined the time course of E-rosette inhibition activities in skin diseases and compared them with those in SLE. The activities of sera from SLE patients tended to be high and persisted for several months after initial diagnosis (Fig. 3). On the contrary, the inhibitory activities of sera from skin diseases disappeared approximately 10–20 days after diagnosis.

## DISCUSSION

Using erythrocyte rosette inhibition test, we have examined sera from patients with viral and related skin diseases. This assay system was developed to detect human naturally occurring T lymphocytotoxic antibody (Hu-NTA), the human analog of NTA found in NZB mice (3). We found naturally occurring T lymphocytotoxic antibodies in the sera from several skin diseases. Most of SLE sera showed positive activity (82%), as described previously (3, 13). Sera from patients with exanthema suspected of viral infection, infectious mononucleosis, rubella and pityriasis rosea were positive in 28%, 44%, 8% and 28% respectively. In contrast, none of the sera from herpes zoster and erythema infectiosum were positive. Although all diseases tested in this study are considered to have an association with viral infection (14, 15), these results indicate that natural T lymphocytotoxic antibodies occur in some sort of diseases (Fig. 1). One could postulate that the four skin diseases which showed positive sera share similar mechanism to produce natural T lymphocytotoxic antibody in their etiology. We analyzed the character of naturally occurring T lymphocytotoxic antibodies detected in these skin diseases and found that these antibodies were mostly in IgM subclass and cold reactive (Tables I, II, Fig. 2). Since these characters of the T lymphocytotoxic antibodies are similar to those of SLE patients, it it suggested that these antibodies are produced in the same mechanisms. However, there was a difference in the duration of titers between the patients with SLE and skin diseases. Natural T lymphocytotoxic antibodies in SLE patients tended to continue high activities over a period of several months (Fig. 3). On the contrary, natural T lymphocytotoxic antibodies in skin diseases were relatively low in titer and disappeared within 10–20 days after diagnosis.

It has been reported that natural T lymphocytotoxic antibody in SLE has biological effects on lymphocyte function and it might be related to the development of the progressive T-cell abnormality in this disease (13). In contrast, since the natural T lymphocytotoxic antibodies in skin diseases are temporary, these antibodies may not severely affect the immune competent cells. Actually, no patient with significant immune disorders among skin diseases was observed in our study (data not shown). From these facts, it is postulated that the etiological role of the antibodies detected in the patients with these skin diseases is different from that in SLE.

Recently, Furukawa and colleagues (10) reported the occurrence of lymphocytotoxic antibody in one out of nine patients with pemphigus. They suggested that the presence of lymphocytotoxic antibody in pemphigus may be a consequence of a viral infection, by chance. Our results also indicate that some viral and related skin diseases showed natural T lymphocytotoxic antibodies (Fig. 1). However, since not all viral and related skin diseases tested in this study showed positive serum, the occurrence of naturally occurring T lymphocytotoxic antibody might be dependent on etiology of diseases including virus.

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