Inhibition of lymphocyte DNA synthesis by plasma from patients with Kawasaki disease

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Abstract. Plasma obtained from patients with Kawasaki disease during the acute phase markedly inhibited DNA synthesis in autologous peripheral blood lymphocytes (PBLs) stimulated by phytohaemagglutinin-P (PHA-P) or concanavalin A (Con A). The inhibition became less marked with the progression of the disease and there was no effect on DNA synthesis in PBLs stimulated by pokeweed mitogen (PWM).

The plasma also inhibited DNA synthesis in PBLs obtained from healthy adults. The postulated suppressors markedly inhibited DNA synthesis in PBLs from healthy adults stimulated by PHA-P, Con A, purified protein derivative (PPD) or mixed lymphocyte culture reaction (MLR) but they had little effect on the DNA synthesis stimulated by PWM or protein A.

With respect to the mechanism, the suppression was found to be potentiated by an increase in the concentration of the patients’ plasma, and not to be associated with cytotoxicity nor with a deficiency of factor(s) indispensable for PBL proliferation. It was also evident that the suppression was not related to the concentration of the stimulant, to the lengths of the culturing period nor to the presence of prostaglandins.

Key words: Immunosuppressive factor - Kawasaki disease - Lymphocyte DNA synthesis - Patient’s plasma

Introduction

Kawasaki disease (mucocutaneous lymph node syndrome: MCLS), first described by Kawasaki in 1967 [8], is a syndrome which presents multiple signs and symptoms. About 1% of patients with this syndrome die due to cardiovascular complications [23]. Although the involvement of allergic reactions is suspected, the aetiology remains unknown.

Material

Sixteen children, aged between 4 months and 7 years, diagnosed as having Kawasaki disease according to the criteria established by Kawasaki [8], were used in this study. Blood samples were withdrawn two to seven times from each patient on different occasions. The acute phase lasted for 5–10 days after onset (average: 8.4 ± 2.6 days). Neither adrenocortical hormones nor aspirin were used during this stage. No clinical signs or symptoms were present during the convalescent period, which lasted 30–40 days (average: 36 ± 9.3 days). Only aspirin was permitted during this stage. One year (11–13 months, average: 12.7 ± 1.7 months) after onset of the disease, the use of all drugs was discontinued. The following 13 children, aged between 6 months and 9 years, were used as controls: 5 healthy children, 4 with streptococcosis, 1 with sepsis, 1 with bacterial pneumonia and 1 with viral pneumonia.

Healthy adult volunteers (20–31 years of age) were recruited from employees of this hospital.

Methods

All plasma samples were aseptically isolated from venous blood to which 20 units/ml preservative-free heparin was added. Portions of patients’ autologous plasma were used for the culturing of PBLs, and the residual plasma was stored at −20°C. Plasma was collected from the ten healthy adults. Stored plasma was treated for 30 min at 56°C and passed through 0.45 µm millipore filters (Millipore Filter Co., Bedford, MA) for sterilization before use.

PBLs were isolated from heparinized venous blood by Ficoll-Hypaque (Pharmacia Fine Chemicals AB, Uppsala, Sweden) density centrifugation [1], and suspended in RPMI 1640 culture medium (Nissui Seiyaku Co., Ltd., Tokyo, Japan) containing 100 units/ml penicillin and 100 µg/ml streptomycin.
Assay of patients' PBL proliferation was carried out following the procedure described previously [9]. The above-mentioned RPMI1640 medium containing 10% fetal calf serum (FCS, GIBCO, control No. 260617) or autologous plasma was used for culture medium, and PBLs were stimulated with 10 μg/ml PHA-P (Difco Laboratories, Detroit, MI), 10 μg/ml Con A (Sigma Chemical Co., St. Louis, MO) or 2.5 μg/ml PWM (GIBCO, Grand Island, NY). Each experiment was performed in triplicate and the mean ± standard deviation (SD) was calculated for the cpm (amount of DNA synthesis). In the determination of healthy adults' PBL proliferation, a microculture technique [22] was used. In brief, PBLs were stimulated with PHA-P 50 μg/ml, Con A 50 μg/ml, PWM 5 μg/ml, protein A (Pharmacia Fine Chemicals) 50 μg/ml, PPD (Japan BCG Laboratory, Tokyo, Japan) 5 μg/ml or allogeneic cells (5 x 10⁴/well) which had been treated with mitomycin C (Kyowa Hakko Co., Ltd., Tokyo, Japan). These were cultured for 5 days, except in an experiment conducted to determine the culturing period, in the RPMI1640 medium containing 10% plasma from the patients obtained during the acute phase of the disease, or control children, or 10% pooled plasma. Experiments using PPD stimulation and MLR were incubated for 7 days. Each experiment was conducted in triplicate or quadruplicate and the cpm (mean ± SD) was calculated.

Evaluation of the suppressive activity of the patients' plasma was made using the following equation and expressed as % inhibition.

\[
% \text{ inhibition} = \left(1 - \frac{P_s - N_s}{P_c - N_c}\right) \times 100
\]

Ps: mean cpm obtained in the stimulant-added experiments using the plasma from the patients or control children
Pc: mean cpm obtained in the stimulant-free experiments using the plasma from the patients or control children
Ns: mean cpm obtained in the stimulant-added experiments using the pooled plasma
Nc: mean cpm obtained in the stimulant-free experiments using the pooled plasma.

In experiments to determine the relationship between the concentration of patients' or control children's plasma and suppressive activity, various concentrations of plasma samples were added to the assay system consisting of PBLs 5 x 10⁴/well, Con A 50 μg/ml and 5% pooled plasma, and the suppressive activity of each sample was evaluated by using the above equation.

The possibility that the suppressive effect of plasma was derived from prostaglandins was investigated using the following procedure: 1 μg/ml indomethacin (Sigma), which was the prostaglandin-inhibitor, was added to the assay system consisting of PBLs 5 x 10⁴/well, Con A 50 μg/ml and 10% patients' plasma, and the ability of indomethacin to counteract the suppressive activity was examined.

The amounts of protein were determined by the method of Lowry et al. [14].

Cell viability was evaluated by the trypan blue dye exclusion test.

Statistical analysis was done using Student's t-test.

Results

When FCS was added to the culture, DNA synthesis in PBLs was comparable in all phases of the disease when stimulated by PHA-P, Con A or PWM. In contrast, when the autologous plasma was used, DNA synthesis by PBLs stimulated by PHA-P or Con A was significantly inhibited during the acute phase of the disease. However, DNA synthesis gradually recovered with the progression of the disease, and 1 year after onset of the disease (Table 1) there were no significant differences between the autologous plasma group and the FCS group under stimulation by PHA-P or Con A.

DNA synthesis in PBLs from the healthy adults stimulated by PHA-P, Con A, PPD or MLR was significantly suppressed by the addition of plasma from the patients. Suppression was very slight with stimulation by PWM and protein A. Suppression was seen only infrequently and to a much lesser extent when plasma from the control children was added (Fig. 1).

| Table 1. Time course of changes in DNA synthesis in PBLs of patients with Kawasaki disease stimulated by different mitogens: comparison between autologous plasma and FCS added to the cultures |
|-----------------|-----------------|-----------------|
| **Acute Phase** | **Convalescent phase** | **After 1 year** |
| Autologous plasma | FCS | Autologous plasma | FCS | Autologous plasma | FCS |
| (cpm) | (cpm) | (cpm) | (cpm) | (cpm) | (cpm) |
| PHA-P | 78634 ± 38474 | 181831 ± 53640 | 126524 ± 82160 | 175561 ± 75645 | 194321 ± 23399 | 186955 ± 68078 |
| (n = 8) | (n = 8) | (n = 8) | (n = 8) | (n = 5) | (n = 5) |
| Con A | 27902 ± 24217 | 133808 ± 61921 | 63585 ± 28743 | 140727 ± 51762 | 146208 ± 23685 | 186955 ± 35856 |
| (n = 8) | (n = 8) | (n = 8) | (n = 8) | (n = 5) | (n = 5) |
| PWM | 36631 ± 22137 | 42015 ± 33753 | 31305 ± 10790 | 40174 ± 21942 | N.D. | N.D. |
| (n = 8) | (n = 8) | (n = 8) | (n = 8) | (n = 5) | (n = 5) |

a: 3H-thymidine incorporation, mean ± SD
b: Not significant, autologous plasma addition compared to FCS addition
N.D.: not done
* P < 0.001, autologous plasma addition compared to FCS addition
** P < 0.01, autologous plasma addition compared to FCS addition