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Studies on Antigenic Variation in the Welcome Strain of *Trypanosoma gambiense* II. On the First Relapse Appearing in Mice Treated with Human Plasma

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SUMMARY

During mouse passage of *Trypanosoma gambiense* (Welcome strain), a first relapse variant (serotype Rc) of an unstable nature was isolated. Within several mouse passages this variant was liable to change its antigenic character and react with another type of antiserum (anti-Ra serum).

Utilizing the single cell isolation technique, this unusual phenomenon was analysed genetically. Antigenic variability appears to be due to the unstable state of the peculiar Rc type parasites from which the RaRc combination type organisms arise.

INTRODUCTION

In the preceding paper (Inoki, in press), it has been shown that if a mouse infected with the Welcome strain is treated with human plasma, a relapse appears regularly after a temporary clearance of parasites from the peripheral blood. Because of this, the combination of both mice infected with the Welcome strain and human plasma therapy is very suitable for studing antigenic variation in *Trypanosoma gambiense.*

Employing this system, therefore, observations were made on the serotype which appears during the first relapse in the mice treated with human plasma and on when the relapse would occur after treatment.

MATERIALS AND METHODS

The same Welcome strain of *T. gambiense* was used as in the first report (Inoki, in press). The pure original strain was designated 0w to differentiate it from the original TG 1 strain 0. It was passed through albino mice by intraperitoneal injection and the resulting infections were treated intraperitioneally with 0.5 ml of human plasma. All the first relapse strains that appeared in these treated mice were isolated separately into normal mice to determine their serotypes. The first relapse strain obtained was named Ra; another relapse strain, which was serologically different from Ra, was named Rb. The method used for serotype

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determination has been reported by Inoki *et al.* (1952). Altogether, four different serotypes, named Ra, Rb, Rc and Rd, were isolated in mice as first relapse strains produced from different mice infected with strain Ow. Other techniques used in this study were described in the preceding paper (Inoki, in press).

EXPERIMENTS

Pure lines of the original strain and of the first relapse variants were maintained by serial passage in mice, and antisera were routinely prepared and stored in a deep freeze. Table 1 shows the serotypes of the first relapse strains which appeared in the treated mice during successive mouse passages. These form part of the results obtained from experiments performed by the author at Indiana University. Studies lasted for about 7 months, from November 5, 1953 through June 22, 1954. In this period, 108 animal passages were carried out employing over one thousand mice. As shown in Table 1, in the first 60 mouse passages only Ra appeared as the first relapse type in the treated mice. Relapse type Rc emerged in the 62nd generation of treated mice. Subsequently a high frequency of Rc alone and in various combinations such as RaRbRc, RbRc and RaRc appeared. Usually after many mouse passages the serotype of the first relapse parasites had a tendency to be Rc rather than Ra. This shift can be explained on the adaptation theory advocated by Inoki and his associates (Inoki, 1952; Inoki *et al.*, 1952a, 1952b, 1956, 1957, 1959, Osaki, 1959), namely that the antigenic type is transformed under the direct action of antiserum. On this theory, the observations could be explained if the Ra antigenic substance were produced in the original Ow type parasite during the course of mouse passage. That this had occurred was proved by detecting the presence of anti-Ra body in the peripheral blood of mice previously infected with Ow parasites, subsequently treated with human plasma, and showing a relapse infection.

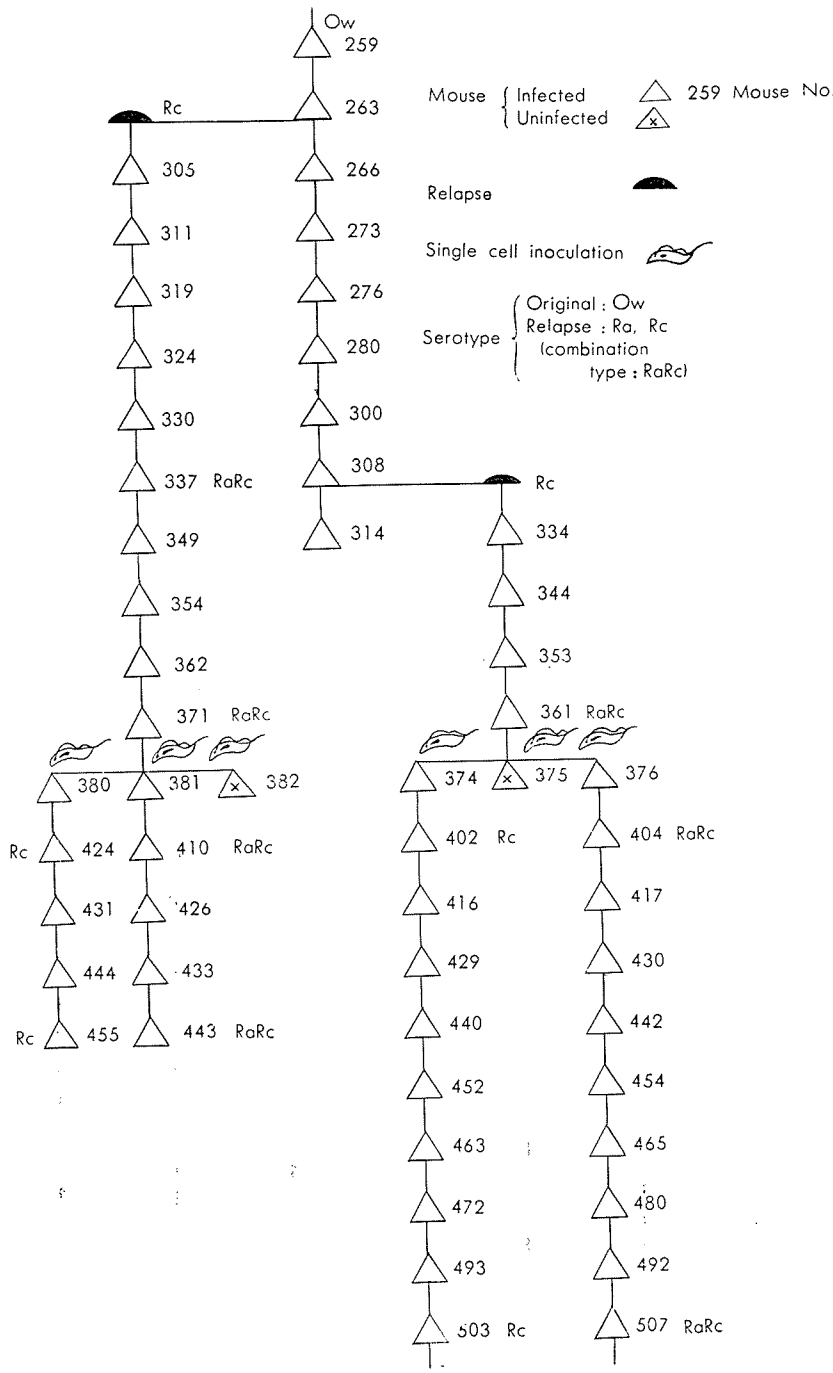
First relapse type Rc parasites obtained from treated mouse No. 263 were subinoculated into two normal mice (Nos. 304 and 305) for determination of the serotype using four standard antisera (anti-Ra, anti-Rb, anti-Rc and anti-Rd). The parasites which subsequently appeared in these two inoculated mice were all shown by the agglomeration test to be type Rc. After five subsequent mouse passages, however, it was found that the parasites could not only agglomerated with anti-Rc, but also simultaneously with anti-Ra. Actually, the parasites from mouse No. 337 shown in Fig. 1 were agglomerated to the same extent by anti-Rc and by anti-Ra sera.

As Fig. 1 indicates, the descendants of the relapse strain Rc, isolated from infected mouse No. 263 immediately after the relapse, also showed a positive agglomeration reaction with anti-Ra serum. From this strain with apparently a double serotype RaRc (mouse No. 371), were isolated two strains established by single parasite inoculation. One of these proved to be a pure strain Rc. Taking this into account, it seems likely that there is a period when new relapse strains may not be stable and the serotype of a strain isolated from such relapse strains can apparently become double or multiple after some generations in mice. Another example of this phenomenon is shown in the same Figure.

Table 1. Serotype of the first relapse appearing in mice treated with human plasma

Date of inoculation	Mouse number	Generation of mouse passage	Serotype of the first relapse	Date of inoculation	Mouse number	Generation of mouse passage	Serotype of the first relapse
5/XI	18	4	Ra	20	267	63	Ra
9	22	7	Ra	22	273	64	Ra
13	25	7	Ra	22	274	64	Ra
16	26	7	Ra	26	280	66	Rb
18	34	9	Ra	26	281	66	Ra
28	49	13	Ra	28	300	67	Rc
28	50	13	Ra	28	301	67	Rc
30	55	14	Ra	30	308	68	Rc
30	56	14	Ra	30	309	68	RaRbRc
3/XII	60	15	Ra	1/IV	314	69	Rc
3	61	15	Ra	1	315	69	Ra
5	64	16	Ra	4	320	70	Rc
5	65	16	Ra	4	321	70	Rc
8	70	17	Ra	6	326	71	RbRc
10	75	18	Ra	8	331	72	Rc
4/I	93	28	Ra	8	332	72	Rc
4	94	28	Ra	10	338	73	Rc
4	95	28	Ra	10	339	73	Ra
4	96	28	Ra	14	357	75	Ra
4	97	28	Ra	14	358	75	Rc
6	100	30	Ra	18	373	77	Rc
15	110	33	Ra	20	385	78	Rc
15	111	33	Ra	20	386	78	Rc
18	114	34	Ra	20	387	78	Rc
20	119	35	Ra	25	411	79	Rc
22	123	36	Ra	25	412	79	Rc
29	134	39	Ra	27	420	80	RaRc
31	139	40	Ra	29	434	81	Rc
4/III	224	56	Ra	3/V	457	83	Rc
4	225	56	Ra	5	468	84	Rc
5	228	56	Ra	13	508	88	Rc
5	229	56	Ra	15	521	89	RaRc
7	234	57	Ra	17	531	90	Rc
7	235	57	Ra	21	551	92	Rc
14	255	60	Ra	29	587	96	Rd
14	256	60	Ra	29	588	96	Rc
18	263	62	Rc	22/VI	718	108	RbRc

Fig. 1. Unstable relapse type (Rc)



DISCUSSIONS

In this study, mice infected with the Welcome strain of *Trypanosoma gambiense* were treated with human plasma, and the serotype of every first relapse strain appearing after a temporary cure was examined by the agglomeration test. The Ra serotype appeared exclusively as a first relapse variant up to the 60th generation of mouse passage. However, another serotype Rc first appeared in the 62nd generation of mouse passage, and in the subsequent passages many serotypes such as Rc, RaRbRc, Rb, *etc.*, developed as first relapse variants. Two relapse strains of type Rc isolated from treated mice during this period were found to have a tendency to change their antigenic characters within a few subsequent mouse passages. This phenomenon suggests why the serotypes of certain relapse strains were difficult to determine at the time of isolation, but were easily determined after a few mouse passages. Genetically, there are two possibilities to be clarified. One is that the seemingly unstable relapse variant under discussion may be not pure, but a mixture of two different serotypes, Rc and RaRc. On this assumption, the antigenic change occurring during mouse passages would be explained by overgrowth of the Rc parasites by an originally small number of the RaRc type organisms in the population of the first relapse strain. These may have been too few in number to be detected initially by the agglomeration test. The other possibility is that first relapse parasites were exclusively of type Rc, but that they had a particular potentiality to generate a new serotype RaRc, either by mutation or by the so-called adaptation process. Either of these two assumptions could explain the above phenomenon. The latter may constitute a rather reasonable explanation of how the parasites could survive in treated mice even though they were continuously in contact with antibody. Further analytical observations are required to give a definite answer to this problem.

In addition, the above results raised the problem why does the serotype of the first relapse strain change from one type Ra to another Rc in the course of mouse passage. This may be explained by assuming that Ra antigenic substance appears in the original Ow type parasites.

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