Carbohydrate Epitope of Sperm Immobilizing Human Monoclonal Antibody H6-3C4 and Common Carbohydrate Antigen Expressed on Sperm and Trophoblast

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The correlation between sperm immobilizing antibodies (SI-Abs) found in the sera of women and sterility with unknown cause is well accepted, but the mechanism to induce sterility in vivo by antibodies is not well clarified.\(^1\) We have established human-mouse heterohybridoma H6-3C4 which secreted a monoclonal antibody (Mab) with extremely high titers of SI and sperm agglutinating (SA) activities, by fusing peripheral lymphocytes from a sterile woman having SI-Abs, with mouse myeloma cells (NS/1 or P3U).\(^2\) This human hybridoma was very stable and secreted constantly human Mab (IgM, \(\lambda\)) for almost four years. The Mab H6-3C4 was an ideal antibody to identify a sperm antigen which is relevant to generate SI-Ab in women. Human spermatozoa could absorb dose-dependently an SI-activity of Mab H6-3C4, but those from other species, except boar, did not. The drop of SI-activity of Mab H6-3C4 was parallel to that of the SA activity after absorption. The binding of \(^{125}\)I Mab H6-3C4 to sperm was completely inhibited by Mab H6-3C4 and partially inhibited by the patient's serum, the lymphocytes of which were used to establish the hybridoma H6-3C4; however, binding was not inhibited by the control serum from a woman without
SI-Ab. The binding was also inhibited with RCA and WGA lectines. This implies that an antigen epitope corresponding to Mab H6-3C4 might be a carbohydrate containing Galactose (Gal) and N-acetyl Glucosamine (Glc) residues. In order to identify an exact structure of carbohydrate epitope, the reaction of Mab H6-3C4 to various standard glycolipids were examined by radio immuno-assay. The glycolipids tested are shown in Table I. Mab H6-3C4 reacted with norhexaosylceramide, 2-3 sialyl, 2-6sialyl nor hexaosylceramide, lactonorhexaosylceramide and 2-3sialyl lactonorhexaosylceramide. No other glycolipid reacted with Mab H6-3C4 in Table I. These results are summarized in Figure 1 (see the column of Mab H6-3C4).

Table I

<table>
<thead>
<tr>
<th>Glycolipid</th>
<th>Reaction with Mab H6-3C4</th>
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<tbody>
<tr>
<td>nHex = Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (lacto-N-norhexaosylceramide)</td>
<td>+</td>
</tr>
<tr>
<td>2,3SsHex = SA2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (2,3-sialosyl-lacto-N-norhexaosylceramide)</td>
<td>+</td>
</tr>
<tr>
<td>2,6SsHex = SA2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (2,6-sialosyl-lacto-N-norhexaosylceramide)</td>
<td>+</td>
</tr>
<tr>
<td>PG = Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (paragloboside)</td>
<td>+</td>
</tr>
<tr>
<td>2,3SPG = SA2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (2,3-sialosylparagloboside)</td>
<td>+</td>
</tr>
<tr>
<td>2,6SPG = SA2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide (2,6-sialosylparagloboside)</td>
<td>+</td>
</tr>
<tr>
<td>6B = SA2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-1ceramide + 3Fucβ1 + 3Fucβ1 (sialosyl di-Yα,6B)</td>
<td>+</td>
</tr>
</tbody>
</table>

These results showed that the definite specificity of Mab H6-3C4 must be to (Gal β 1-4GlcNAcβ1) 2, regardless of internal location in the carbohydrate chain. The antigen epitope of sperm corresponding to Mab H6-3C4 was identified, but carbohydrate structure expressed on fresh human sperm must be confirmed. In order to determine carbohydrate structures expressed on human sperm, various anti-
carbohydrate Mabs were applied for the reaction to human sperm. Mabs NUH-2 and H6-3C4 reacted to human fresh sperm but 1B2, C6 and anti-i serum Dench could react only to Sialidase treated sperm. Neither Mabs recognized fucosylated lactosamine nor globo type carbohydrate chains reacted to human sperm (Figure 1). These data suggested not only that human sperm surface carbohydrates might compose of lactosamine unit (Gal β 1-4GlcNac β 1-3)n, but also of repeating extension and branching with terminal 2-3 sialization. Our proposal concerning carbohydrate structure on human sperm is shown in Figure 2.

Figure 1

The binding portions of Mabs NUH-2 and H6-3C4 to sperm are also indicated in Figure 1. The carbohydrate structure on human sperm seemed to be highly configurated structure and to possess mutivalent antigenicities. However, we suppose that
terminal 2-3 sialic acid might cover a large part of antigenicity of the poly-lactosamine structure, because animal could hardly make antibodies to terminal 2-3 sialyl lactosamine. On the other hand, Mab H6-3C4 has an interesting characteristics which react to sialyl lactosamine. Mab H6-3C4 recognized a particular epitope which seems to localize in an internal image of Sialyl-Gal β 1-4GlcNac β 1-3Gal β 1-4GlcNacβ1-3 structure, because the reactivity of Mab H6-3C4 to this carbohydrate chain was not affected, regardless of terminal sialic acid. These particular reactivity allowed Mab H6-3C4 to bind the surface polylactosamine structure on human sperm regardless of the terminal 2-3 sialic acid.

The characteristic sialyl lactosamine structure on human sperm and binding mechanisms of Mabs NUH-2 and H6-3C4 to human sperm were elucidated, however the mechanism how to induce infertility in women by SI-Abs is unknown.

Figure 2

Presumable Carbohydrate Structure Expressed on Sperm Surface
(highly developed 2,3 sialic poly-neolactosamine structure)

H6-3C4 recognizes every sequence of -O-O-

NUH2 recognizes -O-O-

Terminal 2,3 sialic acid ● blocks binding of usual anti-poly-neolactosamine antibodies such as Dench, 1B2 and C6
It was supposed that lactosamine might play a key role to compose an antigen corresponding to SI-Abs because Mab H6-3C4 was an antibody recognizing lactosamine structure on human sperm. Recently we found that glycolipids extracted from full term human placenta contained a large amount of 2-3 sialic lactosamine and extremely a small amount of 2-6 sialyl or non sialyl (neutral) lactosamines (data is not shown). The dominant 2-3 sialyl lactosamine in the placenta seemed to be similar as a carbohydrate composition of sperm as described here.

The trophoblast cells of placenta directly exposed to mother's blood flow, therefore SI-Abs in sera may directly access to the trophoblast cells. Therefore, we examined whether there may be antigenic similarities between human sperm and trophoblast cells. First trimenster placenta villi were examined for lactosamine antigen expression using various Mabs including Mab H6-3C4.

The results obtained by ABC immunostaining are summarized in Table II. Mabs NUH-2 and H6-3C4 reacted significantly to trophoblast cells (Figure 3).

<table>
<thead>
<tr>
<th>monoclonal antibodies</th>
<th>human sperm</th>
<th>human trophoblast</th>
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<tbody>
<tr>
<td></td>
<td>non treated</td>
<td>sialidase treated</td>
</tr>
<tr>
<td>H6-3C4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NUH-2</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>iB2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C6</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>iB9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dench</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 3, ABC immunostaining of first trimester placenta Villi trophoblasts by (A) H6-3C4, (B) NUH-2.
No other Mabs against lactosamine showed reactivities to trophoblast cells unless the tissues were treated with sialidase. These results seem to be very similar as the results in human sperm using same Mabs. It is clear that both human trophoblast and sperm expressed 2-3 sialyl lactosamine as a dominant carbohydrate structure on the surface. Therefore it is assumed that some kinds of anti-lactosamine antibodies such as Mab H6-3C4 may react not only to human sperm, but also to human trophoblast. Some particular anti-lactosamine antibodies may possibly be raised under some immunological imbalance (bacteria or virus infection), and such particular lactosamine antibodies may cross-react to human sperm and human trophoblast, then they may induce sterility or early embryo lossess as a result.

References