A Case Infected with *Rickettsia tsutsugamushi* Accompanied by Transient Depression of Delayed-Type Hypersensitivity

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Abstract: Transient depression of intradermal skin test to purified phytohemagglutinin (PHA) and purified protein derivative (PPD) was observed in a case infected with *Rickettsia tsutsugamushi* (*R. tsutsugamushi*). This transient depression has already been observed in some of infectious diseases as well as in viral vaccine in human, but not in *R. tsutsugamushi* infection. In this case of R. tsutsugamushi infection the intradermal skin test became normal after 40 days of proper therapy for this infection. In immunological analysis of lymphocytes in peripheral blood OKT3 and OKT4 decreased and OKIal increased, while lymphocyte proliferation test using PHA, concanavalin A (ConA) and pokeweed mitogen (PWM) was almost normal and suppression of immunoglobulins in serum was not detected. Although the microorganism of *R. tsutsugamushi* were not isolated, the diagnosis was done from the clinical symptoms and laboratory data. However, the mechanism of this transient depression of delayed-type hypersensitivity (DTH) is not yet well known.

Key words: *Rickettsia tsutsugamushi*, Delayed-type hypersensitivity

INTRODUCTION

In infectious diseases, such as viral, bacterial, mycobacterial or fungal infection, and after the administration of viral vaccine a transient depression of DTH to tuberculin in human has been reported (Brody *et al.*, 1964; Starr and Berkovich, 1964; Reed *et al.*, 1972; Haider *et al.*, 1973; Heiss & Palmer, 1974; Mangi *et al.*, 1974). The mechanism is not yet known, although in infectious mononucleosis the phenomenon has been correlated with lymphocyte function in vitro and with quantitative alteration in T cells, B cells and atypical lymphocytes (Mangi *et al.*, 1974). In influenza virus infection in mice it has also been demonstrated that transient depression of DTH mediated by suppressor T cells develops during the infection (Liew and Russell, 1980). In animal model with *R. tsutsugamushi* it has been pointed out that defense mechanism of the host is related with cell me-

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diated immunity (Nancy and Osterman, 1979; Jerrells and Osterman, 1982). In this paper we report immunological status investigated in a person infected with *R. tsutsugamushi* who had transient depression of DTH during the exacerbation.

MATERIALS AND METHODS

Case presentation. A 46-year-old man was admitted in the lst department of surgery of Nagasaki university hospital because of high fever.

He was well until November 15, 1984. On November 16 he developed high fever (39°C) accompanied with headache, general fatigue and eczema on the whole body. But he did not visit any doctors. On November 22, 6 days after the onset, he visited the hospital, because of the persistence of remittent fever. Besides the above symptoms the patient had also lymphadenopathy. Though antibiotics (amoxicillin, cefazolin and piperacillin) were administered to the patient, the remittent fever and other symptoms did not subside. Thus, he was introduced and admitted in our department in November 26.

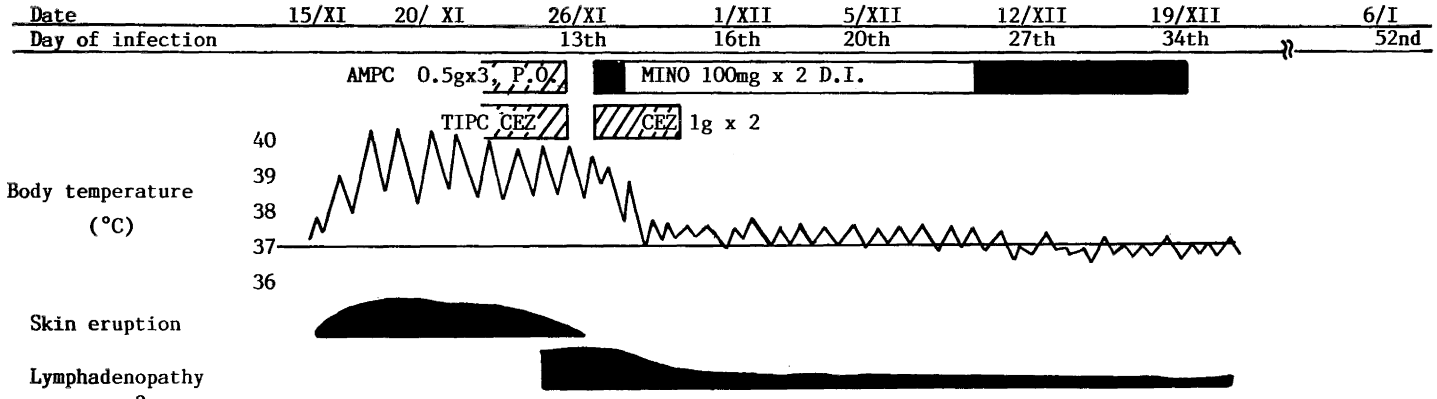
On admission body temperature was 38.5°C, pulse 80/min, respiration 28/min and blood pressure 100/66mmHg. Cervical, axillary and inguinal lymph nodes were enlarged, mobile and tender. A eschar was visible at the right side of cervical region. Eczema was only detected on the anterior part of the chest.

Urine was normal. White blood cell (WBC) count was 16,900/mm^3, with 28 percent of band neutrophils, 14 percent of normal lymphocytes and 20 percent of atypical lymphocytes in the differential. The hematocrit was 40.3 percent, red blood cell $431 \times 10^4/mm^3$, hemoglobin 13.2g/dl, C-reactive protein 5 positive, muco-protein 140mg/dl, erythrocyte sedimentation rate 8mm/1hr, and the total protein 5.5g/dl (albumin 2.9g/dl and globulin 2.6g/dl), urea nitrogen 16mg/dl. Weil-Felix reaction was negative for OXK, OX2 and OX19. The level of IgM and IgG specific antibodies to *R. tsutsugamushi* was over than 1:640 and 1:1,280 in the immune peroxidase method (Suto, 1983), respectively. The findings of an electrocardiogram was within normal limits. X-ray films of the chest showed normal appearance of the lung and heart.

From the above clinical findings and serological data the patient was diagnosed to have *R. tsutsugamushi* infection.

Clinical course. As shown in Fig. 1 minocycline was administered from November 26, and 48hr later his temperature decreased and became normal within 2 weeks. All the clinical symptoms disappeared within 2 weeks. C-reactive protein and other inflammation reactions also became normal within 2 weeks. Lymphnodes decreased gradually and attained normal size within 2 months.

Antibody to R. tsutsugamushi. *R. tsutsugamushi* specific antibody was determined by immune peroxidase method using Gilliam strain as antigen (Suto, 1983).



Date	15/XI	20/ XI	26/XI	1/XII	5/XII	12/XII	19/XII	6/I
Day of infection			13th	16th	20th	27th	34th	52nd
WBC (/mm ^{3})			16,900	9,500	7,700	6,500	7,700	8,400
Defferential (%)								
Band neutrophile			28	3	1	1	2	3
Lymphocytes			14	58	67	72	56	50
			(2,366)*	(5,510)	(5,159)	(4,600)	(4,312)	(4,200)
Atypical lymphocyte			20	1	2	1	1	0
			(3,380)**	(95)	(154)	(65)	(56)	(0)
Weil-Felix								
Proteus OXK			negative		1: 20	1:40	1:40	
R.tsutsugamushi								
Specific IgG antibody			1:1,280		1:1,280		1:1,280	
Specific IgM antibody			1:640		1:640		1:640	
Immunoglobulin in serum								
IgG (mg/dl)			1,804		2,002		2,063	1,752
IgM (mg/dl)			492		438		396	268
IgA (mg/dl)			412		387		230	208

* : Total number of lymphocytes /mm^3. ** : Total number of atypical lymphocytes.

Fig. 1. Case: T,Nishimura, 46 years old, Male infected with Rickettsia tsutsugamushi.

Intradermal skin test. Intradermal skin test for DTH was tested with two antigens which were PPD and PHA. The concentration of PPD and PHA was 0.05μg/0.1ml and 5μg/0.05ml, respectively. At 48 hours the transverse diameter of induration was measured and recorded in millimeters. The intradermal skin tests were considered positive if the transverse diameter of induration was over than 10mm for PPD and 25mm for PHA (Tamaki, 1984).

Subclasses of lymphocytes. Human T lymphocyte subclasses were enumerated by flow cytometric analysis using each monoclonal antibody reacting with the cells in the lymphocyte (Hoffman *et al.*, 1980). The reactivity of each monoclonal antibody for enumeration of lymphocytes in peripheral blood is as follows: OKT 3 reacts with pan T lymphocytes; OKT 4 reacts with helper/inducer T cells; OKT 8 reacts with suppressor/ cytotoxic T cells; OKIal is expressed on the surface of B lymphocytes, monocytes, a subset of Null cells and activated human T lymphocytes (Reinherz *et al.*, 1979).

Lymphocyte proliferation test. Lymphocyte proliferation test was determined by the method of microculture technique (Itoh *et al.*, 1982). For the test, mitogens such as PHA, ConA and PWM were used. The results in counts per minute per million lymphocytes were expressed as counts per minute in the stimulated culture divided by counts in the nonstimulated culture (stimulation index).

RESULTS

Lymphocytes in peripheral blood.

As shown in Fig. 1 the total number of normal lymphocytes in peripheral blood was maximally elevated on the 16th day of infection and thereafter the number of the lymphocytes gradually decreased to normal limits.

Atypical lymphocytes in peripheral blood.

The percentage in differential and total number of atypical lymphocytes in peripheral blood was shown in Fig. 1. The number of atypical lymphocytes was 3,380/mm^3 (20% in differential) on 13th day of infection. Thereafter, the atypical lymphocytes rapidly decreased in number and disappeared on 52nd day of infection.

Subclass of lymphocytes in peripheral blood.

Analysis of lymphocytes in peripheral blood was as shown in Table 1. The percentage of OKT 3 and OKT 4 decreased on 13th day of infection, although that of OKT 8 was within normal limits. OKIal increased during clinical course of infection. Until on 52nd day of infection when we concluded our investigation the percentages of these lymphocytes did not attain normal range, though there was a tendency of improvement.

Lymphocyte proliferation test.

As shown in Table 1 the stimulation index of peripheral lymphocytes was 284 for

Table 1. Cell-mediated immunity of a case infected with *Rickettsia tsutsugamushi*

	Normal values	Day of infection		
		13th	41st	52nd
Subclass of lymphocytes in peripheral blood				
OKT3	59.7–76.9%	53.2		58.0
OKT4	35.5–46.9%	22.1		20.5
OKT8	21.0–32.0%	22.1		24.2
OKIal	8.8–20.6%	25.3		29.5
Stimulation index of lymphocyte proliferation				
PHA	>296	284		283
ConA	≥211	190		184
PWM	≥148	156		152
Intradermal skin test				
PPD	10×10mm	0×0	10×11	11×12
PHA	25×25mm	2×3	20×20	35×40

PHA, 190 for ConA and 150 for PWM. The level of stimulation index was invariant during 52 days of infection.

Intradermal skin test.

Results of PPD and PHA in skin test were shown in Table 1. On the 13th day of infection the reactions for PPD and PHA were negative. The reactivity to PPD became positive on 41st day of infection, although that of PHA was still negative. While, both of the skin tests became positive on 52nd day of infection.

Immunogobulins in serum.

As shown in Fig. 1 concentration of immunoglobulin G (IgG) in serum was 2,002 mg/dl and 2,063mg/dl on the 20th and the 27th days of infection respectively. On 52nd day of infection the concentration of IgG became normal. Concentration of immunoglobulin M (IgM) was already maximum on 13th day of infection and thereafter showed a tendency of normalization. Concentration of immunoglobulin A (IgA) was within normal range during the clinical course and it's highest concentration was detected on the 13th day of infection.

DISCUSSION

This study demonstrated that *R. tsutsugamushi* infection may accompany a temporary depression of DTH during the clinical course as well as other infectious diseases (Olson *et al.*, 1968; Reed *et al.*, 1972; Haider *et al.*, 1973; Heiss and Palmer, 1974; Mangi *et al.*, 1974), although immunological status of the case was investigated from 13th day of infection. Our case was determined as a primary infection of *R. tsutsugamushi*, because high level of *R. tsutsugamushi* specific IgM antibody was detected (Bourgeois *et al.*,

1982).

Atypical lymphocytes were detected on analysis of WBC in peripheral blood before proper therapy was administered for *R. tsutsugamushi* infection. After the administration of minocycline atypical lymphocytes decreased and rapidly disappeared, while normal lymphocytes increased and attained normal limits. Thus, it was assumed that appearance of the atypical lymphocytes might have related with *R. tsutsugamushi* infection, though the isolation of rickettsia in blood was not done. Mechanism of the appearance of atypical lymphocytes is not yet established. Furthermore, we don't have any proof that *R. tsutsugamushi* can grow in lymphocytes, though it had been proved that *R. tsutsugamushi* grows only in living cell.

To understand that relationship between *R. tsutsugamushi* and lymphocytes, subclasses of lymphocytes and lymphocyte function in peripheral blood, DTH and immunoglobulins in serum were investigated.

Bourgeois *et al.* (1982) pointed out that no significant change in pan T lymphocytes was noticed. In the present case infected with *R. tsutsugamushi* pan T lymphocytes decreased during clinical course. The decrease of pan T lymphocytes in our case might have been influenced by severity of the disease caused by late diagnosis.

In a case infected with *R. tsutsugamushi* transient depression of intradermal skin reaction to PPD and PHA was detected during exacerbation, though this transient depression of DTH has already been reported in other infectious dseases (Starr and Berkovich, 1964; Olson *et al.*, 1968; Reed *et al.*, 1972; Haider *et al.*, 1973; Heiss and Palmer, 1974; Mangi *et al.*, 1974). Kinetics of the transient depression of DTH in human is still unclear, though it is reported that the appearance of depression of DTH depends on a viable virus. This was investigated on live measles vaccine in human (Fireman *et al.*, 1969).

Furthermore, lymphocyte proliferation test using PHA and ConA stimulate mainly T lymphocytes while PWM stimulates both B and T lymphocytes, the stimulation index of PHA and ConA was just below normal but that of PWM was just above normal during the clinical course (Itoh *et al.*, 1982).

Dissociation between intradermal skin reaction and lymphocyte function was observed in this case infected with *R. tsutsugamushi*. This dissociation has already been reported in congenital rubella (Olson *et al.*, 1968), measles (Smithwick and Berkovich, 1966), measles vaccine (Fireman *et al.*, 1969) and influenza (Reed *et al.*, 1972) in human. While, the exact reason for the dissociation is still not understood.

It was pointed out that the level of total IgM in serum increased and decreased during clinical course in *R. tsutsugamushi* infection (Bourgeois *et al.*, 1982). In our case not only the level of IgM but also the level of total IgG in serum increased and thereafter decreased to normal limit. In the investigation using live measles vaccine in human in which transient depression of DTH was observed suppression of humoral antibody was not detected (Fireman *et al.*, 1969). Therefore, it was assumed that humoral immunity in our

case is intact.

It was suggested that cellular immunity might be transiently impaired during *R. tsutsugamushi* infection, although only one case was studied. More investigation will be necessary to clarify whether transient depression of DTH is always detected in *R. tsutsugamushi* infection.

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遅延型皮膚反応の低下を伴ったつつがむし病の一症例

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つつがむし病の一症例において，その増悪期間中に遅延型皮膚反応の低下が一過性に検出された．この現象は麻疹，インフルエンザ，ムンプス，風疹，重症結核，全身性真菌症，伝染性単核球症，水痘ではすでに観察されているが，つつがむし病においては未だ報告されていない．我々の症例の遅延型皮膚反応は，発症13日で陰転化し，40日目で正常に復した．遅延型皮膚反応低下中の免疫学的分析では，末梢血中リンパ球のうち，OKT3 およびOKT4 が減少し，OKIal が増加していたが，PHA，ConA および PWM に対するリンパ球増殖反応は正常で，さらに血清中の免疫グロブリン（IgG，IgM，IgA）産生量にも抑制は認められなかった．これらの成績より，遅延型皮膚反応のメカニズムを説明することは困難であるが，つつがむし病増悪期間中に細胞性免疫が低下し，ひいては細胞性免疫に関与する感染症発現の可能性が示唆された．

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