Trypanosoma gambienseの抗原変異について：感染家児に再発する抗原変異型原虫に対する抗体の検出。

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Antigenic Variation of *Trypanosoma gambiense*:

I. Tracing of antibodies against antigenic variants relapsing in an infected rabbit

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Abstract: Mode of relapse of *Trypanosoma gambiense* (Tg) was studied. Antigenic variants were derived from a Tg clone of original antigen type (Tg-O) in 500 ddO mice as the first relapse. Variable antigenic type (VAT) of those variants was determined by agglutination tests with a series of rabbit sera taken from a Tg-O infected rabbit every 2–3 days along the time course of infection. In VAT determination procedure, antibodies in the rabbit sera were traced with those variants derived in mice. Immunologically distinct 27 VATs except Tg-O were isolated and consequently antibodies against them were detected in the rabbit sera. Antibodies against 5 and 2 different VATs were newly detected in the rabbit serum sample collected on day 9 and 11 respectively and antibodies against 4 VATs, on day 14, 16 and 18. In the first relapse in mice two particular VATs were most predominantly expressed and homologous antibodies to them were detected in the rabbit sera in the earliest next to anti-Tg-O antibodies.

*Key words: Trypanosoma gambiense, Antigenic variation, Mode of relapse*
INTRODUCTION

Salivarian trypanosomes evade the immune response of their mammalian host changing antigen type of their surface coat which entirely covers trypanosome cell membrane (Vickerman, 1969; Vickerman and Luckins, 1969). The phenomenon is called antigenic variation. In trypanosome infected mammalian host undulating parasitaemia is observed as different antigen type of trypanosomes increase one after another. Even a single clone of trypanosomes may express more than a hundred variable antigenic types (VATs). VATs are serologically determined and each one results from a variant specific antigen (VSG) which is identified as glycoprotein uniformly dispersed in the surface coat (Cross, 1975).

The largest number of immunologically distinct VATs that can arise from a single trypanosome clone, ever documented, is 101 reported for Trypanosoma equiperdum (Capbern et al., 1977). The maximum potential number is unknown though. With all such a great diversity, VATs seem to be expressed in a loosely ordered manner (Ritz, 1916; Gray, 1962, 1965a,b, 1975). The mechanism controlling this mysterious antigenic variation of trypanosomes is not elucidated, however, approach by molecular genetics of VSG-gene controlling mechanism is making progress as reviewed by Cross (1982) and Turner (1982).

In the present study we tried to survey the mode of relapse in a Trypanosoma gambiensc infected rabbit to find out how many VATs appear every 2 or 3 days and estimate the maximum number of VATs in the course of infection.

MATERIALS AND METHODS

Parasite

Salivarian trypanosome Trypanosoma gambiensc (Tg), Wellcome strain was used. Tg clone of most stable and predominant antigen type which resulted by serial passage through albino mice for a long time was called Tg-O in the meaning of original antigen type of Tg. Trypanosomes or clones expressing different variable antigenic type (VAT) other than Tg-O were generally called variant. Any antigen type of Tg was maintained by serial syringe passage at 3-4 days interval through mice and submitted to operation of cloning just before use if necessary. The cloning procedure was as follows. A single trypanosome was sucked up through very thin tapered capillary pipette from a tiny drop of 0.2% glucose phosphate buffered saline (GPBS) (pH 7.2) containing a very small number of trypanosomes after dilution in a glass slide well under observation with an inverted microscope at the magnification of ×100. The trypanosome was transferred into 0.5 ml of GPBS in a 1 ml syringe and inoculated into the peritoneal cavity of a mouse.

Mice

Without distinction of sex, ddO mice, 7-10 weeks old, were used for different purposes, Tg maintenance, cloning of Tg, preparation of VAT-specific mouse antiserum
and derivation of variants from Tg-O clone.

**Rabbit serum series**

Cloned Tg-O trypanosomes from an infected mouse were freed of host blood components by chromatography on DEAE-cellulose by the method of Lanham and Godfrey (1970) and collected after washing by centrifugation. A male rabbit, 3 kg of body weight, was inoculated with $1 \times 10^7$ Tg-O trypanosomes suspended in 0.5 ml of GPBS subcutaneously in the left thigh. Eight ml of venous blood was taken from the ear every 2 or 3 days from day 5 until 79 postinoculation, on the 80th day the animal died. A series of rabbit serum samples, expected to contain anti-Tg-O as the first and serial anti-variants antibodies, was obtained from those blood samples.

**Preparation of antigen type-specific mouse antiserum**

The method is schematically outlined in Fig. 1. In order to get Tg-O- or VAT-specific mouse antiserum $1 \times 10^7$ cloned trypanosomes were intraperitoneally (ip) inoculated into mice. The next day, the mice showing parasitaemia of $1-5 \times 10^8$/ml were treated by ip injection of 0.1 ml of normal human serum (NHS) to temporarily eliminate parasitaemia, otherwise the mice would die within 12 hours. NHS has been found to be trypanocidal to brucei-group trypanosomes (Rifkin, 1978; Hawking, 1979). Five days after the treatment, the mice were sacrificed for collection of VAT-specific mouse antiserum. The serum exhibited higher than 1 : 1,000 of agglutination titer against trypanosomes of homologous VAT.

**Derivation of variant Tg from Tg-O clone**

When mice were inoculated with $1 \times 10^7$ trypanosomes of Tg-O, treated with NHS just the same as above and left alive, parasitaemia which had disappeared came up around 8 days after the treatment as reported by Inoki (1960) due to relapse by variant (Fig. 2). Variants were repeatedly derived from Tg-O through this procedure as the first relapse in mice and their VAT was determined. Trypanosomes of newly found VAT were maintained, purified by cloning, inoculated into mice for preparation of VAT-specific mouse antiserum and cryopreserved at $-196^\circ$C in liquid nitrogen.

**Agglutination test for VAT determination of variant**

Extremely small quantity of antiserum was placed on a glass slide using a thin capillary tube and cover-slip placed on top. From the edge of the cover-slip trypanosomes in the mouse blood of heavy parasitaemia ($>10^9$/m) were introduced directly from the tail tip. The mixture was examined under a microscope. The reaction was judged positive when big clumps of trypanosomes were produced.

**Nomenclature of variable antigenic type**

Names of VATs and VAT groups are listed in Table 1. A set of VATs of variants of which trypanosomes agglutinated with the rabbit serum samples collected on and after the 9th day was called $V_9$-group. The VAT initially found among those of $V_9$-group was
Fig. 1. Method of preparation of antigen type specific mouse antiserum. Mice were inoculated with $1 \times 10^7$ cloned trypanosomes intraperitoneally (ip) and temporarily cured by treatment with 0.1 ml of normal human serum (NHS). Five days after the treatment mice were bled for collection of antigen type-specific mouse antiserum. The estimated curves of parasitaemia (--) and agglutination titer (-----) in the mouse blood are shown schematically.

Fig. 2. Method of derivation of variant from original antigenic type of Trypanosoma gambiense (Tg-O). Mice were inoculated with $1 \times 10^7$ Tg-O trypanosomes intraperitoneally (ip) and temporarily cured by treatment with 0.1 ml of normal human serum (NHS). Around the 8th day after the treatment variant parasitaemia (>10^8/ml) relapsed. At this time variable antigenic type (VAT) was determined and maintained by mouse passage. The estimated curves of parasitaemia (---) and agglutination titer (-----) in the mouse blood were shown schematically.

designated as $V_9_{-1}$. $V_9_{-2}$ and $V_9_{-3}$ were found second and third respectively. All other groups and VATs were designated in conformity to the formula adopted based on the time scale of infection as the examples explained above.

**Design of experiment**

Tg infection of rabbits pass in chronic state showing relapses but mice inoculated with even a single cell of this strain of Tg die within a week from a severe parasitaemia. On that account, in order to derive variants in Tg-O infected mice, treatment is necessary before the mice die to make them live longer. When variant trypanosomes derived from Tg-O and increased enough to make the peak of parasitaemia (>10^8/ml) in a mouse, agglutination tests were carried on with a series of rabbit serum samples as from the sample collected on the 5th day of Tg-O infection, in which anti-Tg-O antibodies were detected, one by one in accordance with the time of collection. This was done in order to determine the VAT group of the variant by detecting the earliest serum sample to agglutinate with the variant. The specificity of the VAT and corresponding antiserum (mouse serum) was checked by the use of those variants and antisera isolated earlier before being given a new identity following the criteria adopted here. Trypanosomes of
a newly isolated VAT were cryopreserved after collection of VAT-specific mouse anti-serum.

As the above program was repeatedly carried on, following results could be simultaneously obtained. Immunologically distinct VATs and corresponding mouse antisera were isolated increasing in number. Antibodies against the isolated VATs were traced in the rabbit serum samples using variants of those VATs raised in mice. Through the existence of traced antibodies the homologous variants to them were most reliably estimated to have appeared in the rabbit blood earlier than they.

RESULTS

VATs were named according to the time course of infection by detecting antibodies in a series of rabbit serum samples. This is expected to offer the advantage for comparison of the mode of relapse in a rabbit with that in other laboratory animals because from the name of the VAT the order of its expression is readily realized.

Variants derived from Tg-O clone in 500 ddO mice were examined. Among them 27 immunologically distinct VATs except Tg-O were isolated as far as the first relapses were concerned. The isolated VATs are listed in Table 1. From the results it is realized that antibodies against as many as 27 VATs were detected in a series of rabbit serum samples collected during 9th to 31st day of infection. And in a natural consequence it was revealed that trypanosomes of not less than 27 VATs must have appeared in the infected rabbit blood earlier than increment of corresponding antibodies.

From the fact that 5 VATs in Vg-group, 2 VATs in V11-group and 4 VATs in V14-, V16-, and V18-group were newly found, it is proposed as a remarkable finding that at least 4 VATs were expressed every 2 or 3 days during the first 3 weeks of infection. From the same fact that above, another finding might be proposed that populations of different VATs must have constituted an apparent parasitaemia in relapses, granting that

Table 1. List of variable antigenic types (VATs) determined by serum samples taken from a Tg-O* infected rabbit with intervals of 2-3 days

<table>
<thead>
<tr>
<th>Day when serum was taken</th>
<th>9</th>
<th>11</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>21</th>
<th>23</th>
<th>27</th>
<th>31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Determined group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vg-group</td>
<td>Vg-1</td>
<td>Vg-2</td>
<td>Vg-3</td>
<td>Vg-4</td>
<td>Vg-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V11-group</td>
<td>V11-1</td>
<td>V11-2</td>
<td>V11-3</td>
<td>V11-4</td>
<td>V11-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V14-group</td>
<td>V14-1</td>
<td>V14-2</td>
<td>V14-3</td>
<td>V14-4</td>
<td>V14-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V16-group</td>
<td>V16-1</td>
<td>V16-2</td>
<td>V16-3</td>
<td>V16-4</td>
<td>V16-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V18-group</td>
<td>V18-1</td>
<td>V18-2</td>
<td>V18-3</td>
<td>V18-4</td>
<td>V18-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V22-group</td>
<td>V22-1</td>
<td>V22-2</td>
<td>V22-3</td>
<td>V22-4</td>
<td>V22-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V31-group</td>
<td>V31-1</td>
<td>V31-2</td>
<td>V31-3</td>
<td>V31-4</td>
<td>V31-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Trypanosoma gambiense of original antigen type.
The nomenclature is explained in materials and methods.
Table 2. Frequency of variable antigenic type (VAT) derived from Tg-O* as the first relapse in mice

<table>
<thead>
<tr>
<th>VAT Frequency</th>
<th>Incidence (%)</th>
<th>VAT Frequency</th>
<th>Incidence (%)</th>
<th>VAT Frequency</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vg-1</td>
<td>174</td>
<td>Vg-1</td>
<td>3</td>
<td>Vg-1</td>
<td>1</td>
</tr>
<tr>
<td>Vg-2</td>
<td>158</td>
<td>Vg-2</td>
<td>4</td>
<td>Vg-2</td>
<td>1</td>
</tr>
<tr>
<td>Vg-3</td>
<td>12</td>
<td>Vg-3</td>
<td>2</td>
<td>Vg-3</td>
<td>1</td>
</tr>
<tr>
<td>Vg-4</td>
<td>10</td>
<td>Vg-4</td>
<td>3</td>
<td>Vg-4</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-5</td>
<td>1</td>
<td>Vg-5</td>
<td>0.2</td>
<td>Vg-5</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-6</td>
<td>46</td>
<td>Vg-6</td>
<td>1</td>
<td>Vg-6</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-7</td>
<td>1</td>
<td>Vg-7</td>
<td>0.2</td>
<td>Vg-7</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-8</td>
<td>36</td>
<td>Vg-8</td>
<td>1</td>
<td>Vg-8</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-9</td>
<td>21</td>
<td>Vg-9</td>
<td>2</td>
<td>Vg-9</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-10</td>
<td>10</td>
<td>Vg-10</td>
<td>1</td>
<td>Vg-10</td>
<td>0.2</td>
</tr>
<tr>
<td>Vg-11</td>
<td>5</td>
<td>Vg-11</td>
<td>1</td>
<td>Vg-11</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*Trypanosoma gambiense of original antigen type.

There was little difference depending upon VATs in growth rate of trypanosomes and way of host's immune responses to them. For example, the second parasitaemia following Tg-O parasitaemia must have consisted of at least 5 variants of different VATs.

Incidences of VATs expressed in 500 mice were shown in Table 2. Vg-1 and Vg-2 were expressed in high incidences of 34.8% and 31.6% respectively. These values are considerably higher than the others. It is impossible to explain here why these two were expressed most predominantly. However, VATs expressed early (in the first relapse) in mice were also expressed earlier in the rabbit in an indication of some organised order in VAT expression.

DISCUSSION

On the number of VATs some reports have been presented. Osaki (1959) described 23 VATs of a strain of *T. gambiense*. It was the largest number until 1977 when Capbern et al. reported that they isolated 101, so far greater number, of distinct VAT of *T. equiperdum* from infected rabbit. In this study variants of 27 VATs except Tg-0 were obtained. This number is a little larger than the former but far smaller than the latter. If we had examined further relapses following the first relapse in mice, we could have obtained much more VATs and detected corresponding antibodies in all rabbit serum samples collected. Since approximately 4 VATs were expressed every 2 or 3 days in this study, 120 VATs \([(4/2.5) \times (80-5)]\) might be estimated to be expressed in the rabbit throughout the infection course when the total number is simply calculated using average frequency of VAT appearance. This number is comparable to 101 isolates of *T. equiperdum* as reported by Capbern et al. (1977).

The infected rabbit could have lived longer than 80 days without any artificial
damage in addition to trypanosome inoculation since the rabbit died not only because of virulent effect of Tg but greater damage due to so many times of blood-drawings. In this case far much more VATs than 120 could have been expressed.

Though little attention was given to the frequency of development of new antigens, similar results to those shown here were reported. Strains of *T. brucei* produced new VATs at intervals of 2–3 days in infected sheep, goats and laboratory rodents until infected animal died (Gray, 1965a). For *T. equiperdum*, a new VAT was observed at 3 days intervals in infected rabbits (Capbern et al., 1977).

The fact that clones or strains of trypanosomes would generate variants in reproducible order of VATs have been reported by some workers (Osaki, 1959; Gray, 1962, 1965a, 1975; Wilson and Cunningham, 1972; Capbern et al., 1977). In our results on incidence of VATs appeared in 500 mice, it is clear that two variants, V9-1 and V9-2, developed in remarkably higher rate than the others as the first relapse. Antibodies against these two VATs also detected in the earliest in rabbit serum samples next to anti-Tg-O antibodies. This coincidence suggests some ordered sequence in VAT expression.

By serial inoculation of some quantity of blood from an infected rabbit to mice, variants in cyclic relapse in the rabbit may be directly detected. In this way, however, there is possibility to select only the most predominant variant because a number of different variants probably appear in relapses in a mixed population as proposed in the preceding paragraph.

It is reasonably pointed out that variant trypanosomes derived from Tg-O in mice might have been heterogeneous in respect of VAT before cloning was done. This problem would be the subject for future study.

**REFERENCES**


Trypanosoma gambiense の抗原変異について

I. 感染家兎に再発する抗原変異型原虫に対する抗体の検出.

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Trypanosoma gambiense 感染家兎での変異型原虫の出現様式を検討した。抗原型についてオ
リジナルな型を Tg-0 とし、Tg-0 クローンを dd0 マウスに感染させ、そのマウスに抗原変異
型原虫（Variant）を Tg-0 から派生させた。派生してきた Variant の変異抗原型（VAT）を、
Tg-0 感染家兎より 2 ～ 3 日おきに連続して得られた一連の血清を用いて、凝集反応により決定
した。この一連の家兎血清で Variant の VAT を同定することは、Variant を用いて VAT に対
する家兎血清中の抗体を検出することにもなる。

以上のことを 500 匹のマウスについて行ったが、結果として Tg-0 以外に、特異性の異なる 27
の VAT を得た。このことはこれら 27 の VAT に対する抗体が上記 Tg-0 感染家兎血清に検出
されたことになる。Tg-0 感染 9 日目の血清には 5 種、11 日目のものには 2 種、14 日目 16 日目そ
して 18 日目の血清にはそれぞれ 4 種の VAT に対する抗体が新しく検出された。

500 匹のマウスに初発型として派生してきた Variant には、特に二種の VAT が他を圧して
高頻度に表現されており、これら二種の VAT に対する抗体は家兎血清中にも抗 Tg-0 抗体の次
に最も早く検出された。

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