Electron Microscopic Observation of Interaction between Normal Mononuclear Cells and Platelets from ITP Children

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Summary: We used radioimmunoassay to detect platelet-associated IgG and also observed with the electron microscope the interaction between normal mononuclear cells and platelets from ITP children as well as the effect of SPA treatment on the interaction. It was confirmed that ITP was related to the increase of platelet-associated IgG and that platelet-associated IgG did exist on ITP platelets. It was also made clear preliminarily that platelet-associated IgG was bound to ITP platelets mainly through its Fab terminus with its Fc fragment exposed freely.

Key words: platelet, mononuclear cells, idiopathic thrombocytopenic purpura (ITP), staphylococcal protein A (SPA)

It is now believed that idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease, which is mainly related to the increase of platelet-associated IgG. In order to make a further investigation into the pathogenesis of ITP and to explore the possible mechanism in binding platelet-associated IgG to platelets, we have measured platelet-associated IgG values with radioimmunoassay and at the same time we have also observed under electron microscope the interaction between normal mononuclear cells (MC) and platelets from ITP children with increased platelet-associated IgG, as well as the effect of staphylococcal protein A (SPA) on the interaction.

MATERIALS AND METHODS

Detection of platelet-associated IgG

The detecting method was introduced by Shaw et al.[3]. Preparation of $^{125}$I-SPA was supplied by Xuanwu Hospital in Beijing. The values of platelet-associated IgG in 40 normal children, 40 normal adults and 62 ITP children were measured.

Mononuclear cell-platelet binding assay

Pure normal MCs were harvested from the blood of normal children according to the method described by Bøyum[4] and Perper[5], and the cell-binding system was established following the method introduced by Court et al.[6]. Two samples were prepared from each subject for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) respectively. At least 50 normal MCs should be observed for each sample. 8 normal and 12 ITP subjects were studied.

SPA treatment of the platelets

100 μl of SPA (1.4 μg/ml) was added to 100 μl platelet suspension (300 000—500 000 μl), which was then incubated at 37°C for 30 min and washed...
twice with barbital-buffered EDTA. The washed platelets were mixed with normal MCs at the ratio of 1:10 (normal MCs:platelets)\(^{10}\). The samples for SEM were prepared according to the above-mentioned method. In this study 5 ITP children were evaluated.

RESULTS

Values of platelet-associated IgG

Platelet-associated IgG values were expressed as SPA molecules bound to each platelet (SPA/platelet) because a definite ratio between IgG and SPA molecules exists in their interaction\(^{3,7}\). Table 1 summarizes the platelet-associated IgG values in the three different groups. The statistical data showed that there was no significant difference between normal children and adults in the mean platelet-associated IgG values, and that 57 out of 62 ITP children had higher values than normal subjects (the normal range being from 0 to 940 SPA/platelet in our lab).

Electron microscopic observation

1. Total number of bound platelets

The term refers to the whole number of platelets bound to every hundred normal MCs counted under SEM. Table 2 shows that it was evidently larger in ITP group than in normal controls.

2. Relationship between platelet-associated IgG and bound rate of normal MCs

The bound rate of normal MCs means the number of normal MCs bound by more than one platelet in 100 normal MCs counted under SEM. We have tried to analyse the relationship between bound rate of normal MC and platelet-associated IgG values with linear regression. The results proved that there was a positive correlation between them (fig.1), that is, the higher the platelet-associated IgG values, the higher the bound rate of normal MC seemed to be.

![Fig. 1. Relationship between platelet-associated IgG and bound rate of normal MC.](image)

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\begin{align*}
\text{BRMC} & = 22.27 + 0.6227 \times \text{pAMA} \\
& (r=0.6227, P<0.05)
\end{align*}
\]

3. Morphologic characteristics

Both SEM and TEM revealed that the normal MCs observed were mainly T-lymphocytes with relatively smooth cellular surfaces, whereas the cells bound

Table 1. Values of platelet-associated IgG in three groups

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>(\bar{X})</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
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<tbody>
<tr>
<td>Normal children</td>
<td>40</td>
<td>575</td>
<td>222</td>
<td>571</td>
<td>170—1011</td>
</tr>
<tr>
<td>Normal adults</td>
<td>40</td>
<td>609</td>
<td>229</td>
<td>597</td>
<td>81—1447</td>
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<tr>
<td>ITP children</td>
<td>62</td>
<td>2601</td>
<td>1817</td>
<td>2555</td>
<td>212—8261</td>
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</table>

Table 2. Total number of bound platelets (TNBP)

<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Platelet-associated IgG</th>
<th>Total no. of bound platelets</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Range</td>
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<tr>
<td>Normal children</td>
<td>8</td>
<td>529</td>
<td>171—754</td>
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<tr>
<td>ITP children</td>
<td>12</td>
<td>2269</td>
<td>1339—6946</td>
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