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

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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)

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

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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.

Lympocyte activation by SLO. Mononuclear leukocytes were separated from heparinized venous blood by the Ficoll-Conray density gradient method and resuspended at a concentration of $1 \times 10^6$ 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, 1μCi of $[^3H]$ deoxy thymidine ($[^3H]$ dThd, New England Nuclear, 1 mCi/ml, 19.3 Ci/mmol) was added, and the incorporation of $[^3H]$ dThd in lymphocytes was measured with a liquid scintillation counter. The effects of the allogeneic plasma were expressed as % enhancement and calculated as follows:

$$% \text{ enhancement} = \frac{[\text{cpm of lymphocytes cultured in RPMI-1640 supplemented with test plasma}}{-\text{cpm of lymphocytes cultured in RPMI-1640 supplemented with FCS] - 1}] \times 100}{,}$$

where Δcpm = $[^3H]$ dThd incorporation (cpm) in stimulated lymphocytes $- [^3H]$ dThd incorporation (cpm) in non-stimulated lymphocytes.

Results

Lympocyte 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 $[^3H]$ dThd incorporation was $13,310 \pm 22,090 \text{ (Δcpm, mean±SD)}$. However, a significantly higher response (p < 0.001) was observed in patients with MCLS (44,201 ± 32,671) and normal controls older than 6 years (36,080 ± 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).

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 $[^3H]$dThd incorporation and expressed as $\Delta$cpm 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. ●, $\Delta$cpm in mucocutaneous lymphnode syndrome (MCLS) patients; ○, $\Delta$cpm 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 $= \left\{ \frac{[^3H]}{dThd\; incorporation\; in\; lymphocytes\; cultured\; in\; RPMI-1640\; supplemented\; with\; allogeneic\; plasma} - \frac{[^3H]}{dThd\; incorporation\; in\; lymphocytes\; cultured\; in\; RPMI-1640\; supplemented\; with\; fetal\; calf\; serum} \right\} \times 100$. 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 Propioni-
acterium acnes variant (3) and EB virus
(4) are well-known, but unconfirmed, can-
didates.

We have studied the response of lymphoc-
eytes 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 inhi-
bition had no correlation with serum ASO
or ASK titer, serum and plasma with a high
titer of ASO or ASK inhibited the DNA syn-
thesis by lymphocytes profoundly.

At present, the reason for greater re-
response of lymphocytes to SLO in patients
with MCLS than in age-matched normal con-
trols has not been clarified. Lea et al. (10)
described the response of lymphocytes to
SLO as consisting of two immunological re-
actions: a non-specific mitogenic reaction
and specific antigenic stimulation, and point-
ed out that responder cells are T cells which
require accessory cells to respond optimal-
ly. 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 streptococ-
cus, because lymphocytes from cord blood
respond to SLO (11). There were no differ-
ces in the DNA synthesis by lymphocytes
induced by phytohemagglutinin, pokeweed mi-
togen or concanavalin A between normal con-
trols older than 6 years old and those young-
er than 6 years old.

Furthermore, patients with MCLS show-
ed 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 re-
mains obscure, it seems that the inhibitory
factor (s) would not merely be the antibody
to SLO, since both the betaglobulin and gam-
maglobulin 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 inhibi-
tory factor (s) in most patients with MCLS
may be given: 1) There may be common an-
tigens 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 com-
plexes 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 lympho-
cytes to SLO almost completely. Therefore,
such circulating immune complexes are prob-
ably not the cause. 3) MCLS may develop
in infants who do not have the inhibitory fac-
tor (s) which might otherwise protect them
from infection by the pathogen of MCLS. In
any case, further studies are required to
elucidate these problems.

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