Aerobic and Anaerobic Bacterial Interactions in the Development of Anaerobic Bacterial Pneumonia

Jun GOTO

Department of Internal Medicine, Oita Medical University

Present address: Department of Respiratory Disease, National Oita Hospital

(Received: July 22, 1992)

(Accepted: October 20, 1992)

Key words: anaerobic bacterial pneumonia, *Bacteroides fragilis*, mixed infection, phagocytosis

Summary

The interactions between aerobic and anaerobic bacteria in the development of anaerobic bacterial pneumonia were studied by introducing *Bacteroides fragilis* and *Escherichia coli* alone or in combination into guinea pigs by tracheal infusion. The lung lesions induced by *B. fragilis* were mainly located near the pleura, unlike those induced by *E. coli*, and were accompanied by pneumonia, lung abscess, and pleuritis. The lung lesions produced by mixed infection with *B. fragilis* (10^9 cfu) and *E. coli* (10^7 cfu) were significantly more severe than those induced by either microbe alone, and the redox potentials at the foci of inflammation were markedly reduced (max: -330 mV). Analysis of lung lesions after treatment with aztreonam and clindamycin and neutrophil phagocytic activity suggested that *E. coli* was primarily responsible for the lung lesions and that *B. fragilis* promoted the accompanying inflammation, resulting in increased pathogenicity of the mixed infections.

Introduction

Non-spore forming anaerobes (anaerobic bacteria) are important pathogens in respiratory infections such as lung abscess, pyothorax, and aspiration pneumonia, which are often encountered in clinical practice¹⁾²⁾. Anaerobic bacterial infections are usually found as mixed infections in combination with aerobic bacteria¹⁾, and synergism between them has been found in both *in vitro* and *in vivo* experiments³⁾⁴⁾. In animals, anaerobic bacterial infections are often induced by intraperitoneal, subcutaneous or intracutaneous inoculation⁵⁾⁶⁾⁷⁾, but experimental pulmonary infections are scarce, probably because of the high oxygen pressure and rich blood flow in the lung.

In this study, we induced pneumonia by directly infusing bacterial suspensions into the guinea pig trachea, using *Bacteroides fragilis* strains which are the most frequent pathogens implicated in anaerobic bacterial infections. The interactions between aerobic and anaerobic bacteria in formation of lung lesions were evaluated, using *Escherichia coli* as a representative aerobe. Mixed bacterial pneumonia was found to be more severe and more resistant to antibiotic treatment than pneumonia due to either *B. fragilis* or *E. coli* alone, indicating synergism between the two bacterial species in lung lesions. *B. fragilis* is assumed to enhance the pathological changes caused by *E. coli*.

Materials and Methods

1. Bacterial strains

The anaerobic bacteria employed in this study were superoxide dismutase-producing *B. fragilis* N-17, *B. distasonis* N-1, and *B. thetaiotaomicron* N-79, maintained in our department, which exhibited potent pathogenicity in a previous guinea pig intracutaneous infection study⁸⁾. *E. coli* MCO 85036 isolated from a sputum sample preserved in our department was used as a representative aerobic microbe.

2. Animals

Female Hartley white guinea pigs weighing 350–400 g were used.

3. Bacterial inoculation

The anaerobes were incubated on GAM agar medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) in an anaerobic chamber (Forma Scientific, Inc., Marietta, Ohio, USA) at 37°C for 48 hr. *E. coli* was incubated aerobically on Mueller-Hinton agar medium (MH agar medium, Becton Dickinson Microbiology Systems, Cockeysville, Md, USA) at 37°C for 18 hr. The resulting colonies were suspended in 5 ml of sterilized physiological saline, washed and centrifuged at $1,400 \times g$, for 10 min. The sediment of bacteria was resuspended in 3 ml of physiological saline containing 5% mucin (Difco Laboratories, Detroit, Mich, USA), and adjusted to final concentrations of 10^{10} , 5×10^9 , 10^8 , 5×10^7 , 10^6 and 5×10^5 cfu/ml by using a spectrophotometer (UV-120-01, Shimadzu Seisakusho, Kyoto, Japan) calibrated in advance for counting bacteria. The inoculum size and six combinations of anaerobic/aerobic bacteria inoculated are shown in Table 1. The volume of inoculation was 0.2 ml.

Lung inoculation was performed by tracheal infusion using a modified version of Kannangara's method⁹⁾. Guinea pigs were anesthetized by intraperitoneal injection of 1.0 ml of a mixture of 10 ml of a solution of 500 mg of ketamine hydrochloride (Ketalar 50®, Sankyo Company, Ltd., Tokyo, Japan) and 1.25 ml of 2% xylazine hydrochloride (Ceractal®, Bayer Ltd., Tokyo), and were disinfected with povidone iodine (10% Isodine® solution, Meiji Seika Kaisha, Ltd., Tokyo) from the neck to the abdomen. The trachea was exposed by median incision of the neck, and punctured with a 23-G disposable venular needle (Top Company, Ltd., Tokyo); the outer tube was then advanced into the distal bronchial tree of the right lung. A sterilized syringe containing the bacterial suspension was connected to the outer tube, and 0.2 ml was slowly infused into the bronchus, after which the outer tube was extracted, and the puncture hole was sutured.

4. Evaluation of lung lesions

The lung lesions were evaluated immediately after death. The surviving animals were killed on the 7th day after infusion of the bacterial suspension. After gross examination, the right lung was aseptically removed, and a part of it was fixed with formalin for histopathologic examination. Another part was homogenized in 1 ml of sterile physiological saline, diluted serially with saline, cultured on GAM agar medium for anaerobes or MH agar medium for aerobes, and bacterial counts were determined.

Lung lesions were scored according to the severity of inflammation on a scale of 0–7 as follows: 0 for normal; 1 for a condition in which few neutrophils are observed, and aeration of the alveolar space is almost completely retained despite thickening of the alveolar wall due to edema; 2 for milder inflammation in which fewer inflammatory cells are observed, but effusion is present in the alveolar space; 3 for severe inflammation in which the alveolar wall is thickened due to edema, and the alveolar space is filled with inflammatory effusion. Two points each were added for abscesses and pleuritis. This made the maximum score 7.

5. Measurement of the redox potential (Eh)

The Eh was measured in right lung tissue by a potentiometer (No. 6029, Horiba Seisakusho, Kyoto) immediately after thoracotomy.

6. Analysis by chemotherapy

(1) Antibiotics and bacterial strains

Clindamycin (CLDM, Japan Upjohn Ltd., Tokyo) and aztreonam (AZT, Eisai Company, Ltd., Tokyo) were used for inhibition and treatment of developing pneumonia in guinea pigs infected with *B. fragilis* N-17 (10^9 cfu) and *E. coli* MCO 85036 (10^7 cfu). The minimal inhibitory concentrations (MICs) of CLDM and AZT against *B. fragilis* N-17 were 0.05 $\mu\text{g/ml}$ and >100 $\mu\text{g/ml}$, respectively, and those against *E. coli* MCO 85036 were 50 $\mu\text{g/ml}$ and 0.025 $\mu\text{g/ml}$, respectively.

(2) Chemotherapy

After a mixture of *B. fragilis* and *E. coli* was infused into the guinea pig trachea, the animals were divided into 4 groups consisting of 6–15 animals each: A control group not given any antibiotics, an AZT group, a CLDM group, and an AZT + CLDM group. The dose of CLDM was 12 mg and that of AZT was 10 mg per animal. The antibiotics were administered intraperitoneally immediately after inoculation of the bacteria, thereafter every 12 hr for 4 days, and every 24 hr for the final 3 days. As is previously mentioned, the animals were killed after 7 days and lung lesions were evaluated.

(3) Serum and lung tissue concentrations of antibiotics

Three animals in each group were given both AZT (10 mg) and CLDM (12 mg) intraperitoneally. They were sacrificed 30 min and 1, 2, 4, 6 and 8 hr after administration, and their blood was collected. Lungs were removed and homogenized in 0.1 M phosphate buffer, pH 7.4, and the AZT and CLDM concentrations were determined by bioassays using *E. coli* ATCC 27166 and *Micrococcus luteus* ATCC 6638, respectively, as indicator strains¹⁰⁾.

7. Neutrophil phagocytosis

The rates of phagocytosis of *B. fragilis* N-17 and *E. coli* MCO 85036 by neutrophils were determined by the method of Hasui et al.¹¹⁾ using blood from 5 healthy human adults (2 males and 3 females) aged 25–32 years. Bacteria grown on the appropriate agar medium were suspended in physiological saline, washed 3 times, adjusted to 10^9 cfu/ml, and stained for 30 min with 5% propidium iodide (PI; Sigma Chemical Co., St., Louis, Mo, USA) solution. The percent phagocytosis was calculated by flow cytometry (FACScan®; Becton Dickinson Co., San Jose, Calif., USA).

The culture filtrates of *B. fragilis* in GAM broth (Nissui Pharmaceutical Co., Ltd., Tokyo) and of *E. coli* in Mueller-Hinton broth (MHB; Difco) incubation at 37°C for 24 hr were sterilized by passage through a Millipore filter (0.22 μm , Nihon Millipore Ltd., Tokyo), a 100 μl of the filtrate was added to 200 μl of a mixture of whole blood and PI-stained bacterial suspension, and the percent phagocytosis was determined. The pH of each broth suspension was adjusted to that of the culture filtrate with 0.1 N HCl, and the percent phagocytosis was similarly determined for the control.

8. Statistics

The differences between groups were tested for significance by the unpaired Student's *t*-test, and the results were expressed as the mean and standard deviation.

Results

1. Findings of lung inflammation in single bacterial infection

As shown in Table 1, no deaths were occurred in the groups inoculated with a single bacterial strain, although pneumonia was observed in all animals inoculated with 10^9 cfu of each bacterium. Abscess formation was noted in 2 of the 6 animals inoculated with *B. fragilis* at 10^9 cfu and in 1 of the 6 animals inoculated with *E. coli* at 10^9 cfu. Pleural damage was observed in the animals inoculated with *B. fragilis* at 10^9 cfu and *B. distasonis* at 10^9 cfu, but none was noted in any *E. coli* group. Among the groups inoculated with anaerobic bacteria, the lung lesion score was the highest in the *B. fragilis* group at 10^9 cfu (score 3.33

Table 1 Findings in the lung of guinea pigs on the seven days after infection

Bacteria ^{a)}	Inoculum size (cfu ^{b)})	No. of guinea pigs	Mortality ^{c)} (n)	Pneumonia (n)	Abscess formation(n)	Pleuritis (n)
Single infection						
<i>B.f</i>	10 ⁹	6	0	6/6	2/6	4/6
	10 ⁷	6	0	2/6	0	0
<i>B.d</i>	10 ⁹	4	0	4/4	0	2/4
<i>B.t</i>	10 ⁹	4	0	4/4	0	0
<hr/>						
<i>E.c</i>	10 ⁹	6	0	6/6	1/6	0
	10 ⁷	6	0	5/6	0	0
	10 ⁵	4	0	2/4	0	0
<hr/>						
Mixed infection						
<i>B.f/E.c</i>	10 ⁹ /10 ⁷	6	4/6	6/6	6/6	6/6
	10 ⁹ /10 ⁵	6	0	6/6	2/6	2/6
	10 ⁷ /10 ⁷	6	0	6/6	0	0
<i>B.d/E.c</i>	10 ⁹ /10 ⁷	6	2/6	6/6	4/6	2/6
<i>B.t/E.c</i>	10 ⁹ /10 ⁷	6	0	6/6	0	0

a) *B.f* : *Bacteroides fragilis* N-17, *B.d* : *B. distasonis* N-1,

B.t : *B. thetaiotaomicron* N-79, *E.c* : *Escherichia coli* MCO 85036

b) cfu : colony forming unit

c) Animals that died within 7 days were immediately autopsied and evaluated.

Fig. 1 Findings in the lungs of guinea pigs infected with *Bacteroides* sp. and *E. coli* alone or in combination. *B.f*: *Bacteroides fragilis* N-17; *B.d*: *B. distasonis* N-1; *B.t*: *B. thetaiotaomicron* N-79; *E.C*: *E. coli* MCO 85036; cfu: colony forming unit. Inflammation score of the lung is explained in the text.

± 0.9), followed by the *B. distasonis* group at 10⁹ cfu, and the *B. thetaiotaomicron* group at 10⁹ cfu. Among the groups inoculated with *E. coli*, inflammation was the severest at 10⁹ cfu (score 3.0 ± 0.4), with milder changes at 10⁷ and 10⁵ cfu (Fig. 1).

2. Features of lung inflammation induced by mixed infection

As shown in Table 1 and Fig. 1, in the group inoculated concomitantly with *B. fragilis* at 10⁹ cfu and *E. coli* at 10⁷ cfu, 4 of the 6 animals died within 7 days, and marked pneumonia and pleuritis were noted in all the animals (score 7.0). In the group concomitantly inoculated with *B. fragilis* at 10⁹ cfu and *E. coli* at 10⁵ cfu, no deaths occurred, but pneumonia was noted in all animals, and abscess formation in 2 and pleuritis in 2 of the 6 animals (score 3.5 ± 1.1). In the group inoculated with *B. fragilis* at 10⁷ cfu and *E. coli* at 10⁷ cfu, pneumonia only was demonstrated in all the animals (score 1.13 ± 0.2). In the group inoculated with *B. distasonis* at 10⁹ cfu and *E. coli* at 10⁷ cfu, 2 of the 6 animals died, pneumonia was marked, and abscess and pleuritis was demonstrated in 2 (score 4.5 ± 0.9). In the group inoculated with *B. thetaiotaomicron* at 10⁹ cfu and *E. coli* at 10⁷ cfu, lung lesions were relatively mild (score 2.5 ± 0.2). The score was generally higher in

Table 2 Redox potentials of the lungs of guinea pigs infected with *B. fragilis* and/or *E. coli*

Score ^{a)}	Redox potential (mV, mean±SD)					
	<i>B. fragilis</i> ^{b)}	(n)	<i>E. coli</i> ^{c)}	(n)	Mixed ^{d)}	(n)
0~1	-42.6±77	(7)	-10.8±54	(5)	-66.8±30	(5)
2~4	-180 ±23	(3)	-56 ±16	(6)	-150.8±43	(12)
5~7	-205 ±10	(2)	-68(1)		-235.3±51	(11)

^{a)} The scores were explained in the text.

^{b)} The lungs infected with *B. fragilis* N-17 10⁹ cfu (n=6) and 10⁷ cfu (n=6).

^{c)} The lungs infected with *E. coli* MCO 85036 10⁹ cfu (n=6) and 10⁷ cfu (n=6).

^{d)} The lungs infected with *B. fragilis* group 10⁹~10⁷ cfu and *E. coli* MCO 85036 10⁷~10⁵ cfu (n=28).

^{e)} p<0.05, ^{f)} p<0.01

Table 3 Bacterial recovery from the lung of guinea pigs infected with *B. fragilis* 10⁹ cfu and *E. coli* 10⁷ cfu alone or in combination on day 7

Bacteria ^{a)}	Inoculum size (cfu)	No. of guinea pigs (n)	Bacterial recovery (n)		
			<i>B. fragilis</i> alone	<i>E. coli</i> alone	<i>B. fragilis</i> and <i>E. coli</i>
<i>B. fragilis</i>	10 ⁹	6	0	0	•
<i>E. coli</i>	10 ⁷	6	0	0	•
<i>B. fragilis</i>	10 ⁹	6	1 ^{b)} /6	3 ^{c)} /6	1 ^{d)} /6
<i>E. coli</i>	10 ⁷				

^{a)} *B. fragilis* : *Bacteroides fragilis* N-17, *E. coli* : *Escherichia coli* MCO 85036,

Limit of viable count of bacteria is <10² cfu/g.

^{b)} The viable count of *B. fragilis* was 3.5×10⁸ cfu/g.

^{c)} The viable count of *E. coli* was 1.5×10⁶, 5.1×10⁵, 9.8×10³ cfu/g, and all animals died.

^{d)} The viable count of *B. fragilis*/*E. coli* was 3.1×10⁵/2.8×10⁴ cfu/g, and the animal died.

the mixed infection groups than in the single infection groups, being the highest in the group inoculated with *B. fragilis* at 10⁹ cfu and *E. coli* at 10⁷ cfu.

3. Local Eh of the lung

As shown in Table 2, the Eh value was -68 to +60 mV in lungs infected with *E. coli*, and no correlation was observed between the severity of pneumonia and Eh. In the *B. fragilis* single infection groups, Eh tended to decrease as the severity of the pneumonia increased, and at the same score it tended to be lower in the mixed infection groups than in the single infection ones.

4. Recovery of bacteria

The counts of viable bacteria from lung lesions are shown in Table 3. In the groups inoculated with *E. coli* or *B. fragilis* alone, clearance of bacteria from the lung was complete. In the mixed infection groups, the bacteria were recovered from some animals, all of which died except one from the *B. fragilis* recovered animals.

5. Analysis of mixed infection groups by chemotherapy

As shown in Table 4, the concentration of AZT exceeded its MIC against *E. coli* for 6 hr in the serum and for 1 hr in lung tissue, but the level was less than its MIC against *B. fragilis*. Conversely, the concentration of CLDM exceeded its MIC against *B. fragilis* for 6 hr in the serum and for 8 hr in the lung tissue, but it was less than its MIC against *E. coli*. Two (18%) of the 11 animals treated with CLDM died, but no deaths occurred in the animals treated with AZT or AZT + CLDM (Table 5). The lung lesion scores

Table 4 Mean serum and lung tissue concentrations of aztreonam (AZT) and clindamycin (CLDM) in three guinea pigs injected intraperitoneally

Antibiotics ^{a)}		Levels of antibiotics in serum ($\mu\text{g/ml}$) and lung tissue ($\mu\text{g/g}$) at indicated time (h) after injection					
		0.5	1	2	4	6	8
AZT	Serum	38.3	23.9	2.33	0.46	0.05	<0.03
	Lung	0.88	0.58	<0.01	<0.01	<0.01	<0.01
CLDM	Serum	1.78	0.85	0.5	0.18	0.11	<0.03
	Lung	7.67	5.81	5.0	1.77	1.58	1.1

^{a)} Dose : AZT 10mg/animal, CLDM 12mg/animal

Table 5 Findings in the lung of guinea pigs concomitantly infected with *B. fragilis* N-17 at 10^9 cfu and *E. coli* MCO 85036 at 10^7 cfu after 7 days of antibiotic administration

Antibiotics ^{a)}	No. of guinea pigs	Mortality (n)	Pneumonia (n)	Abscess (n)	Pleuritis (n)
None	6	4/6	6/6	6/6	6/6
AZT	9	0	9/9	2/9	1/9
CLDM	11	2 ^{b)} /11	11/11	3/11	2/11
AZT+CLDM	15	0	10/15	0	0

^{a)} AZT : aztreonam. CLDM : clindamycin.

Treatment schedule is shown in the text.

^{b)} The dead animals were autopsied and evaluated immediately.

Table 6 Bacterial recovery from the lung of guinea pigs concomitantly infected with *B. fragilis* N-17 at 10^9 cfu and *E. coli* MCO 85036 at 10^7 cfu after 7 days of antibiotic administration

Antibiotics ^{a)}	Guinea pigs (n)	Bacterial recovery ^{b)} (n)		
		<i>B. fragilis</i> alone	<i>E. coli</i> alone	<i>B. fragilis</i> and <i>E. coli</i>
none ^{c)}	6	1/6	3/6	1/6
AZT	9	3 ^{d)} /9	0	0
CLDM	11	1 ^{e)} /11	2 ^{f)} /11	1 ^{g)} /11
AZT+CLDM	15	0	0	0

^{a)} AZT : aztreonam, CLDM : clindamycin. The treatment schedule is shown in the text.

^{b)} Isolates positives (n)/number of guinea pigs inoculated.

^{c)} The viable count of bacteria is shown in Table 3.

^{d)} The viable count of *B. fragilis* was 1.3×10^3 , 2.2×10^4 and 4.4×10^2 cfu/g.

^{e)} The viable count of *B. fragilis* was 5.9×10^4 cfu/g.

^{f)} The viable count of *E. coli* was 2.5×10^3 and 2.9×10^3 cfu/g, of which the one animal died.

^{g)} The viable count of *B. fragilis/E. coli* was $5.8 \times 10^3/1.6 \times 10^6$ cfu/g, and the animal died.

were 3.0 ± 2.1 in the CLDM group, 2.6 ± 1.9 in the AZT group, and 1.3 ± 0.5 in the AZT + CLDM group, the latter being the lowest (Fig. 2). No bacteria were recovered from the lungs in the AZT + CLDM group. In the AZT group, *B. fragilis* was recovered from 3 of the 9 animals. In the CLDM group, *B. fragilis* was recovered from 1, *E. coli* from 2 and both from 1 of the 11 animals (Table 6). The viable counts are shown in the footnote to Table 6.

Fig. 2 Findings in the lungs of guinea pigs concomitantly infected with *B. fragilis* N-17 10^9 cfu and *E. coli* MCO 85036 10^7 cfu, after antibiotic treatment. AZT: aztreonam; CLDM: clindamycin. The treatment schedule is shown in the text.

Fig. 3 Effect of *B. fragilis* N-17 culture filtrate on phagocytosis of *E. coli* MCO 85036 by human neutrophils. % phagocytosis (mean ± SD): a) 89.3 ± 3.3, b) 74.8 ± 15.2, c) 40.2 ± 9.6, d) 42.6 ± 17.1, e) The pH of the *B. fragilis* culture filtrate was 5.4.

Fig. 4 Effect of *E. coli* MCO 85036 culture filtrate on phagocytosis of *B. fragilis* N-17 by human neutrophils. % phagocytosis (mean ± SD): a) 49.2 ± 5.6, b) 52.6 ± 8.3, c) 52.7 ± 10.6, d) 59.6 ± 10.2. The differences are statistically not significant. e) MHB: Mueller-Hinton Broth. f) The pH of the *E. coli* culture filtrate was 5.2.

6. Interaction between *B. fragilis* and *E. coli* in human neutrophil phagocytosis

The percent phagocytosis of *E. coli* ($89.3 \pm 3.3\%$) by human neutrophils was significantly higher than that of *B. fragilis* ($49.2 \pm 5.7\%$, $p < 0.002$). The percent phagocytosis of *E. coli* showed no significant changes when GAM broth (pH 7.2) was added, but it was significantly reduced when the culture medium of *B. fragilis* (pH 5.4) or GAM broth adjusted to pH 5.4 was added ($p < 0.002$, Fig. 3). The percent phagocytosis of *B. fragilis* was not affected significantly by the addition of MHB (pH 7.2) culture medium of *E. coli* (pH 5.2), or MHB adjusted to pH 5.2 (Fig. 4).

Discussion

There has been no established model of anaerobic bacterial infection of the lung in animals. Kannangara et al.⁹⁾ inoculated rabbits with various anaerobic bacteria by tracheal infusion, and reported that lung abscesses could be produced by anaerobic bacteria isolated from the oral cavity. Using this method, we infected female Hartley guinea pigs by tracheal infusion of *E. coli* and three *B. fragilis* group

organisms (*B. fragilis*, *B. distasonis*, and *B. thetaiotaomicron*) alone or in combination, and the interaction between aerobic and anaerobic bacteria in lung infections was investigated. *E. coli* and *B. fragilis* strains are not very common pathogens in community-acquired lung infections, but they are often responsible for secondary lung infections in patients with severe underlying diseases²⁾⁽¹²⁾⁽¹³⁾.

In the animals infected with *E. coli* alone, pneumonia was more notable than pleural lesions. On the other hand, in most animals infected with *B. fragilis* and *B. distasonis*, marked cell infiltration and tissue damage were noted in the visceral pleura, although the pneumonia was milder. Abscess formation was noted in about one-third of the animals inoculated with *B. fragilis*, and marked inflammation was observed around the abscesses and peripheral lung regions. Thus, the main lung lesions induced by *E. coli* and *B. fragilis* are considered to be different. One possible reason for this difference may be that peripheral lung regions, in which aeration is reduced by inflammatory effusion and secretion, is advantageous for anaerobic bacteria. In the animals infected with a single bacterial species, no bacteria were recovered from the lungs 7 days after inoculation despite findings of pneumonia, abscess formation, and pleuritis. Thereby, lung inflammation is considered to be established early after inoculation, and bacteria to have been cleared by alveolar macrophages, airway cilia, and other physiologic protective mechanisms of the lung. Out of the three *Bacteroides* species, *B. fragilis* was the most virulent. The inflammatory findings in the mixed infection groups were more severe than those in the single infection ones.

In the group infected with *B. fragilis* at 10^9 cfu and *E. coli* at 10^7 cfu, inflammatory findings were markedly stronger than those in other mixed infection groups, deaths were observed within the first 4 days after infection, and *E. coli* was recovered from the lungs, but *B. fragilis* was never detected alone. The Eh values in the lung lesions were mostly 100 mV or less in animals of the mixed infection group in which the inflammation score was 2 or above, suggesting that the foci of inflammation were in the anaerobic environment advantageous for *B. fragilis*. In the antibiotic chemotherapy study, neither bacterial species was recovered and inflammation was the mildest in the animals treated with AZT + CLDM. In those treated with AZT, *E. coli* was not recovered, but *B. fragilis*, which is resistant to the drug, was recovered, and lung lesions were accompanied by abscesses and pleuritis, which were considered to have been primarily caused by *B. fragilis*. In animals given CLDM, *E. coli* and *B. fragilis* persisted in some, and lung lesions were the most severe, suggesting that *E. coli* might synergistically act with *B. fragilis*. Synergism between anaerobic and aerobic bacteria was shown by the intra-abdominal infection study of Weinstein et al⁶⁾. They explained the process of the mixed infection as a biphasic infection⁴⁾⁽¹⁴⁾⁽¹⁵⁾. In the present study, *E. coli* was primarily responsible for pneumonia, and abscesses and pleuritis were notable among lung lesions induced by *B. fragilis*. However, it was uncertain whether this clearly demonstrated the biphasic theory. Further studies are needed concerning this phenomenon. The phagocytic activity of human neutrophils against *E. coli* was significantly reduced by the addition of the culture filtrate of *B. fragilis* (the pH of this culture medium was approximately 5.2–5.4). Rotstein et al.¹⁶⁾ reported that neutrophil phagocytosis and superoxide production are markedly suppressed by the metabolism of anaerobic bacteria. Since *B. fragilis* was less susceptible to neutrophil phagocytosis than *E. coli*, mixed infections caused by the two organisms are considered to enhance the pathogenicity of *B. fragilis* as well as of *E. coli* by facilitating the survival of *B. fragilis* at the foci of inflammation in the lung and reducing neutrophil phagocytosis of *E. coli*. This study suggests that *E. coli* and *B. fragilis* interact synergistically in the lung as well as in intra-abdominal infection, with anaerobic bacteria enhancing the pathogenicity of aerobic bacteria.

Acknowledgments

I would like to thank Miss Masumi Ikuta and Miss Hatsumi Kuroki for their technical assistance, and doctors of the respiratory study group, Second Department of Internal Medicine, Oita Medical University, for their cooperation.

References

- 1) Finegold, S.M.: General aspects of anaerobic infection. *In* Anaerobic infections in humans. (Finegold, S.M. and George, W.L. ed.) p. 137—153, Academic Press, San Diego, California, 1989.
- 2) Bartlett, J.G.: Anaerobic bacterial infections of the lung. *Chest* 91: 901—909, 1987.
- 3) Miyazaki, S., Ishii, T., Tsuji, A., Kitaya, S., Kaneko, Y., Ogawa, M. & Goto, S.: Pathogenic factors in mixed infection due to *Bacteroides fragilis* and other species of bacteria in mice. *Jap. J. Bacteriol.* 41: 611—617, 1986.
- 4) Gorbach, S.L.: Interactions between aerobic and anaerobic bacteria. *Scand. J. Infect. Dis.* 31 (Suppl.): 61—67, 1982.
- 5) Onderdonk, A.B., Cisneros, R.L., Finberg, R.W., Crabb, J.H. & Kasper, D.L.: Animal model system for studying virulence of and host response to *Bacteroides fragilis*. *Rev. Infect. Dis.* 12: s169—177, 1990.
- 6) Weinstein, W.M., Onderdonk, A.B., Bartlett, J.G. & Gorbach, S.L.: Experimental intra-abdominal abscesses in rats: Development of an experimental model. *Infect. Immun.* 10: 1250—1255, 1974.
- 7) Maskell, J.P.: The pathogenicity of *Bacteroides fragilis* and related species estimated by intracutaneous infection in the guinea pig. *J. Med. Microbiol.* 14: 131—140, 1981.
- 8) Nasu, M., Goto, J., Goto, Y., Tashiro, T. & Akashi, M.: An evaluation of the pathogenicity of *Bacteroides fragilis* in the intracutaneous infection of the guinea pig. *J.J.A. Inf. D.* 58: 512—517, 1984.
- 9) Kannangara, D.W., Thadopacci, H., Bach, V.T. & Webb, D.: Animal model for anaerobic lung abscess. *Infect. Immun.* 31: 592—597, 1981.
- 10) Edberg, S.C.: The measurement of antibiotics in human body fluids: Techniques and significance. *In* Antibiotics in laboratory medicine 2nd ed. (Lorian, V. ed.) p. 381—476, Williams & Wilkins, Baltimore, MD, 1986.
- 11) Hasui, M., Hirabayashi, Y. & Kobayashi, Y.: Simultaneous measurement by flow cytometry of phagocytosis and hydrogen peroxide production of neutrophils in whole blood. *J. Immunol. Methods* 117: 53—58, 1989.
- 12) Bartlett, J.G.: Respiratory tract and other thoracic infections. *In* Anaerobic infection in humans. (Finegold, S.M. and George, W.L. ed.) p. 311—331, Academic Press, San Diego, California, 1989.
- 13) Finegold, S.M.: Aspiration pneumonia, lung abscess, and empyema. *In* Respiratory infections: Diagnosis and management 2nd ed. (Pennington, J.E. ed.) p. 264—275, Raven Press, New York, 1989.
- 14) Onderdonk, A.B., Weinstein, W.M., Sullivan, N.M., Bartlett, J.G. & Gorbach, S.L.: Experimental intra-abdominal abscesses in rats: Quantitative bacteriology of infected animals. *Infect. Immun.* 10: 1257—1259, 1974.
- 15) Onderdonk, A.B., Bartlett, J.G., Oouie, T., Sullivan-Seigler, N. & Gorbach, S.L.: Microbial synergy in experimental infection-abdominal abscess. *Infect. Immun.* 13: 22—26, 1976.
- 16) Rotstein, O.D., Vittorini, T., Kao, J., McBurnei, M.I., Nasmith, P.E. & Grinstein, S.: A Soluble *Bacteroides* by product impair phagocytic killing of *Escherichia coli* by neutrophils. *Infect. Immun.* 57: 745—753, 1989.

嫌気性菌性肺炎の成立における好気性菌と嫌気性菌の相互作用

大分医科大学第2内科

後 藤 純

現住所：国立大分病院呼吸器科

(平成4年7月22日受付)

(平成4年10月20日受理)

要 旨

嫌気性菌性肺炎の成立における嫌気性菌と好気性菌との相互作用を研究する目的で、*Bacteroides fragilis* グループの3菌種 (*B. fragilis* N-17, *B. distasonis* N-1, *B. thetaiotaomicron* N-79) と喀痰から分離された *Escherichia coli* MCO 85036 の単独菌および混合菌液をモルモット気管内注入法により接種し、肺病変を観察した。*B. fragilis* グループのうち、*B. fragilis* が最も強い肺病変を惹起させ、その病変部位は *E. coli* のそれとは異なり胸膜近くにあり、肺炎、膿瘍、胸膜炎がみられた。*B. fragilis* 10^9 cfu と *E. coli* 10^7 cfu の混合感染は、単独菌感染よりも強い肺病変を示し、炎症所見の酸化還元電位は低下した (max: -330mV)。

混合感染による肺病変を aztreonam と clindamycin 投与による解析をしたところ、肺病変は *E. coli* によるものが主体であり、*B. fragilis* は *E. coli* との共存によって炎症所見に残存し易くなり、炎症を惹起していることが推察された。ヒト好中球による *E. coli* 貪食率は、*B. fragilis* 培養濾液の添加により有意に低くなり ($p < 0.002$)、*B. fragilis* 貪食率は、*E. coli* 培養濾液の添加にて影響を受けなかった。すなわち、*B. fragilis* による肺病変は *E. coli* との共存下でより強い炎症像を示し、*B. fragilis* は *E. coli* による肺病変を協力的に助長させ、主に膿瘍と胸膜炎を形成していることが示唆された。