Coronary Arteritis in Mice after Systemic Injection of Bacterial Cell Wall Peptidoglycan

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We examined cell wall peptidoglycan (PGL) derived from group A streptococcus and other bacteria for possible induction of coronary arteritis in mouse strains. The histological finding of the main trunk of the coronary arteries of BALB/c, DBA/1J, C57BL/6 and DBA/2 mice, which were given an intravenous injection of sonicated PGL fragments of st. pyogenes at 500 µg per mouse 4 times at intervals of 1 week, showed diffuse cellular infiltration in the vascular wall as well as perivascular space. Marked hyperplasia of the endothelial cells was noted and necrosis of the medial smooth muscle of the coronary artery also was observed. The elastica stain clearly demonstrated fragmentation and degeneration of the elastic fibers. The histological change of the originating site of the aorta also noted swelling or hyperplasia of the endothelial cells and perivascular cellular infiltration. PGL fragments of st. mutans, st. sanguis and s.aureus did not cause any heart lesions. Coronary arteritis induced by st. pyogenes PGL could be very useful as an experimental animal model of Kawasaki's disease.

A single intraperitoneal injection of an aqueous suspension of cell wall fragments or C-polysaccharide-peptidoglycan (PGL) complex from streptococcal group A, but not group D, induced carditis in mice.

The systemic injection of an aqueous suspension of sonicated PGL derived from group A streptococcus and Lactobacillus casei also induced acute joint lesions in mouse strains.

In the present study, we examined PGL derived from group A streptococcus and other bacteria for possible induction of coronary arteritis in mouse strains.

Key words:
- Streptococcus pyogenes
- Dose response
- Mouse H-2 complex
- Intravenous injection

MATERIALS AND METHODS

Animals: BALB/c, DBA/1J, C3H/He, DBA/2, C57BL/6 and AKR female mice, 6 weeks old, were obtained from Nitsuseizai (Tokyo). They were maintained in plastic cages and fed a standard chow diet.

Isolation of cell walls and extraction of PGL: Cell walls were prepared by the method previously described from the following bacteria: streptococcus pyogenes (type 12, strain A374), streptococcus mutans (serotype c, strain MT 8148), streptococcus mutans (serotype d, strain B13), streptococcus mutans (serotype h, strain MF2 28), streptococcus mutans (serotype g, strain 6715), streptococcus sanguis (serotype I, N0395) and staphylococcus aureus (ATCC 25923).

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St. pyogenes PGL was extracted from the cell wall as described previously. St. mutans and St. sanuis PGL also were extracted from the cell wall. Briefly, the cell walls were digested with Pronase E at 37°C for 2 h. After washing, the cell walls were suspended in 5% trichloroacetic acid (TCA) and incubated at 4°C for 14 h to remove polysaccharide. The cell walls were washed with distilled water and suspended in 10% TCA.

The reaction mixture was incubated at 60°C for 3 h with stirring. After centrifugation (at 15,000 xg for 20 min), the pellets were washed with distilled water. The pellets were lyophilized and used as PGL. The treatment of TCA was repeated three times on residual cell walls.

Inoculation: PGLs were solubilized by a sonic oscillator (UR 200, Tomy Seiko, Tokyo) at 20 KW for 40 min. Mice were injected intravenously into the tail with sonicated PGL fragments at a dose of 500 μg in 0.5 ml per mouse 4 times at intervals of 1 week unless otherwise specified.

Histological findings: Mice were sacrificed 1 week after the last injection in most experiments. Heart tissue specimens were fixed in 10% buffered formalin, embedded in paraffin by the conventional procedure, and stained with hematoxylin and eosin and elastica stain.

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RESULTS

Histological features on the lesions of coronary artery by PGL fragments from *Streptococcus pyogenes*: Mice were given an intravenous (i.v.) injection of the PGL fragments of *Streptococcus pyogenes* at 500 μg per mouse 4 times at intervals of 1 week. Fig. 1 shows the main trunk of coronary arterial trees of a BALB/c mouse which was given the PGL. Histological finding showed diffuse cellular infiltration in the vascular wall as well as perivascular space. Fig. 2 is a higher magnification of Fig. 1. Marked hyperplasia of the endothelial cells was noted and necrosis of the medial smooth muscle of the coronary artery also was observed. A marked increase of neutrophils as well as macrophages was noted in the perivascular space. The elastica stain clearly demonstrated fragmentation and degeneration of the elastic fibers (Fig. 3). These changes are typical histological patterns which were classified as three positives (+++ in this study. When the infiltration of neutrophils and macrophages in the perivascular spaces was observed, but this infiltration was much less compared to the cases of the three positive groups, and fragmentation and degeneration of the elastic fibers was devoid, it was classified as one positive (+). Fig. 4 shows the intracardiac vessel which of a DBA/1J mouse. It was noted that the swelling or hyperplasia of the endothelial cells, thickening of the endothelium and narrowing of the vessel resulted from marked cellular infiltration. Moreover, striking perivascular cellular infiltration was noted. However, the myocardium per se was preserved almost intact.

The histological change of the originating site of the aorta in the C57BL/6 mouse was also noted swelling or hyperplasia of the endothelial cells and perivascular cellular infiltration (Fig. 5). The elastica stain of the vessel shows the proliferation or hyperplasia of the endothelial cells as well as degeneration and fragmentation of the elastic fibers (Fig. 6). When no striking morphological alteration of the myocardium was noted and only slight focal cellular infiltration in the interstitium of myocardial fibers was noted, we classified it as the equivocal group (±).

Susceptibility of mouse strains to heart lesions induced by the PGL fragments of cell wall of *Streptococcus pyogenes*: Various inbred mouse strains were examined for susceptibility and resistance to heart lesions induced by the PGL of *Streptococcus pyogenes*. Each animal received an i.v. injection of sonicated PGN fragment at 500 μg per mouse 4 times at intervals of 1 week. BALB/c, DBA/1J, C57BL/6 and DBA/2 mice were susceptible to coronary arteritis, whereas C3H/He mice showed only weak susceptibility and AKR mice failed to produce coronary arteritis (Table I).

Dose responses of PGL fragments to induce heart lesions: BALB/c mice were given an i.v. injection of the PGL fragments of *Streptococcus pyogenes* at graded doses (500, 250, 100 and 50 μg per mouse) 4 times at intervals of 1 week. When mice were injected intravenously with PGL, more than 250 μg per mouse was effective in the induction and severity of coronary arteritis (Table II).

Induction of heart lesions by PGL fragments
TABLE I  INCIDENCE AND SEVERITY OF CORONARY ARTERITIS IN VARIOUS STRAINS OF MICE GIVEN INTRAVENOUS INJECTION OF STREPTOCOCCUS PYOGENES PEPTIDOGLYCAN (PGL)

<table>
<thead>
<tr>
<th>Strains (H-2 haplotype)</th>
<th>Dose (μg/mouse)</th>
<th>Number of injection times</th>
<th>Severity of coronary arteritis</th>
<th>Incidence of No./total</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c (d)</td>
<td>500</td>
<td>4</td>
<td>+++ − ++</td>
<td>5 / 5</td>
</tr>
<tr>
<td>DBA/1J (q)</td>
<td>500</td>
<td>4</td>
<td>++ − +</td>
<td>5 / 5</td>
</tr>
<tr>
<td>C3H/He (k)</td>
<td>500</td>
<td>4</td>
<td>+</td>
<td>3 / 5</td>
</tr>
<tr>
<td>C57BL/6 (b)</td>
<td>500</td>
<td>4</td>
<td>+++ − +</td>
<td>3 / 5</td>
</tr>
<tr>
<td>DBA/2 (d)</td>
<td>500</td>
<td>4</td>
<td>+++ − +</td>
<td>2 / 5</td>
</tr>
<tr>
<td>AKR (k)</td>
<td>500</td>
<td>4</td>
<td>−</td>
<td>0 / 5</td>
</tr>
</tbody>
</table>

Mice, weighing 13 to 18g, 6 weeks old at the initiation of the experiments were used, and injected with PGL 4 times at intervals of 1 week. Range: from −, absence of abnormalities, to +++ maximum severity (see RESULTS).

TABLE II  DOSE RESPONSE OF STREPTOCOCCUS PYOGENES PGL ON THE DEVELOPMENT OF HEART LESIONS IN BALB/c MICE

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose (μg/mouse)</th>
<th>Number of injection times</th>
<th>Treatment</th>
<th>Histopathological severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Aorta</td>
</tr>
<tr>
<td>BALB/c</td>
<td>500</td>
<td>4</td>
<td>iv</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>4</td>
<td>iv</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
<td>iv</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4</td>
<td>iv</td>
<td>−</td>
</tr>
</tbody>
</table>

Female BALB/c mice, weighing 15 to 18g, 6 weeks old at the initiation of the experiments were used. Mice were injected with PGL 4 times at intervals of 1 week. Range: from −, absence of abnormalities, to +++ maximum severity (see RESULTS).

from various bacteria: PGL fragments derived from st. pyogenes were capable of producing severe arteritis, however, PGL fragments of st. mutans, st. sanguis and s. aureus did not cause lesions (data not shown).

DISCUSSION

In the mouse, it has previously been documented that cell walls (C-polysaccharide-PGL complex) of st. pyogenes are able to produce lesions similar to rheumatic cardiac lesions by i.p. injection. However, these histological changes show mainly myocarditis, and significantly lesions were not caused in the originating site of the aorta and/or the main trunk of the coronary artery. The present study clearly demonstrated that an i.v. injection of an aqueous suspension of cell wall PGL derived from st. pyogenes is capable of inducing coronary arterial lesions in mice. This discrepancy may be caused by the difference of the injection route.

Coronary arteritis of the BALB/c, DBA/1J, C57BL/6 and DBA/2 mice was more severe than in C3H/He and AKR mice. It is suggested that complement components of C5 to C9 are not involved in inducing lesions of the coronary artery, because DBA/2 mice are known to be C5 deficient. However, as shown in Table I, mice which have the H-2k haplotype appeared to have low susceptibility to PGL.

PGL of st. pyogenes was very active in inducing coronary arteritis but PGL of other bacterial species was not capable of inducing such heart lesions. PGL derived from st. pyogenes contained 0.2% rhamnose and was partially characterized regarding amino acid content in the previous report. However, we have not yet analyzed the components of PGL of other species. There is a possibility that particles of PGL other than st. pyogenes PGL become much smaller in size than st. pyogenes PGL by sonic

oscillator; therefore, these particles of PGL have no effect in inducing the lesions. Another possible reason is that the special structure(s) of PGL complex may also be important in inducing coronary arteritis.

Coronary arteritis induced by PGL could be very useful as an experimental animal model of Kawasaki's disease (MCLS).

REFERENCES