Systemic candidiasis from *Candida albicans* colonizing the gastrointestinal tract of mice

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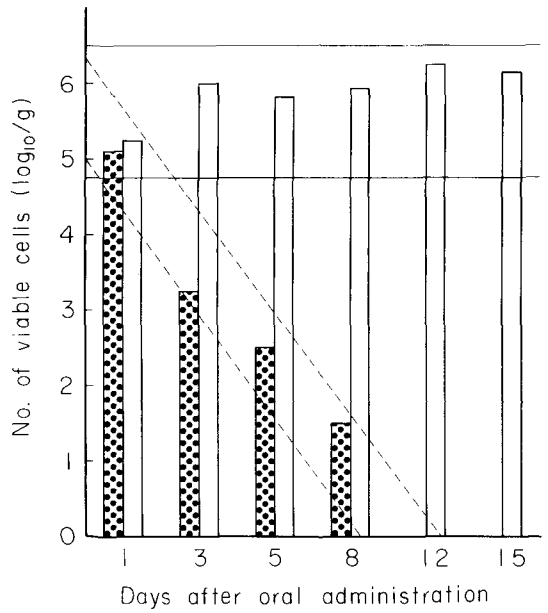
Summary. Reproducible induction of systemic *Candida* infection was achieved by treating mice in which *Candida* colonization had been established in the gastrointestinal tract by aminobenzylpenicillin treatment. Systemic candidiasis was induced in these mice by X-ray irradiation followed by immunosuppressive doses of dexamethasone or X-ray irradiation followed by immunosuppressive doses of trypan blue. Macrophages seem to play an important role in this systemic infection.

The importance of cellular immunity in defence against *Candida* infection has been stressed recently[1,2]. However, details of the mechanism by which *Candida* induces systemic infection are still unclear. Krause et al.[3] reported the occurrence of fungaemia in man after oral ingestion of *Candida* and stressed the importance of *Candida* colonization in the gastrointestinal tract (GIT) as the presumptive source of systemic *Candida* infection. In their opinion, *Candida* that had colonized the GIT may spread to the inner organs of the host by 2 alternative ways: 1st, by persorption through the intestinal wall and, 2nd, by penetrative growth[3,4]. In a previous study of ours, penetrative growth appeared more likely than persorption of *Candida* in the GIT of mice[5]. The present mouse experiments were undertaken to examine the relationship between the growth of *Candida* in the GIT, the defence activity of the host and the occurrence of systemic candidiasis.

Materials and methods. 6-week-old DDI mice weighing approximately 20 g were divided into 12 groups of 35. 6 groups of mice received various immunosuppressive treatments as shown in table 1 after oral administration of aminobenzylpenicillin (AB-PC, 25 mg/mouse). The remaining 6 groups were pretreated with phosphate buffered saline (PBS) instead of penicillin and received the same immunosuppressive treatments as the former 6 groups. For immunosuppression, the mice received either X-ray irradiation (650R) alone, i.p. injection of dexamethasone (0.2 mg/mouse) alone, i.v. injection of trypan blue (4 mg/mouse) alone or the combination of X-ray irradiation and administration of either immunosuppressant. *Candida albicans* (serotype A), grown on Sabouraud's agar for 24 h at 37 °C, was collected, suspended in PBS and 0.1 ml of the suspension (1.5×10^8 cells per mouse) was administered p.o. to each mouse 24 h after X-ray irradiation, simultaneously with the 1st dose of dexamethasone which was given successively for 4 days, or 24 h before trypan blue injection. The number of viable *Candida* cells in the feces and organs (brain, heart, lungs, spleen, liver and kidneys) was examined by colony counting on Sabouraud's agar at various time intervals (table 1, figure).

Results and discussion. When 1.5×10^8 *Candida albicans* was given p.o. to mice, a fairly large count of *Candida albicans* was found throughout the observation period in the feces of the AB-PC treated mice, whereas in mice that did not receive the antibiotic, the number of fecal *Candida* decreased with time and became undetectable 12 days after administration (figure). Treatment with dexamethasone seemed to induce a small increase of *Candida* growth in the GIT of antibiotic treated mice. However, the number of fecal *Candida* in these mice was not statistically significant when compared with those of mice receiving other treatments (data not shown).

Candida was not detected in the brain, heart, lungs, liver, spleen or kidneys throughout the observation period in any



Number of viable *Candida albicans* in feces of mice after oral administration of 1.5×10^8 *Candida*. ▩, Mean number of viable fecal *Candida* from 3 mice without AB-PC. □, Mean number of viable fecal *Candida* from 3 mice with AB-PC. ⊥, Range of viable Candida number of AB-PC non-treated mice with X-ray irradiation, dexamethasone or trypan blue administration. —, Range of viable *Candida* number of AB-PC treated mice with X-ray irradiation, dexamethasone or trypan blue administration.

Table 1. Incidence of the growth of *Candida* from liver or kidneys of mice which received immunosuppressive treatment after oral *Candida* administration

Treatment				Number of positive culture/Number examined						
AB-PC	X-ray	Dexa	Tryp	1[b]	2	4	6	8	12	15
+				0/5 (0/5)[a]	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)
+	+			0/5 (0/5)	0/5 (ND)	0/5 (0/5)	0/5 (0/5)	ND[c] (ND)	ND (ND)	ND (ND)
+	+	+		0/5 (0/5)	0/5 (0/5)	1/5 (0/5)	3/5 (0/5)	ND (ND)	ND (ND)	ND (ND)
+	+		+	0/5 (0/5)	0/5 (0/5)	1/5 (0/5)	4/5 (0/5)	ND (ND)	ND (ND)	ND (ND)
+		+		0/5 (0/5)	ND (ND)	0/5 (0/5)	0/5 (0/5)	ND (ND)	ND (ND)	0/5 (ND)
+			+	ND (ND)	0/5 (0/5)	0/5 (0/5)	0/5 (0/5)	ND (ND)	ND (ND)	0/5 (ND)

[a] 1.5×10^8 *Candida* cells were administered orally to all 12 groups of mice. The results obtained with the 6 groups which received PBS instead of AB-PC are listed in parentheses. [b] Days after oral *Candida* administration. [c] ND: Not done.

of the mice receiving the various immunosuppressive treatments but no AB-PC (table 1, figures in parentheses). However, in the mice that received combined treatments of AB-PC, X-ray irradiation and dexamethasone or trypan blue, *Candida* became detectable in the liver and kidneys at 4 and 6 days after oral administration (tables 1 and 2). The isolates from the liver and kidneys were identified with *C. albicans* subtype A. This suggested that the yeast detected in these organs was not a contaminant or endogenous organism but had spread to the organs from the GIT.

These findings support the idea of penetrative growth of *Candida* rather than persorption through the intestinal wall which would have occurred within a few hours of oral *Candida* administration. They also stress the importance of both *Candida* growth in the GIT and the suppressed state of the host defence mechanism.

Macrophages are known to be rather resistant to sublethal irradiation doses of X-rays which induce severe damage to T cells, B cells and polymorphonuclear leukocytes of mice[6]. However, the suppression of macrophages can be induced by dexamethasone[7] or trypan blue treatments[8]. The fact that the spread into the organs did not follow after single sublethal irradiation but followed after the combination of X-ray irradiation and dexamethasone or trypan blue, suggests an important role of macrophages in the defence against systemic *Candida* infection. Using *Candida* administered orally, several authors have already obtained colonization of the GIT of germ free, specific pathogen free, or antibiotic treated conventional mice, but the colonizing *Candida* did not spread to the inner organs through the intestinal wall[9,10]. The present study shows that *Candida* colonizing the GIT of mice can be the source of systemic *Candida* infection under immunosuppressive conditions brought about by X-ray irradiation combined with dexamethasone or trypan blue administration.

Table 2. Number of viable *Candida* in liver and kidneys of mice that received immunosuppressive treatments after oral *Candida* administration

Treatment			Liver		Kidneys	
			4[a]	6	4	6
AB-PC	X-ray	Dexa	3.4[b] ± 1.2	3.8 ± 0.6	3.7 ± 1.1	4.3 ± 0.8
AB-PC	X-ray	Tryp	-[c]	3.3 ± 0.2	3.5 ± 0.9	4.5 ± 0.5

[a]Days after oral *Candida* administration. [b]Number of viable *Candida* ($\log_{10}$ per organ). [c]Less than 10 *Candida* cells per organ.

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High levels of transition metals in dinoflagellate chromosomes

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Summary. X-ray microanalysis of fixed, sectioned chromosomes of the dinoflagellates *Glenodinium foliaceum, Prorocentrum micans* and *Amphidinium carterae* has revealed high levels of iron, nickel, copper and zinc. We report high levels of these transition metals in association with chromosomes in intact eukaryote cells.

Since Dodge[2] proposed, over a decade ago, that dinoflagellates were evolutionary intermediates between prokaryotes and eukaryotes, considerable attention has been focussed on the fine structure and composition of their nuclei[3-7]. As reported here, our use of X-ray probe microanalysis to investigate the elemental composition of dinoflagellate nuclei has revealed new information about the chemical composition of the nuclei, and in particular the chromosomes. High levels of the transition metals iron, nickel, copper and zinc were found to be associated with the chromosomes of 3 species of marine dinoflagellates. Although transition metals have been reported previously in small amounts in extracted mammalian DNA[8] and deoxyribonucleoprotein (DNP)[9], this is the 1st report of high levels of a wide range of transition metals in association with chromosomes in intact cells.

Materials and methods. 3 marine dinoflagellate species were examined (table). The cells were cultured in Cambridge medium A.E. 50 and prepared for X-ray microanalysis by fixing in 1% glutaraldehyde (buffered by 0.1 M sodium cacodylate, pH 7.2) for 1.5 h, followed by rinsing in buffer, dehydration in an ethanol series, and embedding in Spurr resin[10]. Ultrathin sections (~ 200 nm) were cut, collected on nylon grids and lightly coated with carbon. The sectioned cells were examined completely unstained in an aluminium grid holder using a Corinth analytical electron microscope (Cora-AEI Kratos Ltd, UK). Cora was operated at 60 kV, counting for 500 sec with a spot size of 0.75 µm. Examination of the processing solutions used in the preparation of the cells, by atomic absorption spectrophotometry, showed no detectable contamination by transition metals.

Results and discussion. For each species, 5 cells were analysed in detail, and for each cell X-ray emission spectra were obtained for chromosomes, nucleoplasm, cytoplasm and regions of adjacent resin (control). The elements found in the analyses, and their occurrence in the areas examined in each species, are shown in the table. A typical series of spectra, from 1 cell, showing the 3 regions studied and an adjacent resin control is illustrated in the figure.

The techniques used in preparing the cells (fixation, dehydration and embedding) would result in the majority of weakly bound elements being lost from the cells[11]. Detectable elements retained by these cells were probably closely associated with, if not an integral part of, structural components of the cell.

The chromosomes were remarkable for the high levels (figure) and wide range (table) of elements detected. The transition metals were of particular interest since there are