responses by oseltamivir treatment in mice infected with influenza A virus. Microbes Infect. 12:778–783.
40. **Tsuji M, et al.** 2008. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. Immunity 29:261–271.
41. **Tumpey TM, Renshaw M, Clements JD, Katz JM.** 2001. Mucosal delivery of inactivated influenza vaccine induces B-cell-dependent heterosubtypic cross-protection against lethal influenza A H5N1 virus infection. J. Virol. 75:5141–5150.
42. **Uematsu S, et al.** 2008. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat. Immunol. 9:769–776.
43. **Yamada T, et al.** 2010. Poly(I:C) induces BLyS-expression of airway fibroblasts through phosphatidylinositol 3-kinase. Cytokine 50:163–169.
44. **Yamada T, Zhang K, Yamada A, Zhu D, Saxon A.** 2005. B lymphocyte stimulator activates p38 mitogen-activated protein kinase in human Ig class switch recombination. Am. J. Respir. Cell Mol. Biol. 32:388–394.
45. **Yamaya M, et al.** 2010. Clarithromycin inhibits type A seasonal influenza virus infection in human airway epithelial cells. J. Pharmacol. Exp. Ther. 333:81–90.

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ORIGINAL ARTICLE

Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) severe respiratory failure in Japan

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Abstract
Purpose To evaluate procedures and outcomes of extracorporeal membrane oxygenation (ECMO) therapy applied to 2009 influenza A(H1N1) severe respiratory failure patients in Japan.
Methods This observational study used database information about adults who received ECMO therapy for H1N1-related severe respiratory failure from April 1, 2010 to March 31, 2011.
Results Fourteen patients from 12 facilities were enrolled. Anti-influenza drugs were used in all cases. Before the start of ECMO, the lowest PaO_2/FiO_2 was median (interquartile) of 50 (40–55) mmHg, the highest peak inspiratory pressure was 30 (29–35) cmH_2O, and mechanical ventilation had

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been applied for at least 7 days in 5 patients. None of the facilities had extensive experience with ECMO for respiratory failure (6 facilities, no previous experience; 5 facilities, one or two cases annually). The blood drainage cannula was smaller than 20 Fr. in 10 patients (71.4 %). The duration of ECMO was 8.5 (4.0–10.8) days. The duration of each circuit was only 4.0 (3.2–5.3) days, and the ECMO circuit had to be renewed 19 times (10 cases). Thirteen patients (92.9 %) developed adverse events associated with ECMO, such as oxygenator failure, massive bleeding, and disseminated intravascular coagulation. The survival rate was 35.7 % (5 patients).

Conclusion ECMO therapy for H1N1-related severe respiratory failure in Japan has very poor outcomes, and most patients developed adverse events. However, this result does not refute the effectiveness of ECMO. One possible cause of these poor outcomes is the lack of satisfactory equipment, therapeutic guidelines, and systems for patient transfer to central facilities.

Keywords ECMO · Influenza · Respiratory failure · Mortality

Introduction

The World Health Organization reported individuals infected with a novel swine-origin influenza virus 2009 influenza A(H1N1) in Mexico and the United States in April 2009 [ ]. This report was quickly followed by a worldwide pandemic. The severity of infection was the same as that of seasonal influenza in many cases, but more than a few patients developed severe respiratory failure of a kind that was unlikely to have resulted from conventional seasonal influenza.

Serious cases in which oxygenation could not be maintained by conventional mechanical ventilation were managed with extracorporeal membrane oxygenation (ECMO), often yielding excellent outcomes. According to reports from Australia and New Zealand, 68 patients received ECMO therapy during the 2-month period at the height of the epidemic, and the survival rate exceeded 70 % [ ]. The Extracorporeal Life Support Organization (ELSO) reported a survival rate of more than 60 % for 323 patients [ ]. According to a report from the United Kingdom, treatment outcomes in very severe cases were better when ECMO was applied than when only conventional mechanical ventilation was employed [ ]. Utilization of the ECMO network system and transfer of patients to the ECMO center were considered to be among the factors that resulted in better treatment outcomes [–]. The ECMO Center Karolinska, Sweden, reported a survival rate of more than 90 % [ ].

In Japan, however, no network system or center for ECMO therapy is available, and ECMO has been applied only at individual medical facilities in cases where this therapy was indicated. No data are as yet available in Japan regarding the outcomes of ECMO therapy for 2009 influenza A(H1N1). The present study was undertaken to analyze the procedures and outcomes of ECMO therapy applied to adult patients, using information on patients infected with H1N1 and admitted to intensive care units (ICUs). These data were collected by the Committee of Crisis Control, the Japanese Society of Respiratory Care Medicine and the Committee of Pandemic H1N1 Surveillance, the Japanese Society of Intensive Care Medicine.

Methods

The study involved adults who received ECMO therapy for severe respiratory failure associated with H1N1 influenza from April 1, 2010 to March 31, 2011. A database was created using patient information that had been collected from attending physicians of the facilities participating in this study; the information was provided at the physicians' own discretion in response to a public notification (data collection on ICU patients infected with H1N1) issued by the Japanese Society of Respiratory Care Medicine and the Japanese Society of Intensive Care Medicine. Informed consent from individual patients was obtained by each reporting physician and facility. Data collection pertaining to the findings before hospitalization and upon admission included age, sex, body weight, body mass index (BMI), body temperature, Acute Physiology and Chronic Health Evaluation (APACHE) II score, underlying disease, and vaccination. During treatment, information was collected about complications, Sequential Organ Failure Assessment (SOFA) score, type of anti-influenza drug, mechanical ventilation, blood gas analysis, and continuous renal replacement therapy. In addition, data were collected about the duration of mechanical ventilation, duration of ICU stay, hospitalization period, and patient outcome.

From this database, adult patients who had received ECMO therapy were extracted for analysis. The physician in charge at each facility that provided the ECMO therapy was requested by e-mail or telephone to supply additional detailed information with regard to the following: the equipment used for ECMO therapy, cannula size, site of approach with the cannula, duration of ECMO therapy, duration of mechanical ventilation before the start of therapy, adverse events, cause of death, and previous ECMO experience.

With respect to the ECMO therapy procedures, detailed information was collected on each survey item. The

survival and non-survival groups were compared using the Mann–Whitney test, Fisher's exact test, or chi-square test. Statistical analyses were performed using SPSS II (Abacus Concepts, Berkeley, CA, USA). All values are reported as median (interquartile), and all p values <0.05 are considered statistically significant.

Results

Patient background and treatment course (Table)

Fourteen patients from 12 facilities were enrolled in this study. The survival rate was as low as 35.7 % (5 patients). Weaning from ECMO was impossible in all the patients who later died.

Most patients were male (85.7 %). The mortality rate predicted from the APACHE II score was 24.9 %, but the actual mortality rate (64.3 %) was 2.5 times higher. None of the patients had chronic respiratory failure, chronic heart failure, or immunological diseases as underlying disorders. Anti-influenza drugs were used in all cases: peramivir in 78.6 %, oseltamivir in 42.9 %, and zanamivir in 7.1 %; two drugs were used in each of four cases.

All patients received mechanical ventilation with endotracheal intubation. Airway pressure release ventilation (APRV) was used for mechanical ventilation in 92.9 % of cases. The causes of death were multiple organ failure (MOF) in four cases, respiratory failure in three cases, MOF and uncontrollable bleeding in one case, and concomitant respiratory and circulatory failures in one case. One of the discharged patients had respiratory sequelae.

ECMO equipment and cannula (Tables ,)

ECMO equipment manufactured by Terumo Corporation (Tokyo, Japan) was used in 11 patients (78.6 %). This equipment consists of a console, circuit, oxygenator, and centrifugal pump. The blood drainage cannula was smaller than 20 Fr. in 10 patients (71.4 %), and the maximum size was 21.5 Fr.

ECMO therapy (Table)

All patients received venovenous ECMO therapy, and none required a switch to venoarterial ECMO therapy. None of the facilities had extensive experience with ECMO therapy for respiratory failure. At five facilities, ECMO was used for the first time. Six facilities had previously applied this therapy to one or two cases per year. One facility had used ECMO in at least five cases a year.

Before the start of ECMO therapy, mechanical ventilation had been applied for more than 7 days in two cases from the survival group and three cases from the non-survival group (13, 15, and 20 days, respectivley). The duration of ECMO therapy was 8.5 (4.0–10.8) days, ranging from 1 day (outcome, death) to 39 days (outcome, death).

The duration of each ECMO circuit was only 4.0 (3.2–5.3) days. The ECMO circuit was renewed a total of 19 times among 10 cases. The reasons for renewal were reduced oxygenating capability owing to oxygenator failure (nine times), thrombus attachment to the oxygenator (three times), circuit obstruction with thrombus (three times), poor blood drainage flow (twice), pump head trouble (once), and hemolysis (once). The duration of ECMO therapy for the four cases that did not require circuit renewal was 6 days (outcome, survival), 4 days (death), 4 days (death), and 1 day (death).

Adverse events associated with ECMO therapy (Table)

Excluding 1 patient who died on the first day of ECMO therapy, all patients developed adverse events associated with ECMO (92.9 %). Direct adverse events developed in 11 patients (78.6 %); reduced oxygenating capability owing to oxygenator failure (50 %) was the most frequent. Indirect adverse events developed in 12 patients (85.7 %). The most frequent complication was disseminated intravascular coagulation (DIC, 71.4 %). All these adverse events were associated with the coagulation and fibrinolytic system (DIC, massive bleeding, thrombus, etc.). One patient underwent a surgical procedure to achieve hemostasis.

Discussion

During the 2010–2011 season in Japan, the survival rate of patients with 2009 influenza A(H1N1) severe respiratory failure following ECMO therapy was only 35.7 %. Most patients developed adverse events associated with this therapy.

Several reports have demonstrated the effectiveness of ECMO therapy for H1N1-related severe respiratory failure [, ,]. According to a report from the United Kingdom, the survival rate following ECMO therapy was 76 %, which is significantly higher than that following conventional mechanical ventilation (48 %), and thus demonstrates the effectiveness of ECMO [ ]. In the present study in Japan, the survival rate was only 35.7 %, and the mortality rate was 2.5 times that predicted from the APACHE II score. The survival rate was 64 % in H1N1-related

Table 1 Patient background and treatment course

	All cases (14 patients)	Survival group (5 patients)	Non-survival group (9 patients)
Age (years)	54 (43–60)	54 (35–58)	54 (41–62)
Sex (male/female)	12/2	4/1	8/1
Weight (kg)	70 (64–80)	69 (54–86)	70 (64–80)
Obesity			
35 > BMI ≥ 25	7	3	4
BMI ≥ 35	1	0	1
Body temperature (°C)			
At first examination	38.8 (37.1–39.1)	38.8 (36.8–39.0)	38.8 (37.3–39.4)
Maximum	39.4 (38.7–39.8)	39.2 (39.0–39.7)	39.5 (38.1–39.9)
APACHE II score	17 (12–25)	16 (12–24)	17 (12–28)
Predicted death rate (%)	24.9 (14.6–54.1)	23.5 (15.5–49.7)	26.2 (14.6–61.5)
Maximum SOFA score	15.5 (12.0–19.3)	12.0 (10.0–15.0)	19.0 (14.5–20.5)*
Underlying condition			
Drug abuse	1	0	1
Pregnancy	1	1	0
Vaccination (H1N1 + seasonal)	1	0	1
Complications			
Acute renal failure	7	2	5
Acute hepatic failure	3	0	3
Culture-confirmed infection	4	2	2
Shock	4	1	3
Medical treatment			
Peramivir	11	4	7
Oseltamivir	6	2	4
Zanamivir	1	0	1
Antibiotics	14	5	9
Steroid			
High + low dose	6	3	3
High dose	3	0	3
Low dose	2	2	0
Sivelestat	4	1	3
Vasoactive drugs	13	4	9
Rescue therapies and adjunctive therapies			
Prone position	3	1	2
APRV	13	5	8
Nitric oxide	1	0	1
CRRT	7	2	5
Respiratory severity and ventilator parameters			
Lowest PaO_2/FiO_2 (mmHg)	50 (40–55)	49 (43–53)	50 (40–60)
Highest PEEP (cmH_2O)	24 (17–30)	22 (15–28)	28 (18–30)
Highest PIP (cmH_2O)	30 (29–35)	29 (23–42)	30 (30–35)
Ventilator days (days)	19 (9–25)	24 (16–37)	10 (6–25)
Length of stay in ICU (days)	17 (9–26)	24 (16–31)	10 (6–25)
Hospitalization (days)	25 (12–53)	69 (35–83)	15 (6–25)**

Data expression, median (interquartile)

BMI body mass index, *APACHE* Acute Physiology and Chronic Health Evaluation, *SOFA* Sequential Organ Failure Assessment, *DIC* disseminated intravascular coagulation, *APRV* airway pressure release ventilation, *CRRT* continuous renal replacement therapy, *PEEP* positive end-expiratory pressure, *PIP* peak inspiratory pressure, *ICU* intensive care unit

* $p = 0.004$ (survival group vs. non-survival group); ** $p = 0.036$ (survival group vs. non-survival group)

Table 2 Extracorporeal membrane oxygenation (ECMO) equipment used

Equipment	Number of cases
Console	
CAPIOX SP-101	11
Bio-Console 560	1
Stöckert SCP system	1
MERA HAP-31	1
Circuit	
CAPIOX Custom Pack	11
Unknown	3
Oxygenator	
LX or SX	9
BIOCUBE 6000	4
MERA HP Exelungprime	1
Centrifugal pump	
CX-SP45	11
COBE revolution	1
Unknown	2

CAPIOX SP-101, CAPIOX Custom Pack, LX, SX, CX-SP45 (Terumo, Tokyo, Japan), Bio-Console 560 (Medtronic, Minneapolis, MN, USA), Stöckert SCP system (SORIN Group, Germany), MERA HAP-31, MERA HP Exelungprime (Senko Medical Instrument, Tokyo, Japan), BIOCUBE 6000 (NIPRO, Osaka, Japan), COBE revolution (SORIN Group, Italy)

Table 3 Cannula size, approach site, and proximal position for ECMO

Drainage	Number of cases
Size (Fr.)	
18	6
19.5	4
21	3
21.5	1
Approach site	
Femoral vein	14
Proximal position	
Inferior vena cava	10
Right atrium	4
Return	**Number of cases**
Size (Fr.)	
12	1
15	9
16	2
16.5	1
21	1
Approach site	
Right jugular vein	12
Femoral vein	2
Proximal position	
Superior vena cava	8
Right atrium	4
Inferior vena cava	2

severe respiratory failure treated with mechanical ventilation without ECMO for season of 2010–2011 in Japan [8, 9].

In addition, underlying diseases were present in only two patients in this study, and none of the patients had complications involving respiratory, cardiac, or immunological disorders. Anti-influenza drugs had been used in all cases. In particular, during this season, peramivir, a drug for intravenous administration, was newly available on the market and had been used in 11 patients (78.6 %). This drug was used as a more reliable means of treatment than oseltamivir because it is less likely to cause poor absorption via the digestive tract from such problems as vomiting. This finding suggests that the lives of many of these patients could have been saved if appropriate management with ECMO had been applied.

The blood drainage cannula size is considered an important factor for maintaining appropriate flow during ECMO therapy [10]. According to a report from the ECMO Center Karolinska, blood drainage cannulas with sizes between 23 and 29 Fr. were used for patients with a median body weight of 88 kg [5]. In the present study, the drainage cannula size was <20 Fr. in 70 % of the patients, who had a median body weight of 70 kg (range, 51–90 kg) and height of 146–190 cm (estimated from body weight and BMI). According to the previous reports, the achieved ECMO blood flow rates are generally 4–5 l/min [2, 5, 6]. We did not have any ECMO blood flow data in this study, but the cannulas used for these Japanese patients appear to have been too small in diameter. The use of a blood drainage cannula with too small a diameter is more likely to cause adverse events such as inadequate flow (from poor blood drainage flow), hemolysis (due to the need for a sufficiently high pump rotation rate to achieve satisfactory flow), and a hemorrhagic tendency (caused by platelet consumption).

Recently, adverse events arising from ECMO therapy have been clearly decreasing thanks to advances in component technology and techniques [11]. However, in the present study, adverse events associated with ECMO therapy developed in all patients, except for one who died on the first day of this therapy, and the incidence of adverse events was remarkably high compared with that in previous reports [2, 4–7, 11–14]. Among other adverse events, such disorders of the coagulation and fibrinolytic system as massive bleeding, DIC, and thrombus formation, which are complications that require close attention during ECMO therapy [15], developed in most patients. Problems with the equipment and the excessively small diameter of the

Table 4 ECMO therapy

	All cases (14 patients)	Survival group (5 patients)	Non-survival group (9 patients)
Ventilator days before ECMO (days)	5.0 (0.8–8.5)	3.0 (0.5–7.0)	6.0 (0.5–14.0)
Length of ECMO therapy (days)	8.5 (4.0–10.8)	9.0 (6.5–12.5)	8.0 (3.5–11.5)
Number of circuits used	2.0 (1.0–3.0)	2.0 (1.5–2.5)	2.0 (1.0–3.5)
Duration of each circuit (days)	4.0 (3.2–5.3)	5.0 (3.3–6.8)	4.0 (2.1–4.3)

Table 5 Adverse events related to ECMO therapy

Event	Number of cases (%)
Directly related to the ECMO circuit	11 (78.6)
Oxygenator failure	7 (50.0)
Blood clots	4 (28.6)
Oxygenator	3 (21.4)
Other circuit	1 (7.1)
Cannula-related problems	3 (21.4)
Pump head complications	1 (7.1)
Indirectly related to the ECMO circuit	12 (85.7)
Massive bleeding	8 (57.1)
Surgical site bleeding	4 (28.6)
Upper digestive tract hemorrhage	4 (28.6)
Cannulation site bleeding	2 (14.3)
Pulmonary hemorrhage	1 (7.1)
Hemolysis	2 (14.3)
Disseminated intravascular coagulation	10 (71.4)
Venous thrombus	2 (14.3)

cannulas were probably involved in the development of many of the adverse events associated with ECMO therapy in this study. This view is supported by the observation that the duration of each circuit was only 4 days. The life of the oxygenator was extremely short, and this was a major factor in necessitating circuit renewal only 4 days after the start of use. The recommended period of use is only 6 h for the most frequently employed ECMO circuit and oxygenator in Japan, the CAPIOX Custom Pack (Terumo, Tokyo, Japan), according to its package insert (written in Japanese). The cavity of the circuit used in the present study usually had a volume of 500–600 ml. Every time the circuit was renewed, the same volume of blood was lost, and blood transfusion or intravenous fluid infusion was carried out to compensate for the discarded blood. This procedure is a major source of stress for patients. It would appear to be necessary to review the ECMO equipment used in Japan.

Factors that possibly raised the mortality rate following ECMO therapy include central nervous system injury, gastrointestinal or pulmonary hemorrhage, and renal dysfunction [16]. We found, however, no particular differences in any of these factors between the survival and non-survival groups. The maximum SOFA score during treatment was higher in the non-survival group, which reflects the tendency for a more severe disease course in the non-survival group. The only difference between the two groups is that the non-survival group included some patients who were given mechanical ventilation for a period much longer than 7 days before beginning ECMO. When started within 6 days after initiating mechanical ventilation, ECMO therapy offers a high survival rate [3, 6, 10, 11, 17–20]. It is also possible that initiation of ECMO was delayed because Japanese physicians are unfamiliar with this therapy. In addition, it seems that Japanese physicians had not understood or implemented such routine therapeutic strategies as the ELSO guidelines. Instead, a specific form of ventilation that maintained a high average airway pressure, such as APRV, was employed in many cases. Although the setting for mechanical ventilation during ECMO therapy was not sufficiently clear from the data, it appears likely that a high-pressure setting for mechanical ventilation was adopted even during ECMO therapy, and this may be one of the factors responsible for the high mortality rate. It is necessary for physicians to develop a proper understanding of the ECMO treatment strategy.

The survival rate of adults with severe respiratory failure following ECMO therapy is reported to be usually 61 % [15]. It has also been reported that when ECMO therapy is applied to patients with severe respiratory failure, transfer to a central facility, such as an ECMO center, is likely to yield better outcomes [4–6, 11, 12, 14]. During the 2009–2010 season, ECMO therapy was applied to 16 patients with H1N1-related severe respiratory failure in Sweden; 13 (81 %) of these patients were transferred to the ECMO Center Karolinska, and the result was successful weaning from ECMO in all cases [5]. In Italy, establishment of the ECMO network resulted in a high survival rate [6]. Both the effectiveness of ECMO therapy for H1N1-related severe respiratory failure and treating many cases at the central facility were reportedly major factors contributing to the high survival rate [4]. The facilities in Japan have very little experience with ECMO therapy for patients with severe respiratory failure. At most Japanese facilities in the present study, ECMO for severe respiratory failure had been applied to only one or two respiratory failure cases a year or even less frequently; about half of the facilities had no previous experience with this therapy. H1N1-related severe respiratory failure has a high probability of recovery in response to ECMO. Thus, adopting

ECMO therapy should be given due consideration. However, because the number of patients with this condition is not particularly large, transferring patients to central facilities for this therapy is anticipated to improve treatment outcomes because the physicians at such centers can gain experience through dealing with a larger number of cases. ECMO may also be indicated for H5N1 (avian influenza), an outbreak of which is now a great concern. A recent report has shown that ECMO should be performed at centers with high case volumes, established protocols, and clinicians who are experienced in its use [11]. Facilities serving as centers for this therapy should be established in Japan as soon as possible.

The present study has a limitation in that the survey did not cover all patients who received ECMO therapy. According to a report by the Ministry of Health, Labour and Welfare of Japan, there were 15 deaths among the adults who were given ECMO therapy (the number of survivors has not been made public) [21]. In the present study, 9 of the patients died, which would suggest that more than half of all Japanese patients who received ECMO therapy were covered by this survey.

The survival rate for patients with H1N1-related severe respiratory failure following ECMO therapy in the present study was very low. However, this result does not refute the effectiveness of ECMO therapy for H1N1-related severe respiratory failure; the result is instead attributable to the lack of experience and lack of preparedness of Japanese facilities to provide ECMO therapy. To improve the outcomes of ECMO therapy not only in Japan but also in other countries inexperienced with ECMO therapy, efforts should be made along the following lines: (1) supply ECMO equipment suitable for treatment of severe respiratory failure; (2) promote a full understanding of the ECMO treatment strategy by physicians and other medical staff; and (3) transfer patients to central facilities established for this therapy.

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Conflict of interest All authors have no conflict of interest to disclose.

References

1. World Health Organization. Influenza-like illness in the United States and Mexico. http://www.who.int/csr/don/2009_04_24/en/index.html. Accessed 11 Nov 2011.
2. The Australia and New Zealand Extracorporeal Membrane Oxygenation (ANZ ECMO) Influenza Investigators. Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) acute respiratory distress syndrome. JAMA 2009;302:1888–95.
3. Extracorporeal Life Support Organization (2011) H1N1 ECMO Registry. http://www.elso.med.umich.edu/H1N1Registry.html. Accessed 11 Nov 2011.
4. Noah MA, Peek GJ, Finney SJ, Griffiths MJ, Harrison DA, Grieve R, Sadique MZ, Sekhon JS, McAuley DF, Firmin RK, Harvey C, Cordingley JJ, Price S, Vuylsteke A, Jenkins DP, Noble DW, Bloomfield R, Walsh TS, Perkins GD, Menon D, Taylor BL, Rowan KM. Referral to an extracorporeal membrane oxygenation center and mortality among patients with severe 2009 influenza A(H1N1). JAMA. 2011;306:1659–68.
5. Holzgraefe B, Broomé M, Kalzén H, Konrad D, Palmér K, Frenckner B. Extracorporeal membrane oxygenation for pandemic H1N1 2009 respiratory failure. Minerva Anestesiol. 2010;76:1043–51.
6. Patroniti N, Zangrillo A, Pappalardo F, Peris A, Cianchi G, Braschi A, Iotti GA, Arcadipane A, Panarello G, Ranieri VM, Terragni P, Antonelli M, Gattinoni L, Oleari F, Pesenti A. The Italian ECMO network experience during the 2009 influenza A(H1N1) pandemic: preparation for severe respiratory emergency outbreaks. Intensive Care Med. 2011;37:1447–57.
7. Roch A, Lepaul-Ercole R, Grisoli D, Bessereau J, Brissy O, Castanier M, Dizier S, Forel JM, Guervilly C, Gariboldi V, Collart F, Michelet P, Perrin G, Charrel R, Papazian L. Extracorporeal membrane oxygenation for severe influenza A (H1N1) acute respiratory distress syndrome: a prospective observational comparative study. Intensive Care Med. 2010;36:1899–905.
8. Committee of Crisis Control Japan Society of Respiratory Care Medicine, Committee of Neonatal and Pediatric Intensive Care The Japanese Society of Intensive Care Medicine, Committee of 2010 Pandemic H1N1 Surveillance The Japanese Society of Intensive Care Medicine. Database analysis of critically ill patients admitted to ICU with 2010 influenza. Nihon Shuuchuutiryouigakukai Zasshi (J Jpn Soc Intensive Care Med). 2012;19:107–11 (in Japanese).
9. Committee of Crisis Control Japan Society of Respiratory Care Medicine, Committee of Neonatal and Pediatric Intensive Care The Japanese Society of Intensive Care Medicine, Committee of 2010 Pandemic H1N1 Surveillance The Japanese Society of Intensive Care Medicine. Database analysis of critically ill patients admitted to ICU with 2010 influenza. Jinkou Kokyuu (Jpn J Respir Care). 2011;28:203–6 (in Japanese).
10. Extracorporeal Life Support Organization (2009) ELSO Guidelines. http://www.elso.med.umich.edu/Guidelines.html. Accessed 21 Nov 2011.
11. Brodie D, Bacchetta M. Extracorporeal membrane oxygenation for ARDS in adults. N Engl J Med. 2011;365:1905–14.
12. Linden V, Palmér K, Reinhard J, Westman R, Ehrén H, Granholm T, Frenckner B. High survival in adult patients with acute respiratory distress syndrome treated by extracorporeal membrane oxygenation, minimal sedation, and pressure supported ventilation. Intensive Care Med. 2000;26:1630–7.
13. Freed D, Henzler D, White CW, Fowler R, Zarychanski R, Hutchison J, Arora RC, Manji RA, Legare JF, Drews T, Veroukis S, Kesselman M, Guerguerian AM, Kumar A, The Canadian Critical Care Trials Group. Extracorporeal lung support for patients who had severe respiratory failure secondary to influenza A (H1N1) 2009 infection in Canada. Can J Anesth. 2010;57:240–7.
14. Peek GJ, Mugford M, Tiruvoipati R, Wilson A, Allen E, Thalanany MM, Hibbert CL, Truesdale A, Clemens F, Cooper N, Firmin RK, Elbourne D, CESAR Trial Collaboration. Efficacy and economic assessment of conventional ventilatory support

versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomized controlled trial. Lancet. 2009;374:1351–63.
15. Bartlett RH, Gattinoni L. Current status of extracorporeal life support (ECMO) for cardiopulmonary failure. Minerva Anestesiol. 2010;76:534–40.
16. Brogan TV, Thiagarajan RR, Rycus PT, Bartlett RH, Bratton SL. Extracorporeal membrane oxygenation in adults with severe respiratory failure: a multi-center database. Intensive Care Med. 2009;35:2105–14.
17. Beiderlinden M, Eikermann M, Boes T, Breitfeld C, Peters J. Treatment of severe acute respiratory distress syndrome: role of extracorporeal gas exchange. Intensive Care Med. 2006;32: 1627–31.
18. Mols G, Loop T, Geiger K, Farthmann E, Benzing A. Extracorporeal membrane oxygenation: a ten-year experience. Am J Surg. 2000;180:144–54.
19. Lewandowski K, Rossaint R, Pappert D, Gerlach H, Slama KJ, Weidemann H, Frey DJM, Hoffmann O, Keske U, Falke KJ. High survival rate in 122 ARDS patients managed according to a clinical algorithm including extracorporeal membrane oxygenation. Intensive Care Med. 1997;23:819–35.
20. Pranikoff T, Hirschl RB, Steimle CN, Anderson HL III, Bartlett RH. Mortality is directly related to the duration of mechanical ventilation before the initiation of extracorporeal life support for severe respiratory failure. Crit Care Med. 1997; 25:28–32.
21. Ministry of Health, Labour and Welfare of Japan. Influenza reports released April 1, 2011. http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou01/rinshou.html. Accessed 11 Nov 2011.

●原 著

肺膿瘍・膿胸7例における歯周病細菌PCR検査の臨床的意義の検討

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要旨：肺膿瘍，膿胸の発症には歯周病が関与し，起炎菌は *Streptococcus anginosus* group や偏性嫌気性菌などが知られている．今回我々は，肺膿瘍，膿胸の病巣からの検体で歯周病細菌PCR検査を施行した7例（肺膿瘍3例，膿胸4例）を経験した．1例のみで気管支洗浄液から嫌気性菌が分離されたが，経気管支生検や胸水からの検体の培養検査では嫌気性菌は培養されず，PCR検査では6例に歯周病細菌が検出された（*Porphyromonas gingivalis* 3例，*Tannerella forsythensis* 1例，*Treponema denticola* 1例，*Prevotella intermedia* 1例）．うち1例ではまだ病原性が明らかでない *T. denticola* が検出された．歯周病細菌PCR検査は，培養診断が困難な嫌気性菌性呼吸器感染症の起炎菌の同定に有用な検査と考えられた．

キーワード：肺膿瘍，膿胸，歯周病細菌，PCR法，*Treponema denticola*
Lung abscess, Empyema, Periodontal bacteria, PCR assay, *Treponema denticola*

緒 言

近年，歯周病などの口腔内感染症と全身性疾患の関連が注目されているが，そのなかで誤嚥性肺炎，肺膿瘍，膿胸といった呼吸器感染症については比較的以前から関連性が指摘されており，特に嫌気性菌の関与が重要視されてきた．山下らは呼吸器感染症67例における嫌気性菌検出率は膿胸で80%，肺膿瘍で43%，誤嚥性肺炎で42%であったと報告している[1]．

一方，実際の臨床現場では，気道系検体における嫌気培養の困難さから起炎菌培養同定までに至らない症例が多く，口腔内嫌気性菌の関与は過小評価されている可能性が高いと考えられる．

最近，歯科領域において歯周病細菌の検出にreal-time PCRによる定量法が応用され，実地臨床でも用いられるようになってきた[2][3]．real-time PCR法は従来のPCR法と比し定量性に優れるため，コンタミネーションなどの問題も克服できると考えられる．

今回我々は，肺膿瘍と膿胸の病巣から採取した検体を用い歯周病細菌PCR法による感染症診断を試み，その有用性を確認できたので報告する．

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対象と方法

対象は2009年6月から2011年11月までに順天堂大学医学部附属浦安病院呼吸器内科に入院した肺膿瘍または膿胸の7例であり，肺膿瘍の症例では気管支鏡検査を行い，経気管支生検（transbronchial biopsy：TBB），気管支洗浄を施行し，膿胸の症例では経皮的に胸水を穿刺した．採取された検体において培養検査と歯周病細菌遺伝子検査を施行した．培養検査については，胸水は血液培養用の嫌気用レズンボトル（BBL）と滅菌スピッツに採取し，肺組織は嫌気ポーター（テルモ・クリニカルサプライ株式会社），気管支洗浄液は滅菌スピッツに入れて，速やかに細菌検査室に搬送した．提出検体はBTB乳糖寒天培地，血液寒天培地，チョコレート寒天培地（BBT），ブルセラHK寒天培地（極東製薬）を使用し分離した．BTB乳糖寒天培地と血液寒天培地は35℃・18時間好気培養，チョコレート寒天培地は35℃・18時間炭酸ガス培養，ブルセラHK寒天培地は嫌気バッグ法（三菱化学メディエンス）にて35℃・48時間（発育不良時96時間まで延長）培養した．嫌気性菌の同定は，微生物学検査マニュアルの臨床嫌気性菌'97[4]に準じてレベル1a・2aの方法で同定を行い必要に応じてRapid ANA II System（アムコ）を使用した．歯周病細菌遺伝子検査は生検した肺組織と胸水を用いて，株式会社ミロクメディカルラボラトリー（長野県佐久市）に依頼し，歯周病細菌遺伝子検査はTaqManプローブ法を使用したreal-time PCR法で定量的に目的菌遺伝子（*Aggregati-*

Table 1 Characteristics of the 7 cases

Case	Age/ gender	Diagnosis	History of periodontal disease	Culture of sputum	Culture of TBB, BLF, or effusion (source)	PCR analysis (source)	Copy number of the targeted pathogen (proportion of total bacterial load)	Treatment
1	69/F	lung abscess	+	normal flora	no growth (TBB and BLF)	*Treponema denticola* (TBB)	11,040 copies/8 mm^3 (10.3%)	CAM + CLDM (6 weeks)
2	55/M	Empyema	+	*Klebsiella pneumoniae*	*Streptococcus constellatus* (effusion)	*Porphyromonas gingivalis* (effusion)	40,800 copies/ml (20.52%)	CTRX + CLDM (4 weeks)
3	60/M	lung abscess	+	normal flora	no growth (TBB and BLF)	*Tannerella forsythensis* (TBB)	1,280 copies/8 mm^3 (42.1%)	ABPC (7 weeks)
4	54/M	Empyema	−	normal flora	no growth (effusion)	*Porphyromonas gingivalis* (effusion)	6,000 copies/ml (0.01%)	ABPC/SBT (4 weeks)
5	83/M	Empyema	−	methicillin sensitive *Staphylococcus aureus*	no growth (effusion)	*Porphyromonas gingivalis* (effusion)	5,600 copies/ml (0.09%)	ABPC + CLDM (4 weeks)
6	55/F	Empyema	+	normal flora	*Streptococcus intermedius* (effusion)	*negative* (effusion)	not detected (0.0%)	TAZ/PIPC (3 weeks)
7	68/M	lung abscess	−	normal flora	*Prevotella oralis* (BLF)	*Prevotella intermedia* (TBB)	1,000 copies/8 mm^3 (0.1%)	TAZ/PIPC (2 weeks) → ABPC/SBT (2 weeks)

BLF, bronchial lavage fluid; GNR, gram negative rod; CAM, clarithromycin; CTRX, ceftriaxone sodium hydrate; CLDM, clindamycin hydrochloride; ABPC: ampicillin; ABPC/SBT, ampicillin/sulbactam; TAZ/PIPC, tazobactam/piperacillin.

bacter actinomycetemcomitans, *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Prevotella intermedia*) の検出を行った．方法は目的菌の菌種に特異的な 16S rRNA をターゲットにしたプライマーで増幅を行い，TaqMan プローブにて検出し，既知量のコピー数の DNA 溶液をスタンダードとして定量的に測定した．総菌数は 16S rRNA 領域の共通配列で PCR 法による増幅を行い，同様に既知のコピー数の検量線から定量的に測定した．また検出菌の総菌数に対する相対的比率を補正はせず算出した．対象7例について，歯科疾患の既往，喀痰培養，肺組織と気管支洗浄液または胸水の培養，PCR 検査，治療内容を検討した．

成　績

症例一覧を Table 1 に示した．4 例に歯科疾患の既往を認めた．喀痰検査では陽性 2 例，陰性 5 例であり，嫌気性菌はいずれの症例でも分離されなかった．病巣部の培養では，症例 2 の胸水から *Streptococcus constellatus*，症例 6 の胸水から *S. intermedius*，症例 7 では気管支洗浄液のみから *Prevotella oralis* が検出されたが，他4例（症例 1，3 の気管支洗浄液および肺組織，症例 4，5 の胸水）は陰性であった．一方，病巣部の検体を用いた PCR 検査では 7 例中 6 例と，嫌気性歯周病細菌の検出において培養と比べ高率に陽性であり，明らかな歯科疾患の既往がない症例でも陽性となった．肺膿瘍 3 例の肺組織からはそれぞれ *T. denticola*（症例 1），*T. forsythensis*（症例 3），*P. intermedia*（症例 7）が検出され，膿胸の 3 例からは全例 *P. gingivalis* が検出されたが，いずれの症例も 1 菌種のみが陽性であった．また症例 3 では病理組織中にグラム陰性桿菌のコロニーが鏡検されたが，培養では同定できず，PCR 検査で *T. forsythensis* が 1,280 コピー（総菌数の 42.1%）と高い優占率で認められた．症例 1，2，3 の歯周病菌数あるいは総菌数に対する割合は高かったが，症例 4，5，7 では総菌数に比して歯周病菌の割合が低かった．症例 7 では気管支洗浄液の培養では *P. oralis* が分離され，肺組織の PCR 検査でも少数ながら *Prevotella* 属の *P. intermedia* が検出された．以下に症例 2 例を提示する．

【症例 1】
患者：69 歳，女性．
主訴：発熱，血痰．
既往歴：高血圧症，子宮筋腫．
生活歴：喫煙歴なし，機会飲酒．

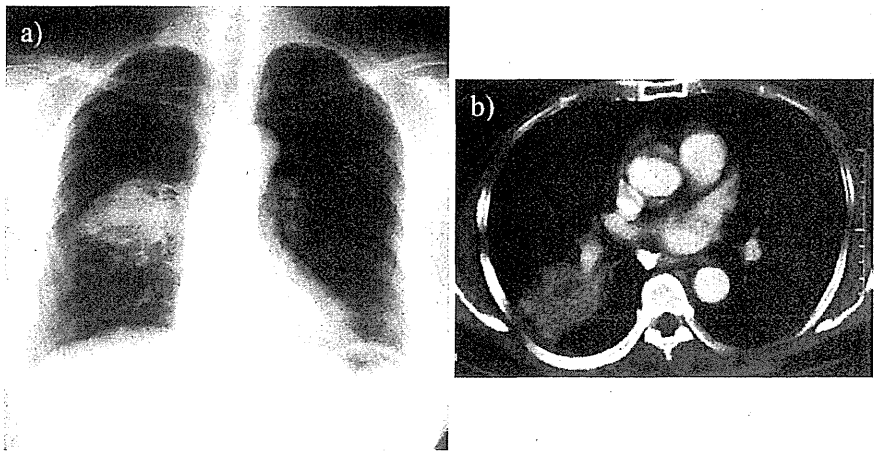

Fig. 1　(a) Chest radiograph on admission, showing a nodular shadow in the right-middle lung field. (b) Chest CT scan on admission, revealing an irregularly shaped mass lesion of the right lower lobe (Case 1).

現病歴：2009 年 4 月 11 日から感冒症状があり，1 週間後発熱，血痰が出現し近医を受診した．胸部 CT 上右 S6 に浸潤影を認め肺膿瘍が疑われたが，痰や気管支洗浄液の培養は陰性であった．メシル酸ガレノキサシン（garenoxacin mesilate hydrate：GRNX）400 mg/日 が 3 週間投与されたが，発熱と血痰を繰り返し，腫瘤影も残存していたため，6 月 11 日当科紹介初診となった．

初診時現症：身長 142.0 cm，体重 49.2 kg，血圧 130/70 mmHg，脈拍 73/min（整），体温 36.7℃，SpO_2 98%（room air），胸部聴診所見は異常なし．口腔内所見として齲歯と動揺歯を認めたが未治療であった．

入院時検査所見：白血球数 11,000/μl（好中球 77.7%），赤沈 76 mm/h，CRP 5.2 mg/dl と炎症反応を認めた．血清学的検査では腫瘍マーカーは正常内，カンジダ，アスペルギルス，クリプトコッカスの抗原，β-D-グルカンはいずれも陰性．喀痰の細菌培養，抗酸菌検査も陰性であった．

画像所見：胸部 X 線では右中肺野に均一な濃度の楕円形の腫瘤影を認め（Fig. 1a），胸部 CT では右 S6 に約 6 cm 大の内部が一部低吸収の腫瘤影を認めた（Fig. 1b）．

臨床経過：気管支鏡検査を施行し TBB と気管支洗浄を行った．病理所見では炎症細胞浸潤と肺胞壁の破壊を認め肺膿瘍と診断した．しかし TBB の組織培養，気管支洗浄液の塗抹・培養検査はいずれも陰性であった．難治性の肺膿瘍と診断，嫌気性菌のほか *Rhodococcus* や *Legionella* の可能性も考え，クリンダマイシン（clindamycin hydrochloride：CLDM）1,200 mg/日を 4 週間，クラリスロマイシン（clarithromycin：CAM）400 mg/日を 6 週間投与し，第 27 病日で炎症反応は陰性化した．臨床症状も消失し，胸部 X 線で膿瘍は著明な縮小を認めた．その後齲歯治療も行ったところ再発しなかった．

歯周病細菌 PCR 検査：本症例は齲歯と動揺歯を認めたことから，歯周病の感染巣の菌が起炎菌である可能性を疑った．TBB 検体を用いて歯周病細菌 PCR 検査を施行し，*T. denticola* が検出された．

【症例 2】

患者：55 歳，男性．

主訴：呼吸困難，背部痛．

既往歴：高血圧症．

生活歴：喫煙歴　20 本/日×35 年間，飲酒歴　日本酒 2 合・ビール 2 本/日．

現病歴：2009 年 8 月 3 日より 38℃の発熱，8 月 5 日から背部痛と呼吸困難が出現したため，救急車で来院し緊急入院となった．

初診時現症：身長 158.4 cm，体重 66.7 kg，血圧 110/60 mmHg，脈拍 85/min（整），体温 37.0℃，SpO_2 96 %（room air），胸部では右呼吸音減弱．吸気時と体動時に背部痛を認めた．

入院時検査所見：白血球数 20,700/μl（好中球 87.2%），CRP 19.0 mg/dl と炎症反応を認めた．胸水は好中球優位の滲出性で，胸水培養から *S. constellatus* が分離された．

画像所見：胸部 X 線で右胸水を認め（Fig. 2），胸部 CT で右 S5 の浸潤影と右胸水を認めた．

臨床経過：入院時に喀痰と胸水を採取した．喀痰培養で *Klebsiella pneumoniae* が検出され，セフトリアキソン（ceftriaxone sodium hydrate：CTRX）2 g/日　と CLDM 1,200 mg/日を開始した．第 7 病日に胸水培養から *S. constellatus* が分離され膿胸の併発と診断し，胸水

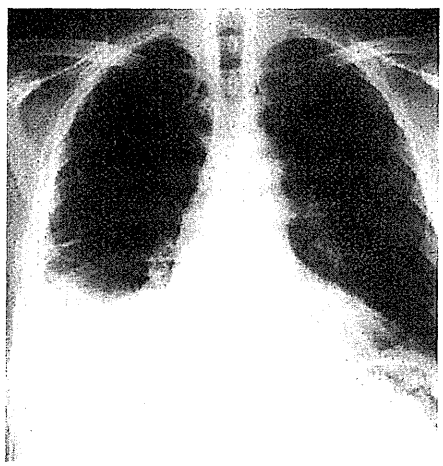

Fig. 2 Chest radiograph on admission showing right pleural effusion (Case 2).

ドレナージと胸腔内洗浄を施行した．改善を認め第16病日にドレーンを抜去．CTRXとCLDMを19日間投与した後，アンピシリン/スルバクタム（ampicillin/sulbactam：ABPC/SBT）1,175 mg/日の内服に変更し外来で治療を継続した．退院1週間後にCRPは陰性化し，1ヶ月後の胸部X線では胸水は消失した．

歯周病細菌PCR検査：喀痰から*K. pneumoniae*，胸水から*S. constellatus*を認めたことから誤嚥の関与を疑った．2週間前に齲歯治療を受けていたため，胸水検体で歯周病細菌PCR検査を施行したところ，*P. gingivalis*が検出された．

考　察

歯牙，歯周疾患と呼吸器感染症の関連については，Finegold[5]が，嫌気性菌性胸膜・肺感染症143例で基礎疾患・発症誘因を検討し，歯牙・歯周疾患と誤嚥の重要性を指摘している．我が国では古西と三笠[6]が，嫌気性菌性呼吸器感染症の基礎疾患として歯牙・歯周疾患が41%と最多であると報告している．

嫌気性菌性呼吸器感染症における起炎菌同定率の向上のため，以前よりさまざまな検査法が試みられてきた．Bartlettら[7]は，経皮経気管吸引による病巣からの無菌的な検体採取の有用性を報告しており，近年ではCTガイド下病巣穿刺の有用性も示されている[8][9]．しかしこれらをもってしても，起炎菌同定に至らない症例も存在する．

そこで，抗菌薬投与後の培養陰性例や嫌気性菌，遅発育菌，あるいはトレポネーマなどの人工培地での発育困難な菌など，培養診断ができない感染症に対し，大楠と江崎は補助診断としてPCR法による菌の同定を試みており，臨床的に有効性が示されている[10]．またKawanamiらは細菌感染関連胸水において16S rRNAを用いた網羅的解析を行い，培養法で診断困難な症例でも起炎菌診断が可能であり，またより高い頻度で嫌気性菌を認めたことを報告している[11]．

歯牙・歯周疾患に罹患した歯周組織の細菌の分布は，正常のグラム陽性通性嫌気性菌主体の分布とは異なり，*P. gingivalis*，*T. forsythensis*，*T. denticola*，*P. intermedia*，*Fusobacterium nucleatum*，*A. actinomycetemcomitans*といった偏性嫌気性グラム陰性桿菌を中心とした菌群が優勢になっている．これらの細菌は歯周炎の発症，進展に特に重要とされているが，誤嚥性肺炎や膿胸，肺膿瘍の病巣でも分離される頻度が高く，病原菌と認識されている．こうした背景から我々は，歯周病細菌PCR検査は嫌気性菌性呼吸器感染症の診断の一助となりうるのではないかと考えた．

当院での病巣の検体を用いた検索では，嫌気性菌培養陰性の6例中，5例でPCR検査が陽性であり，気管支洗浄液の培養で嫌気性菌を認めた1例はPCR検査でも陽性であった．齲歯治療直後の抗酸菌症のTBB検体1例，肺癌のTBB検体1例と非感染性の胸水1例で歯周病細菌PCR検査を試みたがいずれも陰性であり，症例数は少ないが細菌感染疾患に特異的に陽性であった．また検体採取に伴う定着菌のコンタミネーションについては，4例の胸水は無菌的に採取しているため問題はないが，TBB検体については，real-time PCRによる定量法での検出菌の総菌数に対する比率が，症例1では10.3%，症例3では42.1%と高く，偽陽性の可能性は低いと思われた．症例7ではPCRでも気管支鏡でも*Prevotella*属が検出され，口腔内嫌気性菌群が起炎菌であると推測されたが，*P. intermedia*はPCRでの菌数や比率から主要菌ではなく，混合感染あるいは疑陽性の可能性が考えられた．また症例3において病理組織で鏡検されたグラム陰性桿菌が，組織培養では同定できず，PCR検査で*T. forsythensis*を認め，網羅的解析でないため主要な起炎菌と断定はできないものの，検出の有効性は期待できるものと思われた．さらに結果報告までの平均日数が8.3日（7〜10日）であり，迅速性の点では従来の嫌気培養と比較して劣らないものであった．培養法は薬剤感受性を確認できるなど細菌感染症には不可欠な検査であり，現時点ではPCR検査は特に嫌気性菌において有用な補助診断と考えられ，また凍結保存検体でも検査できる点からも培養困難な際には施行する価値があると思われる．

さらに，今回の検討で嫌気性菌性呼吸器感染症の発症や進展にかかわると考えられる興味深い所見を2点認めた．一つは，症例2において，培養とPCR法の併用に

より，誤嚥を発症機序とした *S. anginosus* group と *P. gingivalis* の混合感染であったことが示唆されたことである．*S. anginosus* group はしばしば他の嫌気性菌との混合感染における相乗作用が示されており，新里ら[12]はマウス肺炎モデルの研究で，*S. constellatus* と *P. intermedia* の混合感染群では，それぞれの単独感染群に比し，肺病理像の炎症が非常に強く，膿瘍形成率および致死率が有意に上昇したと報告している．症例2の結果は，これまで *S. anginosus* group の単独感染と認識されていた症例の中に嫌気性菌との混合感染も含まれていたことを示す1例と考えられ，同様の結果は Kawanami らの手法で検索された報告例にもみられる[11]．

二つ目は，現時点ではヒトにおける病原性が定かではない *T. denticola* が，症例1の肺膿瘍の病巣から検出されたことである．*T. denticola* はスピロヘータ科に属する口腔内常在細菌で，発育には TYGVS 培地を用いた嫌気的条件下での培養といった特殊な培養環境を必要とする．細胞付着活性や宿主組織進入能を有し，侵襲性の歯周疾患の病巣で増加していると報告されている．ヒトにおいては *T. denticola* による肺感染症の報告例は文献上みられず病原性が明らかでないが，動物実験では病原性を示唆する報告がある．君塚[13]は，マウスの気管内に *P. gingivalis* と *T. denticola* を混合感染させて，BALF 中の TNF-α，IL-1β，IL-6 などの炎症性サイトカイン産生量が単独感染に比し増加することや膿瘍形成率および致死率が有意に上昇することを報告している．今回，肺膿瘍の病巣から *T. denticola* が検出されたことから，*T. denticola* が肺膿瘍の起炎菌の一つとして働いていた可能性が推測される．かつては *S. anginosus* group や嫌気性菌も口腔内に常在する病原性を持たない菌とされ，後に肺感染症の起炎菌と認識された経緯があり，*T. denticola* のヒトにおける病原性に関しても，臨床的な症例蓄積と微生物学的見地からの検討を進めていく必要があると思われる．

今回，培養診断が困難な呼吸器感染症に対し，歯周病細菌 PCR 検査を用いた同定を行い，有用性を示す結果が得られた．今後さらに症例を蓄積し，嫌気性菌性感染症診断の一方法としての臨床的意義を検討する必要があると考えられる．

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引用文献

1) 山下裕子，河野　茂，田中研一，他．嫌気性菌による呼吸器感染症―その検体採取法の検討を含めて―．感染症誌 1994; 68: 631-63.
2) 高松伸博．歯周初期治療における *Bacteroides forsythus*，*Porphyromonas gingivalis*，*Actinobacillus actinomycetemcomitans* の変動：DNA プローブ法と PCR 法による測定．口腔病会誌 1997; 64: 534-543.
3) 藤瀬　修．歯周病細菌遺伝子検査の臨床へのアプローチ　歯周病関連細菌の PCR による定量法の臨床応用（歯周病細菌遺伝子検査の臨床へのアプローチ）．日本歯周病学学会会誌 2001; 43（春季特別号）: 43.
4) 上野一恵（監修）．微生物検査マニュアル―臨床嫌気性菌検査法 '97．日臨微生物誌 1997; 7（Suppl 1），1-115.
5) Finegold SM. Lung abscess. In: Mandell GL, Douglas RG, Bennett JE. ed. Principles and Practice of Infectious Disease. 5th ed. New York: Churchill Livingstone. 2000; 751-755.
6) 古西　満，三笠桂一．偏性嫌気性菌感染症の基礎と臨床　臨床編　呼吸器感染症．臨床と微生物 2008; 35: 371-5.
7) Bartlett JG, Rosenbaltt JE, Finegold SM, et al. Percutaneous transtrancheal aspiration in the diagnosis of anaerobic pulmonary infection. Ann Intern Med 1973; 79: 535-40.
8) Ghaye B, Dondelinger RF. Imaging guided thoracic interventions. Eur Respir J 2001; 17: 507-28.
9) 宮本　篤，坪井永保，高谷久史，他．経皮的病巣穿刺にて *Haemophilus parainfluenzae* および *Streptococcus intermedius* が分離された肺膿瘍の1例．日呼吸器会誌 2006; 44: 561-6.
10) 大楠清文，江崎孝行．感染症診断における遺伝子解析技術の適応．日臨微生物誌 2008; 18: 163-76.
11) Kawanami T, Fukuda K, Yatera K, et al. A higher significance of anaerobes: The clone library analysis of bacterial pleurisy. Chest 2011; 193: 600-8.
12) 新里　敬，重野芳輝，斎藤　厚，他．嫌気性菌の "*Streptococcus milleri*" group 呼吸器感染症に及ぼす影響（第2報）．嫌気性菌感染症研 1992; 22: 66-9.
13) 君塚隆太．マウス肺炎モデルにおける歯周病原性菌混合感染による病態解析．歯科学報 2003; 103: 905-11.

Abstract

Clinical evaluation of PCR assays for detection of periodontal bacteria in patients with lung abscesses or empyemata

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Current evidence suggests that periodontal disease may be associated with the development of a lung abscess or an empyema. Some microbes, including the *Streptococcus anginosus* group and obligate anaerobic bacteria, are recognized as important pathogens. We analyzed periodontal disease bacteria by polymerase chain reaction (PCR) assays of transbronchial biopsy (TBB) specimens, bronchial lavage fluid (BLF), or pleural effusion obtained from 7 cases (lung abscess, 3; and empyema, 4). In 1 case, anaerobes were isolated from BLF culture, but periodontal pathogens were detected in TBB specimens or pleural effusion by PCR assays in 6 cases (*Porphyromonas gingivalis*, 3; *Tannerella forsythensis*, 1; *Treponema denticola*, 1; and *Prevotella intermedia*, 1). In 1 case *T. denticola* of unknown pathogenicity was detected in the TBB specimens. PCR assays targeting periodontal disease bacteria are potentially useful for identifying the causes of respiratory infectious diseases, which are difficult to diagnose by anaerobic culture.

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CXCR4-Tropic, But Not CCR5-Tropic, Human Immunodeficiency Virus Infection Is Inhibited by the Lipid Raft-Associated Factors, Acyclic Retinoid Analogs, and Cholera Toxin B Subunit

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Abstract

Development of an effective low-cost anti-acquired immunodeficiency syndrome (AIDS) drugs is needed for treatment of AIDS patients in developing countries. Host cell lipid raft microdomains, which are enriched with cholesterol, glycolipids, ceramide, and gangliosides, are important for human immunodeficiency virus type 1 (HIV-1) entry. Retinoid analogs have been shown to modulate ceramide levels in the cell membrane, while cholera toxin B subunit (CT-B) specifically binds to the ganglioside GM1. In this study, we found that the acyclic retinoid analogs geranylgeranoic acid (GGA) and NIK-333 as well as CT-B efficiently attenuate CXCR4-tropic, but not CCR5-tropic, HIV-1 vector infection. We also found that GGA and NIK-333 suppress CXCR4-tropic HIV-1 infection by attenuating CXCR4 expression. CT-B also attenuated CXCR4-tropic HIV-1 infection, but did not suppress CXCR4 expression. These results suggest a distinct role for lipid raft microdomains in CXCR4- and CCR5-tropic HIV-1 infections and illuminate novel agents for the development of AIDS therapy.

Introduction

HIGHLY ACTIVE ANTIRETROVIRAL therapy (HAART), which suppresses human immunodeficiency virus type 1 (HIV-1) reverse transcriptase, protease, and integrase, has been found to be an effective treatment against acquired immunodeficiency syndrome (AIDS). In fact, many patients infected with HIV-1 do not progress to AIDS in developed countries due to implementation of HAART. However, HIV-1/AIDS continues to be a serious problem, as many HIV-1-infected patients in developing countries do not have access to effective anti-HIV-1 drugs due to the prohibitive cost of the therapy, and thus, the numbers of HIV-1-infected patients are increasing worldwide. In addition, HIV-1 variants resistant to current drugs have appeared.[1] To resolve these problems, novel, low-cost drugs that inhibit HIV-1 infection are critical.

Lipid raft microdomains of target cell membranes are required for HIV-1 infection.[2–6] Lipid rafts are enriched with cholesterol, glycolipids, and ceramide.[7] Extraction of cholesterol from cell membranes,[4,6] binding of cholesterol with various factors,[2,8] and inhibition of biosynthesis of cholesterol[9,10] or glycolipids[11–13] suppress HIV-1 infection, suggesting that cholesterol and glycolipids may be targets for novel anti-HIV-1 drugs. In this study, we examined the effects of lipid raft-associated factors, which were isolated from natural products, on HIV-1 vector infection.

Retinoic acid and its analogs modulate ceramide levels in cell membranes.[14–20] Retinoid analogs may inhibit HIV-1 infection by altering ceramide levels of the target cell membrane. In fact, an all-trans retinoic acid[21] and a weak nuclear retinoid receptor agonist, *N*-(4-hydroxyphenyl) retinamide (4-HPR),[22] inhibit HIV-1 infection[19,23]; however, because 4-HPR has severe toxicities, such as induction of vitamin A deficiency

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symptoms, clinical application of 4-HPR is restricted.[24] Geranylgeranoic acid (GGA), which is a natural acyclic retinoid analog present in medicinal herbs,[25] serves as a weak agonist for retinoid receptors, similar to 4-HPR.[26, 27] NIK-333, which is an artificial acyclic retinoid analog with a structure similar to GGA (Fig. 1), prevents recurrence of hepatocellular carcinoma following oral administration without any obvious side effects in clinical studies of liver cancer patients.[28,29] We analyzed the effects of the acyclic retinoid analogs GGA and NIK-333 on HIV-1 vector infection.

Cholesterol is enriched in lipid raft microdomains and requires their structural maintenance. Extraction of cholesterol from cell membranes by methyl-β-cyclodextrin (MβCD),[4, 6] inhibition of cholesterol synthesis by statin,[9,10] or binding of amphotericin B methyl ester to cholesterol[8] suppresses HIV-1 infection. Plant sterols are cholesterol analogs that reduce serum cholesterol levels by replacing cholesterol.[30] Therefore, plant sterols may function as anti-HIV-1 agents.

Because cholera toxin B subunit (CT-B) specifically binds to the ganglioside GM1, this subunit is frequently used as a lipid raft marker.[4,6] The cytopathic determinant of cholera toxin is subunit A, which has the poly(ADP) ribosylation activity of G-proteins.[31] In contrast, the B subunit has no cytopathic effect. GM1 is enriched in raft microdomains and has been reported to bind HIV-1 envelope (Env) glycoprotein.[32] Additionally, CD4-positive lymphocytes that have elevated levels of another gangliosides, GM3, are highly susceptible to HIV-1 fusion and entry.[11] Therefore, CT-B may inhibit HIV-1 infection without cytopathic effects.

In this study, we examined the effects of these raft-associated factors on HIV-1 vector infection. Our results showed that acyclic retinoid analogs and CT-B efficiently suppressed CXCR4-tropic HIV-1 vector infection, providing novel strategies for the development of CT-B or acyclic retinoid analog treatment for AIDS patients. In contrast, these factors did not affect CCR5-tropic HIV-1 vector infection, suggesting that raft microdomains are involved differently in CXCR4- and CCR5-tropic HIV-1 infections.

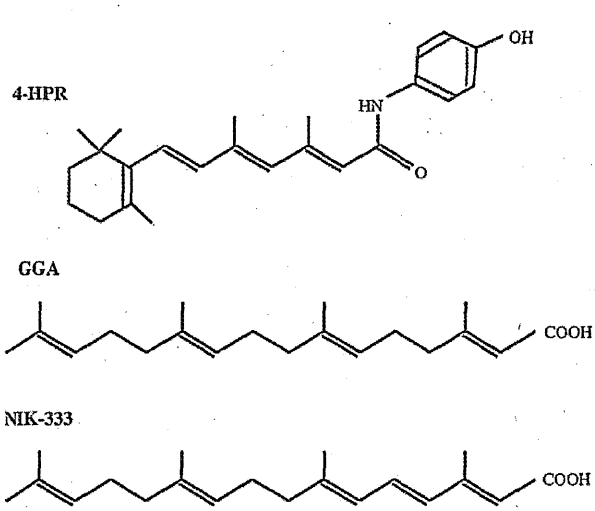

FIG. 1. Chemical structures of 4-HPR, GGA, and NIK-333.

Materials and Methods

Cells

COS7, 293T, NP2, TE671, and HeLa cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) (Wako) supplemented with 8% fetal bovine serum (Biosource) at 37°C in 5% CO_2. NP2 cells expressing CD4 and CXCR4 (NP2/CD4/X4) or CD4 and CCR5 (NP2/CD4/R5) were kindly provided by Dr. H. Hoshino.[33] NP2 cells expressing CD4 and C-terminally HA-tagged CXCR4 (NP2/CD4/X4-HA) were constructed as previously reported.[34] TE671, HeLa, and 293T cells expressing CD4 (TE671/CD4, HeLa/CD4, and 293T/CD4) were constructed with a CD4-encoding murine leukemia virus (MLV) vector as previously reported.[35] MAGIC5 cells, which are derived from HeLa cells, express CD4 and CCR5 and contain the β-galactosidase (β-Gal) gene under control of the HIV-1 long terminal repeat.[36]

Expression plasmids

CXCR4-tropic HXB2 and CCR5-tropic JRFL HIV-1 Env expression plasmids were kindly provided by Dr. Y. Yokomaku (National Hospital Organization Nagoya Medical Center). A VSV-G expression plasmid and expression plasmids required for LacZ reporter gene-containing HIV-1 vector construction were obtained from Invitrogen. An expression plasmid encoding C-terminally HA-tagged CXCR4 was constructed as already reported.[34]

Transduction assay

To obtain HIV-1 vector particles, COS7 cells were transfected with the HIV-1 vector construction plasmids using Fugene transfection reagent (Roche). The transfected cells were washed with D-MEM medium 24 h after transfection and maintained in fresh medium for 24 h. Target cells were either left untreated or pretreated with the retinoid analogs 4-HPR (Sigma-Aldrich), GGA, or NIK-333 for 2 days or with CT-B (Sigma-Aldrich) or stigmasterol (Sigma-Aldrich) for 1 day. GGA and NIK-333 were synthesized by Kowa Company, Ltd. (Tokyo, Japan). The cells were inoculated with culture supernatants from the transfected COS7 cells and then stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Nacalai) 2 days after inoculation. Blue cells were counted to estimate transduction titer. Approximately 10^4, 10^4, and 10^6 infected cells were detected among cells inoculated by the HXB2 Env-, JRFL Env-, and VSV-G-containing vectors, respectively. To normalize transduction titers, the VSV-G vector was diluted 100 times with medium.

Flow cytometry

To analyze cell surface CD4 expression, suspended cells were either left untreated or treated with an anti-CD4 antibody conjugated with FITC (Sigma-Aldrich). Cell surface expression of CXCR4 or CCR5 was analyzed in suspended cells treated with rat anti-CXCR4 (A80) or anti-CCR5 (T312) monoclonal antibody.[37] As a control, cells were treated with a rat serum. The cells were then washed three times with phosphate-buffered saline (PBS) and treated with an FITC-conjugated anti-rat IgG antibody (Sigma-Aldrich). The stained cells were quantified using a flow cytometer (BD Biosciences).

Western immunoblotting

NP2/CD4/X4-HA cells were treated with the retinoid analogs, and cell lysates were prepared. The cell lysates were subjected to SDS polyacrylamide gel electrophoresis (Bio-Rad) and transferred onto a PVDF membrane (Millipore). The membrane was treated with a mouse anti-HA monoclonal antibody (Covance), and then with an HRP-conjugated anti-mouse IgG antibody (Bio-Rad).

Vector particle binding to target cells

Target cells were incubated with culture supernatants from the HIV-1 vector-producing cells for 1 h at 4°C. The cells were washed three times with PBS, and cell lysates were prepared. HIV-1 Gag p24 levels were measured with a p24 enzyme-linked immunosorbent assay (ELISA) (ZeptoMetrix) to estimate the numbers of HIV-1 vector particles bound to the target cells.

Cell fusion assay

The 293T cells were transfected with the HXB2 Env expression plasmid, which also encodes the Tat protein. As a control, 293T cells were transfected with a Tat expression plasmid. The transfected cells were cultured with MAGIC5 cells 24 h after transfection, and cell lysates were prepared from the cells 24 h after the mixed culture. Upon cell fusion, the Tat protein induced β-Gal expression. β-Gal activity in the cell lysates was measured to estimate cell fusion capability.

Statistical analysis

Differences between two groups were determined by the Student's *t*-test. The difference was considered statistically significant if the *p*-value was <0.05 for all tests.

Results

Acyclic retinoid analogs and CT-B inhibit CXCR4-tropic HIV-1 vector infection

To assess whether retinoid analogs inhibit HIV-1 vector infection, target cells were pretreated with 4-HPR, GGA, or NIK-333 for 2 days. The chemical structures of the analogs are shown in Fig. 1. NP2 cells expressing CD4 and CXCR4 (NP2/CD4/X4), NP2 cells expressing CD4 and CCR5 (NP2/CD4/R5),[33] and HeLa cells expressing CD4 (HeLa/CD4)[35] were used as target cells. All of the retinoid analogs inhibited infection by a CXCR4-tropic HXB2 Env-carrying HIV-1 vector (Fig. 2A). Previous reports indicated that 4-HPR inhibits HIV-1 infection,[23] and this result is consistent with our findings. In addition, cell viability was not affected by the analog treatment under these conditions. These results indicate that the acyclic retinoid analogs GGA and NIK-333 as well as 4-HPR inhibit CXCR4-tropic HIV-1 infection.

VSV-G-mediated infection is independent of lipid rafts,[4,6] so we assessed whether VSV-G-pseudotyped HIV-1 vector infection is also attenuated by the retinoid analogs. VSV-G-pseudotyped HIV-1 vector infection was not significantly affected by the retinoid analogs (Fig. 2B). Similarly, infection by HIV-1 vector pseudotyped with the Env protein of the CCR5-tropic JRFL strain was not inhibited by the retinoid analogs (Fig. 2C). These results indicate that the retinoid analogs specifically suppress CXCR4-tropic HIV-1 Env-mediated infection but not VSV-G- and CCR5-tropic HIV-1 Env-mediated infection and that the retinoid analogs inhibit CXCR4-tropic HIV-1 infection by a mechanism other than a cytopathic effect.

We next assessed whether CT-B inhibits HIV-1 vector infection. CD4-expressing TE671 (TE671/CD4), HeLa/CD4, NP2/CD4/X4, and NP2/CD4/R5 cells were pretreated with CT-B for 24 h and then inoculated with HXB2 Env- or JRFL Env-bearing HIV-1 vector in the absence of CT-B. CT-B significantly attenuated CXCR4-tropic Env-mediated infection but not VSV-G-pseudotyped HIV-1 vector infection in TE671/CD4 (Fig. 3A), HeLa/CD4 (Fig. 3B), and NP2/CD4/X4 cells (Fig. 3C). However, CT-B did not inhibit CCR5-tropic Env-mediated infection in NP2/CD4/R5 cells (Fig. 3C). If CT-B inhibited cell growth, this toxin should also suppress VSV or CCR5-tropic vector infection; however, CT-B did not affect cell growth as analyzed by microscopic observation. These results indicate that CT-B specifically suppresses CXCR4-tropic HIV-1 infection by a mechanism other than cell growth inhibition.

Additionally, we assessed whether a plant sterol, stigmasterol, inhibits HIV-1 vector infection. The target cells were pretreated with stigmasterol (80 μg/ml) for 24 h. The transduction efficiency of the HIV-1 vector was not affected by the treatment (data not shown).

Retinoid analogs inhibit CXCR4 cell surface expression

As the acyclic retinoid analogs inhibited CXCR4-tropic HIV-1 vector infection, we next assessed whether these retinoid analogs suppressed cell surface expression of the HIV-1 infection receptors, CD4, CXCR4, and CCR5. 4-HPR did not affect CD4 cell surface expression in HeLa/CD4 cells (Fig. 4A). GGA and NIK-333 treatment elevated CD4 expression, though the acyclic retinoid analogs inhibited CXCR4-tropic HIV-1 vector infection. In contrast, all of these retinoid analogs reduced cell surface CXCR4 expression. Similar results were observed in NP2/CD4/X4 cells, in which CXCR4 is artificially expressed (data not shown). Furthermore, these retinoid analogs did not affect CCR5 expression (Fig. 4B). These results suggest that the retinoid analogs inhibit CXCR4-tropic HIV-1 infection by suppressing CXCR4 cell surface expression.

When NP2 cells expressing C-terminally HA-tagged CXCR4 were treated with the retinoid analogs, expression levels of the HA-tagged CXCR4 were not altered, analyzed by Western immunoblotting using an anti-HA antibody (Fig. 4C). This result suggests that the retinoid analogs inhibit the trafficking of CXCR4 to the cell surface, but do not inhibit CXCR4 expression.

CT-B also inhibited CXCR4-tropic HIV-1 vector infection but not CCR5-tropic HIV-1 vector infection; however, CT-B did not affect cell surface expression of CCR5 (Fig. 4B), CXCR4, or CD4 (Fig. 4C). These results indicate that CT-B inhibits CXCR4-tropic infection by a mechanism other than suppression of CXCR4 expression.

Retinoid analogs and CT-B do not affect HIV-1 particle binding to host cells

We analyzed the effects of the retinoid analogs and CT-B on CXCR4-tropic HIV-1 vector particle binding to the target cells by p24 ELISA. The amount of p24 protein

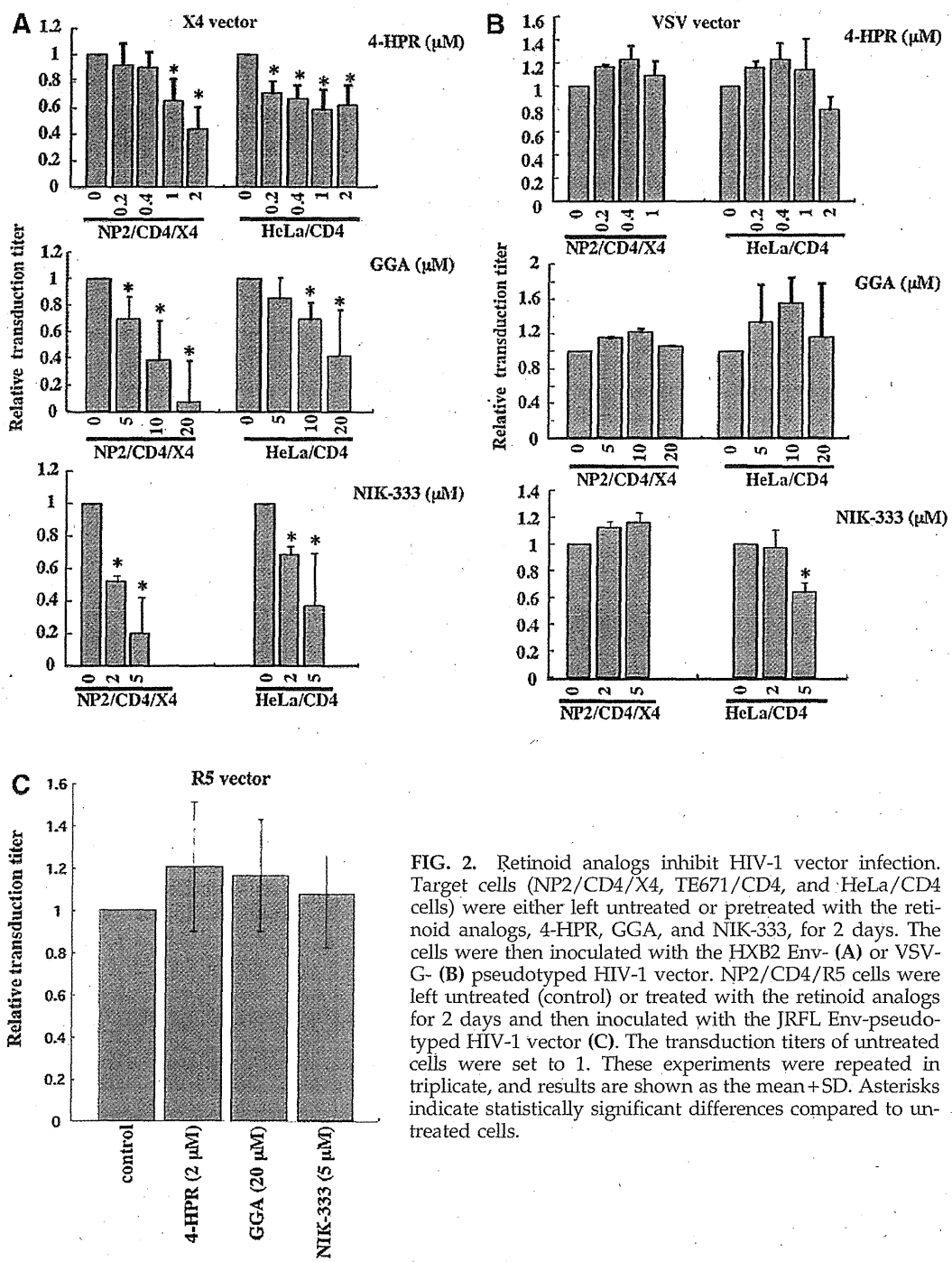

FIG. 2. Retinoid analogs inhibit HIV-1 vector infection. Target cells (NP2/CD4/X4, TE671/CD4, and HeLa/CD4 cells) were either left untreated or pretreated with the retinoid analogs, 4-HPR, GGA, and NIK-333, for 2 days. The cells were then inoculated with the HXB2 Env- **(A)** or VSV-G- **(B)** pseudotyped HIV-1 vector. NP2/CD4/R5 cells were left untreated (control) or treated with the retinoid analogs for 2 days and then inoculated with the JRFL Env-pseudotyped HIV-1 vector **(C)**. The transduction titers of untreated cells were set to 1. These experiments were repeated in triplicate, and results are shown as the mean+SD. Asterisks indicate statistically significant differences compared to untreated cells.

bound to CD4-expressing HeLa cells was higher than that bound to CD4-negative HeLa cells, indicating that vector particle binding is CD4-dependent (Fig. 5A). None of the retinoid analogs (Fig. 5B) or CT-B (Fig. 5C) affected HIV-1 vector particle binding to the CD4-expressing target cells. These results show that the retinoid analogs and CT-B inhibit CXCR4-tropic HIV-1 infection by a mechanism other than suppression of CD4-dependent virion binding to target cells.

Retinoid analogs and CT-B inhibit membrane fusion activity of HIV-1 Env protein

To assess whether the retinoid analogs or CT-B inhibit HIV-1 Env-mediated membrane fusion activity, we analyzed the effects of these agents on HIV-1 Env-induced syncytium formation. HEK293T cells transfected with the plasmid encoding the HIV-1 HXB2 Env and Tat proteins were cocultured with MAGIC5 cells for 24 h, and β-galactosidase activity was

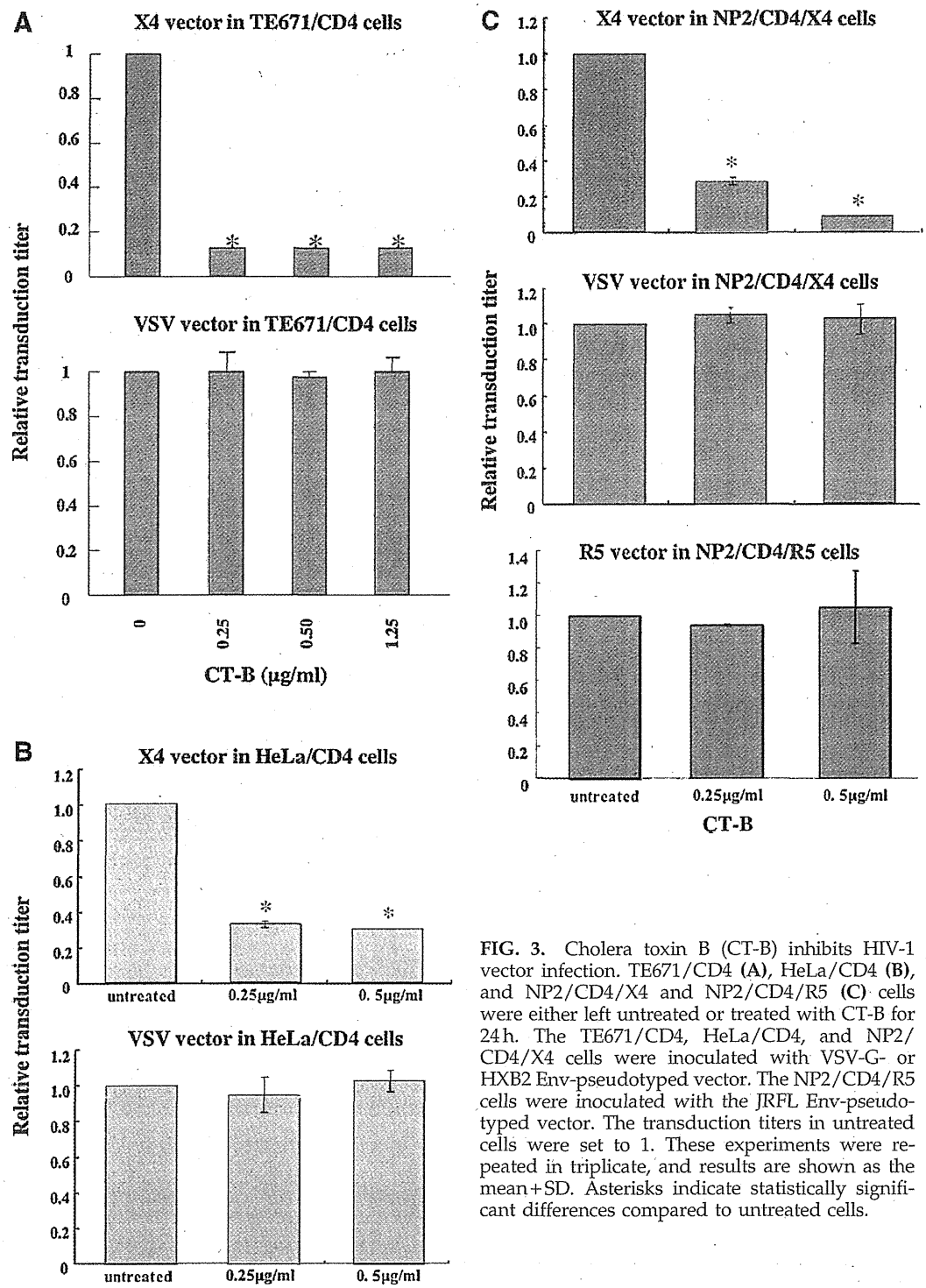

FIG. 3. Cholera toxin B (CT-B) inhibits HIV-1 vector infection. TE671/CD4 **(A)**, HeLa/CD4 **(B)**, and NP2/CD4/X4 and NP2/CD4/R5 **(C)** cells were either left untreated or treated with CT-B for 24 h. The TE671/CD4, HeLa/CD4, and NP2/CD4/X4 cells were inoculated with VSV-G- or HXB2 Env-pseudotyped vector. The NP2/CD4/R5 cells were inoculated with the JRFL Env-pseudotyped vector. The transduction titers in untreated cells were set to 1. These experiments were repeated in triplicate, and results are shown as the mean+SD. Asterisks indicate statistically significant differences compared to untreated cells.

measured in the cell lysates. The retinoid analogs (Fig. 6A) and CT-B (Fig. 6B) suppressed syncytium formation. Direct inhibition of the HIV-1 Env-mediated membrane fusion reaction by these factors would suppress both CXCR4- and CCR5-tropic HIV-1 infections; however, the factors did not affect CCR5-tropic HIV-1 infection (Fig. 2C). Taken together, these results support the hypothesis that retinoid analogs inhibit CXCR4-tropic HIV-1 infection by attenuating CXCR4 expression, although CT-B may affect the HIV-1 entry process between vector particle binding to target cells and membrane fusion.