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Cryptosporidium and Pneumocystis in the Immunosuppressed Mouse

EDWARD D. WAGNER AND MERCY R. PRABHU DAS

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Cryptosporidium is an opportunistic coccidial protozoan parasite of the intestinal tract. It was first described by Tyzzer (1907) from a laboratory mouse. It has been found in a wide range of vertebrate animals (Tzipori, 1983). Diarrhea and malabsorption are the outstanding clinical manifestations of the disorder produced in humans (Current, *et al,* 1983). The infection is self-limited in normal, healthy individuals (Babb *et al,* 1982; Blagburn and Current, 1983). Severe symptoms, including excessive water loss, may lead to death in the immunocompromised person (Sloper, *et al,* 1982) including those with AIDS – Acquired Immunodeficiency Syndrome (Guarda, *et al,* 1983). Experimental infections have been reported in mice and rats by Reese, *et al* (1982).

It had previously been shown that when rats and mice are immunosuppressed with drugs, the opportunistic parasite, *Pneumocystis carinii* develops in the lungs and causes disease (Walzer, *et al,* 1979). These findings implied that the mechanism involves a reactivation of latent infection. Both parasites, *Cryptosporidium* and *Pneumocystis,* are seen in AIDS patients and they are life-threatening. Air-borne transmission is the common method of dissemination of *Pneumocystis,* (Walzer, *et al,* 1979) whereas in cryptosporidiosis it is by ingestion of fecally-contaminated material containing the infective stage oocyst (Reese, *et al,* 1982).

The results of the experiments reported here are derived from tests designed to determine whether or not laboratory mice, when immunosuppressed, will develop cryptosporidiosis and pneumocystosis without the introduction of the infective stage oocysts or cysts.

Materials and Methods

The AKR/J mice (Jackson Laboratories, Bar Harbor, Maine) were used because it is a strain that was shown by Walzer, *et al* (1979) to be susceptible to the development of pneumocystosis when immunosuppressed. The mice used were females weighing between 18 and 20 grams. They were maintained on regular 23% protein pelleted mouse diet and tap water for the acclimatization period indicated for each of the two experiments described. Thereafter, the control mice were kept on that diet and water provisions, whereas the test mice were placed on a low (8%) protein diet and were given drinking water ad libitum containing dexamethasone (1 mg/l) to suppress the immune system, and tetracycline (1 mg/ml) to inhibit the growth of bacteria.

In Test I the mice were separated, 5 to 6 mice per cage. They were housed in a room along with other strains of mice. They were given the regular mouse feed and tap water for a period of two weeks. Thereafter, the test mice were given the low (8%) protein pelleted

Department of Microbiology, Loma Linda University, Loma Linda, California 92350

mouse feed, and the water containing the dexamethasone and tetracycline. All the control and test mice were checked for the presence of oocysts of *Cryptosporidium* in the fecal pellets on the day of arrival, following the acclimatization period, after 13 days into the immunosuppression schedule, and on a weekly basis thereafter. This was done by staining the smears by the modified Ziehl-Neelsen procedure. Of the 17 test animals, small intestine tissues were available from 16, and lung tissue for histological sectioning and staining from 15. All 5 of the control animals were checked in the same manner, including fecal and histological material.

In Experiment II, the mice were received from the supplier in protective-filter containers and were placed in filtered-air gnotobiotic isolators. The 35 mice were separated into seven cages with 5 mice per cage. Of these, 5 mice served as control animals. All of the mice were given the regular mouse feed and tap water for a period of 1 week. Thereafter, the test mice were placed on the same immunosuppression schedule as for Test I. Fecal pellets were collected and examined for the presence of the oocysts of *Cryptosporidium* on the day of arrival, after a 1-week period, and twice a week thereafter. When mice became moribund, they were killed so that portions of the small intestine and the lungs could be obtained for histological sectioning and staining. The intestinal sections were stained by two methods, namely, hematoxylin and eosin and the May-Grunwald Giemsa stains. Some portions of lung sections were stained with Gomori's methenamine silver, and other portions with hematoxylin and eosin.

Lymphocyte transformation tests were performed on 5 control and 6 test mice in order to determine their immune status. The general immunocompetence of the spleen-cell population

Table 1 Occurrence of cryptospordiosis in female AKR/J mice on an immunosuppression drug/diet regimen

Mouse No.	At arrival	2 weeks on control diet	Number of days on regimen and infection status: positive (+) and negative (–)								
			13	20	27	34	42	49	55	62	64
1	–	–	+	–	+	–	+	Killed			
2	–	–	–	–	–	+	+	K			
3	–	–	–	–	–	+	+	K			
4	–	–	+	–	–	+	+	+	K		
5	–	–	–	–	–	+	+	+	Died		
6	–	–	+	–	–	+	+	+	K		
7	–	–	–	–	–	–	+	K			
8	–	–	–	–	–	+	–	+	+	–	–K
9	–	–	–	–	–	–	+	K			
10	–	–	–	–	–	–	+	+	+	–	–K
11	–	–	–	–	–	–	D				
12	–	–	–	–	–	–	+	+	+	+	K(d60)
13	–	–	–	–	–	–	+	+	+	–	+K
14	–	–	–	–	–	–	+	+	+	–	+K
15	–	–	–	+	–	–	–	+	+	+	K(d56)
16	–	–	–	+	–	–	–	+	K		
17	–	–	–	+	–	–	–	+	K		
Controls											
18	–	–	–	–	–	–	–	–	–	–	–K
19	–	–	–	–	–	–	–	–	–	–	–K
20	–	–	–	–	–	–	–	–	–	–	–K
21	–	–	–	–	–	–	–	–	–	–K	
22	–	–	–	–	–	–	–	–K			

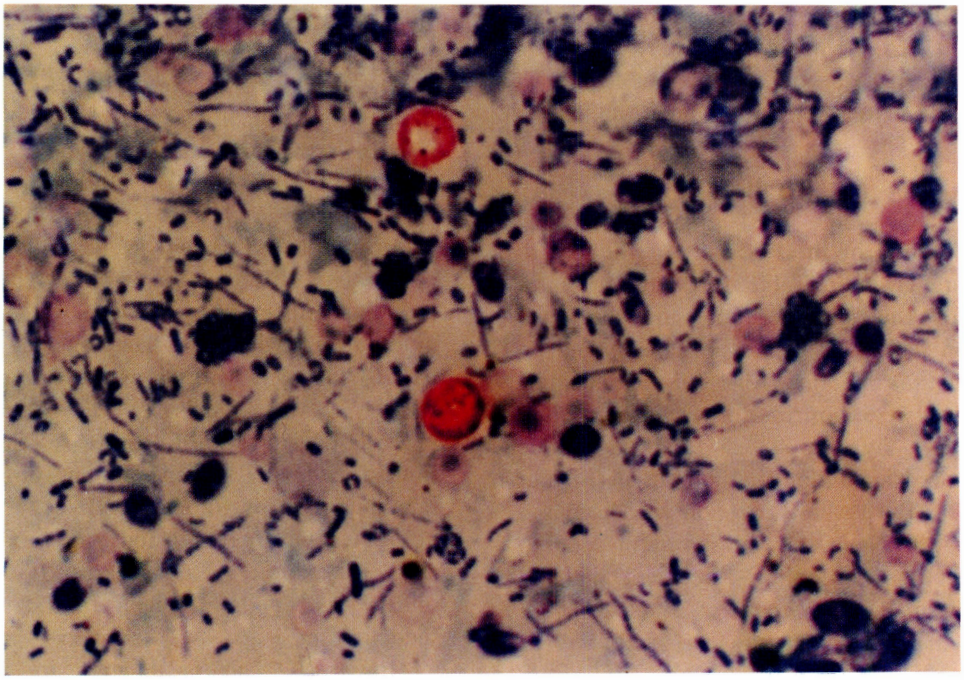

Fig. 1 *Cryptosporidium* oocysts in a mouse fecal smear stained with the modified Ziehl-Neelsen procedure. The red-staining oocysts each contain a vacuole and one or more black-staining granules. (1000x magnification)

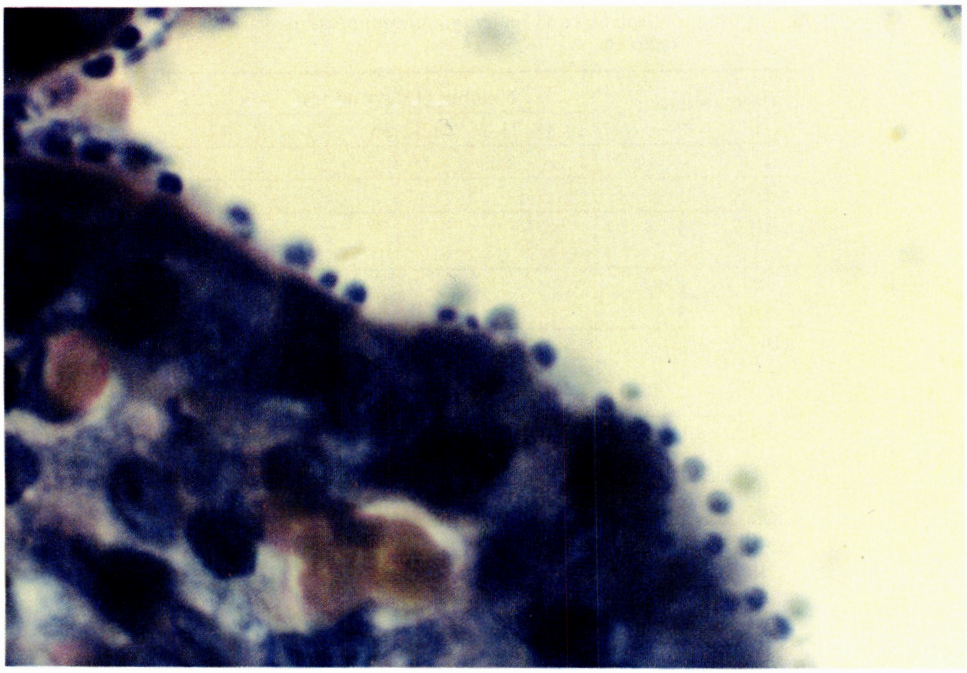

Fig. 2 Mouse ileum intestinal section showing endogenous stages of *Cryptosporidium* on the brush border. The stain used was May-Grunwald Giemsa. (1000x magnification)

was monitored with the mitogen phytohemagglutinin (PHA, 0.1 micrograms/well). Data were expressed as mean counts per minute for each mouse as well as a stimulation index.

Results

The results of the first part of the experiment are given in Table 1. Of the 17 mice used for the immunosuppression test, two died, on days 42 and 62, presumably due to *Pneumocystis* infection. All 15 of the remaining test mice were positive for *Cryptosporidium* as revealed by the stated fecal and tissue diagnostic examination methods used (Figures 1 and 2). The earliest infection was diagnosed by day 13. None showed symptoms due to the infection. This is in agreement with an observation by Sherwood *et al* (1982). All 15 of the mice that showed symptoms of pneumonitis became moribund, and were killed and found to be positive for *P. carinii*. None of the control mice had detectable infection by either parasite as determined by using the same diagnostic procedures.

The results of the second part of the experiment in which the mice were placed in isolators revealed that over a period of 50 days, 17 out of 30 mice (56.7%) became positive for *Cryptsporidium* at some time during the course of the experiment. None of the control mice was positive for this parasite. The results are tabulated in Table 2. There was only one mouse (VI-2) which was shedding oocysts from day 14 to day 39 during every test day until the day it was killed (day 42). All 5 of the mice in Cage VII were positive for the infection. The day of positive finding varied considerably. Except for cage VII, oocysts were not detected in every mouse in a particular cage. The number of oocysts seen in the fecal smears was very low, ranging from 3 to 4 per slide.

Mouse VI-2 had the infection for a longer period of time than any of the others. All the rest of the mice were either positive during one

Table 2 Occurrence of *Cryptosporidium*-positive mice kept in isolators and on an immunosuppression drug/diet regimen

Cage No.	Mouse No.	Number of days on test									
		7	14	18	21	25	28	32	35	39	42
I*											
II	1		+								
III	4	+									
	5	+	+				+				K†
IV	1									+	+K
	5								+	+	+K
V	1				+						–K(d50)
	4								+		
	5						+				–K(d48)
VI	1				+						
	2		+	+	+	+	+	+	+	+	+K
	4						+				–K
	5							+		D‡	
VII	1			+							–K(d46)
	2				+			+	+		–K
	3							+			–K(d46)
	4							+			D(d46)
	5		+		+					+	

* Cage 1, control mice, all negative
† K=killed, K(d no.)= killed on test day
‡ D=died, D(d no.)= died on test day

check and negative during the next or were not found to be infected. Intestinal histologic sections were negative for *Cryptosporidium*. No *Pneumocystis carinii* infection was detected in the lung tissue sections of these same mice. Among the immunosuppressed mice, several became moribund and died. They were seen at necropsy to have pulmonary hemorrhage, probably due to a viral infection.

Results of the lymphocyte transformation test showed that the test mice were immunosuppressed when the mean counts per minute were compared. It was also found that the spleens of the immunosuppressed mice were markedly reduced in size when compared to the controls. It was found that the spleen weights of the immunosuppressed mice ranged in a ratio of 1/5 to 1/50 those of the controls. This is due to the effect of the corticosteroid which resulted in a reduction in the size of various lymphoid tissues, for example, the spleen and lymph nodes (Claman, 1975).

Discussion

The immunosuppressed mice in Test I developed cryptosporidiosis without the introduction, such as by gastric gavage, of the infective stage parasite. This implies that the parasite was present in a latent form. When the immune status was depressed, the infection became patent, as seen by the appearance of oocysts in the feces and also the presence of endogenous stages in the intestinal tissues. Pneumocystis developed as a result of immunosuppression. The infective stage may have been latent and/or airborne on an ongoing basis during the test period.

When the mice were kept in filtered-air isolators (Test II) and placed on the same immunosuppression regimen as in Test I, 17 out of 30 mice developed a low-level infection with *Cryptosporidium*. None developed the pneumocystis infection. These findings may indicate that in Test I, the mice had a latent infection with *Cryptosporidium,* and when isolated in cages and immunosuppressed, they developed the patent infection. In Test II, which was done a year later, only a few of the mice had the latent infection and these developed a light *Cryptosporidium* infection.

In Test I, the mice either had latent *Pneumocystis carinii* infection which was reinactivated due to immunosuppression and/or they became infected via air-transmission. In Test II, when the mice were received from the supplier in filtered-air cartons, and placed in gnotobiotic isolators, it may be that the mice did not have the latent *Pneumocystis carinii* infection and therefore there was no reinactivation of the infection. If this is true, this would have important clinical significance with regard to the longevity of the infectious stage of *P. carinii,* filtered air in a hospital setting, and the immunosuppressed patient.

Summary

When AKR/J mice were immunosuppressed with dexamethasone and a low (8%) protein diet and were kept caged in an animal room where other caged mice were housed, all developed *Pneumocystis* and *Cryptosporidium* infections. Another group of the same strain and sex of mice was housed in filtered-air isolators in a room free of other animals. They were placed on the same immunosuppression drug/diet regimen as above, and were shown by the lymphocyte transformation test to have become immunocompromised. A low-grade infection with *Cryptosporidium* developed in 56.7% of these mice but none of them developed pneumocystis. We conclude that *Cryptosporidium* is an opportunistic parasite which can occur in a latent state in the host until activated by a reduced immune state. *Pneumocystis carinii*, which has an air-borne mode of transmission, may also be latent in the respiratory tract. However, when the transmission of the infective stage is interrupted, as in the test in which the mice were kept in filtered-air isolators for a period of 50 days and none developed pneumocystosis,

the development of pneumocystosis did not occur due to the low level of infective stages which remained low because of the filtered incoming air. These findings may suggest a clinical application with respect to the immunosuppressed patient and these opportunistic parasites.

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免疫抑制マウスにおける *Cryptosporidium* と *Pneumocystis*

EDWARD D. WAGNER AND MERCY R. PRABHU DAS

(*Department of Microbiology, Loma Linda University, California, USA*)

AKR/J マウスにデキサメタゾンと低蛋白飼料を与えて免疫抑制を起こさせ，他のマウスと同室で飼育すると，すべてのマウスは Pneumocystis(P) と Cryptosporidium(C) の感染を誘発した．一方，フィルター付アイソレーター中で飼育し上述の方法で同様に免疫抑制を起こさせたマウス群では，その56.7%に低レベルのCの感染がみられ，Pの感染はすべてのマウスにみられなかった．これらの結果より，Cは宿主の免疫力低下によって再燃するまでは潜伏状態にある日和見感染寄生虫である．空気を介して伝播されるPも気道に潜伏感染しているが，本実験におけるように50日間アイソレーター中で飼育することにより，その伝播が止められると，Pの発症は起こらない．これらの知見は，免疫抑制患者とその日和見感染寄生虫に関する臨床応用に役立つであろう．