Acta Medica Okayama

Volume 40, *Issue* 6 1986 *Article* 2

DECEMBER 1986

Hyperreactivity of lymphocytes to streptolysin O and lack of plasma inhibitory factor (s) in patients with mucocutaneous lymphnode syndrome.

Hiroshi Wakiguchi[*] Mikiya Fujieda[†] Kenji Matsumoto[‡]
Yuji Ohara[**] Akiko Wakiguchi[††] Taisuke Shiraishi[‡‡]
Takanobu Kurashige[§] Isamu Kitamura[¶]

[*]Kochi Medical School,
[†]Kochi Medical School,
[‡]Kochi Medical School,
[**]Kochi Medical School,
[††]Kochi Medical School,
[‡‡]Kochi Medical School,
[§]Kochi Medical School,
[¶]Kochi Medical School,

Hyperreactivity of lymphocytes to streptolysin O and lack of plasma inhibitory factor (s) in patients with mucocutaneous lymphnode syndrome.*

Hiroshi Wakiguchi, Mikiya Fujieda, Kenji Matsumoto, Yuji Ohara, Akiko Wakiguchi, Taisuke Shiraishi, Takanobu Kurashige, and Isamu Kitamura

Abstract

Lymphocyte activation by streptolysin O (SLO) and factors in the plasma which inhibit the response to SLO were examined in 19 patients with mucocutaneous lymphnode syndrome (MCLS), 54 age-matched (6 months-6 years) normal children, 41 normal children older than 6 years and 10 normal adults. In normal children younger than 6 years, the response to SLO was weak and in many cases no response was seen. On the other hand, in the patients with MCLS, the response of lymphocytes to SLO was high and comparable to the response in adults and children older than 6 years. The DNA synthesis of lymphocytes stimulated by SLO was inhibited almost completely by autologous or allogeneic plasma of many of the normal children and adults. The plasma of patients with MCLS did not inhibit, but rather enhanced the response to SLO. These results suggest that the increased response of lymphocytes to SLO and the lack of plasma inhibitory factors in patients with MCLS may be due to the immune response to the pathogen of MCLS, as yet undiscovered.

KEYWORDS: mucocutaneous lymphnode syndrome, streptolysin O, lymphocyte activation, plasma inhibitory factor (s)

---

*PMID: 3825591 [PubMed - indexed for MEDLINE]

Acta Med Okayama 40 (6) 285-289 (1986)

Hyperreactivity of Lymphocytes to Streptolysin O and Lack of Plasma Inhibitory Factor (s) in Patients with Mucocutaneous Lymphnode Syndrome

Hiroshi Wakiguchi, Mikiya Fujieda, Kenji Matsumoto, Yuji Ohara, Akiko Wakiguchi, Taisuke Shiraishi, Takanobu Kurashige and Isamu Kitamura

Department of Pediatrics, Kochi Medical School, Kochi 781-51, Japan

Lymphocyte activation by streptolysin O (SLO) and factors in the plasma which inhibit the response to SLO were examined in 19 patients with mucocutaneous lymphnode syndrome (MCLS), 54 age-matched (6 months-6 years) normal children, 41 normal children older than 6 years and 10 normal adults. In normal children younger than 6 years, the response to SLO was weak and in many cases no response was seen. On the other hand, in the patients with MCLS, the response of lymphocytes to SLO was high and comparable to the response in adults and children older than 6 years. The DNA synthesis of lymphocytes stimulated by SLO was inhibited almost completely by autologous or allogeneic plasma of many of the normal children and adults. The plasma of patients with MCLS did not inhibit, but rather enhanced the response to SLO. These results suggest that the increased response of lymphocytes to SLO and the lack of plasma inhibitory factors in patients with MCLS may be due to the immune response to the pathogen of MCLS, as yet undiscovered.

Key words : mucocutaneous lymphnode syndrome, streptolysin O, lymphocyte activation, plasma inhibitory factor (s)

Mucocutaneous lymphnode syndrome (MCLS, Kawasaki disease) is a disease of unknown etiology. Because of the frequent association with serious cardiac complications, its pathogenesis and preventive measures have been extensively studied. Hemolytic streptococcus (1), mites (2), a variant of *Propionibacterium acnes* (3) and EB virus (4) have been suggested to be possible pathogens to cause MCLS. Previously, we reported the detection of the plasma factor (s), which is inhibitory to the streptolysin O (SLO)-induced DNA synthesis by lymphocytes, in most of the normal individuals tested (5). In the present study, the SLO-induced DNA synthesis by lymphocytes in patients with MCLS, and the effects of plasma of the patients on the response to SLO were investigated.

Materials and methods

Patients. Nineteen patients, 7 months to 6 years of age, admitted to Kochi Medical School Hospital from March 1982 to December 1983 were studied. Lymphocytes and plasma were collected from each patient 1 to 3 months after the onset of MCLS. None of the patients with MCLS showed any significant elevation of the antistreptolysin O (ASO) or antistreptokinase (ASK) titer.

Fifty-four normal children aged 6 months to 6

years were studied as age matched normal controls. Forty-one children aged 6 years to 14 years and 10 normal adults were studied also.

Lymphocyte activation by SLO. Mononuclear leukocytes were separated from heparinized venous blood by the Ficoll-Conray density gradient method and resuspended at a concentration of 1×10^6/ml in RPMI-1640 supplemented with penicillin G, streptomycin, glutamine, sodium bicarbonate and 10% fetal calf serum (RPMI-10% FCS). Then, 100 μl of the mononuclear cell suspension were cultured with 0.1 unit of SLO and 10 μl of fetal calf serum or test plasma serum for five days in 5% CO_2 at 37°C. Eighteen hours before stopping the culture, 1 μCi of [^{3}H] deoxy thymidine ([^{3}H] dThd, New England Nuclear, 1 mCi/ml, 19.3 Ci/mmol) was added, and the incorporation of [^{3}H] dThd in lymphocytes was measured with a liquid scintilation counter. The effects of the allogeneic plasma were expressed as % enhancement and calculated as follows:

% enhancement = [(Δcpm of lymphocytes cultured in RPMI-1640 supplemented with test plasma/Δcpm of lymphocytes cultured in RPMI-1640 supplemented with FCS)$-1]\times 100$, where Δcpm = {[^{3}H] dThd incorporation (cpm) in stimulated lymphocytes}$-${[^{3}H] dThd incorporation (cpm) in non-stimulated lymphocytes}.

Results

Lymphocyte activation by SLO. The SLO-induced DNA synthesis by lymphocytes of nineteen patients with MCLS within three months from the onset of the disease was compared with that of 54 normal children younger than 6 years and 51 normal controls older than 6 years (Fig. 1). DNA synthesis was slight in lymphocytes of the normal infants and children younger than 6 years old, and [^{3}H] dThd incorporation was $13,310\pm 22,090$ (Δcpm, mean$\pm$SD). However, a significantly higher response ($p < 0.001$) was observed in patients with MCLS ($44,201\pm 32,671$) and normal controls older than 6 years ($36,080\pm 33,931$). There was no difference in the amount of DNA synthesis by SLO stimulated lymphocytes between normal children older than 6 years and normal adults (data not presented).

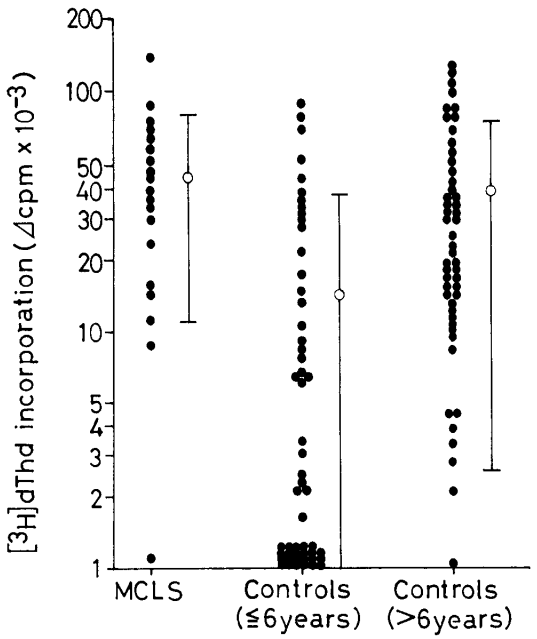

Fig. 1 Streptolysin O-induced DNA synthesis by lymphocytes in mucocutaneous lymphnode syndrome (MCLS). Response of lymphocytes was tested by [^{3}H] dThd incorporation and expressed as Δcpm {([^{3}H] dThd incorporation in stimulated lymphocytes (cpm))$-$([^{3}H] dThd incorporation in non-stimulated lymphocytes (cpm))}. Open circles and error bars indicate means and standard deviations.

Effects of plasma on the response of autologous lymphocytes to SLO. The effects of plasma on SLO-induced DNA synthesis by autologous lymphocytes were determined in 18 patients with MCLS, 50 normal children and 10 normal adults whose indices of response to SLO were higher than 5,000 Δcpm (Fig. 2). Among the normal cases, there were 35 in which DNA synthesis was inhibited almost completely by addition of autologous plasma, and there were 25 in which the plasma enhanced or did not inhibit the DNA synthesis by lymphocytes. On the other hand, the response of lymphocytes was not inhibited by the plasma of patients with MCLS.

Effects of plasma on the response of allogeneic lymphocytes to SLO. The effects of

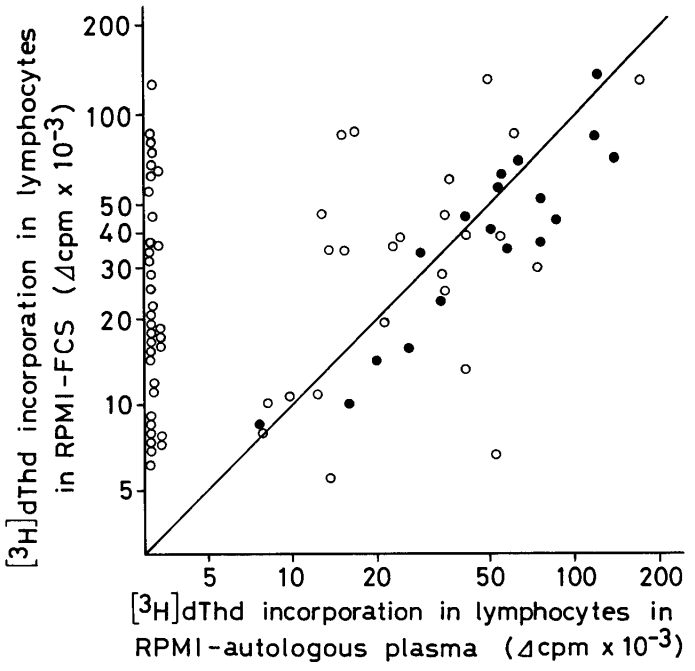

Fig. 2 Effects of plasma on DNA synthesis by autologous lymphocytes stimulated by streptolysin O. DNA synthesis was tested by [^{3}H] dThd incorporation and expressed as Δcpm as in Fig. 1. DNA synthesis by lymphocytes cultured in RPMI-1640 supplemented with fetal calf serum and that of lymphocytes cultured in RPMI-1640 supplemented with autologous plasma were compared. ●, Δcpm in mucocutaneous lymphnode syndrome (MCLS) patients; ○, Δcpm in normal subjects.

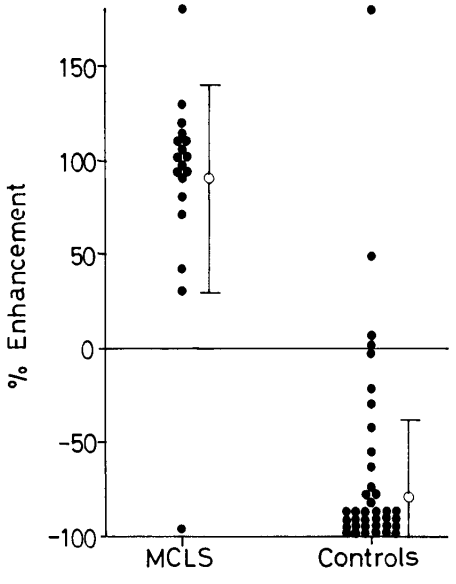

Fig. 3 Effects of plasma on DNA synthesis by allogeneic lymphocytes stimulated by streptolysin O in mucocutaneous lymphnode syndrome (MCLS). The effects was expressed as % enhancement = [([^{3}H] dThd incorporation in lymphocytes cultured in RPMI-1640 supplemented with allogeneic plasma/[^{3}H] dThd incorporation in lymphocytes cultured in RPMI-1640 supplemented with fetal calf serum)−1]× 100. Open circles and error bars indicate means and standard deviations.

fresh frozen plasma on SLO-induced DNA synthesis by allogeneic lymphocytes were tested in 18 patients with MCLS and 39 age-matched normal controls (Fig. 3). Though the plasma of the normal group produced more than 50% suppression in 31 cases, the patients' plasma produced more than 50% suppression in only 1 out of 18 cases, and pronounced enhancement was seen in 15 cases. The % enhancement of the normal group was $-74 \pm 41\%$ (mean $\pm$ SD), and that of the MCLS group was $83 \pm 55\%$. The difference was statistically significant ($p < 0.001$).

Discussion

A number of immunological studies have been performed concerning MCLS: increased immunoglobulin levels (6), the presence of immune complexes (7), enhanced T suppressor activity (8) and the presence of serum and plasma inhibitory factor (s) against DNA synthesis of lymphocytes stimulated by phytohemagglutinin or concanavalin A (9)

have been shown in MCLS patients. As to the etiologic agent of the disease, hemolytic streptococcus (1), mites (2), a *Propionibacterium acnes* variant (3) and EB virus (4) are well-known, but unconfirmed, candidates.

We have studied the response of lymphocytes to SLO and plasma inhibitory factors (5) and have found that the plasma of most of the normal individuals tested contained factors which inhibited the DNA synthesis by lymphocytes. Though the degree of inhibition had no correlation with serum ASO or ASK titer, serum and plasma with a high titer of ASO or ASK inhibited the DNA synthesis by lymphocytes profoundly.

At present, the reason for greater response of lymphocytes to SLO in patients with MCLS than in age-matched normal controls has not been clarified. Lea *et al.* (10) described the response of lymphocytes to SLO as consisting of two immunological reactions: a non-specific mitogenic reaction and specific antigenic stimulation, and pointed out that responder cells are T cells which require accessory cells to respond optimally. It is difficult to explain the reason for weak response of lymphocytes to SLO in normal controls younger than 6 years old by weak sensitization to hemolytic streptococcus, because lymphocytes from cord blood respond to SLO (11). There were no differences in the DNA synthesis by lymphocytes induced by phytohemagglutinin, pokeweed mitogen or concanavalin A between normal controls older than 6 years old and those younger than 6 years old.

Furthermore, patients with MCLS showed no hyperreactivity in lymphocyte response to phytohemagglutinin, pokeweed mitogen or concanavalin A. From these facts, it is suggested that the patients with MCLS may be sensitized (or infected) with a pathogen that shares a common antigen with SLO, and consequently show the lymphocyte response to SLO comparable to that of adults.

Although the significance of the inhibitory factor (s) to the response of lymphocytes to SLO in the plasma of normal individuals remains obscure, it seems that the inhibitory factor (s) would not merely be the antibody to SLO, since both the betaglobulin and gammaglobulin fractions contain the inhibitory factor (s) (5) and sera with negative ASO activity can contain the inhibitory factor (s). Three explanations of the lack of the inhibitory factor (s) in most patients with MCLS may be given: 1) There may be common antigens between the inhibitory factor (s) and the causative pathogen (s) of MCLS, so that the antibody against the pathogen (s) would inactivate the inhibitory factor (s) that were present in the plasma of patients with MCLS. 2) Circulating immune complexes which increase in the course of MCLS (7) may also alter the activity of the inhibitory factor (s). However, the plasma of patients with autoimmune disease, such as systemic lupus erythematosus or rheumatic disease, inhibits the response of lymphocytes to SLO almost completely. Therefore, such circulating immune complexes are probably not the cause. 3) MCLS may develop in infants who do not have the inhibitory factor (s) which might otherwise protect them from infection by the pathogen of MCLS. In any case, further studies are required to elucidate these problems.

References

1. Matsumi F and Ueno T: Streptococci as a causative agent for Kawasaki disease (MCLS). Jpn J Sci Biol (1979) **32**, 247-249.
2. Furusho K, Ohba T, Soeda T, Kimoto K, Okabe T and Hirota T: Possible role for mite antigen in Kawasaki disease. Lancet (1981) **2**, 194-195.
3. Kato H, Fujimoto T, Inoue O, Kondo M, Koga Y, Yamamoto S, Shingu M, Tominaga S and Sasaguri Y: Variant strain of *Propionibacterium acnes*: a clue to the aetiology of Kawasaki disease. Lancet (1983) **2**, 1383-1387.

4. Kikuta J, Mizuno F, Osato T, Konno M, Ishikawa N, Noro S and Sakurada H: Kawasaki disease and unusual primary infection with Epstein-Barr virus. Pediatrics (1984) **73**, 413-414.
5. Kuramitsu M, Tone Y, Kuroiwa Y, Morita H, Ogura H, Wakiguchi H, Kurashige T and Kitamura I: Immunoregulatory mechanism in various disease —— suppressive effect of serum on blastogenic response of lymphocytes to SLO. (abstr.) J Jpn Pediatr Soc (1982) **86**, 1648 (in Japanese).
6. Yanase Y, Takayama J, Aso S and Imada Y: Serum immunoglobulin and delayed type hypersensitivity skin tests in the patients with MCLS. J Jpn Pediatr Soc (1982) **86**, 424-435 (in Japanese).
7. Sawa H: Circulating immune complexes in the serum of the patients with mucocutaneous lymphnode syndrome. J Jpn Pediatr Soc (1979) **83**, 493-498 (in Japanese).
8. Matsuoka Y: Lymphocyte function in the patients with Kawasaki disease —— activity of T_G suppressor cells —— J Jpn Pediatr Soc (1983) **87**, 648-655 (in Japanese).
9. Hirao J, Izumi N, Nagai Y, Seike I and Ichimura T: Analytic studies of immunosuppressive factor in plasma of patients with Kawasaki disease (MCLS). Allergy (1981) **30**, 969-975 (in Japanese).
10. Lea T, Michaelson TE and Rosmussen AU: Characterization of the mitogenic and antigenic stimulatory properties of a purified streptolysin O preparation. Clin Exp Immunol (1982) **50**, 139-147.
11. Kitamura I, Nasu K, Kurashige T, Nishibayashi Y, Hayashi H and Kunitomi T: Dynamics of immune serveillance cells. Jpn J Neonatol (1971) **7**, 266-271 (in Japanese).

Received: August 19, 1985
Accepted: October 1, 1986

Reprint requests to:
Hiroshi Wakiguchi
Department of Pediatrics
Kochi Medical School
Kohasu Oko-cho
Nankoku 781-51, Japan