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J Epidemiol 2012;22(2):86-90
doi:10.2188/jea.JE20110085

Review Article

Infection Control in Healthcare Settings in Japan

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Received August 15, 2011; accepted December 14, 2011; released online February 4, 2012

ABSTRACT

In Japan, the practice of infection control in healthcare settings has a short history of less than 3 decades. Before that, infection control practices were far from perfect and even ignored. This review summarizes changes in infection control in Japan since the 1980s and offers some comparisons with practices in foreign countries, especially the United States. Infection control is far better now than 25 years ago, but there remain fundamental issues that limit the development of better infection control practices. These problems include insufficient funding and human resources due to the socialized healthcare insurance system in Japan and the lack of interest in infection control research.

Key words: healthcare-associated infection; infection control; healthcare insurance system

INTRODUCTION

In Japan, infection control in the healthcare setting was not an organized endeavor until the founding of a society for infection control (Japanese Society for Environmental Infections, JSEI) in 1986.[1] During my medical education in the mid-1980s, there was no instruction in infection control. The idea of universal precautions, which became popular in healthcare settings in Western countries, had not yet been introduced to Japan, and procedures with some risk of exposure to blood-borne pathogens, such as phlebotomy and peripheral line insertion, were often done without gloves. This was during the time when methicillin-resistant *Staphylococcus aureus* (MRSA) became prevalent throughout Japan, especially in postoperative patients. Many had diarrhea with a MRSA-positive stool culture, which was diagnosed as MRSA enterocolitis. This disease entity is still debated, but that situation led to the development of a relevant infection control strategy in Japan, something that had never existed before. The key events since 1986 are listed in Table 1. In this article, I describe the changes in infection control in Japan during the last 25 years and discuss the present situation and challenges we currently face.

Table 1. Key events in infection control in Japan

1980s	Increase in the incidence of healthcare-associated MRSA infection
1986	Founding of JSEI
1993	Infection control department established at The University of Tokyo Hospital (first in Japan)
1999	Nationwide surveillance of surgical site infections by JSEI
2000	Nationwide surveillance of multidrug-resistant organisms by MHLW
2000	832 MD and PhD staff certified as Infection Control Doctors (ICD)
2001	18 nurses certified as Infection Control Nurses (ICN)
2004	Mandatory assignment of dedicated infection control personnel at Advanced Treatment Hospitals
2010	Revision of medical reimbursement system: implementation of additional reimbursement for advanced infection control management

The infection control team

For effective infection control, it is necessary to combine personnel who spend a specified fraction of their time—usually expressed in full-time equivalents (FTEs)—on infection control. Therefore, teamwork among infection control personnel is very important. The infection control team (ICT) is a very popular concept among healthcare workers in Japan, in contrast to the situation in the United States, where members of each profession (physicians, nurses, pharmacists, microbiologists, etc) work independently for infection control and are responsible for their designated, specialized areas. Although Japanese law does not require hospitals to have ICTs, they must have an infection control committee (ICC) that includes the chairperson and executive officers of the hospital. The ICC makes the final decisions regarding infection control programs, but this is often just an endorsement.

Program establishment and practice are led by the ICT, which conducts surveillance of multidrug-resistant pathogens and device/procedure-associated infections such as bloodstream infection (BSI), urinary tract infection (UTI), and surgical site infection (SSI). Other activity includes ward audit (rounds), education of healthcare personnel, adherence

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monitoring of hand hygiene and other infection control practices, and investigation of possible outbreaks. The ICT develops and revises infection control policy in their hospital. The work volume is too great for a single individual; thus, the ICT is essential for infection control activities.

Surveillance

Until the mid-1990s, interest in surveillance was very low. Only a small group of people who learned and were inspired by US infection control practices conducted surveillance at the hospital level. In 1998, the JSEI established a surveillance system in Japan, the Japanese Nosocomial Infections Surveillance (JNIS). The system was based on the US National Nosocomial Infection Surveillance (NNIS) system with some modifications and initially focused on SSIs. Eight hospitals participated initially, and more hospitals joined later. Currently, approximately 50 hospitals send their data to the system each year. Aggregated data are analyzed, and feedback is sent to the hospitals by emails that provide detailed data on the respective hospitals and the overall system. Aggregated data are presented at US meetings such as those of the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Discussion of data collection, analysis, and other issues related to SSI surveillance takes place at an SSI surveillance meeting, which has been held twice a year since 2002.

The Japanese Ministry of Health, Labour and Welfare (MHLW) administers another surveillance system, the JApan Nosocomial Infection Surveillance (JANIS), which was developed as a public health service and mainly focuses on collecting data on nosocomial infection by multidrug-resistant organisms (MDROs) such as MRSA, although the system was based on similar components in the NNIS system. The main elements are a laboratory component and a hospital-wide MDRO component. For the intensive care unit (ICU) component, hospitals collect device-associated infection data. The denominator of the infection rate was initially device-days but was changed to patient-days of the targeted ICUs. This change was based on data showing that the infection rate was similar using device-days and patient-days as the denominator in this surveillance system.[2] However, this modification made Japanese data incompatible with data from other countries, such as the United States, where device-days remains the denominator in this form of surveillance.

The JNIS system was renamed JHAIS (Japanese Healthcare-associated Infections Surveillance) in 2008 and began monitoring device-associated infection (ie, central line-associated BSI, catheter-associated UTI, and ventilator-associated pneumonia) as well. Approximately 20 hospitals participate in this system.

The professional community

JSEI was established in 1986 with only 231 members. In 2011, only 25 years after its founding, it has more than 6000 members. Among the 3984 members who disclose their occupation to the Society, most are registered nurses (42%). The proportions of physicians, pharmacists, and clinical laboratory technologists are 19%, 16%, and 8%, respectively. Recent annual meetings have attracted more than 5000 attendees, which exceeds the attendance of any of the 3 major Western healthcare epidemiology organizations, ie, the Association for Professionals in Infection Control and Epidemiology (APIC), SHEA in the United States, and the Hospital Infection Society (HIS) in the United Kingdom.

JSEI activity includes an annual meeting (in February) and publication of a journal (6 issues per year). It also has several committees, including editorial, educational, and international committees. The first training course for healthcare epidemiology was held in 2009 by the educational committee.

The organization responsible for the diagnosis and treatment of infectious diseases in Japan is the Japanese Society for Infectious Diseases. The Japanese Society for Chemotherapy oversees antimicrobials, and the Japanese Society for Microbiology is responsible for clinical microbiology.

Certification

Japan has 4 specialized certifications in infection control for different occupations.

Certified Nurse for Infection Control (CNIC)

The Japanese Nursing Association accredits this certification, which requires 6 months of intensive study at a designated educational institution in Japan and a passing grade on the certification examination. Eighteen nurses were certified after the first examination, in 2001, and as of July 2011 there are 1364 CNICs in Japan. In 2011, 10 institutions offer education for this certification. The curriculum in infection control is comprehensive and includes surveillance, practice, microbiology, and planning of infection control programs in healthcare facilities.

Infection Control Doctor (ICD)

The Committee for the ICD accredits this certification. Candidates must be a medical doctor (MD), or have a PhD in a healthcare field, for more than 5 years, be a member of a society approved by the Committee, have experience in infection control in a healthcare setting, and have proof of participation in educational meetings or scientific conferences. An examination is not necessary for certification. A certified ICD is expected to lead infection control activities in a hospital, with the support of the members from each healthcare profession (ie, physicians, nurses, pharmacists, and microbiologists) and administrative staff. In the first year, 2000, a total of 832 persons were certified; as of January 2010, 6815 have been certified.

Board-Certified Infection Control Pharmacy Specialist (BCICPS)

The Japanese Society of Hospital Pharmacists (JSHP) accredits BCICPS certification. Candidates for certification

must: be a licensed pharmacist, be a member of the JSHP, be a certified ICD, be named as an author in 3 abstracts for designated scientific conferences in the pharmacy field (and as first author in at least 1), have 2 publications in the field of infection control (and as first author in at least 1), and pass an examination. Forty pharmacists were certified in the first year, 2005, and 219 have been certified as of April 2011.

This certification system was aimed to designate pharmacists who are routinely involved in infection control activities, provide instruction to the next generation of pharmacists in infection control, and conduct research in the field. However, because the requirements for certification are very high, a new category was created in 2008, namely, Board-Certified Pharmacist in Infection Control (BCPIC), which is less advanced than the BCICPS. Ninety-four pharmacists were certified in the first year, and 364 have been certified as of October 2010.

Infection Control Microbiological Technologist (ICMT)

The Japanese Society for Clinical Microbiology (JSCM) accredits ICMT certification. Candidates must be a clinical technologist and a member of the JSCM and be active in infection control practices in a healthcare setting, among other requirements. In the first year, 2006, 253 technologists were certified, and 411 have been certified as of January 2011.

Public organizations and agencies

The Centers for Disease Control and Prevention (CDC) in the United States and the Health Protection Agency (HPA) in the United Kingdom are 2 of the most famous organizations in the world. At the national level in Japan, infection control in healthcare settings is under the jurisdiction of the Medical Service Division, Health Policy Bureau, MHLW. This division has technical and administrative officers whose main role is to manage rules and regulations, such as laws and bylaws. The National Institute of Infectious Diseases (NIID) is expected to support the MHLW both technically and scientifically and is equivalent to the US CDC and HPA. NIID is designed to function as a research laboratory for various pathogens, a reference laboratory for nationwide research on microbiology, and a laboratory for investigation of numerous drugs, blood products, and vaccines. There is no designated section in NIID for infection control in healthcare settings. It is therefore impossible to create official guidelines for healthcare-associated infections (HAIs) or lead HAI surveillance. Regarding epidemiologic investigation of HAIs, the Field Epidemiology Training Program (FETP) in the NIID investigated approximately 10 HAI outbreaks caused by pathogens such as vancomycin-resistant *Enterococcus*, multidrug-resistant *Pseudomonas aeruginosa* (MDRP), and *Clostridium difficile*. The FETP is a 2-year intensive course in field epidemiology.

Laws and rules

The Japanese healthcare system is regulated by the Medical Service Act (*Iryou-hou* in Japanese). In the 2007 version of the Act, healthcare safety is a primary goal for every hospital and clinic. In addition, in the Ordinance for Enforcement of the Medical Service Act (*Iryou-hou shikou kisoku*), prevention of HAIs is expressly included as part of healthcare safety. Health centers must establish an HAI policy in each facility, form a committee for HAI prevention, educate employees, and take part in HAI surveillance and reporting. It also requires advanced treatment hospitals (ATHs) and teaching hospitals to establish an HAI prevention department and designate a person(s) to staff the department. This regulation is mandatory, and penalties may apply in cases of intentional violations.

Reimbursement

The costs of HAI prevention are paid by hospitals, and, until recently, no reimbursement was given for superior HAI prevention practices. In addition, reimbursement for treatment of HAIs was equal to that of the respective infectious disease, which meant that there was no incentive to implement better HAI prevention practices.

In 1996, hospitals with good infection control practices began to receive an additional reimbursement of 50 yen (0.6 USD) per patient per day. The requirements for this additional reimbursement were minimal, so, within 2 years, 70% of Japanese hospitals had applied for it. In 2000, this reimbursement policy was discontinued and replaced with a new system of penalties for hospitals with insufficient infection control practices. This policy was also discontinued, in 2006.

In 2010, as part of healthcare safety, a reimbursement of 1000 yen (12 USD) per patient per admission was introduced. Before this policy was begun, most hospitals in Japan did not give physicians or CNICs a designated time period for infection control. If a hospital wishes to receive the reimbursement, it must pay the annual cost for the designated work hours for a physician (a salary of 0.5 full-time equivalents [FTEs], about 4 million yen) and a nurse (a salary of 0.8 FTEs, about 4 million yen), which equals approximately 8 million yen (Table 2). This is roughly equal to the amount that would be reimbursed for a hospital with 300 beds and an average length of stay of 15 days. Therefore, generally speaking, this incentive is attractive for hospitals with more than 300 beds but not for those with fewer beds. There are no data on the number of hospitals that have applied for this reimbursement.

Guidelines

Due to the situation regarding the public organization that oversees infection control, there is no official guideline published by the government. The MHLW has a research fund that it is distributed to selected research groups, which create documents similar to guidelines. These are usually prepared based on guidelines published by the CDC with

Table 2. Requirements for additional reimbursement (April 2010)

Division of infection control
Infection control team in division, consisting of the following:
(1) At least 1 infection control nurse with designated training experience and at least 1 infection control physician with designated infection control experience
(1 with >80% FTE; the other with >50% FTE)
(2) Infection control pharmacist and infection control microbiology technologist, both with experience in infection control
Policy regarding duties of infection control team
Hospital infection control policy must be distributed to all wards and divisions
Educational lecture for all staff, at least twice a year
Antimicrobial stewardship program

some modifications. Furthermore, research in this field is limited in Japan, so the country has very few data of its own. Therefore, documents published by these groups are more like expert opinions.

Scientific societies and professional organizations have important roles to play. In 1990, the JSEI published *A Guide for the Prevention of Hospital Infection*, the first publication of its kind in Japan. In 2001, the Japanese Nursing Association published *A Guidebook for Infection Control*, which was followed by the *Guideline For Hospital Infection Control* and the *ICD Textbook*, published by the Committee for National University Hospitals and the Committee for the ICD, respectively. These publications are updated regularly and are widely used in the Japanese infection control community. In addition, numerous commercial-based documents have been published.

Education

In general, infection control personnel in hospitals are certified, experienced, and knowledgeable and are responsible for teaching infection control practices to other healthcare workers. The Health Service Act mandates education sessions in every hospital and clinic, and a supplemental document published by the MLHW specifies that the sessions should be held more than twice a year. To fulfill this requirement, large hospitals hold education sessions within their facility; however, in smaller hospitals and clinics, this might be difficult. As an alternative, they can subsidize healthcare workers to attend seminars hosted by a local government, society, university hospital, or even a private company. There are numerous seminars on infection control throughout Japan. However, because many are held in large metropolitan areas, their geographical distribution is uneven.

Education for certification in infection control is provided by each accrediting body, but there is no education in healthcare epidemiology in Japan. Many universities regard infection control as a clinical practice rather than a field of study and do not have a department of infection control in their (graduate) school of medicine. As a result, healthcare epidemiology is made light of by the medical community and is not regarded as fundamental to infection control. The JSEI held its first healthcare epidemiology training course in Japan in 2009. About 50 infection control professionals attended the session, and it will now be held annually. This might increase the number of professionals who are able to conduct high-quality studies in infection control.

Research

Few publications by Japanese researchers in infection control have been published in English. There are several possible reasons for this, namely (1) not many universities have a department of infection control, which means that even in university hospitals, there is inadequate staffing, funding, and time allocated for research, (2) Japan's socialized health insurance system limits staffing and funding resources for infection control, (3) there are few educational opportunities in healthcare epidemiology, and (4) although research groups receive funding from the MHLW, this funding is closely related to government policy, and the areas of interest are limited.

The media and the general public

The media often react hysterically to clusters or outbreaks of multidrug-resistant organisms. For example, in September 2010, a university hospital in Tokyo had an outbreak of multidrug-resistant *Acinetobacter baumannii*. The media reported the case and related issues in the headlines for 1 week and then suddenly stopped covering it, presumably because they had become disinterested. The reporting during that week was full of sensationalism and lacked a scientific understanding of the situation. Indeed, the outbreak was investigated by a scientific body and the police.[3] The Japanese authorities have a tendency to investigate events (and not only HAIs) from a punitive rather than a scientific perspective, and the general public has the same tendency. After the events described above, there were many anonymous online comments criticizing the university hospital.

Challenges in infection control practices in healthcare settings

The most serious fundamental problem in infection control is the lack of personnel assigned to infection control in hospitals. Under the socialized medical insurance system, hospitals tend to assign healthcare personnel to areas that produce direct revenue, and infection control is not such an area.

In the United States, the standard ratio of infection control personnel is about 1 per 250 beds. The figure among hospitals participating in the National Nosocomial Infections Surveillance system is about 1 per 115 beds.[4] However, there are limited data on personnel assigned to infection control. The MHLW conducted a survey of advanced treatment hospitals (*Tokutei Kinou Byouin*). There are 83 ATHs in

Japan, and most are affiliated with faculties of medicine. According to the *Disclosure of Practice Report From ATHs*, which was published in 2009,[5] there were 159 designated personnel in 83 ATHs that had a total of 72 178 beds. This means that, on average, there is 1 infection control specialist per 454 beds, which is far lower than the standard ratio in the United States. Among the 83 ATHs, only 10 had more than 1 specialist per 250 beds. ATHs with more than 1000 beds often had only 1 infection control specialist.

Another survey of healthcare facilities[6] found that, as of October 2008, there were 468 hospitals with more than 500 beds. Among them, 204 (44%) had 1 or more infection control specialist with an FTE of 0.8 or greater (ie, a person almost completely concerned with infection control), and 253 (54%) had 1 or more personnel with an FTE of between 0.2 and 0.8. Eleven hospitals (2%) had no designated personnel. These data show that even in ATHs, which have the resources to assign personnel working in the faculty of medicine (ie, people not employed by the hospital) to infection control in the hospital, few personnel were actually assigned to infection control. The situation in non-AHTs is likely to be much worse in terms of human resources.

Beginning in April 2010, a new reimbursement system came into effect. This gives a hospital about 12 USD per patient if it fulfils MHLW requirements regarding infection control, which mandate an infection control nurse and infection control physician (one at 0.5 FTE; the other at 0.8 FTE), a pharmacist (0.5 FTE), and a microbiologist (0.5 FTE). This revision favors larger hospitals, which are better able to fulfill the requirement and thus receive the reimbursement. Considerable changes in infection control practices are anticipated.

Summary

The present author's experience visiting many US hospitals suggests that infection control practices in Japanese hospitals are as good as those in US hospitals. However, Japan is far behind in terms of research and data collection. More attention and funding are therefore required in these areas.

ACKNOWLEDGMENT

Conflicts of interest: None declared.

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Epidemiol. Infect. (2011), **139**, 317–325. © Cambridge University Press 2010
doi:10.1017/S0950268810000981

A norovirus outbreak associated with environmental contamination at a hotel

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(*Accepted 5 April 2010; first published online 30 April 2010*)

SUMMARY

In December 2006, an outbreak of gastroenteritis occurred involving 372 guests and 72 employees at a hotel after a guest vomited in corridors on the third (F3) and 25th (F25) floors. Norovirus with identical genotype was confirmed by real-time reverse transcription–polymerase chain reaction in faecal samples from guest cases and employees. Spread of the outbreak on F25 was compared with that on F3. The attack rate in the guests who visited F25 alone (15·0%, 106/708 guests) was significantly higher than in those who visited F3 alone (3·5%, 163/4710 guests) (relative risk 4·3, 95% confidence interval 3·4–5·5, $P<0·001$). The outbreak on F3 ended within 2 days, while that on F25 extended over 7 days. The environmental ratios of F3 to F25 were 7·4 for volume, 6·9 for floor area and 7·6 for ventilation rate. This outbreak suggests that environmental differences can affect the propagation and persistence of a norovirus outbreak following environmental contamination.

Key words: Environmental management, gastroenteritis, infectious disease control, infectious disease epidemiology, Norwalk agent and related viruses.

INTRODUCTION

Noroviruses are major causes of outbreaks of viral gastroenteritis. They are transmitted primarily through the faecal–oral route, either by direct person-to-person spread or by consumption of contaminated food or water [1–4]. In a closed environment they may be spread by airborne viral particles originating from vomitus [5–12]. A review of the attack rates (AR) in relation to the location of vomit and vomit-contaminated formites suggests that inhaling and swallowing dust from desiccated vomit and faeces are important factors [12]. Noroviruses have been detected in environmental swabs by reverse transcription–polymerase chain reaction (RT–PCR) [13, 14]. This suggests that environmental contamination is a major source of norovirus outbreaks in closed or semi-closed settings [13–17]. However, it is not sufficiently known how the viruses spread in buildings or what affects the development of an outbreak.

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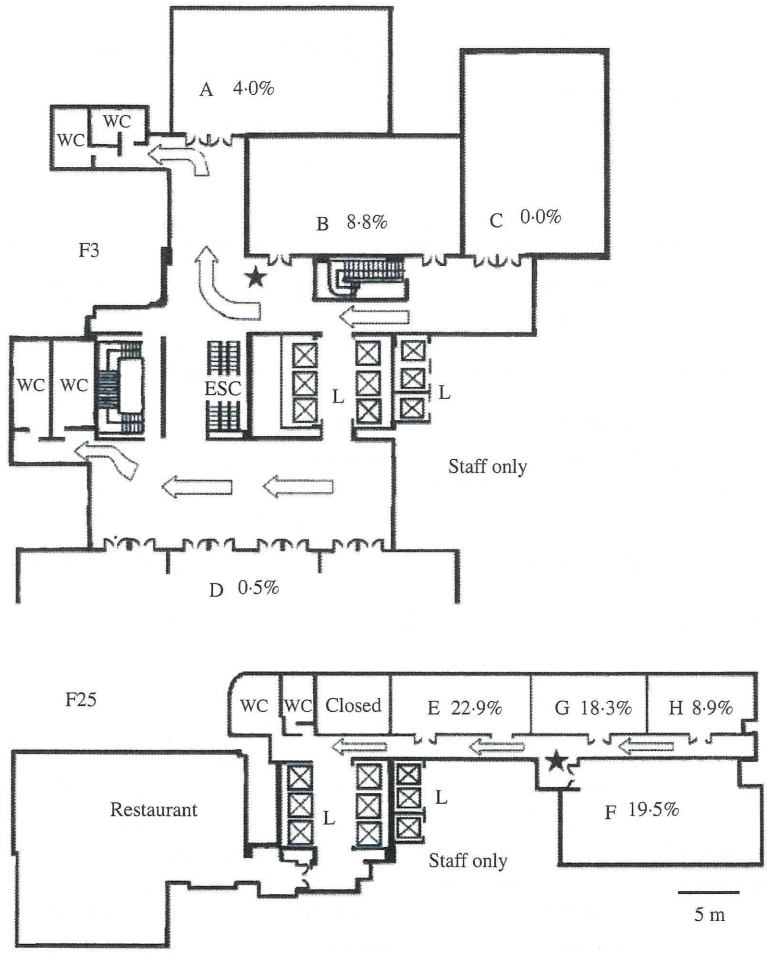

Fig. 1. Relationship between the spatial distribution of gastroenteritis in the hotel guests and the vomit locations on the two different floors at a hotel. L, Lift; ESC, escalator; WC (water closet/toilet). The black stars indicate the locations on the third floor (F3) and the 25th floor (F25) where a guest vomited before mid-day on 2 December 2006. The figures indicate the attack rate in the guests who visited the hall between 2 and 10 December. 'Staff only' areas are marked. Arrows indicate the approximate air current produced by the air-conditioning system. The air supplies were installed on the ceiling board of the corridor, and the air vents of the air conditioners were installed in the toilets of each floor.

We encountered an outbreak of norovirus in a hotel that occurred following vomiting incidents by a guest on two floors with different environments. This outbreak is an example of how environmental factors, such as space or ventilation, can affect the propagation and persistence of norovirus outbreaks following environmental contamination.

METHODS

Epidemiological investigation

Description of the outbreak

On 5 December 2006, a hotel notified the Public Health Centre that several party guests had complained of developing nausea, vomiting and diarrhoea 1–2 days following their visit to the hotel. Disinfection of restaurants, kitchens, and toilets in the staff area was performed on and after 5 December. The main kitchen on floor 3 (F3) and the restaurant on floor 25 (F25) were closed between 6 and 13 December. On 7 December, we obtained some information from various employees. Before mid-day on 2 December a female guest (index case) had vomited in the corridor in front of hall B (waiting room) on F3 (Fig. 1). She then went to a wedding reception on F25 and vomited again in the corridor near to the entrance of hall F. A waiter on F25 (employee B) attended to her and cleaned up the vomit using table napkins, which he discarded in a

waste bin within the staff area on F25. The waste was incinerated that night. The guest used toilets on both the floors after vomiting and left the hotel without returning to the reception. A cleaner (employee A) quickly removed the carpet stains on F3 and then F25 using a brush and soapsuds while wearing rubber gloves. On 7 December, we conducted a thorough disinfection of the environment including the vomit locations and toilets for guests. The last date of onset among the guests was 10 December, then the outbreak ended.

Environmental investigation and meal service in the hotel

This hotel had 25 floors. Floors 2, 3, 4 and 25 contained halls for receptions. Halls on the two floors were often jointly utilized for one wedding reception. Floors 5 to 24 contained 815 bedrooms. Floor 3 had lifts, an escalator to the ground floor (F1) and a staircase to floor 4 (F4), but F25 had only lifts except for an emergency staircase. All the floors of the corridors and halls were covered with carpet. The corridor on each floor was cleaned every night using vacuum cleaners that were also used in the halls. Separate toilets and lifts were provided for guests and employees. Toilet facilities for guests had automatic taps and the entrance did not have a door. The corridors and halls were separately ventilated. The volume of the corridor on F3 was 2128 m^3 (floor area 733·7 m^2, ceiling height 2·9 m, width 7·2 m) and that on F25 was 288 m^3 (floor area 106·7 m^2, ceiling height 2·7 m, width 2 m). The corridor of F3 had eight air outlets within the toilets and 20 air inlets on the ceiling board, whereas the corridor of F25 had three air outlets and one air inlet. Ventilation volumes of F3 corridor were 11 860 $m^3\ h^{-1}$ and, of F25, 210 $m^3\ h^{-1}$. The ventilation rate, i.e. the ratio of the air volume entering the room per hour to the room volume, equalled the exhaust airflow (the ventilation volume) divided by the room volume [18]. The ventilation rate for the corridor of F3 was 5·6 h^{-1} and that of F25 was 0·7 h^{-1}. The ratios of F3 to F25 were 7·4 for volume, 6·9 for floor area, and 7·6 for ventilation rate.

All the meals for parties at halls on floors 2, 3, 4 and 25 were prepared within the main kitchen on F3 and supplied to each floor via lifts used only by staff. Restaurants on F1, floor 2 (F2) and F25 prepared meals for their own guests. A cafeteria for staff was in the basement (B1).

Hotel guests

The hotel reported the number of guests who complained of gastrointestinal symptoms, the date of their visit to the hotel, and the floor number and the hall or restaurant that they used. Any guest who developed acute gastroenteritis (vomiting and/or diarrhoea) within 1–3 days of their visit to the hotel between 2 and 10 December 2006 was defined as a guest case. Lists of the party schedules, including the number of participants, the hall used, the timetable and the menus were sought from the hotel. Many guests who attended the wedding reception or year-end party did not stay overnight. For guest cases, we administered a questionnaire, including details of age, sex, food history and onset time and duration of symptoms. We failed to ask them which toilets they had used and the routes they had taken within the hotel.

To determine the source of infection, party guests were classified into two groups: those with vomiting who had accessed F3 and/or F25, and those without vomiting who had accessed F2 and/or F4. To study the environmental factors that might have affected the extent and progress of the outbreak, the guests who had accessed F3 and/or F25 were classified into three groups (Table 1): those who had accessed F3 alone but not F25; those who had accessed F25 alone but not F3, and those (including the index case) who had accessed both floors. The attack rate (AR) in the guests was calculated for each group. Furthermore, the AR in guests who had accessed each hall was also calculated (Fig. 1). The guests who visited hall E on F25 included those from hall D on F3. The guests who visited hall F on F25 included those from halls A or B on F3 (Fig. 1, Table 1). To address the possible date of exposure to virus to the floor, we used the date of visit to the hotel (not the onset) as x axes in the epidemic curves (Figs 2, 3).

Hotel employees

The hotel provided us with the number of employees who had worked between 2 and 10 December 2006, details of their sections and whether or not they had become ill. Those who had worked during this period and developed nausea, diarrhoea and vomiting were defined as employee cases. The hotel also reported whether these cases had consumed any meals from the staff cafeteria.

Table 1. *Attack rate and relative risk on the different floors with a vomiting incident between 2 and 10 December 2006*

Guests visited floor	F3 alone				Both F3 & F25		F25 alone	
Ill guests/total guests	163/4710				82/267		106/708	
Attack rate (%)	3·5				30·7*		15·0*	
Relative risk (95% CI)	1				8·9 (7·0–11·2)		4·3 (3·4–5·5)	
Guests visited hall	A	B	C	D	E†	F†	G	H
Ill guests/total guests	18/446	134/1530	0/536	11/2198	70/306	83/426	26/142	9/101
Attack rate (%)	4·0	8·8	0·0	0·5	22·9	19·5	18·3	8·9
Relative risk (95% CI)	1 (1·3–3·5)	2·2 (–)	0·0 (0·06–0·26)	0·12	5·7 (3·4–9·3)	4·8 (3·0–7·9)	4·5 (2·6–8·0)	2·6 (1·4–4·9)

CI, Confidence interval.
* Significantly different from the attack rate in the guests who visited floor 3 (F3) alone ($P<0·001$, χ^2 test).
† The hall includes the guests who visited both floors 3 and 25 (Both F3 & F25). Of the 30 guests who visited both halls D and E, eight became ill. Of the 237 guests who visited halls A or B and hall F, 74 became ill.

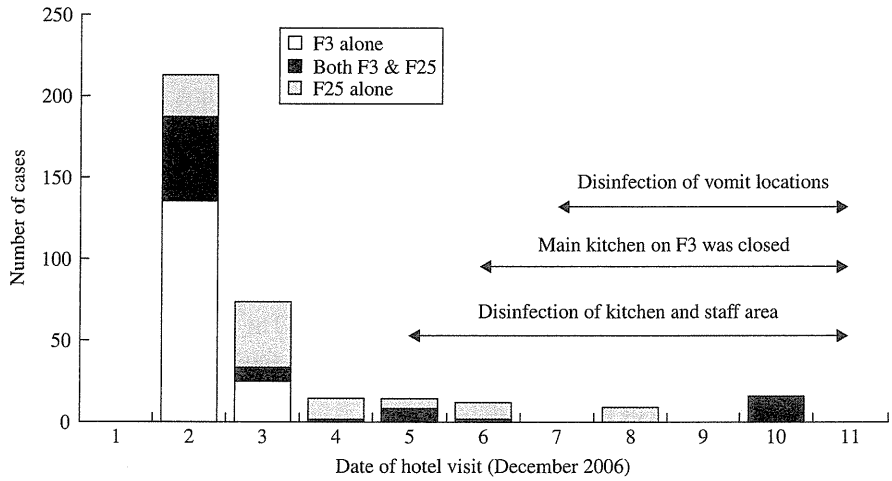

Fig. 2. Epidemic curves for the number of the gastroenteritis cases among the party guests who visited the hotel between 2 and 10 December 2006.

Laboratory investigations

Stool specimens from 92/372 guest cases and from 98 employees (including 85 without symptoms) were examined for 11 bacteria, including *Salmonella* and *Shigella* and norovirus. Of the 98 employees, 79 were food handlers, 18 were waiters and one was a cleaner (Table 2). Of the 79 food handlers, 60 worked in the main kitchen, 10 in the restaurant on F25, and nine in the staff cafeteria. Environmental swabs from 44 positions in the main kitchen and 27 samples from the residual food served at parties on 2 and 3 December 2006 were examined for bacteria. Eight cold samples, including sliced raw fish and sweets, were examined for norovirus. However, we did not collect environmental swabs [13–17] for norovirus from the corridor area with a vomiting incident (carpets, female toilets and lift buttons) or from the dust of the vacuum

Table 2. *Results of the real time-PCR performed on the 98 employees*

PCR	Clinical symptoms ...	Positive*		Negative		Total
		Positive	Negative	Positive	Negative	
Food handlers (AR 5·1 %)						
Party foods (main kitchen)		0	0	3	57	60
Restaurant (F25) for guests		4	0	0	6	10
Cafeteria (B1) for stuff		0	0	1	8	9
Waiters (AR 50·0 %)		5	4	2	7	18
Cleaner		0	0	0	1	1
Total		9†	4	6†‡	79	98

PCR, Polymerase chain reaction; AR, attack rate.
* Onsets of clinical symptoms of employees were between 3 and 10 December 2006.
† 100 % homologous to the pilot strain among the guest cases on F3.
‡ The rate of asymptomatic infection was estimated to be 31·6 % (6/19).

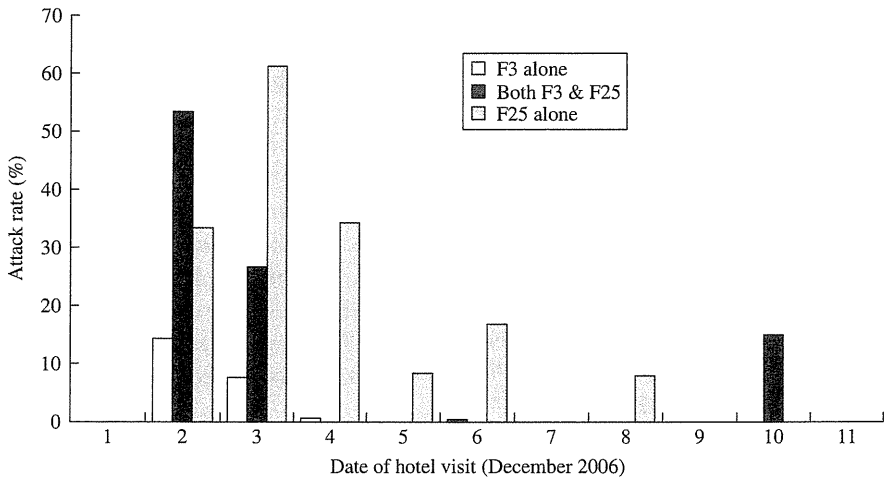

Fig. 3. Attack rate in the party guests who visited different floors with a vomiting incident between 2 and 10 December 2006.

cleaners, because we were technically unable to detect norovirus in environmental swabs. To detect noroviruses from faecal and food samples, a real-time RT–PCR was performed according to the slightly modified protocol of Kageyama *et al.* [19, 20].

The stool specimens from 61/92 guest cases and from all the 98 employees were examined at the Division of Virology, Tokyo Metropolitan Institute of Public Health. The remaining stool specimens from 31 guest cases were examined at other institutes. The employee who tested positive for norovirus but had no clinical symptoms was defined as an asymptomatic employee.

A sequence analysis of the conserved capsid domain (G2SKF/G2SKR) of genogroup II norovirus (GII NV) was further performed on all 59 GII NV-positive specimens (from 44 guest cases and 15 employees). Cycle sequencing was performed with PCR products using a thermal cycler (GeneAmp PCR system 9700) and BigDye Terminator v. 1.1 Cycle Sequencing kit (Applied Biosystems, USA), and the sequences were determined with the ABI Prism 3130 Genetic Analyser. The first GII NV-positive specimen was used as a pilot strain for homology test because no material was obtained from the index case. Phylogenetical analysis of the virus was performed with a method reported by Katayama *et al.* [21]. Sequencing of a hypervariable region encoding the P2 domain [22] was tried on one GII NV-positive specimen.

Statistical analysis

Relative risks (RR) with 95 % confidence intervals (CI) were calculated for the AR in the guests who had visited F25 compared to those who had visited F3

alone, and for the AR in each hall compared to hall A. χ^2 tests were performed on numbers of ill or not ill guests on each floor.

RESULTS

Epidemiological investigation

Hotel guest cases

Of the guests, 372 (including the index case) met the case definition for this outbreak. Of these, one stayed at the hotel and 18 had dinner at the restaurant (four on F1, three on F2, and 11 on F25) but did not attend a party in the halls. Denominators for these cases were unknown. The remaining 353 guest cases were included in the 6846 guests who had mostly attended wedding receptions or year-end parties in the halls. Two (0·2%) of the 1161 guests who had visited F2 and/or F4 (floors without a vomiting incident), and 351 (6·2%) of the 5685 guests who had visited F3 and/or F25 (floors with a vomiting incident), met the case definition. There was a significant difference between the AR on F3 and/or F25 and that on F2 and/or F4 (RR 35·8, 95% CI 8·9–143·7, $P<0·001$).

One hundred sixty-three (3·5%) of 4710 guests on F3 alone, 106 (15·0%) of 708 guests on F25 alone, and 82 (30·7%) of 267 guests on both F3 and F25 met the case definition. Of 163 cases on F3 alone, 161 (99%) occurred in the first 2 days of the outbreak, whereas the onset in cases on F25 alone ranged over 7 days and, on both floors, over 9 days (Fig. 2). The AR in the guests on F25 alone (RR 4·3, 95% CI 3·4–5·5, $P<0·001$) and the guests on both F3 and F25 (RR 8·9, 95% CI 7·0–11·2, $P<0·001$) were significantly higher than those in the guests on F3 alone (Table 1).

There were regional differences in the AR in the guests who had visited halls A, B, C or D on F3, and halls E, F, G or H on F25 (Table 1). The AR in those who had visited hall B was significantly higher than that in those who had visited halls A, C or D. On the other hand, the AR in the guests who had visited hall E was highest and significantly different from that of the guests who had visited hall H. Eight (26·7%) of the 30 guests who visited both hall D on F3 and hall E on F25 became ill. Seventy-four (31·2%) of the 237 guests who visited both halls A or B and hall F became ill (Table 1).

Of 372 guest cases, 199 (response rate 53%) including the index case, returned a completed questionnaire. The index case had not consumed any hotel meals. The interval between the beginning of the party and the onset of gastroenteritis was defined as the incubation period. The first onset of guest cases other than the index case was at midnight on 2 December 2006, at the guest's home. The mean incubation period of 198 guest cases, excluding the index case, was $35·9 \pm 15·5$ (mean $\pm$ S.D.) h. Although the party guests who visited floors 2, 3, 4 or 25 had consumed various meals made by the same food handlers in the main kitchen, the restaurant guests had not eaten any party foods.

Hotel employee cases

Of 838 employees, 72 (AR 8·6%) met the case definition for this outbreak. No staff in the main kitchen and the restaurants had reported any illness on or prior to 2 December 2006. The first onset in an employee was on 3 December. Employee A did not become ill, but employee B became ill on 4 December, 37 h after contact with the vomit. The AR of employees was highest in those in the restaurant on F25 (29·7%, 11/37). The AR in waiters was 11·5% (19/165) and in food handlers 3·7% (6/163). The six food handlers became ill between 4 and 7 December – four of them had worked in the restaurant on F25 (Table 2) and the others in the restaurant on F1 or F2. Of 72 employee cases, 14 (19·4%) reported that they had not consumed any meals in the staff cafeteria.

Laboratory results

GII norovirus was detected by real-time RT–PCR in stool specimens obtained from 71 (77·2%) of 92 guest cases, nine (69·2%) of 13 employee cases, and six (7·1%) of 85 employees without symptoms. The nine symptomatic employees included four food handlers in the restaurant on F25 and five waiters, including employee B (Table 2). Employee A (cleaner) tested negative. The six asymptomatic employees included three food handlers in the main kitchen, another in the staff cafeteria, and two waiters (Table 2). The food handlers in the main kitchen and restaurant on F25 had not consumed food and drink in the staff cafeteria. If the total number of symptomatic and asymptomatic employees is regarded as the total number of infections, the AR for asymptomatic infection is estimated as 31·6% (6/13).

The results of homology test for the PCR products from 58/59 GII NV-positive specimens (from 44 guest cases and 15 employees), showed 100% homology

with the pilot strain (a guest on F3) except for one case (a guest on F25). Forty-four GII NV-positive guest cases included 38 party guests in halls on F3 and/or F25 and six guests at the restaurant on F25. Sequence analysis of the conserved capsid domain (G2SKF/G2SKR) of the virus revealed that the genotype from 58/59 GII NV patients was GII/4 EUb DenHaag/06/NL and another genotype from only one case was GII/12 SaU1/04/JP. The result of sequencing the hypervariable region encoding the P2 domain for a GII NV-positive specimen showed that the genotype was also GII/4 EUb DenHaag/06/NL. No norovirus was detected in the cold foods and no pathogenic bacteria were detected in stool specimens.

DISCUSSION

This paper describes an explosive outbreak of norovirus gastroenteritis following two vomiting incidents by an index case on two different floors ('affected floors') in a hotel. No specimen was obtained from the index case. We compared the AR in the affected and unaffected floors.

The AR and number of gastroenteritis cases in the guests who visited halls on the affected floors (F3 and F25) were overwhelmingly higher than those on the unaffected floors (F2 and F4). Consequently, the two locations with a vomiting incident on the two floors were considered as major infection sources of this outbreak. The strain GII/4 norovirus was isolated from most of the guest cases and the employees. The development of disease and transmission was facilitated by the low infectious dose (i.e. <100 viral particles) and the resistance of these viruses to various environments and the standard cleaning and disinfection agents [2, 3, 8, 10]. Infection in the guest cases spread widely on the two floors after cleaning the vomit locations (Figs 1, 2). We believe that environmental contamination [13–17] played a significant role in sustaining the outbreak, although we were unable to identify the virus in the environmental swabs.

Norovirus with identical genotype was confirmed by real-time RT–PCR in all the positive specimens from 43 guest cases and 15 employees, including four asymptomatic food handlers, except for one guest case on F25. These facts suggest that this outbreak was caused by a single infectious source. The guest with a different virus genotype was probably infected elsewhere.

Although many party guests on the different floors had eaten the party foods made by the same food handlers (including asymptomatic food handlers) within the main kitchen, there was a clear difference between the AR on the affected and unaffected floors. Nevertheless, the 18 guests from the restaurants on F1, F2 and F25 had not had any party foods but still became ill. Furthermore, the genotype of norovirus detected from them was identical to that from the party guests. The food handlers in the main kitchen and restaurant on F25 had not eaten the meals made by an asymptomatic food handler in the staff cafeteria, but the genotypes of norovirus detected from all food handlers was identical (Table 2). Therefore, the cause of this outbreak could not have been a specific menu nor any foods contaminated by asymptomatic food handlers. However, a few of the guest cases who complained of gastroenteritis could have been infected with the same genotype of the virus outside the hotel, because strain GII/4 has been predominant as the cause of outbreaks worldwide since the mid-1990s. To clarify this point, we could have sequenced the hypervariable region encoding the P2 domain [22] but were unable to do so except for one case.

We studied the development of outbreaks on the two affected floors in detail. The main observation in this outbreak was that the AR in the guests who visited F25 was significantly higher than that in those who visited only F3. Furthermore, the outbreak on F3 was rapidly terminated, while the onset of gastroenteritis in the guests who visited F25 spread over 9 days (Figs 2, 3). This suggests that some environmental differences in the two floors had caused this. We hypothesized that the differences in the structure of the building, floor area, volume and ventilation rate of the corridors were the important environmental factors that led to the difference in the durations of infection. At the first day of the outbreak, the amount of noroviruses involved in the vomit residue on both the floors is unknown, but it was clearly sufficient to cause the total number of guest cases on F3 alone ($n=163$), which was comparable to that on F25 ($n=188$).

To explain how norovirus spread in the buildings, we considered two possibilities. First, viruses may be carried by contact with vomit residue or dust. There is a report that carpets may harbour viable virus for at least 12 days and that the virus is not removed by routine vacuum cleaning [23]. People who walked on vomit residue might have carried the viruses

elsewhere on shoes or long dresses. The density of viruses on the floor surface of corridor F3 would more rapidly decrease than that on F25, because more people walked through this corridor, where the floor area was 6·9 times larger than that on F25. The highest AR was in hall B (Fig. 1) on F3; this could be explained by exposure to the directly contaminated area in front of the hall. In contrast, the density of virus on the surface of corridor F25 may have been kept high because of its narrowness. Hall E showed the highest AR (Fig. 1) on F25, but could be visited without passing through the vomit area. The corridor around hall E could have been most contaminated by people walking through the vomit area from halls F, G and H to the toilet or lift.

Second, viruses might be spread as airborne dust. This possibility would be supported by a study that examined 144 environmental swabs using nested RT–PCR. We showed that the highest proportion of positive samples were detected in directly contaminated carpets, but noroviruses were also detected from environmental swabs in elevated sites, such as mantelpieces or light fittings, unlikely to have been touched. This suggests that airborne dissemination occurred [14]. The vomit residue, desiccated in the dry environment of the hotel, would be disseminated as dust, and possibly into the air by people traffic or vacuum cleaning. The airborne dust containing noroviruses might have been moved by airflow to the toilets with outlet of air. The ratios of F3 to F25 for volume and ventilation rate of the corridor are 7·4 and 7·6, respectively. Therefore, the airborne viruses in the corridor air of F3 could have been rapidly diluted with the larger air volume and higher ventilation rate [18]. This hypothesis could also explain the rapid decline of the outbreak on F3 and the highest AR in hall E on F25.

Although the employees had been working in the hotel for a long period, the AR in food handlers in the main kitchen on F3 was remarkably lower than that in waiters. It is speculated that the waiters often accessed the contaminated corridors, while the food handlers were working within the kitchen area away from the corridors on F3 and frequently washed their hands. However, the staff area on F25 might have been contaminated by employee B, who was in direct contact when cleaning up the vomit using napkins, leading to the higher AR in the employees in restaurant F25. The female toilets and lift buttons could also have been directly contaminated by the hands of the index case. From experimentally contaminated surfaces, noroviruses can be readily transferred to other fomites via hands [24]. However, there was no gender difference in the guest cases. The lift button to F25 might have been contaminated by the index case and pushed by many guests going to F25, but we did not investigate this.

This outbreak demonstrates that environmental factors such as floor area, volume and air ventilation in the building can affect the extent and progress in norovirus outbreaks with environmental contamination. This outbreak also taught us that the vomit and the area around it should be thoroughly disinfected before desiccation, as brushing and vacuum cleaning, especially in a closed or semi-closed setting with carpeted floors, releases viruses into the air.

ACKNOWLEDGEMENTS

We thank Yukinao Hayashi and the staff of the Division of Virology, Tokyo Metropolitan Institute of Public Health for their assistance in laboratory data collection and management of this paper.

DECLARATION OF INTEREST

None.

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Hepatology Research

Hepatology Research 2011; **41**: 1216–1222 doi: 10.1111/j.1872-034X.2011.00881.x

Original Article

Suppressor of cytokine signal 3 and IL28 genetic variation predict the viral response to peginterferon and ribavirin

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Aim: The aim of this study was to investigate the relationship among the expression of suppressor of cytokine signaling 3 (SOCS 3) in the liver, the SNPs in the IL28B locus, and the outcome of interferon therapy.

Methods: Prior to interferon treatment, we immunostained 67 liver specimens from chronic hepatitis C (CHC) patients who were receiving peginterferon alpha-2b/ribavirin therapy for suppressor of cytokine signaling 3 (SOCS3), and compared the expression of SOCS3, IL28 polymorphisms and other clinical factors between the patients and compared their eventual outcomes.

Results: Significant differences between the low SOCS3 group and high SOCS3 group were found in age, as well as in the platelet, transaminase, gamma-glutamyl transpeptidase levels. The incidence of high SOCS3 was not significantly different between subjects with the TT genotype and the TG genotype (TT : TG = 71%:29%, $P = 0.250$). In a multivariate analysis, age (≥65 years old) (odds ratio 0.221 [0.120–0.966], $P = 0.045$), IL28B gene (genotype TT) (odds ratio 5.422 [1.254–23.617], $P = 0.024$) and SOCS3 (high) (odds ratio 0.308 [0.104–0.948], $P = 0.040$) were significant predictors of the interferon response. In patients with the TT genotype, those with low SOCS3 immunostaining showed a high sustained virological response (69%), while the sustained virological rate was low (27%) in the patients with high SOCS3 immunostaining.

Conclusions: Using a combination of the SOCS3 immunostained area in the liver and the expression of IL28B single nucleotide polymorphisms might be a useful predictor of hepatitis C virus clearance by interferon therapy.

Key words: hepatitis C virus, IL28B, interferon, suppressor of cytokine signaling 3

INTRODUCTION

APPROXIMATELY 200 MISSION people worldwide are infected with hepatitis C virus (HCV). In Japan, about 2 million people are chronically infected, and HCV is the leading cause of hepatocellular carcinoma (HCC). The current standard care for chronic hepatitis C (CHC) is a combination of peginterferon-α (PEG-IFN) and ribavirin. This treatment is effective in approximately 40–50% of CHC patients with a high viral load of genotype 1.[1–5] This therapy is costly and frequently associated with side effects. Therefore, predicting the outcome of interferon therapy is important.

Several factors, such as gender, body mass index, the presence of steatosis and liver fibrosis, drug adherence and viral factors including the serum quantity of HCV RNA and HCV genotype have been reported to be significantly associated with the treatment outcome.[2,6–11] Among viral factors, Akuta *et al.* recently reported that the substitution of the HCV core amino acid was a predictor for the effect of interferon and ribavirin combination therapy.[2,12] Among the host factors, recent reports showed that genetic variations near the IL28 gene (rs8099917, rs1297860) on chromosome 19 were predictors of the virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals

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Received 4 March 2011; revision 16 July 2011; accepted 24 July 2011.

with HCV, and also affected the clinical outcome, including spontaneous clearance of HCV.[13–15]

We previously reported that the expression of suppressor of cytokine signaling 3 (SOCS3), which is related to insulin resistance, impairs the response to interferon treatment and might be a useful predictor of HCV clearance by interferon therapy.[16]

In this study, we examined the relationship among the expression of SOCS 3 in the liver, single nucleotide polymorphisms (SNPs) in the IL28B locus, and the outcome of interferon therapy.

METHODS

NEEDLE BIOPSIES OF the liver were obtained from 67 patients with positive HCV antibodies prior to interferon treatment at Nagasaki University Hospital and National Hospital Organization (NHO) Nagasaki Medical Center. Twenty of 67 cases were also examined in a previous study.[16] All patients with genotype 1b received weekly injections of PEG-IFN. The clinical data of the patients are summarized in Table 1. Liver biopsy was performed by needle puncture for diagnostic purposes. The diagnosis of each case was independently confirmed histologically by liver pathologists according to the Japanese chronic hepatitis classification criteria (New Inuyama classification). According to these criteria, mild activity was defined as A0 or A1, severe activity as A2 or A3, mild fibrosis as F0 or F1, and severe fibrosis as F2, F3, or F4. Fatty changes in >5% of all areas were defined as steatosis.

Table 1 Clinical backgrounds of the patients

Age	56.8 ± 9.3
Gender	Male : Female = 37:30
BMI (kg/m^2)	23.5 ± 2.9
Viral load (KIU/mL)	2320 ± 1519
White blood cell (/uL)	5074 ± 1713
Hemoglobin (mg/dL)	14.1 ± 1.3
Platelet (×10^3/uL)	167.3 ± 75.6
AST (IU/L)	77.1 ± 45.2
ALT (IU/L)	101.2 ± 56.3
γGTP (IU/L)	70.6 ± 65.5
HCV core 70 wild	40 cases
HCV core 91 wild	50 cases
Steatosis (>5%)	37 cases
A (0–1:2–3)	36:31
F (0~1:2~4)	22:45

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, gamma-glutamyl transpeptidase; HCV, hepatitis C virus.

All patients received PEG-IFN (Schering-Plough, Tokyo, Japan) + ribavirin (Schering-Plough, Tokyo, Japan) therapy for 48 weeks. The patients who were treated with a dose of PEG-IFN or ribavirin reduced by more than 20% were excluded from the study. PEG-IFN (1.5 μg/kg) was administered once per week, and the ribavirin dose was titrated according to body weight. A sustained virological response (SVR) was defined as undetectable HCV RNA at 6 months after the end of interferon treatment.

Of 38 patients who could not achieve an end-of-treatment response, 28 patients required a re-elevation of their viral loads regardless of the fact that the HCV-RNA levels were temporarily negative, and 10 patients did not achieve an HCV negative result during the entire treatment period.

SOCS3 immunohistochemistry

All tissue samples were fixed in 10% neutral buffered formalin and then embedded in paraffin, and 4 μm thick serial sections were cut from each paraffin block. In the immunohistochemical study, an anti-SOCS3 antibody (dilution 1:100, Affinity BioRegents, Golden, CO, USA) was used for SOCS3. Immunohistochemistry was performed with the labeled streptavidin biotinylate antibody (LSAB) method and a commercially available kit (Histofine, SAB-PO(R); Nichirei Corporation, Tokyo, Japan). The area immunostained for SOCS 3 was divided according to the number of immunoreactive cells per unit area. Immunoreactive cases were classified as those with less than 30% of the hepatocellular cells stained (low SOCS3 group) and those with 30% or more of the cells stained (high SOCS3 group), because our previous study showed that staining of more than 30% of the area was a significant predictor of viral clearance.[16]

Genetic variation near the IL28B gene

Genotyping for replication was performed by use of the Invader assay or direct sequencing. In this study, genetic variation near the IL28B gene (rs8099917), which was previously reported to be a predictor of the virological response was investigated.[13]

Statistical analysis

The SPSS 9.0 for Windows statistical software program was used to assess correlations among multiple variables. When appropriate, clinical and laboratory data

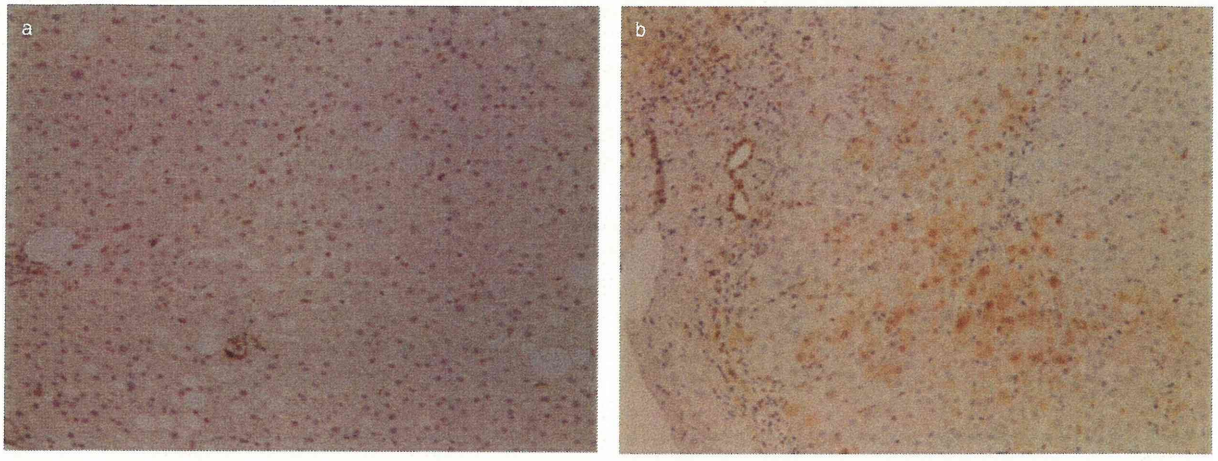

Figure 1 (a) This case showed less than 5% suppressor of cytokine signaling 3 (SOCS3) immunostained areas (low immunostaining). (b) This cases showed about 50% SOCS3 immunostaining areas (high immunostaining).

were compared with the Student's *t*-test or the Mann–Whitney test. A *P*-value of <0.05 was considered to be statistically significant.

RESULTS

Immunostaining of SOCS3 in the liver (Figs 1,2)

IMMUNOSTAINING FOR SOCS3 was mainly seen in the periportal area. Less than 30% SOCS3 immunostained areas were found in 36 cases (54%) and areas with 30% or more immunostaining for SOCS3 were found in 31 cases (46%).

The frequency and distribution of the SOCS3 expression are shown in (Fig. 2)

Correlation between SOCS3 immunostaining and clinicopathlogical factors

A significant difference between low and high SOCS3 groups was found in age (low : high = 54.5 ± 9.8:59.5 ± 8.1, $P = 0.028$), the levels of platelets (low : high = 189.5 ± 90.0:141.6 ± 41.3, $P = 0.009$), aspartate aminotransferase (AST) (low : high = 94.5. ± 56.0:62.1 ± 33.5, $P = 0.003$), alanine aminotransferase; (ALT) (low : high = 85.8 ± 52.4:119.0. ± 56.3, $P = 0.015$), gamma-glutamyl transpeptidase (γGTP) (low : high = 48.8 ± 53.5:94.7 ± 70.6, $P = 0.004$). The incidence of steatosis (low : high = 33%: 81%, $P = 0.001$), severe activity (low : high = 27%: 67%, $P = 0.001$) and sever fibrosis (low : high = 52%: 84%, $P = 0.006$) was significantly higher in the SOCS3 high

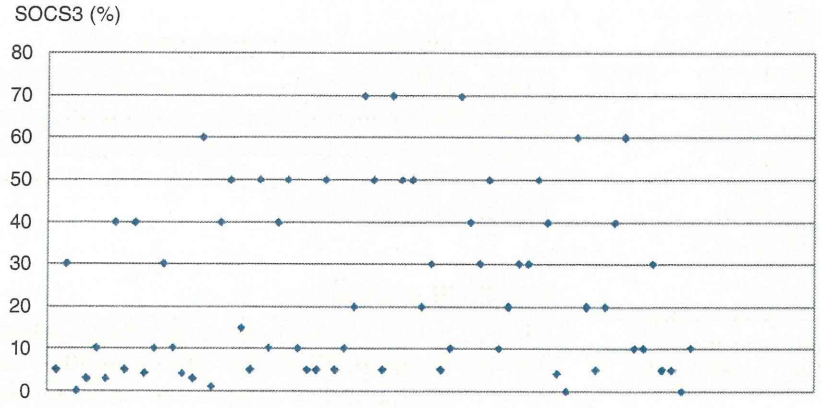

Figure 2 The distribution of the SOCS3 immunostaining area is shown.