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Transmission modes of *Pneumocystis carinii* among rats observed by karyotype analysis*

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Abstract: To observe the transmission patterns of karyotype of *Pneumocystis carinii* (Pc) by rat colonies, three strains of rats, Sprague-Dawley(SD), Wistar(W) and Fisher(F) from various animal vendors, were suppressed of their immunity by injection of methyl prednisolone. They were kept for 5 to 13 weeks in 3 different animal rooms, A, B, and C. The purified organisms were prepared in low melting point agarose gel by embedded lysis method for pulsed field gel electrophoresis. Field inversion gel electrophoresis showed 2 patterns of the karyotype of Pc. The rooms A and C contained SD rats from the source P, and also the room A was used for F and W rats. However, Pc from all of the SD and F rats in the room A showed same karyotypes, the pattern I. The SD rats from different vendors, M and S, were reared in the room B, and shared the same Pc karyotypes, the pattern Ⅱ. The rats of W strain were from the vendor M, and immune-suppressed in the animal room A. Five weeks after the experiment, the Pc showed the karyotype pattern Ⅱ but the pattern became mixed with the type I after 7 to 8 weeks. The findings revealed that the animals born and reared in the same animal quarter harbored Pc with same karyotypes. If the animals were kept under immune-suppression in the same room with heavily infected hosts, they could be infected by Pc from their neighbors. The present experimental findings suggest that Pc is transmitted among rats through the air.

Key words: *Pneumocystis carinii*, albino rats, rat strain, animal room, karyotype, air transmission

INTRODUCTION

Though human cases of *Pneumocystis carinii* (Pc) pneumonia are rapidly increasing throughout the world, the basic research on this organism is not enough for solving its medical problems. Its natural life history is still uncertain as well as its taxonomic position is unsettled (Cushion *et al.*, 1988; Frenkel *et al.*, 1990). Also little is known on its transmission between the hosts due to lack of method to trace any specific isolate of Pc. Only some evidences in the nude mice or nude rats suggested that the transmission might be through the respiratory tract(Walzer *et al.*, 1977; Furuta *et al.*, 1984). However, it is hard to verify if the recovered organisms are the exact Pc of experimental infection because it is not possible to prove its origin.

Analysis of its karyotype by pulsed field gradient gel electrophoresis (PFGE) reveals that Pc is a complex of organisms of various karyo-

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types in spite of same morphology (Yoganathan *et al.*, 1989; Lundgren *et al.*, 1990; Hong *et al.*, 1990 & 1992). Its karyotypes were different not only by the host, human and rat, but also by the strains or colonies of rats. Though the biological significance of the karyotype variation still remains unknown, the karyotype analysis by means of PFGE must be an excellent probe for tracing a specific isolate of the organisms.

In the present study, the karyotype analysis was applied as a tracing method for Pc of a specific karyotype among rats in the same room and therefore to verify the transmission mode of Pc.

MATERIALS AND METHODS

1. Induction of *P. carinii* infection in rats

Adult rats of Sprague-Dawley (SD), Fisher (F) and Wistar (W) strains were suppressed of their immunity by weekly injection of 4 mg or 2 mg methylprednisolone (Depomedrol®, Upjohn Korea Ltd.) for 5 to 13 weeks. Ampicillin was also supplied to them in a dosage of 1 mg/ml tap water. The experimental rats were purchased from 4 different vendors (P, M, S, K) and were kept in 3 separate animal rooms (A, B, C) for the experiment (Table 1).

Table 1. Albino rats used for Pc infection in 3 animal rooms

Strains/ samples	Vendor*	Duration of immune-suppr.	Animal* rooms	Pattern of karyotype
Sprague-Dawley				
SD28	M	9w	B	Ⅱ
SD30	P	13w	A	Ⅰ
SD32	P	10w	C	Ⅰ
SPF1	S	10w	B	Ⅱ
Fisher				
F14, F15**	K	9w	A	Ⅲ
F19	P	8w	A	Ⅰ
Wistar				
W13-3	M	5w	A	Ⅱ
W13-20	M	7w	A	Ⅱ + Ⅰ
W13-32	M	8w	A	Ⅱ + Ⅰ

* P & A: The animal room in the Department of Parasitology, Seoul National University College of Medicine
M & B: The animal laboratory in the basic medical science building, Seoul National Uni versity College of Medicine
C: The animal room in the Institute of Endemic Diseases, Seoul National University College of Medicine
S: The animal laboratory in the Asan Medical Center, Seoul
K: The animal laboratory in the Kwanak campus, Seoul National University
** The rats were under the experiment without Pc infected rats in the same room.

2. Isolation of Pc and preparation of agaroses blocks for PFGE

The rats were sacrificed by ether anesthesia and their lungs were collected. After chopping and homogenizing the lungs, host tissues and cells were removed by cell lysis and filtration through membranes of 10 μm pore size. The numbers of the organisms were counted under the microscope. Contaminating host DNA was digested with DNase I (Boerhinger Mannhaim Co., Germany). The purified organisms were resuspended in 0.25 M EDTA and 1 M sorbitol, and mixed with equal volume of 1.2% low melting point agarose solution. The concentration of Pc in the gel matrix was conditioned to be 10^9 nuclei/120 μl. The gel blocks were treated overnight at 55°C in solution Ⅲ (0.5 M EDTA pH 9.0, 10 mM Tris pH 8.0, 1% sarkosyl) containing 0.1 mg/ml proteinase K (Boerhinger Mannhaim, Germany). The blocks were stored in 0.5 M EDTA (pH 9.0) at 4°C and used for PFGE.

3. PFGE by FIGE (field inversion gel electrophresis)

The agarose blocks were loaded in the trough at the top of agarose gels. The gel running conditions were varied by individual gel. The agarose blocks of a strain of yeast, *Saccharomyces cerevisiae* AB 972, were also loaded on the gels as a DNA size marker. The running buffer was 0.5X TBE (45 mM Tris, 45 mM boric acid, 0.125 M EDTA) at 14°C. The gels were stained in ethidium bromide solution (0.05 mg/ml), and the DNA bands were observed on the UV transil-

luminator. The general procedures followed the method of Hong *et al.* (1992).

RESULTS

The karyotypes of individual Pc isolate were presented in Figs. 1, 2 & 3. The karyotype of Pc from F rats showed 2 different band patterns with minor differences although the rats were kept in the same room A(Fig. 1). The 2 patterns did not show profound differences, but the bands larger than 580 kb showed different lengths. The F rats were supplied from the vendors P or K(Table 1), and the different patterns were made by sources of the rats.

Two different karyotype patterns were also observed from SD rats, supplied by 3 different vendors(P, M, S) but kept in 3 separate rooms (A, B, C) (Fig. 2). They showed two patterns, I or Ⅱ. The SD rats from the vendor P were

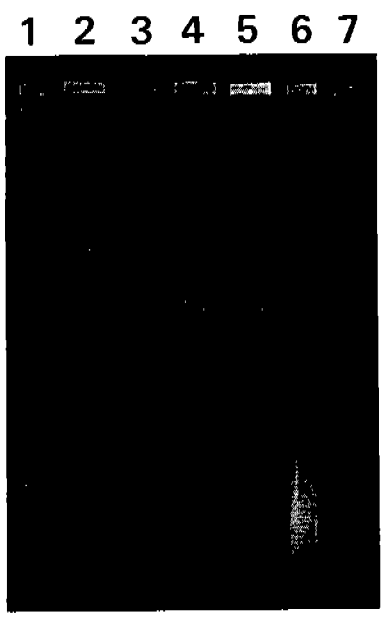

Fig. 1. The FIGE gel run under conditions of 1% agarose, 50 sec forward and 25 sec backward, 110 V, 96 hrs, and 1/2X TBE buffer 14°C. Loaded samples are, 1. Size marker *Saccharomyces cerevisiae* AB 972; 2. F2; 3. F3-1; 4. F12; 5. F13-1; 6. F14; 7. F15. All of the Fisher rats were kept in the room A, but F2, F12, and F13-1 were from the animal room P and F3-1, F14, and F15 were from K.

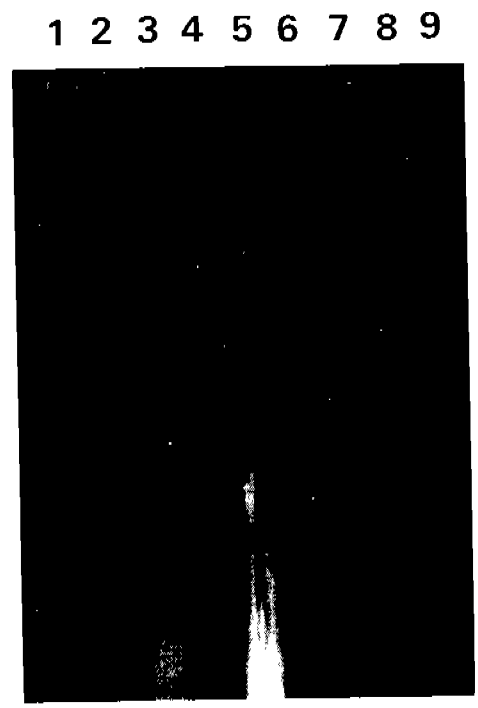

Fig. 2. The FIGE gel run under conditions of 1% agarose, 50 sec forward and 25 sec backward, 95 V, 120 hrs, and 1/2X TBE buffer 14°C. Loaded samples are 1. Size marker *Saccharomyces cerevisiae* AB 972; 2. F19; 3. F14; 4. SD30-6; 5. SD28; 6. SPF1; 7. W13-3; 8. W13-20; 9. Size marker as lane 1.

kept in the rooms A and C, but showed same karyotype of the pattern I. The rats which were suppressed of their immunity in the room B, however, showed the common karyotype of Pc, the pattern Ⅱ, although they were purchased at 2 independent animal vendors(M & S).

The W rats kept in the room A were infected by Pc of the pattern Ⅱ karyotype, which was commonly prevalent among the SD rats from the same vendor M. But later the rats were infected by mixed Pc of the pattern Ⅱ and I karyotypes(Fig. 3). The size of the chromosomal bands from Pc was estimated according to the patterns as schematically presented in Fig. 4.

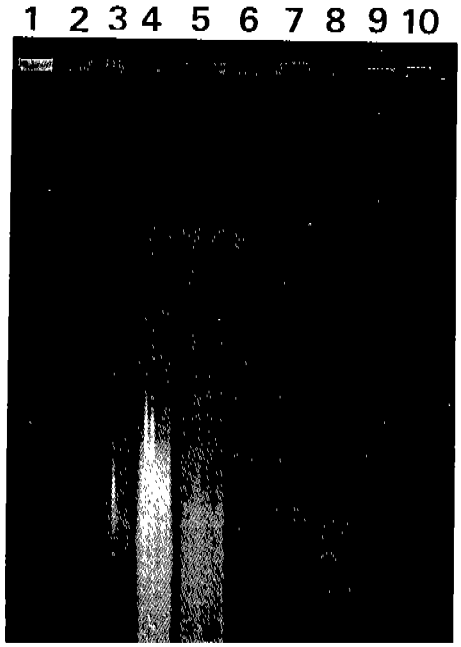

Fig. 3. The FIGE gel run under conditions of 1% agarose, 50 sec forward and 25 sec backward, 95 V, 96 hrs, and 1/2X TBE buffer 14°C. Loaded samples are 1. Size marker *Saccharomyces cerevisiae* AB 972; 2. F19; 3. F2; 4. SD28; 5. SD32; 6. SPF1; 7. D30-6; 8. W13-3; 9. W13-20; 10. W13-32.

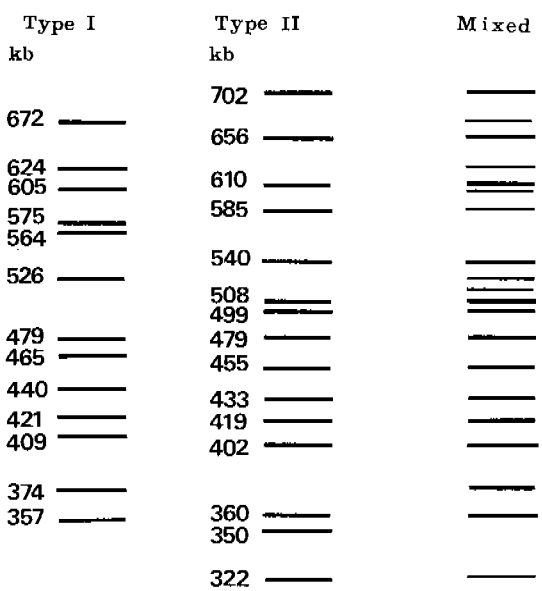

Fig. 4. Karyotypes of *P. carinii* showing the patterns Ⅰ, Ⅱ, and the mixed one. The pattern Ⅰ was found in the room A, and the pattern Ⅱ was in the room B.

DISCUSSION

The karyotype of Pc has been described different according to the authors. However, the chromosomal molecules of size over 1 Mb reported by Yoganathan *et al.* (1989) or Lundgren *et al.* (1990) must not be truely from Pc. In the analyses by Hong *et al.* (1990 & 1992), the large bands were regarded to come from the host DNA. The present study resolved only 13 bands in the pattern Ⅰ and 15 bands in the pattern Ⅱ, which were obtained from rats in the animal rooms A and B respectively. The small band was estimated as 357 kb in the pattern Ⅰ and 322 kb in the pattern Ⅱ. However, the pattern Ⅰ should be similar with that Hong *et al.* (1992) observed because the animal room A contained the rats bred from the ancestral rats of the study. The present gels could not resolve the small-sized chromosomes of size less than 300kb. The difference may be caused mainly by changed running conditions of PFGE. The already published karyotypes gave a strong suggestion that they should not be compared simply of their estimated size. They should be compared only by running in the same gel.

The present karyotypes can be grouped into 2 patterns; Ⅰ and Ⅱ. The 2 patterns were mainly originated by the separated experimental animal rooms A and B respectively. The strain of rats gave little influence on the variation of karyotypes. The pattern Ⅰ is the karyotype of Pc which is prevalent among rats in the animal room of Department of Parasitology, Seoul National University College of Medicine, regardless of the rat strain. At first Hong *et al.* (1992) set rat coloies for Pc in 1990, there found 2 karyotypes differed by rat strains, F and SD, which were supplied by different vendors and were kept in different rooms. However, the karyotype patterns from F and SD rats in the rooms A and C of the present study showed the common karyotype of the pattern Ⅰ. During the past 3 years the 2 strain rats were mixed bred in the same room, and used for Pc infec-

tion, which must have induced the infection by Pc of same karyotype.

The present finding shows two different Pc infection modes among the rats from different sources. The SD, F or W rats which were produced in the conventional animal quarters (vendors P, M and K) were infected by Pc of their own latent infection which might be transmitted among the healthy rats in the quarters. Contrary to this, the rats in the animal room B which were bred in the pathogen-free environment (the vendor S) harbored the Pc of the pattern Ⅱ, which was found in all of the rats of the experimental room B. This finding supports the conventional concept that Pc in latent infection grows fulminantly by immune suppression. If the animal had no latent germs of Pc, it would be infected by Pc from other animals.

The presence of infected rats in a room or not is also important. F rats from the vendor K gave a different karyotype of Pc from that of F rats from P although both of the rats were immune-suppressed in the same room A. The only difference was that there were no infected rats in the room A when the F rats from the vendor K were under the experiment. On the contrary, W rats from the vendor M and kept in the room A revealed Pc infection of the karyotype pattern Ⅱ which was common among the rats from the vendor M and kept in the room B, 5 weeks after the experiment. But the karyotype became mixed of the patterns Ⅱ and Ⅰ after 7 or 8 weeks. The result revealed that the rats were infected by latent Pc at first, but later mixed infected with Pc from highly infected neighbors in the same experimental room. The findings of the W rats and the SD rats from the vendor S stongly suggest that the rats must have been infected by Pc originated from their infected neighbors.

There were no direct communications between rats unless they were in the same cage. The rats from different source were of course kept in separate cages, but the cages were placed side by side in the same animal room. Only the human hands for steroid injection were touching the rats in separate cages. The contact was about 30 seconds a week, which was a hardly imagined vector for Pc transmission. Therefore the present finding can be a strong evidence of Pc transmission through the air. Also production of enough numbers of the organisms takes at least 7 weeks in rats. The space itself shows little influence but presence of severely infected hosts in the same space is a definite source of Pc transmission. This fact means little possibility of Pc transmission through any form of extracorporeal stage in its life cycle.

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=국문초록=

핵형 추적에 의한 카리니주폐포자충의 전파 양상 확인

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현재 국내에서 사육 공급되는 실험용 흰쥐의 계통에 따라 감염되는 *Pneumocystis carinii*(Pc)의 핵형이 다르다는 사실이 밝혀졌다. 특정 핵형의 병원체를 추적하여 전파를 파악하는 연구의 일환으로, 여러 다른 공급처에서 얻은 Sprague-Dawley(SD), Fisher(F), Wistar(W) 흰쥐를 여러 장소에서 사육하면서 면역억제하여 Pc를 발현시키고, 이를 순수하게 모아서 field inversion gel electrophoresis를 사용하여 염색체 분자를 분리 관찰하였다. 한 실험실(A)에서 감염을 유발시킨 F 흰쥐는 두 공급원(P, K)에 따라서 같은 실험실에서 사육하였는데도 다른 핵형을 보였다. SD 흰쥐를 M, P, S 세 군데에서 공급받아 서로 격리된 다른 사육실 세 군데에 (A, B, C) 나누어 사육한 결과 P공급원의 흰쥐를 다른 방 A와 C에서 사육하였는데 같은 핵형(I형)을 보였고, 또한 다른 두 공급원 M과 S에서 구한 SD 계통 흰쥐를 같은 방(B)에서 사육하여 같은 핵형(Ⅱ형)을 얻었다. P에서 공급한 F 흰쥐와 M에서 공급한 W 흰쥐를 A 사육실에서 SD 흰쥐와 함께 실험한 결과, F와 SD는 같은 Pc의 핵형(1형)을 보이고, W는 감염 5주에 B 사육실의 흰쥐와 같은 유형 (Ⅱ형)을 가졌으나 7주 및 8주에는 Ⅱ 유형과 I 유형의 복합형을 나타내었다. 이러한 핵형 변화의 유형으로 이들 숙주가 면역억제될 때에는 잠재적으로 가지고 있는 Pc가 발현되나 같은 환경 내에 다른 증감염된 숙주가 있을 경우에는 이 동물로부터 또한 감염을 받는다는 사실을 확인하였다. 특히 상재성 병원체에 감염되지 않게 생산한 동물은 주위의 동물로부터 감염되는 것이 확실하며, 또한 단순하게 같은 방에서 다른 사육조(animal cage)에 사육을 하는 것으로 Pc의 전파가 일어난다는 사실로 미루어 보면, 이 병원체가 공기를 통하여 전파되는 것으로 추정할 수 있었다. (이 연구는 1990년도 서울대학교 의과대학 발전기금에 의하여 지원되었음)

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