Molecular comparison of bacterial isolates from blood with strains colonizing pharynx and intestine in immunocompromised patients with sepsis

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Most causative organisms of sepsis in immunocompromised patients are the same species as those that colonize their own nasopharynx or intestinal tract. To determine whether the strains recovered from blood originate mainly from patients' own flora, isolates from blood and throat and/or stool were investigated by genomic analyses. Surveillance cultures of throat and stool were taken prospectively from cancer patients being treated with intensive chemotherapy followed by haematopoietic stem-cell transplantation. In those cases of sepsis in which the isolate from blood was the same species as that from the throat and/or stool, the genomic profiles of the isolates were compared by PFGE. Ten cases of blood culture-positive sepsis were documented in six of 14 subjects during a 2 year period; isolates of *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Enterococcus* sp., viridans streptococci and *Fusobacterium* sp. were recovered from blood. In five of seven cases in which the blood isolate was the same species as that from the throat or stool, the genotypes of the isolates from both sites were identical. In the majority of immunocompromised patients, the causative organisms of bloodstream infections originated mainly from their own flora.

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INTRODUCTION

In immunocompromised patients, such as those with malignancy, bone-marrow transplant recipients and low-birth-weight infants, sepsis is rapidly progressive and life-threatening. Prompt empirical antimicrobial therapy is essential for management of potential causative organisms, particularly in profoundly neutropenic patients (those with < 500 neutrophils μl^{-1} ; Hughes *et al.*, 2002; Katz & Mustafa, 1993; Pizzo, 1999).

Organisms predominantly responsible for sepsis among patients with cancer and neutropenia include coagulase-negative staphylococci, viridans streptococci, enterococci, *Pseudomonas aeruginosa* and Gram-negative enteric bacilli (Aquino *et al.*, 1995; Koll & Brown, 1993; Pizzo, 1999). Most of these species colonize the patient's nasopharynx, intestinal tract and skin; these organs may serve as endogenous reservoirs for causative organisms of bloodstream infections. However, as far as we are aware, there has been only one report describing a study that investigated whether these colonizing species are substantial sources of sepsis in immunocompromised patients (Kennedy *et al.*, 2000).

Using molecular typing methods, we systematically investigated the association between the isolates from blood and those from throat and stool in immunocompromised patients with sepsis.

METHODS AND SUBJECTS

Between April 1996 and March 1998, all patients with cancer or haematological disorders who were being treated with chemotherapy followed by haematopoietic stem-cell transplantation (HSCT), such as bone-marrow transplantation (BMT), at the Department of Pediatrics, Asahikawa Medical College Hospital, Asahikawa, Japan, were eligible for entry to the study. Fourteen patients were recruited during this period (Table 1): eight had acute lymphoblastic leukaemia (ALL) and among the remaining six patients, there was one case each of acute mixed-lineage leukaemia, acute myeloblastic leukaemia, yolk-sac tumour, primitive neuroectodermal tumour, malignant lymphoma and aplastic anaemia.

After informed consent was obtained, throat swab and stool cultures were done prospectively once or twice a week during the 2 year study period, regardless of whether the patients presented with symptoms such as fever, or whether they were neutropenic. In cases where sepsis was suspected, blood cultures were performed. Sepsis was defined as a clinical situation with positive blood culture and symptoms of a systemic response, such as fever, tachycardia and increased

Abbreviations: ALL, acute lymphoblastic leukaemia; BMT, bone-marrow transplantation; HSCT, haematopoietic stem-cell transplantation.

Table 1. Details of patients and their underlying diseases

Patient no.	Age (years)	Sex	Underlying disease
1	8	M	Acute lymphoblastic leukaemia
2	3	F	Acute mixed lineage leukaemia
3	12	F	Acute myeloblastic leukaemia
4	11	M	Yolk-sac tumour
5	17	M	Acute lymphoblastic leukaemia
6	11	M	Acute lymphoblastic leukaemia
7	8	M	Acute lymphoblastic leukaemia
8	12	M	Acute lymphoblastic leukaemia
9	7	F	Acute lymphoblastic leukaemia
10	15	M	Primitive neuroectodermal tumour
11	5	M	Malignant lymphoma
12	1	F	Acute lymphoblastic leukaemia
13	11	M	Aplastic anaemia
14	16	M	Acute lymphoblastic leukaemia

respiration rate. Throat swab specimens were inoculated onto 5% sheep blood agar and chocolate agar plates, and stool specimens were inoculated onto deoxycholate–hydrogen sulfide–lactose agar plates (Eiken Chemical Company) and 5% sheep blood agar plates. Each isolate was identified in the clinical microbiology laboratory using standard bacteriological procedures (Murray *et al.*, 1995), and most isolates were freeze-dried and stored until they could be tested. In cases of sepsis where the blood isolate was the same species as the throat and/or stool isolate which had been recovered before, at the time of or shortly after sepsis, the genomic profile of the former was compared with that of the latter by PFGE.

PFGE was performed according to the method described by Maslow *et al.* (1993) with modifications. Briefly, overnight cultures of the isolates in brain-heart infusion broth (Difco) were harvested, washed and resuspended in Pett IV buffer (10 mM Tris/HCl, pH 7.6; 1 M NaCl). The cells were then mixed with low-melting-temperature agarose (FMC BioProducts) and allowed to solidify in a 100 µl mould. The plugs were incubated overnight at 37 °C in lysis solution (6 mM Tris/HCl, pH 7.6; 1 M NaCl; 100 mM EDTA; 0.5% Brij 58; 0.2% sodium deoxycholate; 0.5% sodium lauroyl sarcosine), containing 20 µg RNase ml⁻¹ (Wako Nippon Gene) and 1 mg lysozyme ml⁻¹ (Wako), and 5 U lysostaphin ml⁻¹ (Sigma) for isolates of *Staphylococcus epidermidis*. The solution was replaced with ESP solution (0.5 M EDTA, pH 8.0; 1% sodium lauroyl sarcosine) containing 0.1 mg proteinase K ml⁻¹, and the plugs were incubated overnight at 50 °C. The plugs were then washed and digested with the following restriction enzymes: *Sma*I for *Staphylococcus epidermidis*, *Streptococcus mitis* and *Enterococcus faecium*, and *Spe*I for *P. aeruginosa*. Restriction fragments were separated by PFGE with a CHEF DR II system (Bio-Rad) through 0.9% agarose gel at a field strength of 6 V cm⁻¹ and with initial and final pulse times of 1.0 and 40.0 s, respectively.

RESULTS

All 14 subjects were enrolled in the study. The cases of sepsis are summarized in Table 2; ten episodes of sepsis in which organisms were isolated from blood were documented in six of 14 patients. Patients 5 and 7 had two and four episodes of sepsis, respectively. In all cases, sepsis occurred during the neutropenic period: patient 1 had sepsis 8 days after BMT, and the second episode of patient 5 occurred 5 days after BMT. In the remaining cases, sepsis occurred during intensive chemotherapy regimens before HSCT.

There were seven cases of sepsis in which the isolate from blood was the same species as that from throat and/or stool. In one of the remaining cases, patient 5, the blood isolate in one of two episodes was *Fusobacterium* sp., for which throat and stool cultures were not done. We were unable to keep the blood isolate of *Streptococcus mitis* from the first episode of sepsis in patient 7. In patient 8, *Streptococcus oralis* was isolated from blood, but the pharyngeal strains were stored as viridans streptococci and were found to be *Streptococcus mitis* or *Streptococcus parasanguinis*, not *Streptococcus oralis*.

Fig. 1 shows the genomic profiles of the isolates in seven cases of sepsis; in each case, the blood isolate was the same species as those from throat and/or stool. In five of these seven cases, the genotype of the isolate from blood was identical to that of the pharyngeal or stool isolate. In the second episode of sepsis in patient 7, the genotype of the *P. aeruginosa* strain isolated from blood was shared by those of the strains that were isolated from the throat, 37 and 23 days before the onset of sepsis. Later in this patient, the third and fourth episodes of sepsis were separated by 35 days. Strains of *Staphylococcus epidermidis* were isolated from the blood in both cases; the genotypes of these two strains were the same, and were also identical to those of pharyngeal *Staphylococcus epidermidis* strains isolated 1 month before the first episode, and of the several strains isolated between episodes. The genotypes of *E. faecium* isolated from blood and throat at sepsis in patient 11

Table 2. Cases of blood culture-positive sepsis

Patient no.	Isolate from blood	Specimen from which the same strain from blood was isolated	Clonal identity of blood and throat/stool isolate
1	<i>Staphylococcus epidermidis</i>	Throat, stool	Different
5	<i>Staphylococcus epidermidis</i>	Throat	Different
5	<i>Fusobacterium</i>	NA	NA
7	<i>Streptococcus mitis</i>	NA	NA
7	<i>Pseudomonas aeruginosa</i>	Throat	Identical
7	<i>Staphylococcus epidermidis</i>	Throat	Identical
7	<i>Staphylococcus epidermidis</i>	Throat	Identical
8	<i>Streptococcus oralis</i>	NA	NA
11	<i>Enterococcus faecium</i>	Throat	Identical
12	<i>Streptococcus mitis</i>	Throat	Identical

NA, Not available.

were identical. In patient 12, the genotype of the *Streptococcus mitis* strain isolated from blood was shared by those of the pharyngeal strains isolated 3 days before and at the onset of sepsis.

In patient 1, the genotypes of the pharyngeal and stool strains of *Staphylococcus epidermidis* isolated 18 days after the onset of sepsis were the same, but were different from that of the blood *Staphylococcus epidermidis* isolate. In the first episode of sepsis in patient 5, the genotypes of the blood and pharyngeal strains isolated on the day of sepsis were also different.

DISCUSSION

We found that among most cases of sepsis in immunocompromised patients, the bacterial strains isolated from blood were clonally identical to the strains colonizing the patients' pharynx or intestinal tract. It was confirmed that the causative organisms of bloodstream infections in these patients originated substantially from their own flora.

The range of bacteria isolated from blood cultures in our patients was representative of those agents that commonly cause systemic infection in neutropenic patients (Aquino *et al.*, 1995; Koll & Brown, 1993; Pizzo, 1999). In this study, the following bacteria were also isolated from patients: *Staphylococcus epidermidis* in four cases, viridans streptococci in three cases, and one case each of *Enterococcus* sp. and *P. aeruginosa*.

In most cases of sepsis, the bacteria involved were indistinguishable from the patients' own flora, such as in the nasopharynx, intestinal tract or on skin. To determine whether the blood isolates originated substantially from patients' own flora, it was necessary to compare the genomic profile of the blood isolate with that of the colonizing strain. Using a molecular typing method, PFGE, we systematically examined the relationship between strains in the blood of immunocompromised patients with sepsis, and the pharyn-

geal and intestinal strains collected prospectively. In a case report, Kennedy *et al.* (2000) demonstrated that blood isolates of *Staphylococcus epidermidis* and *Streptococcus oralis* had the same genotype as strains isolated from the oral cavity in a bone-marrow transplant recipient. In our study, the isolate from blood was clonally identical to that from throat or intestinal tract in five of seven cases with sepsis, and the variety of organisms included *P. aeruginosa*, *Staphylococcus epidermidis*, *E. faecium* and *Streptococcus mitis*.

However, in two cases with *Staphylococcus epidermidis* sepsis, the genotype of the blood isolate was different from that of the throat and/or stool isolate. In these two cases, PFGE was performed with another restriction enzyme, *KpnI*, and the blood and colonizing strains were also different in both cases (data not shown). In patient 1, the reason for this may be that the pharyngeal and intestinal strains tested for genotype were isolated 18 days after sepsis. In this case, the *Staphylococcus epidermidis* strain was also isolated from the central venous catheter tip. Although this strain could not be tested for genotype because of a laboratory oversight, its antibiotic-susceptibility spectrum was similar to that of the blood isolate. In another case (patient 5), the pharyngeal strain that was isolated on the day of sepsis was not identical to the blood strain. The genotype of the blood strain in patient 1 was the same as that in the first episode of patient 5. This may be due to nosocomial infection; skin organisms can be spread to other patients by the hands of healthcare workers and, through intravenous catheters, into the bloodstream. Skin and central venous catheter cultures may be also needed to determine the sources of sepsis, as *Staphylococcus epidermidis* is one of the most predominant members of skin flora.

In the present study, the frequency of blood culture-positive sepsis was high. The reason for this may be that the enrolled patients had severe underlying diseases that required intensive chemotherapy followed by HSCT, causing prolonged neutropenia; all eight patients with ALL were those with relapse, and patient 5 (who suffered four episodes of sepsis) had refractory ALL.

Fig. 1. PFGE patterns of the isolates from blood and those from throat and/or stool in cases of sepsis in immunocompromised patients. λ , Molecular mass markers; lanes 1, 2 and 3, *Staphylococcus epidermidis* from blood, throat and stool, respectively, in patient 1; lanes 4 and 5, *Staphylococcus epidermidis* from blood and throat, respectively, in the first episode of sepsis in patient 5; lanes 6 and 7, *P. aeruginosa* from blood and throat, respectively, in the second episode of sepsis in patient 7; lanes 8 and 9, *Staphylococcus epidermidis* from blood and throat, respectively, in the third episode of sepsis in patient 7; lanes 10 and 11, *Staphylococcus epidermidis* from blood and throat, respectively, in the fourth episode of sepsis in patient 7; lanes 12 and 13, *E. faecium* from blood and throat, respectively, in patient 11; lanes 14 and 15, *Streptococcus mitis* from blood and throat, respectively, in patient 12.

The results of bacteriological culture from the nasopharynx and intestine, which are obtained on a particular plate of a particular swab, may not be fully representative of the true flora of the patient at that time. Small numbers of organisms in mucosal sites, which are not identified by routine microbiological culture, may invade the bloodstream, especially if they possess certain virulence mechanisms. It was reported that routine surveillance cultures from anterior nares,

oropharynx, urine and rectum are of little clinical utility in the evaluation or care of cancer patients with fever and neutropenia (Hughes *et al.*, 2002; Kramer *et al.*, 1982; Pizzo, 1993). In the present study, however, in six of ten episodes of sepsis, the same species were isolated from blood and from throat and/or stool, 0–37 days before the onset of sepsis. In five of these six episodes, the blood isolates were found to be the same strain as the pharyngeal or intestinal strains by PFGE typing. Although our study included only a small number of patients and there may be cost-effectiveness problems, surveillance cultures of throat and stool may give clinicians predictive yields of potential sepsis-causing pathogens among immunocompromised patients.

In summary, in immunocompromised patients with sepsis, there is a substantial link between the strains isolated from blood and those that colonize the throat and intestinal tract, confirming that members of the patients' own flora are significant causes of sepsis.

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