Trial of Isolation of a Virus from Sera of Patients with
Kawasaki Disease

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(Received October 31, 1985)

We tried to isolate pathogenic viruses from specimens of patients with Kawasaki disease. Blood clots
and sera, spinal fluid, throat swabs, stool and urine from 24 patients with Kawasaki disease were
studied. The specimens were inoculated into HEL cells and Vero cells. The cells were observed for
one month, but no cytopathic effect (CPE) occurred. Blind passage was performed, but no degenera-
tion of the second series of cells was observed. Fluorescent antibody indirect methods to determine
viral antigens in cells inoculated with patients’ specimens was also negative.

(Key Words: Kawasaki disease, CPE of HEL cells, CPE of Vero cells)

INTRODUCTION
Kawasaki disease is an acute febrile disease in infancy and early childhood. It is a self-limited
disease in most cases, but it may be accompa-
nied by permanent coronary arterial changes
with coronary ischemia in some patients. Although the etiology of this disease is unkown,
it is an epidemic disease. We noted that patients
during the epidemic period had typical symp-
toms, while patients during the non-epidemic period had atypical and less severe symptoms,
in general. Clinical symptoms are also differ-
ent from epidemic to epidemic. During 1982,
most of the patients had severe cheilitis some-
times accompanied by ulcers, which was not
noted previously. Some investigators thought
this was an infectious disease and tried to iden-
tify a pathogenic microorganism. Hamashima
et al found ricketia-like bodies in patients with
Kawasaki disease, Kato et al repored that they obtained Propionibacterium acnes (variant),
and Numasaki et al isolated RS virus from pa-
tients with Kawasaki disease (3-5,12). No
definite association between these microorgan-
isms and Kawasaki disease has yet been estab-
lished. The purpose of this study was to try to
isolate a pathogenic virus from specimens of
children with Kawasaki disease.

MATERIALS AND METHODS
Twenty-four patients who were admitted to
Tokai University Hospital during 1983 and 1984
were studied. The age of the patients ranged
between 4 months and 5 years. Throat swabs
were soaked in Hanks’ solution with PC and
SM. Suspensions of stool and urine, with PC
and SM added, were centrifuged at 10,000 rpm
for 10 minutes and supernatant solutions were
examined. Spinal fluid, blood clots and sera
were examined as they were. A part of the speci-
mens was analysed as soon as the samples were
obtained, and the others were stored at -75°C
until studied.

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Isolation of virus: monolayer cultures of HEL cells originating from human embryo lung and Vero cells originating from green monkey kidney were used. After these cells had grown fully, they were cultured in a maintenance medium (Eagle minimum essential medium with 2% fetal bovine serum, L-glutamine, sodium bicarbonate and PC and SM). The specimens from the patients (0.1 ml/tube) were inoculated into these cells. The cells were cultured in a CO2 incubator with 5% CO2. The maintenance medium of the stool samples was changed after one hour, on the following day and once a week thereafter. The maintenance medium of other samples was changed on the following day and once a week thereafter. The cells were observed for one month to see if CPE was present. Blind passage of cells were also performed once or twice. The cells were observed for one month after each passage.

Fluorescent antibody tests: The specimen was inoculated into a layer of HEL cells cultured on cover glass. After the cells were cultured for 8-20 days, each of the sera from four patients with Kawasaki disease during the convalescent phase (30 days after the beginning of fever) were dripped on the glass. After incubation at room temperature for 30 minutes, the cell sheets were stained with fluorescein-conjugated anti-human immunoglobulins at room temperature for 30 minutes.

RESULTS

Table 1 shows the results of CPE observed on HEL cells inoculated with each specimen. No CPE was observed on cells with sera, blood clots, spinal fluid, throat swabs, stool or urine. In cells for which a blind passage was performed, no CPE was observed. Fresh specimens (five sera samples, one blood clot sample, two spinal fluid samples, five throat swab samples, and three stool samples) were tested. No CPE was observed in the cells inoculated with these fresh samples. The results obtained from fresh samples are included in Table 1.

Table 2 shows the results of CPE observed on Vero cells inoculated with the specimens. No CPE was observed in the cells inoculated with fresh specimens or stored specimens. Table 3 shows the results of fluorescent antibody tests on HEL cells. No specific fluorescence was observed.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Cytopathic effects on HEL cells by specimens from Kawasaki disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Primary</td>
</tr>
<tr>
<td>Serum</td>
<td>0/17*</td>
</tr>
<tr>
<td>Blood clot</td>
<td>0/19</td>
</tr>
<tr>
<td>Spinal fluid</td>
<td>0/22</td>
</tr>
<tr>
<td>Throat swab</td>
<td>0/30</td>
</tr>
<tr>
<td>Stool</td>
<td>0/30</td>
</tr>
<tr>
<td>Urine</td>
<td>0/25</td>
</tr>
</tbody>
</table>

*number of samples tested

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Cytopathic effects on Vero cells by specimens from Kawasaki disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>Primary</td>
</tr>
<tr>
<td>Serum</td>
<td>0/11*</td>
</tr>
<tr>
<td>Blood clot</td>
<td>0/19</td>
</tr>
<tr>
<td>Spinal fluid</td>
<td>0/19</td>
</tr>
<tr>
<td>Throat swab</td>
<td>0/24</td>
</tr>
<tr>
<td>Stool</td>
<td>0/26</td>
</tr>
<tr>
<td>Urine</td>
<td>0/23</td>
</tr>
</tbody>
</table>

*number of samples tested
Table 3  Detection of antigen by the fluorescent antibody technique in cells inoculated with specimens from Kawasaki disease

<table>
<thead>
<tr>
<th>Samples</th>
<th>rate of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
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</tr>
<tr>
<td>Spinal fluid</td>
<td>0/7</td>
</tr>
<tr>
<td>Throat swab</td>
<td>0/11</td>
</tr>
<tr>
<td>Stool</td>
<td>0/7</td>
</tr>
<tr>
<td>Urine</td>
<td>0/6</td>
</tr>
</tbody>
</table>

*number of samples tested

DISCUSSION

Various organisms such as mites, rickettsia, Propionibacterium acnes, streptococcus, RS virus, and EB virus have been reported to be the cause of Kawasaki disease, (1-6,10-16). Previously, one of the authors reported that non-specific increases in anti-mumps antibodies and anti-rubella antibodies along with polyclonal increases in serum immunoglobulins were present in children with Kawasaki disease (7-9). These observations suggested that we can not identify a pathogen of Kawasaki disease only by screening the serum antibody levels. A direct and reproducible demonstration of a pathogen in specimens obtained from patients and not from normal healthy people may be the best way of identification of the causative agent of the disease. We tried to isolate a virus from specimens of children with Kawasaki disease using HEL cells and Vero cells, in which various viruses can grow. We could not observe any definite CPE, even when we continued the study for one month, and performed blind passages. Since RS virus is not stable when it is frozen, we examined fresh specimens. We could not isolate any viable virus. Because EB virus cannot grow on the cells we used, we cannot comment on the relationship between EB virus and Kawasaki disease. It is not possible to isolate a virus from children with Kawasaki disease by the generally used methods, and some special method will be needed when detection of a virus in Kawasaki disease is attempted.

REFERENCES