Agglutinating Serum for Distinguishing \textit{Staphylococcus aureus} of Human Biotype

I. LIVE

\textit{Department of Microbiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19174}

Received for publication 3 February 1975

Antiserum to \textit{Staphylococcus aureus} strain 17 was treated with \textit{S. aureus} strain 61218 until the antibodies against thermostable agglutinogen were removed. The absorbed serum agglutinated phage-typable as well as phage-untypable staphylococci of human biotype, whether recovered from people or from dogs.

In studies on identification of \textit{Staphylococcus aureus} of canine origin by means of an agglutination test, \textit{S. aureus} strain 61218 was found suitable for the production of antiserum, which upon proper absorption resulted in a serum that agglutinated specifically \textit{S. aureus} of canine biotype (13). The specificity appeared to depend upon reaction of the absorbed serum with thermolabile agglutinogen peculiar to \textit{S. aureus} of canine origin.

In the present study the feasibility of distinguishing \textit{S. aureus} of human origin in general by a wide-spectrum agglutinating serum (similar to the characterization of \textit{S. aureus} of canine biotype by absorbed serum 61218) was investigated. This was prompted by the desirability of having available a screening serum for human \textit{S. aureus} in general, as well as for the purpose of serological identification of \textit{S. aureus} of human biotype commonly encountered in dogs (6). For that purpose antiserum to \textit{S. aureus} strain 17 was investigated, which had been reported (13) to possess specific thermolabile agglutinin in addition to thermostable agglutinin shared with culture 61218.

\textbf{Cultures.} Strain 61218 constitutes a canine isolate of \textit{S. aureus} (13), and strain 17 represents one of the type strains of staphylococci of human biotype (12). As previously recommended (1, 13), thermostability of agglutinogens of the cultures was determined by the ability of the bacteria to retain their agglutinating property after autoclaving at 120 °C for 90 min.

The animal cultures were isolated at random from the anterior nares of dogs brought to the veterinary hospital for treatment of various conditions. The human cultures had been recovered from nasal carriers in student surveys.

\textbf{Biochemical tests.} The differential coagulase test and fibrinolysin test with human and with canine plasma were carried out as previously described (6). The phage typing procedure was the same as previously indicated (6).

\textbf{Preparation and absorption of antisera.} The antisera were prepared by injecting two groups of rabbits with \textit{S. aureus} cultures 61218 and 17, respectively (cultures made available by J. Pillet). The immunization procedure and the absorption of immune serum 61218 were carried out as previously described (6). Antiserum 17 was absorbed as follows. Bacteria grown on Trypticase soy agar were used. Ten milliliters of 1:10 dilution of antiserum 17 which agglutinated promptly both the homologous culture and culture 61218 in the live as well as in the autoclaved form was mixed with 24-h bacterial growth from 5 Roux bottles of \textit{S. aureus} strain 61218. The surface growth in each bottle was washed off with 5 ml of 0.85% NaCl; the bacterial suspensions from the five bottles were pooled and centrifuged at 2,000 rpm for 1 h; the supernatant material was discarded, and the sediment was suspended in the 10 ml of diluted serum. The serum-bacteria mixture was incubated at 37 °C for 2 h with shaking every 15 min, after which time it was refrigerated overnight and subsequently centrifuged. The separated serum was tested for agglutinins against live as well as against autoclaved bacteria of the immunizing and of the absorbing strain. At this stage, cells of strain 61218 were not agglutinated by antiserum 17 regardless of whether the bacteria were alive or autoclaved. On the other hand, the absorbed serum agglutinated live bacteria of culture 17 within a few seconds, whereas the autoclaved bacteria of this strain were agglutinated slowly and only to a slight degree. To remove further the reactivity of antiserum 17 with thermostable agglutinogen, a second absorption with culture 61218 was carried out. Subsequently, the twice-absorbed
serum 17 did not agglutinate autoclaved culture 17 but continued to agglutinate the live bacteria promptly. The absorbed serum was designated as serum H (referring to its reactivity with S. aureus of human biotype).

**Agglutination test.** The slide agglutination test was used as previously described (6).

In Table 1 are presented results of serological tests on cultures susceptible to phages of group I, II, or III, as well as on phage-untypable cultures isolated from human beings and from dogs. All but one of 55 such isolates, whether obtained from people or from dogs, were agglutinated by absorbed serum H. On the other hand, none of these cultures was agglutinated by absorbed serum 61218. Thus, these findings indicated a relationship between S. aureus of human biotype (as determined by biochemical tests—coagulation of human plasma with or without coagulation of canine plasma and predominantly fibrinolysin-positive reaction [6]) and absorbed serum H. Another indication that these cultures were of human origin was the fact that staphylococci susceptible to phages of group I, II, or III of the international set of phages had been reported to be of human biotype (8).

In addition, of 27 phage-untypable human isolates, all of which had the biochemical characteristics of S. aureus of human origin, 26 were positive in the agglutination test with absorbed serum H without being agglutinated by absorbed serum 61218. Similarly, 23 phage-untypable canine isolates with biochemical properties of S. aureus of human biotype were agglutinated only by absorbed serum H. On the other hand, all 29 canine isolates possessing biochemical characteristics associated with S. aureus of canine origin (coagulation of canine plasma only and lack of fibrinolytic activity [6]) agglutinated with absorbed serum 61218 and not at all with absorbed serum H.

When cultures of phage type 187 were examined, two different types of reactivities were observed, depending on the species from which the isolates had been obtained (Table 2). All 14 cultures recovered from people possessing biochemical attributes of S. aureus of human biotype were agglutinated only by absorbed serum H, and the four isolates from dogs with biochemical characteristics of S. aureus of canine origin were agglutinated only by absorbed serum 61218. These findings are in accord with previous observations that phage 187 is the only one of the international set of phages to which S. aureus of human as well as of canine origin may be susceptible (6, 8).

The close contact of human beings with dogs is known to be conducive to the interchange of staphylococci between them, as evidenced by the finding of S. aureus of human biotype in dogs and by the isolation of S. aureus of canine origin in man (6). From an epidemiological standpoint, therefore, it would appear important to be able to trace sources of these bacteria transmissible between the two host species.

Development of absorbed serum 61218 made it possible to identify serologically S. aureus of canine biotype, whether encountered in dogs or in people (6, 13). On the other hand, in the past, recognition of S. aureus of human origin in dogs has depended largely on phage typing, since isolates from dogs susceptible to the international set of phages have been found to be of human biotype, except for some lysed by phage 187 (6, 8). However, susceptibility of staphylococcal cultures to phages has been reported to be a variable characteristic (7, 9, 14). Furthermore, a high percentage of S. aureus of human biotype is known to be altogether resistant to the phages and thus unidentifiable by the phages.

**Table 1. Agglutination test with sera H and 61218 on phage-typrable and phage-untypable Staphylococcus aureus of human and of canine sources**

<table>
<thead>
<tr>
<th>Susceptibility to phages</th>
<th>Source of isolates</th>
<th>No. of isolates</th>
<th>No. of isolates positive in agglutination test with serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H 61218</td>
</tr>
<tr>
<td>Groups I, II, III</td>
<td></td>
<td></td>
<td>H 61218</td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24 0</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30 0</td>
</tr>
<tr>
<td>Insusceptible</td>
<td>Human</td>
<td>27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26 0</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23 0</td>
</tr>
<tr>
<td></td>
<td>Canine</td>
<td>29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0 29</td>
</tr>
</tbody>
</table>

<sup>a</sup> Coagulated human plasma and fibrinolytic.

**Table 2. Agglutination test with sera H and 61218 on phage type 187 Staphylococcus aureus of human and of canine sources**

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>No. of isolates positive in agglutination test with serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H 61218</td>
</tr>
<tr>
<td>Human</td>
<td>14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Canine</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Coagulated human plasma and fibrinolytic.

<sup>a</sup> Coagulated canine plasma and nonfibrinolytic.
method (2, 10, 15). Although biochemical criteria have recently proved useful in classifying such phage-untypable cultures with respect to human or canine host specificity (6, 8), these properties, likewise, are considered subject to change (4). The antigenic makeup of staphylococci, on the other hand, has been reported to be a stable characteristic (3, 5, 11) so that an agglutinating serum for distinguishing *S. aureus* of human origin would seem advantageous. Absorption of immune serum 17 solely with *S. aureus* strain 61218 resulted in an agglutinating serum which appeared specific for thermolabile agglutinogen of *S. aureus* of human biotype.

This investigation was supported by a research grant from the World Health Organization.

LITERATURE CITED