Persistence of Polymicrobial Abscesses in the Poorly Controlled Diabetic Host

ALICE N. BESSMAN, FRANCISCO L. SAPICO, MEHER TABATABAI, AND JOHN Z. MONTGOMERIE

SUMMARY

Polymicrobial infections are frequently found in soft tissue infections of the lower extremities in diabetic patients. The relative susceptibility to and persistence of soft tissue polymicrobial infections of diabetic and nondiabetic mice using bacteria commonly found in clinical foot infections were studied. Subcutaneous abscesses were induced in three groups of diabetic and nondiabetic mice using: (1) *E. coli* and enterococcus, (2) enterococcus and *Bacteroides fragilis* (*B. fragilis*), and (3) *E. coli* and *B. fragilis*. Abscesses were removed at 1 and 2 wk for total colony counts. At 1 wk, there was a significantly greater bacterial growth in the abscesses of the diabetic mice compared with the nondiabetic mice only in the group injected with enterococcus and *B. fragilis*. There were significantly higher colony counts in the diabetic compared with the nondiabetic mice in all three groups at 2 wk after injection of the bacteria. Two weeks after injection of inocula containing *B. fragilis*, both in combination with *E. coli* or enterococcus, all nondiabetic mice had eradicated *B. fragilis* from the abscesses, but significant numbers of *B. fragilis* persisted in the abscesses of the diabetic mice. In the diabetic mice, the presence of enterococci was more synergistic for growth of *B. fragilis* than was the presence of *E. coli*.

These studies demonstrate that the bacteria of polymicrobial soft tissue infections persist for a longer period of time in the diabetic compared with the nondiabetic host. In addition, *B. fragilis* has increased pathogenicity in the diabetic compared with the nondiabetic host, particularly in the presence of enterococci.

DIABETES 1986; 35:448–53.

Increased susceptibility of the diabetic patient to a variety of infections has been postulated on the basis of clinical observations.[1,2] Persons with diabetes have been estimated to account for 45–70% of all lower extremity amputations performed.[3] These amputations are made necessary by a combination of vascular disease, neuropathy, and infection, i.e., infected diabetic gangrene. The bacterial flora in these infections has been shown to be polymicrobial and to include anaerobes and aerobes.[4–7]

For many years, there has been the general clinical impression that diabetic patients are more susceptible to many bacterial infections than nondiabetic subjects.[8] Various aspects of the immune system, such as in vitro granulocyte adherence,[9] granulocyte phagocytosis and microbicidal activity,[10–12] and monocyte activity[13] have been found to be deficient in the white blood cells of the uncontrolled diabetic patient. In vivo granuloma formation has been shown to be suppressed in hyperglycemic diabetic mice.[14] However, there remains a paucity of prospective clinical data addressing the question of the relative resistance of the diabetic host, compared with the nondiabetic host, to in vivo bacterial soft tissue infections. To investigate this question, we have studied the development and course of experimentally induced bacterial polymicrobial subcutaneous abscess formation, using a combination of aerobes and anaerobes, in the genetically diabetic mouse and its nondiabetic littermate.

MATERIALS AND METHODS

Female diabetic mice 8–10 wk of age and their nondiabetic female littermates were obtained from the Jackson Laboratory, Bar Harbor, Maine (strain C57B1.Ks-J-*db-m*). Three sets of 12 diabetic and 12 nondiabetic mice were studied. In each set, the mice were housed in groups of six (two diabetic groups and two nondiabetic groups). Each mouse was marked for identification and observed on a daily basis for the following parameters: urine glucose and ketones, size and appearance of abscess, and evidence of systemic symptoms such as lethargy or excessive sweating. The diabetic mice developed glucosuria within 1 wk after delivery

From the Department of Medicine, Diabetes Section (A.N.B.) and Infectious Disease Section (F.L.S., M.T., J.Z.M.), Rancho Los Amigos/University of Southern California Medical Center, Downey, California.

Address reprint requests to A. N. Bessman, M.D., Rancho Los Amigos Medical Center, 256 Harriman Building, 7601 E. Imperial Highway, Downey, California 90242.

Received for publication 22 May 1985 and in revised form 13 September 1985.

at which time the injections of the various combinations of bacteria were made (vide infra).

Each mouse was injected intradermally in the groin area, according to the method of Brook et al.[15] Three organisms commonly found in diabetic foot infections were selected: *E. coli*, enterococcus, and *Bacteroides fragilis*. An *E. coli* from a clinical blood isolate from a patient with pyelonephritis was tested in a trial series of studies. The mice either died (0.1 cm^3, 10^7/ml) or no abscesses developed (0.1 cm^3, 10^6/ml). *E. coli* Yale strain was then used. This strain has been well described[16] and is K-antigen negative, serum resistant, and hemolysin negative. A capsule was demonstrated.[17,18] The *Bacteroides fragilis* was obtained from an abscess cavity of a patient with a deep pressure sore abscess and was capsule positive. The enterococcus was obtained from a blood culture of a patient with enterococcal pyelonephritis and was capsule negative. A total volume of 0.2 cm^3 was injected of the following bacterial suspensions: group 1: E. coli 0.1 cm^3, 10^7/ml and enterococcus 0.1 cm^3, 10^8/ml; group 2: enterococcus 0.1 cm^3, 10^8/ml and *B. fragilis* 0.1 cm^3, 10^8/ml; and group 3: *E. coli* 0.1 cm^3, 10^7/ml and *B. fragilis* 0.1 cm^3, 10^8/ml. The bacterial concentration of *E. coli* was reduced to 10^7/ml because we had found that 0.1 cm^3 of 10^8/ml *E. coli* caused 90% mortality.

After injection of the inocula, the 12 diabetic and 12 nondiabetic mice from each of groups 1, 2, and 3 (vide supra) were randomly divided into subsets of six for killing at 1 and 2 wk. At the end of the 1- and 2-wk intervals after abscess induction, under intraperitoneal nembutal anesthesia, the abscesses were dissected out in toto for quantitative microbiology. Blood was obtained by cardiac puncture at these times for blood glucose levels. Quantitative counts of the abscesses were done by grinding the removed tissue in a Beck's tissue grinder according to methods previously described.[19] The media used were MacConkey and sheep blood agar plates for *E. coli*, Columbia CNA agar and sheep blood agar for enterococcus, and Brucella-Menadione in blood agar and Kanamycin-Vancomycin laked agar for *B. fragilis*. Standard aerobic and anaerobic dilution and incubation techniques were used. The grinding and other procedures were performed in an anaerobic chamber when *B. fragilis* was one of the injected organisms.

RESULTS

After injection of the bacterial suspensions, all of the mice developed discrete palpable abscesses within 24–48 h. The peak size was reached at 72–96 h. At this time, there was moderate local tenderness that uniformly subsided over 2–4 days. The abscess size gradually decreased over the next week, but remained palpable in most animals. After 4–5 days, the consistency of the abscess in the nondiabetic animal was much firmer than that in the diabetic. In the abscesses in which *E. coli* was part of the flora, spontaneous drainage occurred in some of the abscesses (see Table 1). At the time of killing, the abscesses were well-localized, discrete, and easily removed intact. In some of the nondiabetic mice, the abscesses induced by enterococcus and *E. coli* were very hard and white at 2 wk. The diabetic mice in which *E. coli* and enterococcus were injected were clinically "sick" (lethargic, increased sweating) for about 48 h. In the diabetic mice with *E. coli* and enterococcus abscesses, the three with spontaneous drainage were all in the group killed at 2 wk. Two of these were the mice that had no growth and the third had the lowest colony count. In the diabetic mice with *E. coli* and *B. fragilis*, one of the mice with no growth at 2 wk had spontaneous drainage; the other two with no growth did not have spontaneous drainage. These findings are summarized in Table 1; the daily urine glucose and ketone values are also summarized in Table 1. None of the nondiabetic mice had glucosuria or ketonuria. All of the diabetic mice remained 4+ glucosuric but none had ketonuria at any time.

At both 1 and 2 wk there were no significant differences in the blood glucose levels of the diabetic mice injected with the three different bacterial combinations or between the nondiabetic mice of the three different groups. The blood glucose levels are given in Tables 2–4. There did not appear to be any significant differences of blood glucose levels between the diabetic mice with large colony counts and those in which no colonies were grown (see Tables 2–4).

The bacterial counts (reported as log 10) of the abscesses from groups 1, 2, and 3 are given in Tables 2, 3, and 4, respectively. The one-tailed *t*-test was used for statistical analysis because of the prediction, on the grounds of biologic experience, that the diabetic host would have a more severe infection than the nondiabetic.

TABLE 1
Clinical and pathologic characteristics

	E. coli Enterococcus		Enterococcus *B. fragilis*		*E. coli* *B. fragilis*	
	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Urine glucose	0	3–4+	0	3–4+	0	3–4+
ketones	0	0	0	0	0	0
Mortality after injection	0	0	0	0	0	0
Morbidity	0	Excess sweating, lethargy first 48 h 12 of 12	0	0	0	0
Time to maximum abscess size (h)	48	72	48	72	48	72
Avg. maximum palpable size of abscess	3 × 4 cm	3 × 6 cm	3 × 5 cm	3 × 6 cm	2 × 3 cm	3 × 4 cm
Consistency of abscess: firmness	4+	2+	4+	1+	3+	1+
Spontaneous external drainage	7 of 12	3 of 12	0	0	5 of 12	3 of 12

TABLE 2
Blood glucose levels and colony counts* from abscesses induced with *E. coli* and enterococcus

	Diabetic			Nondiabetic		
	E. coli A	Enterococcus B	Blood glucose (mg/dl)	*E. coli* C	Enterococcus D	Blood glucose (mg/dl)
Day 7	5.45	5.64	590	6.90	7.36†	204
	7.00	7.15	716	5.38	5.18†	207
	6.30	6.90	648	4.04	3.75†	218
	3.30	3.45	795	2.30	4.48†	218
	5.53	5.70	618	5.59	5.63†	220
	4.48	5.50	685	6.18	6.36†	196
Total	32.06	34.34	4052	30.39	32.76	1263
Mean	5.34	5.72	675	5.10	5.46	210
SD	1.31	1.31	74.00	1.65	1.30	9.67
No significant differences in colony counts between diabetic and nondiabetic mice.						
	A	B	Blood glucose (mg/dl)	C	D	Blood glucose (mg/dl)
Day 15	7.04	6.90	840	3.95	3.15	200
	7.04	7.00	792	0	0†	180
	4.95	4.48	806	0	0	190
	2.00	3.08†	740	0	0	188
	0	0†	754	0	0†	169
	0	0†	786	0	0	210
Total	21.03	21.46	4718	3.95	3.15	1137
Mean	3.51	3.58	786	0.66	0.53	190
SD	3.28	3.24	36.03	1.61	1.29	1445
$P < 0.05$ comparing A versus C and B versus D.						
$P < 0.005$ comparing A + B versus C + D.						

*Reported as log 10.
†Spontaneous external drainage.

Table 2 summarizes the data from group 1 mice in which *E. coli* and enterococci were injected. At 1 wk, there were no significant differences in colony counts between diabetic and nondiabetic mice. Two weeks after injection, there were significantly higher counts of *E. coli* alone, enterococcus alone, and both organisms in combination in the diabetic compared with the nondiabetic mice. At 2 wk, five of six nondiabetic mice grew no organisms from the abscess site, whereas culture of the abscess sites from only two of six diabetic mice grew no organisms. In all abscesses containing viable bacteria, both *E. coli* and enterococci were recovered.

Table 3 summarizes the data from group 2 mice in which enterococci and *B. fragilis* were injected. At the end of the first week, the cultures from the diabetic mice already demonstrated significantly increased colony counts compared with the nondiabetic mice. *B. fragilis* were not recovered from the abscesses in two of the nondiabetic mice at the end of 1 wk. At 2 wk, the abscesses in diabetic mice showed significantly higher colony counts, comparing enterococcus and *B. fragilis* both individually and in combination. At 2 wk, all of the abscesses in the diabetic mice grew both enterococcus and *B. fragilis*, but in the nondiabetic mice, no abscesses grew *Bacteroides* and one did not grow enterococcus as well.

Table 4 summarizes the data from the mice in which *E. coli* and *B. fragilis* were injected. At 1 wk, there were no significant differences in colony counts. At 2 wk, the abscesses in the diabetic animals showed significantly higher colony counts than those in the nondiabetic mice, comparing *E. coli* and *B. fragilis* alone and the combination of bacteria. By 2 wk, three of six diabetic and all six nondiabetic mice had eradicated both species of bacteria.

Organisms other than those injected were not found in any of the abscess cultures.

DISCUSSION

Controversy regarding whether or not patients with diabetes mellitus are more susceptible to infection than age- and sex-matched nondiabetic control patients has eluded resolution,[8,20] although it has generally been accepted that infections in the diabetic patient are more severe and often more difficult to control.[20,21] Defects in granulocyte function, such as phagocytosis, intracellular killing ability, and granulocyte adherence, have been reported in in vitro studies using white cells from diabetic and nondiabetic patients.[10–13] Using both pneumococci and staphylococci, improvement in these granulocytic functions was shown to correlate with degree of improvement of blood glucose levels. Similarly, Bagdade et al.[9] found that 76–100% of control granulocyte adherence was achieved after treatment of the diabetic state when blood glucose levels were 190 mg/dl or below. When posttreatment hyperglycemia of >200 mg/dl remained, granulocyte adherence remained proportionally decreased. Using both artificially induced and genetic mouse models of diabetes, Mahmoud et al.[14] showed a suppression of granuloma formation in the diabetic host compared with the nondiabetic control. Treatment of the diabetic state resulted in a reversal of this suppression, but if blood glucose levels remained at 225 mg/dl or higher, very little improvement was seen. Abnormalities in other aspects of the immune system[13] and

wound healing[22] have also been demonstrated. The clinical significance of these various findings in relation to bacterial infections remains unclear.

The blood glucose levels of the mice in the three experimental groups were similar at 1 and at 2 wk, all markedly hyperglycemic. The mice with and without positive abscess cultures had similar blood glucose levels. Therefore, the differences in colony counts seen when various combinations of bacteria were used are likely secondary to differences in bacterial synergism rather than differences in degree of hyperglycemia between the groups of individual animals. These findings are consistent with the evidence (vide supra) that near-normal blood glucose levels are necessary for improvement of many altered host defense mechanisms in the hyperglycemic diabetic.

That decreased circulation and abnormal vasculature might contribute to the susceptibility of diabetic patients to various infections, especially lower extremity lesions, has been a matter of speculation. The abscesses in the present study were induced in the soft tissue and not in the extremities. Furthermore, microscopic studies have not revealed vascular abnormalities in the retina and other organs in the *db/db* mouse at the age studied in the current experiments.[23,24]

In the current study, *E. coli* and enterococcus, facultative aerobes, and *B. fragilis*, an anaerobe, were selected as the inciting agents for subcutaneous abscess formation because they are commonly identified bacteria in polymicrobial soft tissue infections in diabetic patients.[5–7] The results demonstrated that, 1 wk after induction of a polymicrobial subcutaneous abscess using these combinations of organisms, there was significantly greater bacterial growth in the diabetic host in only one combination of bacteria, that of enterococcus and *B. fragilis*. However, 2 wk after induction of polymicrobial abscesses using the described combinations of bacteria, the total bacterial counts in diabetic mice were significantly greater with all three combinations of bacteria as compared with nondiabetic mice (see tables). These findings may be relevant to the cited findings of delayed intracellular killing despite engulfment by granulocytes.[12]

In contrast to findings in rats that intra-abdominal abscesses formed only with inocula of both an anaerobe and a facultative aerobe[25] and that abscesses failed to develop with inocula of *E. coli* and enterococci, all groups of mice in the current study developed abscesses with the combinations of bacteria described, including *E. coli* and enterococcus. Encapsulation and capsular material have been shown to enhance abscess formation of various anaerobes and aerobes.[26,27] It has been shown that nonencapsulated organisms may develop the potential for capsule formation and abscess induction when injected with encapsulated organisms.[28] In our studies, both the *B. fragilis* and *E. coli* had capsules. The potential for capsule formation, and/or a more susceptible host, may explain the ability of the *E. coli* and enterococcus to produce abscesses in our model and not in the rat.

The clinical course of the abscesses seen in the current study was similar to that reported by others.[29,30] Spontaneous drainage from the abscesses has been reported[29] and was observed in our mice. In the present studies, no drainage

TABLE 3
Blood glucose levels and colony counts* from abscesses induced with enterococcus and *Bacteroides fragilis*

	Diabetic†			Nondiabetic		
	Enterococcus A	*B. fragilis* B	Blood glucose (mg/dl)	Enterococcus C	*B. fragilis* D	Blood glucose (mg/dl)
Day 7	7.88	7.30	654	5.32	5.64	204
	7.90	7.46	800	5.88	6.48	237
	6.95	5.89	695	4.14	0	220
	8.15	7.53	625	4.15	0	175
	7.66	7.08	746	5.40	6.09	218
				5.04	5.88	204
Total	38.54	35.16	3520	29.93	24.09	1258
Mean	7.71	7.05	704	4.99	4.02	210
SD	0.46	0.67	70.36	0.71	3.12	20.93
$P < 0.001$ A versus C and A + B versus C + D.						
$P < 0.05$ B versus D.						

	A	B	Blood glucose (mg/dl)	C	D	Blood glucose (mg/dl)
Day 15	4.60	2.00	792	3.38	0	190
	4.70	4.78	750	3.08	0	212
	5.00	3.72	838	2.30	0	212
	7.96	7.08	798	2.30	0	190
	5.49	5.60	844	2.30	0	178
				0	0	220
Total	32.75	23.18	4022	13.36	0	1202
Mean	4.63	3.86	804	2.23		200
SD	2.58	2.55	38.25	1.19		16.56
$P < 0.001$ B versus D and A + B versus C + D.						
$P < 0.005$ A versus C.						

*Reported as log 10.
†N = 5 instead of 6 because 2 diabetic mice died before any injections were given.

TABLE 4
Blood glucose levels and colony counts* from abscesses induced with *E. coli* and *Bacteroides fragilis*

	Diabetic			Nondiabetic		
	E. coli A	*B. fragilis* B	Blood glucose (mg/dl)	*E. coli* C	*B. fragilis* D	Blood glucose (mg/dl)
Day 7	7.77	8.03	790	6.70	7.04†	212
	7.18	6.72	675	5.53	5.85	212
	5.18	3.76†	694	3.75	0†	208
	5.11	3.69†	605	5.62	7.11†	146
	7.04	7.97	714	6.11	7.30	180
	6.10	6.13	560	0	2.00†	180
Total	38.38	37.30	4038	27.71	29.30	1138
Mean	6.40	6.22	673	4.62	4.88	190
SD	1.11	2.05	81.50	2.47	3.12	26.18
No significant differences in colony counts between diabetic and nondiabetic mice.						
	A	B	Blood glucose (mg/dl)	C	D	Blood glucose (mg/dl)
Day 15	6.51	8.04	790	0	0	239
	5.82	7.18	792	0	0	190
	6.85	7.98	744	0	0	178
	0	0†	796	0	0	240
	0	0	812	0	0	190
	0	0	742	0	0	180
Total	19.18	23.20	4676	0	0	1216
Mean	3.20	3.87	779			203
SD	3.52	4.25	29.19			28.58
$P < 0.025$ comparing A versus C and B versus D.						
$P < 0.01$ comparing A + B versus C + D.						

*Reported as log 10.
†Spontaneous external drainage.

occurred if *E. coli* was not part of the inoculum. Since the lowest colony counts were associated with spontaneous drainage in the diabetic group of mice with *E. coli* and enterococcal abscesses, and since this same trend was seen in the group with abscesses from *E. coli* and *B. fragilis*, the ability to effect spontaneous drainage may be another impaired host resistance factor in diabetes.

E. coli has been shown to be the organism primarily responsible for the bacteremia seen in the first few days of polymicrobial intra-abdominal abscesses in the rat.[31] In our model, it is probable, as indicated by systemic symptoms, that the diabetic mice injected with *E. coli* and enterococcus were bacteremic for the first 48 h. Neither of the other two inocula resulted in systemic symptoms. These findings suggest that enterococcus may enhance the bacteremic potential of *E. coli* in the diabetic mouse.

In addition to the above evidence that the diabetic host was less effective in diminishing or eradicating the growth of bacteria in the abscesses at 2 wk, the results demonstrate that there is differential bacterial synergy in the diabetic and nondiabetic host. When *B. fragilis* was injected with enterococcus, all of the nondiabetic but none of the diabetic animals had eradicated *B. fragilis* after 2 wk. The enterococcus remained viable in 91% of all animals, diabetic and nondiabetic. When *B. fragilis* was injected with *E. coli*, all of the nondiabetic and 50% of the diabetic animals had eradicated *B. fragilis* at the end of 2 wk. Thus, the presence of enterococci provided more synergism for persistence of growth of *B. fragilis* in the diabetic host than did the presence of *E. coli*. At the end of 2 wk, the anerobe, *B. fragilis*, was eradicated whether injected with enterococcus or *E. coli* in all nondiabetic animals (12 of 12) but in only 22% (3 of 11) in diabetic animals.

In a clinical study of diabetic patients who were septic from infected gangrene, *B. fragilis* was the organism most frequently isolated from the blood.[32] Although *B. fragilis* is isolated from the infected soft tissue of these patients, the most frequently isolated organisms are facultative aerobic gram-negative *Enterobacteriaceae* and enterococcus. The current study reaffirms the decreased resistance of the diabetic host to *B. fragilis*.

The persistence of enterococcus in the abscesses was influenced by whether the infection included *E. coli* or *B. fragilis*. When injected with *E. coli*, enterococcus was eradicated in 5 of 6 nondiabetic animals and in 2 of 6 diabetic animals. When enterococcus was injected with *B. fragilis*, it was eradicated in only one of the nondiabetic mice and in none of the diabetic mice. The findings demonstrate that, in the diabetic and nondiabetic, the presence of *B. fragilis* renders the enterococcus a more persistent pathogen in soft tissue infections than does the presence of *E. coli*. The implications of these findings, that the diabetic hosts are at risk for infections with enterococcus and *B. fragilis*, are consistent with the frequent association of these bacteria in clinical infections.

The pathogenicity of enterococcus has been redocumented recently with the description of enterococcal endocarditis developing on prosthetic valves after sigmoidoscopy.[33] The current study seemed to demonstrate that enterococcus enhanced the bacteremic potential of *E. coli*. In view of these findings, the synergistic role of *B. fragilis* in

relation to the persistence of enterococcus, and the synergistic role of enterococcus in relation to *E. coli* in the diabetic host, assumes increased clinical relevance.

A recent review of soft tissue abscesses in an outpatient nondiabetic population revealed that *Staphylococcus aureus* was the sole causative agent in only 19% of patients. The majority of abscesses were polymicrobial in nature, and healed without antibiotic treatment.[34] In contrast, diabetic patients with polymicrobial abscesses have been reported to require both antibiotics and surgical intervention for healing.[35] The animal data of the current study support the clinical impressions that the diabetic host is less able to effect spontaneous external drainage and to eradicate established infections than is the nondiabetic host.

The diabetic animals in the present study were untreated for their diabetes; the mice were hyperglycemic but not ketotic. Studies showing defective white cell function as well as delayed wound healing have demonstrated partial but not complete correction of these defects with control of the diabetic state. The possibility that treatment of the diabetic state would enable the diabetic host to contain or eradicate these infections in the same manner as the nondiabetic host remains to be studied.

In summary, our studies provide evidence to substantiate the clinical impression that polymicrobial bacterial infections in the diabetic are more difficult to control than in the nondiabetic, and that the diabetic host is less able to suppress or eradicate bacterial growth in polymicrobial soft tissue infections. In addition, in polymicrobial soft tissue infections in the diabetic host as compared with the nondiabetic host, the increased pathogenicity of enterococcus as well as the increased pathogenicity of *B. fragilis* have been demonstrated.

ACKNOWLEDGMENTS

The authors wish to thank Virginia Ginunas and San Leon Sullivan for their skilled technical assistance.

REFERENCES

[1] Wheat, L. J.: Infection and diabetes mellitus. Diabetes Care 1980; 1:187–97.

[2] Casey, J. I.: Host defense and infections. *In* Diabetes Mellitus, Theory and Practice, 3rd Edit. Ellenberg, M., and Rifkin, H., Eds. New Hyde Park, New York, Medical Examination Publishing Co., 1980:670–71.

[3] Most, R. S., and Sinnock, P.: The epidemiology of lower extremity amputations in diabetic individuals. Diabetes Care 1983; 6:87–91.

[4] Bessman, A. N., and Wagner, F. W., Jr.: Non-clostridial gas gangrene. JAMA 1965; 233:958–63.

[5] Louie, T. J., Bartlett, J. G., Tally, F. P., and Gorbach, S. L.: Aerobic and anaerobic bacteria in diabetic foot ulcers. Ann. Intern. Med. 1976; 85:461–63.

[6] Sharp, C. S., Bessman, A. N., Wagner, F. W., Jr., and Garland, D.: Microbiology of deep tissues in diabetic gangrene. Diabetes Care 1978; 1:289–92.

[7] Fierer, J., Daniel, D., and Davis, C.: The fetid foot: lower extremity. Infections in patients with diabetes mellitus. Rev. Infect. Dis. 1979; 1:210–17.

[8] Savin, J. A.: Bacterial infections in diabetes mellitus. Br. J. Dermatol. 1974; 91:481–87.

[9] Bagdade, J. D., Stewart, M., and Walters, E.: Impaired granulocyte adherence, a reversible defect in host defense in patients with poorly controlled diabetes. Diabetes 1978; 27:677–81.

[10] Bagdade, J. E., Nielson, K. L., and Bulger, R. J.: Reversible abnormalities in phagocytic function in poorly controlled diabetic patients. Am. J. Med. Sci. 1972; 263:451–56.

[11] Bagdade, J. D., Root, R. K., and Bulger, R. J.: Impaired leucocyte function in patients with poorly controlled diabetes. Diabetes 1974; 23:9–15.

[12] Nolan, C. M., Beaty, H. N., and Bagdade, J. D.: Further characterization of the impaired bactericidal function of granulocytes in patients with poorly controlled diabetes. Diabetes 1978; 27:889–94.

[13] Kitihara, M., Eyre, H. J., Lynch, R. E., et al.: Metabolic activity of diabetic monocytes. Diabetes 1980; 29:251–56.

[14] Mahmoud, A. A. F., Rodman, H. M., Mandel, M. A., et al.: Induced and spontaneous diabetes mellitus and suppression of cell mediated immunologic responses. J. Clin. Invest. 1976; 57:362–67.

[15] Brook, I., Coolbaugh, J. D., and Walker, R. I.: Antibiotic and clavulanic acid treatment of subcutaneous abscesses caused by *Bacteroides fragilis* alone or in combination with aerobic bacteria. J. Infect. Dis. 1983; 168:156–59.

[16] Guze, L. B., Montgomerie, J. Z., Potter, C. S., et al.: Pyelonephritis. XVI. Correlates of parasite virulence in acute ascending *Eschericia coli* pyelonephritis in mice undergoing diureses. Yale J. Biol. Med. 1973; 46:203–11.

[17] Lennette, E. H., Balows, A., Hausler, W. J., Jr., and Truant, J. P., Eds.: Manual of Clinical Microbiology, 3rd Edit. Washington, D.C., American Society of Microbiology, 1980.

[18] Kasper, D. L.: Chemical and biological characterization of the lipopolysaccharide of *Bacteroides fragilis*, subspecies *fragilis*. J. Infect. Dis. 1976; 134:59–66.

[19] Sapico, F. L., Witte, H. N., Canawati, H. C., et al.: The infected foot of the diabetic patient: quantitative microbiology and analysis of clinical features. Rev. Infect. Dis. 1984; 6:S171–76.

[20] Johnson, J. E.: Infection and diabetes. *In* Diabetes Mellitus, Theory and Practice. Ellenberg, M., and Rifkin, H., Eds. New York, McGraw-Hill, 1970:734–45.

[21] Thornton, G. F.: Infections and diabetes. Med. Clin. North Am. 1971; 55:931–38.

[22] Goodson, W. H., III, and Hunt, T. K.: Wound healing and the diabetic patient. Surg. Gynecol. Obstet. 1979; 149:600–608.

[23] Hummel, K. P., and Coleman, D. L.: Diabetes, a new mutation in the mouse. Science 1966; 153:1127–28.

[24] Coleman, D. L., and Hummel, K. P.: Studies with the mutation, diabetes, in the mouse. Diabetologia 1967; 3:238–48.

[25] Onderdonk, A. B., Bartlett, J. G., Louie, T., et al.: Microbial synergy in experimental abdominal abscess. Infect. Immunol. 1976; 13:22–26.

[26] Kasper, D. L., Onderdonk, A. B., Polk, B. F., and Bartlet, J. G.: Surface antigens as virulence factors in infection with *Bacteroides fragilis*. Rev. Infect. Dis. 1979; 1:278–88.

[27] Onderdonk, A. B., Kasper, D. L., and Bartlett, J. G.: The capsular polysaccharide of *Bacteroides fragilis* as a virulence factor: comparison of the pathogenic potential of encapsulated and unencapsulated strains. J. Infect. Dis. 1977; 136:82–89.

[28] Brook, I., and Walker, R. I.: Infectivity of organisms recovered from polymicrobial abscesses. Infect. Immunol. 1983; 42:986–89.

[29] Joiner, K. A., Onderdonk, A. B., Gelfand, J. A., et al.: A quantitative model for subcutaneous abscess formation in mice. Br. J. Exp. Pathol. 1980; 61:97–107.

[30] Brook, I., and Walker, R. I.: Pathogenicity of anerobic gram-positive cocci. Infect. Immunol. 1984; 45:320–24.

[31] Bartlett, J. G., and Gorbath, S. L.: Experimental intra-abdominal abscesses in rats: quantitative bacteriology of infected animals. Infect. Immunol. 1974; 10:1256–59.

[32] Sapico, F. L., Bessman, A. N., and Canawati, H. C.: Bacteremia in patients with infected lower extremities. Diabetes Care 1982; 5:101–104.

[33] Rodriguez, W., and Levine, J.: Enterococcal endocarditis following flexible sigmoidoscopy. West J. Med. 1982; 140:951–53.

[34] Meislin, H. W., Lerner, S. A., Graves, K. H., et al.: Cutaneous abscesses, anaerobic and aerobic bacteriology and outpatient management. Ann. Intern. Med. 1977; 87:145–49.

[35] Bessman, A. N., Tabatabai, M. F., and Sapico, F. L.: Non-*Staphylococcal aureus* abscesses in the diabetic patient. Abstract. Diabetes 1985; 34 (Suppl. 1):202A.