Microbial Skin Flora of Selected Cancer Patients and Hospital Personnel

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The bacterial flora of the skin from five anatomical sites on 10 leukemia patients, 10 patients with malignant melanoma, and a control group of 10 medical personnel was examined quantitatively and qualitatively. This was done to determine whether malignant disease results in changes in skin flora and to establish carrier rates of gram-negative bacteria on the skin of personnel in hospital environments. Gram-negative bacteria were isolated more frequently (74 isolates from 100 cultures) from the skin of leukemia patients than from either patients with malignant melanoma (8 isolates from 100 cultures) or the medical personnel (9 isolates from 100 cultures). *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were isolated exclusively from leukemia patients. Relative proportions of gram-negative bacteria in total populations were determined. The axilla was the only site with a uniformly high proportion of gram-negative bacteria. From all other sites cultured, gram-negative populations were low (1 to 5 bacteria/cm² of skin), although a high proportion of gram-negative populations occurred randomly throughout all subject groups. It was concluded that leukemia patients tend to carry gram-negative bacteria on the skin. The factors permitting colonization of skin by gram-negative bacteria are discussed.

The increase in hospital infection rates caused by gram-negative bacteria in recent years, particularly in the compromised host, has raised the question of the role of the skin as a source of the infectious material. Whereas the microbial flora of skin is predominantly gram-positive in healthy individuals (19), gram-negative bacteria have been found to be the predominant organisms isolated from the skin of critically ill patients (26), which suggests that with certain systemic disease processes gram-negative bacteria may more readily colonize the skin. Furthermore, it has been postulated that the widespread use of antibacterials in cosmetics, soaps, and other topical preparations selects against gram-positive microbial populations and may result in colonization by gram-negative bacteria (2, 8, 27).

The purpose of this study was to establish whether microbial skin populations are altered on patients with malignant disease, with particular reference to carriage of gram-negative bacteria, and to determine the incidence of isolation of these organisms in normal individuals in a hospital environment. The microbial flora from five anatomical skin sites were determined quantitatively and qualitatively

shortly after the admission of patients with acute leukemia to the hospital, prior to major surgical procedures for patients with malignant melanoma, and were compared with similar cultures taken from personnel working in a hospital environment. Leukemia patients were chosen because of their high incidence of infection with gram-negative bacteria and their serious prognosis, a fact that has been well documented (9, 11). The patients with malignant melanoma provide a group of solid-tumor cancer patients treated at the same institution as the leukemia patients, and the medical personnel serve as a control group that should reflect the influence of a hospital environment on the carriage of gram-negative bacteria on skin.

MATERIALS AND METHODS

Descriptive data of the three groups of subjects, leukemia, melanoma, and hospital personnel, are shown in Table 1. Each group contained 10 subjects. It can be seen that the age range, although overlapping, was not comparable among groups; the leukemia patients were the youngest and the melanoma patients were the oldest. This difference represents the medians expected for these diseases and could not be avoided without selection. It has

TABLE 1. *Subject description*

Subject group	No. of patients	Age (yr)		No. using antibacterial soap	No. using deodorants
		Range	Median		
Control	10	27-32	30	7	9
Melanoma	10	33-79	56	7	6
Leukemia	10	15-52	25	5	6

been reported, however, that once puberty is reached microbial flora remain stable until the seventh decade (24). The use of antibacterial soaps and deodorants was also recorded, and it can be seen that medical personnel were the heaviest users of deodorants whereas usage by melanoma patients and leukemia patients was the same. Fewer leukemia patients (five) used antibacterial soaps than did melanoma patients (seven) or the control group (also seven). All patients were sampled within 24 h of admission to the hospital and prior to receiving treatment of any kind, including antibiotics. None of the patients was in a protected environment. The diagnoses of the patients with malignant disease are given in Table 2. The staging of the patients with malignant melanoma is that used by the M.D. Anderson Hospital and Tumor Institute (14), and the majority of patients included in this study were stage III, which represents patients with regional metastasis. All leukemia patients had the acute form of the disease and, hence, a shorter history of disease (1-17 months) than the melanoma patients (2-156 months).

Sampling procedures. The sites cultured were palms of hands, soles of feet, and back over the scapulae, axillae, and groin. Each area was cultured in duplicate, samples being taken from both right and left sides of the body. This skin sampling method chosen has been evaluated statistically and found to compare favorably with other quantitative skin sampling methods (18). Specimens were collected by delineating an area (16 cm²) with a sterile template and scrubbing the skin in that area with calcium alginate swabs moistened in phosphate-buffered saline, pH 7.2, containing 0.1% Triton X-100. These swabs were suspended in 2.5 ml of the same buffer solution in test tubes (13 by 100 mm) and shaken on a wrist action shaker for 5 min. After suitable dilutions, bacterial counts were made by using pour plates of Trypticase (BBL) soy agar containing 0.1% Tween 80. Quantitation was also done using Casman sheep blood agar and inoculating the surface by using a calibrated dropping pipette. McConkey medium, phenylethyl alcohol agar, and other selective media were used when necessary. Of the original suspension, 0.25 ml was used to inoculate Casman sheep blood agar for qualitative studies, and 0.5 ml was inoculated into thioglycollate broth to detect organisms present in low concentrations. Plates were incubated for 3 days at 31 C. Routine

diagnostic media and procedures were used for identification (5).

Statistical procedures. The mean bacterial population was calculated from duplicate cultures taken from right and left sides of the body from each site in each subject. Significant differences in bacterial skin populations among groups were determined by an analysis of variance followed by calculation of least significant differences when $P < 0.05$.

RESULTS

The culture sites were selected to show both host physiological factors and environmental influence. The hands are directly influenced by the environment since they are closely involved in the occupation of the subject. The back is protected from the environment with clothes and may reflect to a greater extent the resident flora of the host dependent on the physiological activity of the individual. The axillae, groin, and feet were chosen as warm moist areas, since moisture has been shown to be a factor in the proliferation of gram-negative bacteria of skin (20).

Qualitative results. The incidence and numbers of gram-negative bacteria on normal skin is known to be very low (12, 19); hence, an increased frequency of isolation suggests an alteration in the normal controlling factors responsible for maintaining gram-positive flora. In this study the carrier rate was highest in leukemia patients. Gram-negative bacteria were isolated from 6 of the 10 leukemia patients from at least one anatomical site, as compared with 2 and 3 patients from the melanoma and control groups, respectively. The relative importance of different anatomical sites in carriage of gram-negative bacteria is shown in Table 3. Of the five sites sampled, the axilla is colonized most consistently by these organisms for

TABLE 2. *Type of leukemia, stage of melanoma, and duration of disease*

Determination	No. of patients	Duration
Type of leukemia		
Acute myelogenous	5	1 mo-3 mo
Acute lymphocytic	3	2 wk-7 mo
Acute stem cell	1	2 wk
Acute unclassified	1	6 wk
Stage of melanoma		
Stage I	2	3 mo-7 mo
Stage III	7	2 mo-156 mo
Stage IV	1	78 mo

each patient group. The leukemia patients differ from the other two groups in that gram-negative bacteria show a wider distribution and are present with the same frequency on all skin sites except the hands.

When the frequency of isolation of gram-negative bacteria is compared among groups, differences are observed between leukemia patients and the other two groups (Table 4). Seventy-four isolates of gram-negative bacteria were obtained from a total of 100 cultures from all five sites in leukemia patients as compared with 8 isolates from patients with malignant melanoma and 9 from the control hospital personnel. All skin sites were found to be a source of a variety of species of gram-negative bacteria in leukemia patients, whereas the axilla was the main site colonized by these organisms in the other two groups and then usually only by a single species.

The species of gram-negative bacteria and their frequency of isolation from the leukemia, melanoma, and medical personnel groups are listed in Table 5. Certain species, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were isolated solely from leukemia patients, and other genera, such as *Proteus* and *Acinetobacter*, occurred not exclusively but more frequently. *Proteus* spp., *P. aeruginosa*, and *K. pneumoniae* are frequently causes of infection in leukemia patients, and their in-

frequent isolation from healthy individuals is noteworthy. *Enterobacter* spp. were most frequently isolated from melanoma patients whereas the control group carried *Enterobacter* spp., *Escherichia coli*, and *Acinetobacter* spp.

Although the gram-negative bacteria are listed in Table 5 by genera, all were identified as to genus and species when possible. All species of *Proteus* proved to be *P. mirabilis*; all *Enterobacter* spp. from the control and melanoma group were *E. aerogenes*, whereas the leukemia patients yielded other species of *Enterobacter* as well. *K. pneumoniae* was the only *Klebsiella* spp. isolated. The taxonomical position of *Acinetobacter* and *Moraxella* spp. presented problems; since the time this study was initiated, the nomenclature of these organisms has been continually changing. Also, the majority of the biochemical reactions from the species isolated from skin were found to be atypical in some respects from named species, which made it difficult to assign a definite species name.

The frequency of isolation of *Staphylococcus aureus* (Table 6) did not differ appreciably among the three groups.

Quantitative results. The distribution of microbial skin populations from the five anatomical skin sites sampled (Fig. 1) shows that there are low (back and hands) and high (feet)

TABLE 3. Site and frequency of gram-negative bacteria

Site	Frequency of gram-negative bacteria		
	Control ^a	Melanoma	Leukemia
Hand	1	0	2
Axilla	3	2	4
Back	1	0	4
Groin	1	2	4
Feet	1	0	4

^a Each of the subject groups consisted of 10 people.

TABLE 4. Site and frequency of different isolates of gram-negative bacteria^a

Site	Frequency of gram-negative bacteria		
	Control ^b	Melanoma	Leukemia
Hand	1	0	6
Axilla	3	7	19
Back	3	0	16
Groin	1	1	20
Feet	1	0	13

^a Twenty cultures per patient.

^b Subject group.

TABLE 5. Frequency of isolation of gram-negative species from 100 cultures

Species	Frequency of gram-negative species		
	Control	Melanoma	Leukemia
<i>Proteus</i>	0	1	18
<i>Acinetobacter</i>	2	0	15
<i>P. aeruginosa</i>	0	0	10
<i>Enterobacter</i>	3	6	8
<i>Klebsiella</i>	0	0	7
<i>Moraxella</i>	0	0	6
<i>E. coli</i>	3	1	6
<i>Citrobacter</i>	1	0	4

TABLE 6. Site and frequency of isolation of *S. aureus*

Site	Frequency of <i>S. aureus</i>		
	Control ^a	Melanoma	Leukemia
Hand	1	1	0
Axilla	0	0	1
Back	0	0	1
Groin	0	1	0
Feet	0	0	1

^a Each group consisted of 10 subjects.

FIG. 1. Distribution of total microbial skin populations from five sites in leukemia and melanoma patients. Each symbol represents the mean population obtained from duplicate samples from each subject.

and groin) population sites that are uniform for each group. As observed in earlier studies (6), individual differences in microbial populations of the skin from a single site within each subject group are frequently great (e.g., axillary counts in the melanoma group ranged from 0.78 to 5.83 colony-forming units/cm²), which makes statistically significant differences difficult to establish. No statistically significant differences among the three subject groups were found in microbial skin populations from the feet and groin. Although statistically significant differences in microbial skin populations were observed from the back, hands, and axilla, none of the subject groups was consistently higher or lower than another. The means, standard deviation, and *P* values of bacterial skin populations from these areas are given in Table 7. The mean values for the back and the hands of the melanoma group are lower than those of either the leukemia or the control group, significantly so from the hands but only significantly lower than the leukemia group from the back. Conversely, microbial skin populations from the axillae in melanoma patients proved to be significantly higher than the control group. Since the results obtained for the axilla were inconsistent with those from other sites, they were analyzed further by separating the deodorant users from the nonusers to determine whether this was a result of the antibacterial activity of deodorants. The results (Fig. 2) show that, in the melanoma group, those patients who did not use deodorants had the highest bacterial counts, whereas there was no direct correlation between populations

TABLE 7. Comparison of the mean log values of bacterial populations per square centimeter of skin

Determination	Mean	SD	<i>P</i> values
Back			
Control	1.08	0.73	0.0155
Melanoma	1.07	0.88	
Leukemia	1.93	0.42	
Hand			
Control	1.94	0.45	0.0043
Melanoma	1.18	0.68	
Leukemia	2.39	0.98	
Axilla			
Control	2.05	1.57	0.0138
Melanoma	4.05	1.39	
Leukemia	2.81	1.27	

and use of deodorants among the leukemia patients.

The proportion of gram-negative bacteria relative to the total microbial populations for each carrier is shown in Fig. 3 and 4. The axilla (Fig. 3) is the only site where large populations of gram-negative bacteria occur, and even there, in seven of the nine subjects, normal flora were present as well. The only subjects with no normal flora were two members of the control group. Gram-negative populations from the back, hands, feet, and groin were generally low, not exceeding 5 colony-forming units/cm² of skin in most cases. The few carriers with high populations of gram-negative bacteria occurred randomly within the three subject groups, and the leukemia patients did not have

FIG. 2. Distribution of bacterial populations of the axilla in deodorant users and nonusers. Each symbol represents the mean population obtained from duplicate samples from each subject. The arrow represents the mean population of the deodorant users versus that of the nonusers.

FIG. 3. *Relative proportion of population of gram-negative bacteria to total microbial populations in the axillae in nine subjects. Each bar represents data obtained from the mean of duplicate samples in one subject. Only subjects carrying gram-negative bacteria are shown here.*

higher populations of these organisms than did the other two groups.

DISCUSSION

The results presented here show that the overall percent carrier rate for leukemia patients (60%) was seemingly higher than that for melanoma patients or the control personnel, but with such low rates larger groups need to be studied to establish statistical differences. The most important observation made in this

FIG. 4. *Relative proportions of populations of gram-negative bacteria to total microbial populations from four anatomical sites: hands, feet, back, and groin. Each bar represents data obtained from the mean of duplicate samples in one subject. Only subjects carrying gram-negative bacteria are represented here, hence the blank areas for the melanoma group in the charts for hands, feet, and back.*

study was the fact that, when leukemia patients carry gram-negative bacteria on their skin, the bacteria can be isolated from practically all anatomical sites samples as opposed to the melanoma and control groups, which showed a more normal distribution of these organisms in the warm moist areas such as the

axilla and groin. It appears that gram-negative bacteria are capable of proliferating on the skin of some leukemia patients indiscriminately. Furthermore, a greater variety of species was isolated from the leukemia patients than from the other two subject groups. The potentially dangerous organisms *P. aeruginosa* and *K. pneumoniae* were isolated solely from leukemia patients, and *P. mirabilis* occurred in a high incidence as well. By comparison, *Enterobacter* spp. occurred predominantly in the other subject groups. The presence of *Acinetobacter* spp. is of interest, as well, since these species are indicative of an environmental origin such as showers and sinks, which suggests that gram-negative organisms from many sources, the patients themselves as well as the environment, are potentially capable of colonizing the skin.

The predominantly gram-positive bacterial populations that make up the cutaneous flora on normal skin appear to be controlled by both physiological and environmental factors. Moisture has been shown to be the most important of the latter (20), although use of antibacterials has been implicated (2, 8, 27). Gram-negative bacteria are known to be particularly susceptible to desiccation (21) and, when moisture has been supplied by occlusion of a skin site, gram-negative bacteria proliferate (20). Hence, moisture is believed to be responsible for the isolation of gram-negative bacteria on skin sites, such as the axillae and groin, in healthy individuals. The physiological control of cutaneous flora is evidenced by the stability of the gram-positive flora. Several hours after elimination of the normal microbial flora by an antiseptic application, gram-positive populations return (18). Further evidence is supplied by the observation that most gram-negative bacteria do not survive longer than several hours when artificially inoculated on skin (15). Part of this effect is due to desiccation, but some physiological, self-disinfecting activity appears to be present as well. In a review of the early work in this field, Burtenshaw (4) proposed factors that may be responsible for the stability of microflora of normal skin including: (i) bacterial interference (interaction with other microbial species that may be producing antagonists such as bacteriocins), (ii) the presence of antibodies and other immune phenomena, and (iii) the biochemical environment of the skin (e.g., the lipid content in the skin appears to control colonization of gram-positive bacteria) (1). Although some aspects of these theoretical possibilities have been examined (25, 28), the factors that control normal flora are still poorly understood. Until more is learned, it is difficult

to define the changes on the skin of leukemia patients that lead to colonization by gram-negative bacteria.

The control group in this study appears to bear out the current knowledge of distribution of gram-negative bacteria on normal skin, and it is interesting to observe that, even in an environment of potentially high gram-negative bacterial populations, healthy individuals do not carry these organisms to any extent. It is interesting, also, that melanoma patients have an incidence and distribution comparable with the control group. This fact suggests that the carriage of gram-negative bacteria by patients with acute leukemias, early in the evolution of their disease, is disease related, and one might speculate that the disease process may be responsible for changes in the physiology of the skin, resulting in an alteration in the biochemical environment. An immunological deficiency may also play a role since this is known to occur in many patients with leukemia (10).

Total microbial skin populations were studied in the three groups of patients in an attempt to correlate susceptibility to infection with population density. The results, however, did not support this theory, but the leukemia patients did have significantly higher microbial skin populations on the back, an area chosen to show physiological control. The wide range in bacterial population among individuals and the relative stability within individuals have been observed previously (7) and have caused speculation as to the factors involved, a problem that has remained unsolved.

Although widely distributed among the leukemia patients, the populations of gram-negative bacteria isolated were not high, and normal flora were always present. In a previous study on staphylococcal skin carriage (17), it was observed that population size did not correlate with persistence of carriage. The presence of the gram-negative bacteria on skin may be important in prognosis, however, since the majority of the leukemia patients required treatment with antibiotics during their subsequent hospital admissions. These patients are being followed through the course of their disease in an attempt to correlate subsequent clinical infections with known skin flora isolated early in their disease as reported here.

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