

Research Note

Morphological Observations on *Trichomonas vaginalis* Isolated from the Urine of a Patient Infected with Atypical Leukemia

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Trichomonas vaginalis is one of the sexually transmitted protozoa widely distributing in the world. It is known that this species of protozoa easily changes in size and shape depending on the living conditions. Glebski (1969) examined morphologically 300,000 parasites among 198 strains of *T. vaginalis* and found that the size and shape of this species varied according to the source of isolation such as vaginal secretion and urine. The nature of the environmental conditions, for example, the presence or absence of concomitant bacteria often affects the size and shape of trichomonas. We found that *T. vaginalis* isolated from the urine of a leukemia patient showed various morphological changes.

The patient was a man of 68 years and a

bedding retailer living in Neyagawa city, Osaka Prefecture, Japan. On March 19, 1987, he was admitted to the hospital attached to the Research Institute for Microbial Diseases, Osaka University, because of atypical leukemia (MDS; myelodysplastic syndrome), diabetes and oral candidiasis. We observed *T. vaginalis* and *Candida species* in his urine specimen on March 28, 1987, although the patient did not complain of any trouble in urogenital system. He was treated with oral administration of 500 mg/day metronidazole (Flagyl, Shionogi) for 5 successive days beginning on March 30. *Trichomonas* was not found in his urine specimen on and after April 9.

T. vaginalis was isolated from the urine before the treatment with Flagyl and cultured in *Trichomonas* medium (Nissui Seiyaku Co Ltd) at 37°C under the cohabitation of *Candida species*. *Trichomonas* was found to grow slowly in the medium for at least three weeks without subculture to fresh medium. They had been cultivated over two years. For light microscopical observations, the cultured trichomonas was smeared on microscopical slides, fixed in absolute methanol and stained with Giemsa's solution diluted with 1/30 M phosphate buffer, pH 7.2, for 30 min.

T. vaginalis which were found on the stained slides were classified morphologically into 4 types (Table 1). In Table 1, type I shows the most typical feature in comparison with morphology of *T. vaginalis* having been cited by various authors. This type hold 15.6% of the

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total parasites counted. The majority of parasites is of type IV. In the nondividing stage of parasites, type I is pear- or spindle-shaped and always has four anterior flagella arising from a kinetosomal complex from which also arises a recurrent flagellum, the undulating membrane and the costa (Fig. 1A). The undulating membrane runs half the full length of the body. The recurrent flagellum terminates at the end of the undulating membrane without forming a free posterior flagellum. A slender axostyle passes through the central part of the parasite and projects from the posterior end of the body. The resting nucleus is an elongated oval body.

Similar to type I, type II–IV always have four anterior flagella, a recurrent flagellum, an undulating membrane and a costa. But, type II–IV are narrower in width than type I. The width of type II is significantly narrower than the range of width of *T. vaginalis* commonly described in text books. Type III is shorter in length than other types. The ratio of length to width in type III is almost the same as that in type I. But, the length and width of type III are half of those of type I. Figure 1B shows an anucleated form of type II which has a clearly projected axostyle. Figure 1C is a type II trichomonas with a nucleus. Figure 1D is a nucleated trichomonas of type III. Type II and

III parasites seem to be produced from type I by autotomy, that is, detachment of a portion of the cytoplasm, as shown in Figs. 1F and G. Figure 1E illustrates type IV, which contains a nucleus nearly in the central part and two candida at the destructive stage in the posterior part of parasite. The recurrent flagellum, being about as long as the body length is observed in Figs. 1B and D. Therefore, these parasites with a disproportionately long recurrent flagellum might be produced as the result of detachment of the anterior part of the cytoplasm from the original parasites. Figure 1F shows a trichomonas undertaking autotomy, in which the main part of cytoplasm containing a nucleus and many candida is posteriorly cast off; consequently a type II anucleated parasite with a kinetosomal complex, four anterior flagella and a recurrent flagellum is produced. The parasite has a little amount of cytoplasm around the axostyle. Figure 1G also shows the another pattern of autotomy. The posterior part of parasite cytoplasm which contains many candida but not a nucleus, is cast off and a type II nucleated form is just separated. Figure 1H shows phagocytosis of candida by trichomonas. The deep indented cytoplasm contains many candida at various stages of destruction. A candida is almost completely ingested. The

Table 1 The rates of appearance and the measurements of four types of *T. vaginalis*

Type	Rate of appearance (%) [*]		Length [†]			Width			Length/Width [‡]
	N form [§]	AN form	Range (μm)	X [#]	SD	Range (μm)	X	SD	Range
I	15.6	0	12.7–13.9	13.3	0.6	10.1–11.1	10.6	0.5	1.1–1.4
II	16.8	2.0	9.7–10.5	10.1	0.4	2.1– 2.7	2.4	0.3	3.6–5.0
III	12.9	1.5	6.4– 7.2	6.8	0.4	5.1– 5.9	5.5	0.4	1.1–1.4
IV	51.2	0	12.4–13.4	12.9	0.5	4.4– 5.2	4.8	0.4	2.4–3.0

*: Counts on 1,000 randomly selected parasites in stained smears made from the culture.

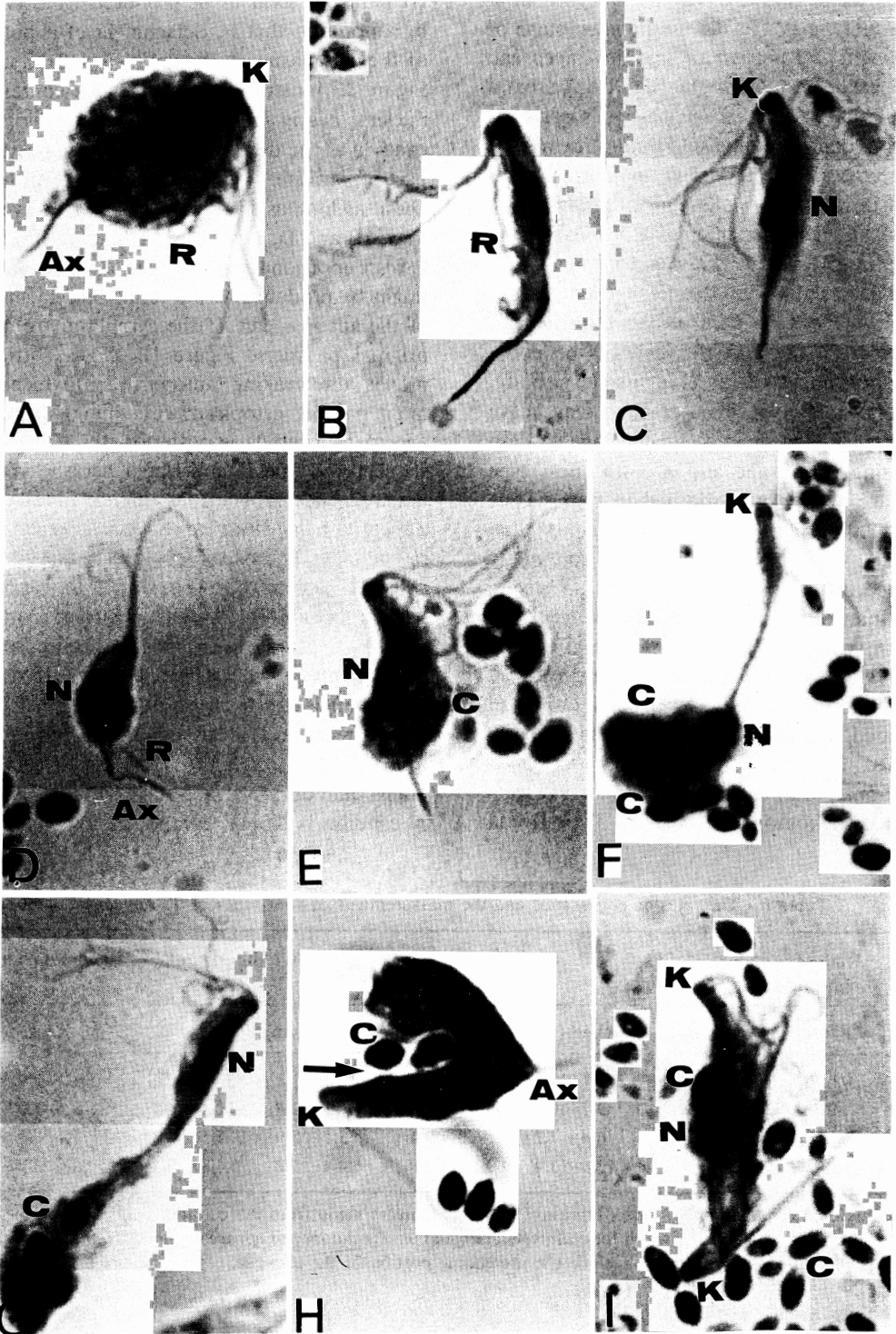
†: Measurements of length and width for each types are based on 30 random and apparently nondistorted organisms. Measurements of length do not include the protruding portion of the axostyle.

‡: Ratio of length to width.

§: Nucleated form.

||: Anucleated form.

#: Sample mean.



trichomonas has a kinetosomal complex, an axostyle projecting from the posterior end of the body and the flagella, which looks like only one flagellum. An abnormal division of trichomonas is observed in Fig. 1I. The axis of usual division in trichomonas is longitudinal. One set of a divided nucleus and one kinetosomal complex migrate toward the opposite position and finally the cytoplasmic division occurs. As shown in Fig. 1I, however, two kinetosomal complexes already have migrated to the opposite position. Anucleated forms of type II and III may be also derived from such abnormal dividing forms.

The autotomy in *T.vaginalis* has been well described by Bland *et al.* (1932), Powell (1936) and Wenrich (1939). But, they did not observe the autotomy of cytoplasm containing candida. Some researchers observed the autotomy in senescent cultures of *T.vaginalis* and speculated that this is a process of eliminations of toxic substances. We also suppose that in the present case the autotomy may be the most efficient method to eliminate a collapsed part of the cytoplasm caused by excessive phagocytosis of candida. The life span of type II and III trichomonas can not be estimated. In the present study, however, these types are always observed in trichomonas culture. The urogenital trichomonas does not appear to have any cytostome. Concerning the ingestion of solids, Wenrich (1939), Bland *et al.* (1932) and Honigberg and

King (1964) found that some strains have the capacity to form the fine and lobose types of filopodia-like cytoplasmic extensions, which are used for ingestion of food particles in an amoeboid manner. But, to find the ingestion of solid particles by *T.vaginalis* is rare. Ingestion of solid particles in amoeboid manner is observed in trichomonas growing in both vaginal secretions and freshly isolated cultures. The ability to ingest solid particles usually disappears after a few weeks of growth *in vitro* (Nielsen, 1975). Trichomonas isolated in this study had a large cleft of the cytoplasm, though no pseudopodia-like extensions. They still kept their capacity for ingestion even after more than two years of cultivation. When we found *T.vaginalis* and *Candida* in a urine specimen of the patient, we did not notice a wide variety of the size and shape of the trichomonas. After this trichomonas strain had been isolated from the urine and then cultivated in the Trichomonas medium at 37°C for a few days, we noticed this variety for the first time.

In the present study, appearance of various types of trichomonas is concerned with concomitant candida which infected the patient. Many cases with a mixed infection of trichomonas and candida in vagina have been known up to now. The Trichomonas medium (Nissui) is commercially available and have been used to isolate *T.vaginalis* for clinical examination. But, no reports have been found about the

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- Fig. 1A-I Light micrographs of *Trichomonas vaginalis* isolated from the urine of a patient. Ax = axostyle projection, C = candida, K = kinetosomal complex, N = nucleus, R = recurrent flagellum.
- Fig. 1A Type I showing the most typical feature of *T.vaginalis*. A parasite has four anterior flagella, a recurrent flagellum, the undulating membrane and costa, arising from a kinetosomal complex. $\times 1,900$
- Fig. 1B Type II anucleated form showing a recurrent flagellum with disproportionate length to the body length. The width of body is particularly narrow. $\times 3,000$
- Fig. 1C Type II nucleated form. $\times 2,500$
- Fig. 1D Type III nucleated form. The length of body is shorter than that of any other types. $\times 2,200$
- Fig. 1E Type IV containing two candida. $\times 2,300$
- Fig. 1F A trichomonas undertaking autotomy. The anterior part is separating from the posterior part of cytoplasm containing a nucleus and many candida. After autotomy, type II anucleated parasite with a kinetosomal complex, anterior flagella and a recurrent flagellum is produced. $\times 1,200$
- Fig. 1G A parasite showing the another pattern of autotomy. Type II nucleated form is just separated. $\times 2,400$
- Fig. 1H Phagocytosis of candida by trichomonas. The deep indented cytoplasm (arrow) contains many candida. A trichomonas has a kinetosomal complex, axostyle projection and the flagellum. $\times 2,200$
- Fig. 1I An abnormal dividing form of trichomonas. Two kinetosomal complexes already have migrated to the opposite position. A nucleus has not divided yet. $\times 2,200$

appearance of various size and shape of *T.vaginalis*. Candidiasis is one of the opportunistic diseases. This patient of trichomoniasis was suffered from oral candidiasis under the immunodeficiency state due to atypical leukemia. It was considered that this candida infection already spreaded to other organs. It was understandable that *T.vaginalis* and *Candida sp* were found in his urine. Both trichomonas and candida isolated from the patient having such background have been cultured together. It was assumed that such coexistence of *T.vaginalis* with *Candida* might induce this *T.vaginalis* strain the morphological changes mentioned above. However, future experimental study will be necessary to give a clear evidence to this assumption.

When a case such as in the present study occurs in the future, we are interested in observing morphology of *T.vaginalis*.

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