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Human Immune Responses to Oral Microorganisms: Patterns of Systemic Antibody Levels to *Bacteroides* Species

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Human systemic antibody levels to oral members of the *Bacteroides* genus were assessed with an enzyme-linked immunosorbent assay. Antibody levels to *B. gingivalis*, two homology groups of *B. intermedius*, *B. melaninogenicus*, *B. denticola*, *B. loescheii*, *B. corporis*, *B. oralis*, *B. buccae*, and *B. gracilis* were determined in subjects with localized juvenile periodontitis, advanced destructive periodontitis, or adult periodontitis and in normal persons. Significantly elevated serum immunoglobulin G (IgG) antibody levels to *B. gingivalis* were seen in adult and advanced destructive periodontitis patients. Serum IgM and IgA antibodies were increased in diseased versus normal subjects, whereas negligible levels of serum IgE antibody were detected to this microorganism. Serum IgG antibody levels to *B. intermedius* were increased in advanced destructive periodontitis patients; however, the frequency of elevated responses were similar among the groups. Extreme antibody levels to the other *Bacteroides* spp. were occasionally observed in this population. Additionally, all of the elevated levels were found in diseased patients. Distribution analyses of the antibody levels indicated that most patients exhibited a pattern of elevated antibodies to a limited number of the oral *Bacteroides* spp. The results suggested that elevated systemic antibody levels to oral *Bacteroides* spp. are more frequently found in periodontal disease patients. These antibody responses presumably reflect a colonization of the patients. The distribution of the responses may indicate the potential pathogenicity of the microorganisms and is consistent with distinctive host-parasite interactions in this disease.

Members of the genus *Bacteroides* have been isolated as a portion of the gram-negative anaerobic microbiota of human periodontal lesions (25, 31, 34, 36, 37). These bacteria have also been cultured from periodontal disease in both primates (11, 27) and dogs (12, 32). Furthermore, the transmissible pathogenicity of *Bacteroides melaninogenicus* has been demonstrated with animal models (15, 29).

Studies have provided evidence for a bacterial specificity in different forms of periodontal disease (28, 30). Bacterial infections are frequently accompanied by an immune response that is specific for the pathogenic microorganism (21). As such, both cell-mediated immune responses (9, 13, 22) and antibody responses (7, 14, 16, 18) to *Bacteroides* species have been examined in studies of human periodontal disease. The purpose of this report is to delineate, by cross-sectional analyses, the association of systemic responses to *Bacteroides* spp. with human periodontal disease.

MATERIALS AND METHODS

Microorganisms. *Bacteroides gingivalis* 381 (Forsyth Dental Center collection, [FDC]), *B. intermedius* 581 (FDC), *B. intermedius* 8944 (L. V. Holdeman, Virginia Polytechnic Institute), *B. denticola* 10043 (L. V. Holdeman), *B. loescheii* ATCC 15930 (L. V. Holdeman), *B. melaninogenicus* 287 (FDC), *B. corporis* 9342 (L. V. Holdeman), and *B. buccae* A628 (FDC) were cultured anaerobically (80% N_2, 10% CO_2) at 37°C on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% sheep blood and supplemented with 5 μg of hemin per ml and 0.3 μg of menadione per ml. *B. gracilis* 1084 (FDC) was cultured anaerobically on unsupplemented 5% sheep blood agar.

The organisms were prepared for antibody analyses by growth under anaerobic conditions at 37°C in mycoplasma broth base (BBL) supplemented with hemin (5 μg/ml) and menadione (0.3 μg/ml). The organisms were harvested by centrifugation (13,000 × *g*; 20 min) and washed three times in phosphate-buffered saline (0.02 M phosphate) containing 1 mM EDTA. The bacteria were killed with 0.5% buffered formal saline by incubation at room temperature for 16 to 18 h. The organisms were again washed three times in phosphate-buffered saline–EDTA and stored at 4°C.

Antisera. Rabbit anti-human immunoglobulin G (IgG; lot D4020), IgM (lot 0919F), IgA (lot D4638), and IgE (lot D4877) were obtained commercially (Calbiochem-Behring, Somerville, N.J.) and used in the development of the enzyme-linked immunosorbent assay (ELISA). Goat anti-rabbit IgG (14.8 mg of antibody per ml; Miles Laboratories, Inc., Elkhart, Ind.) was conjugated to alkaline phosphatase (type VIII; Sigma Chemical Co., St. Louis, Mo.) as previously described (6).

Antibody analysis. An ELISA was used to determine antibody activity in the human serum to the various *Bacteroides* species (3). Briefly, the formalinized microorganisms were suspended in 0.1 M $NaCO_3$ buffer (pH 9.6) containing 0.02% NaN_3 and attached to polystyrene microtiter plates (Linbro) at 37°C for 3 to 4 h. The plates were then stored at 4°C and used in the assay. A preliminary study determined that 4×10^7 to 8×10^7 organisms were attached to the ELISA plates (3). To detect human serum antibody, the antigen-coated plates were incubated for 2 h at room temperature with appropriate dilutions of the human serum. After the plates were washed, rabbit anti-human isotype-specific antiserum was added and incubated for 2 h at room temperature. The reaction was developed by incubation for 16 to 18 h with goat anti-rabbit IgG conjugated to alkaline phosphatase and addition of substrate (*p*-nitrophenylphos-

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TABLE 1. Reference human serum IgG antibody activity and standard curves for *Bacteroides* species

Bacteroides species	Reference standard antibody activity (EU)	Standard curve OD = m ($\log_{10}$ EU) + b		
		m	b	r
B. gingivalis 381	69.0[a]	1.146 (0.993–1.236)[b]	−0.710	0.998
B. intermedius 581	88.1	1.069 (0.967–1.303)	−0.867	0.986
B. intermedius 8944	100	1.317 (1.225–1.401)	−0.933	0.991
B. melaninogenicus 287	75.0	0.960 (0.909–1.108)	−0.847	0.995
B. denticola 10043	53.0	1.166 (0.989–1.206)	−0.843	0.984
B. loescheii 15930	11.2	0.805 (0.799–0.877)	−0.664	0.991
B. oralis 33321	42.0	0.802 (0.743–0.885)	−0.625	0.974
B. gracilis 1084	49.0	0.983 (0.836–1.111)	−0.752	0.979
B. corporis 9342	1.2	0.893 (0.821–0.926)	−0.653	0.997
B. buccae A628	46.8	0.957 (0.915–1.030)	−0.700	0.989

[a] Antibody activity for each organism was determined by equating 1 EU to the same OD for each antigen system.
[b] Range of slopes determined from at least eight different analyses.

phate). The extent of color was determined at 405 nm (ARTEK ELISA reader; ARTEK Industries).

Antibody activity in the human sera is expressed as ELISA units (EU), defined by a reference curve prepared by a linear regression analysis. A single serum pool was used as a reference reactivity to all *Bacteroides* species. To describe a relationship between antibody levels to the different *Bacteroides* species, the arbitrary EU were adjusted for each organism. This was accomplished by using a dilution of the reference serum such that a similar optical density (OD) at 405 nm was obtained after reaction with each microorganism. Thus, the OD reactivity to a certain microorganism could be extrapolated so that the value of 1 EU for each organism was related to the same extent of reaction (OD). The antibody activity (EU) for each experimental serum was determined by comparison with the reference serum curves (Table 1). Each experimental serum was assayed in triplicate at a single dilution. The systems were designed so that the majority of the sera would exhibit an OD within the linear range of the standard curves. If a sample OD was outside the range of the reference, that sample was retested at higher dilutions. The slopes of the regression of IgG antibody binding to the different *Bacteroides* species were quite similar (Table 1), which is consistent with the ability to relate the EU among the microorganisms. Second, the y intercepts of all of the curves were negative, indicating a background EU level associated with the nonspecific binding of the developing reagents to the antigens. Finally, the antibody in the reference standard serum showed a broad range of reactivities with a low antibody content to *B. corporis* and high levels to the *B. intermedius* genotypes. The equalization of the EU by this method provides the capability to relate antibody levels to the different microorganisms within each of the patients.

The Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U test were used to analyze antibody levels. Analyses of the distribution of responding individuals in the disease categories were performed by using the chi-square test, and correlations among the antibody responses were determined by a Spearman rank analysis (24).

Adsorption procedure. To determine the specificity of the antibody responses to the *Bacteroides* species, adsorptions of sera with the formalinized microorganisms were performed. A 1:25 dilution of four human sera (1.0 ml) was incubated with 10^9 organisms in 1.0 ml of phosphate-buffered saline and mixed for 2 h at 37°C. The organisms were removed by centrifugation, and the resulting 1:50 serum dilution was used to test for residual antibody activity. Four additional human serum samples were subjected to three consecutive adsorptions with 10^9 microorganisms. A mean baseline EU value was subsequently determined from these four sera. Positive samples were those with EU values greater than 2 standard deviations above the mean negative level.

Patient samples. All serum samples were obtained from patient populations of the following: Forsyth Dental Center; the School of Dentistry, State University of New York at Buffalo; and the Center for Research in Oral Biology, University of Washington. Blood was drawn by venipuncture, and serum was collected by centrifugation after clotting. The samples were kept frozen (−20°C) until analyzed. The patients selected had radiographic evidence of a previous episode of destructive periodontal disease, may have had previous treatment but not within the previous 6 months, had had no antibiotics within the previous 6 months, and had no history of systemic disease.

Four groups of individuals were used in this study. A localized juvenile periodontitis (LJP) group was composed of 67 patients (age range, 12 to 30 years) exhibiting molar-incisor bone loss as described previously (20, 35). The clinical criteria for an advanced destructive periodontitis (ADP) group (n = 62; age range, 13 to 35 years) have been described previously (5). This group also included both generalized juvenile periodontitis patients (35) and rapidly progressive periodontitis patients (20). Although this group is somewhat heterogeneous, the diagnostic criteria suggesting a more extensive disease were similar enough to incorporate the clinical interpretations for purposes of these studies. The adult periodontitis (AP) group of 69 patients (age range, 40 to 63 years) included AP patients as previously described (20, 35). In general, these patients represented an older population with a generalized bone loss pattern that may be a more chronic type of disease. Eighty-two normal individuals were included (age range, 18 to 53 years) who had some gingivitis and no bone loss.

RESULTS

Serum antibody to *B. gingivalis*. To determine whether humoral antibody levels to any of the *Bacteroides* species were associated with periodontal disease, patient sera were tested in ELISA against each of the *Bacteroides* species. Serum IgG responses to *B. gingivalis* were significantly increased in both frequency and level of activity in adult periodontitis and advanced destructive periodontitis patients when compared to all other diseased or normal individuals

($P < 0.003$; Fig. 1). These findings suggested that colonization of these patients with *B. gingivalis*, as reflected by an immune reactivity, may have been associated with the periodontal diseases.

To further define the antibody patterns of the diseased patients to *B. gingivalis*, IgM, IgA, and IgE isotype antibody levels were determined. Although increased IgM levels were seen in diseased patients when compared with normal patients (Table 2), the association with different classifications of periodontal disease was not as striking as was seen with serum IgG antibody. Serum IgA antibody was also present in the diseased patients (Table 2), but, like IgM antibody levels, the activity did not completely differentiate among the disease types. A randomly selected group of sera representing each of the patient categories was also analyzed for serum IgE antibody. Minimal IgE anti-*B. gingivalis* antibody was found in serum samples from any of the patients or normal individuals tested (Table 2).

The specificity of the serum antibody to *B. gingivalis* was assessed by adsorption studies with heterologous formalinized microorganisms. Although 10^7 homologous organisms removed >80% of the serum IgG activity, treatment of the human sera with 10^9 *B. intermedius*, *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Streptococcus sanguis*, or *Actinomyces viscosus* organisms had a negligible effect on the antibody levels in the sera (Table 3).

Serum antibody response to *B. intermedius*. A cross-sectional analysis of serum antibody levels was performed to examine the association of the antibodies to *B. intermedius* strain 581 with periodontal disease (Table 4). In contrast to *B. gingivalis* responses, only ADP patients exhibited a

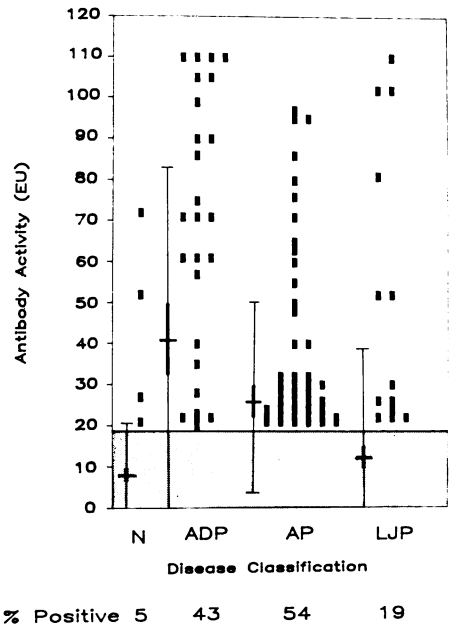

FIG. 1. Human serum IgG antibody responses to *B. gingivalis* 381. The points represent patient samples with antibody activity greater than background reactivity. Also, the remaining subjects in each group (i.e., 78 of the normal group) that have levels of antibody below the threshold are not represented individually. Background reactions were determined by adsorptions of four reactive sera with formalinized *B. gingivalis* organisms. The stippled area represents the mean ± 2 standard deviations of quadruplicate determinations of the four adsorbed sera. Any antibody levels greater than this background were considered positive. The horizontal lines (—) denote the mean value of the group, the thick vertical bar (▮) encloses 2 standard errors of the mean, and the vertical brackets (I) enclose 2 standard deviations. *n* values: normal patients (N), 82; ADP patients, 62; AP patients, 69; and LJP patients, 67

TABLE 2. Serum antibody responses to *B. gingivalis* in periodontal disease patients

Disease category	*n*	Serum antibody activity (EU)		
		IgM[a]	IgA	IgE
LJP	67	38 ± 9[b]	17 ± 6[b]	2.2 ± 1.4
ADP	62	139 ± 46[b,c,d]	26 ± 9[b]	1.9 ± 0.7
AP	69	63 ± 11[b,c]	17 ± 3[b]	3.0 ± 1.0
Normal	82	21 ± 4	6 ± 2	1.3 ± 0.6

[a] Antibody activity determined for IgM at a 1:50 dilution of serum, for IgA at a 1:25 dilution, and for IgE at a 1:10 dilution. Similar to analyses for IgG, reference standard curves for each isotype were created by a linear regression analysis. The samples were measured in triplicate at the dilution stated. The system was adjusted so that the major portion of the sera would have reactivities within the range of the standard curves. If any serum reaction was outside the range of the standard, that sample was retested at a higher dilution.

[b] Statistically different from the normal group at least at $P < 0.05$.

[c] Statistically different from the LJP group at least at $P < 0.05$.

[d] Statistically different from the AP group at least at $P < 0.05$.

TABLE 3. Specificity of human serum response to *B. gingivalis*

Microorganism for adsorption	No. of cells	% Antibody activity removed	
		B. gingivalis	*B. intermedius*
Bacteroides gingivalis 381	10^9	89.1[a]	7.9
	10^8	87.0	NT[b]
	10^7	86.0	NT
	10^6	49.7	NT
	10^5	3.0	NT
Bacteroides intermedius 581	10^9	9.8	97.6
	10^8	NT	93.2
	10^7	NT	79.4
	10^6	NT	38.3
	10^5	NT	11.6
Fusobacterium nucleatum 364	10^9	0	2.5
Actinomyces naeslundii I	10^9	6.4	0
Streptococcus sanguis 254	10^9	4.2	0
Eikenella corrodens 1073	10^9	9.9	1.7
Actinobacillus actinomycetemcomitans Y4	10^9	7.1	4.3
Wolinella recta 371	10^9	3.7	4.1
Campylobacter concisus 484	10^9	6.1	0.9

[a] Percentages are means of quadruplicate determinations for each condition.

[b] NT, Not tested.

TABLE 4. Serum antibody levels to *B. intermedius* 581 in periodontal disease patients

Disease category	*n*	Serum antibody activity, EU (% positive)		
		IgG	IgM	IgA
LJP	67	69 ± 8[a] (31)[b]	31 ± 14 (69)	9 ± 3 (33)
ADP	62	129 ± 25[c] (51)	83 ± 27 (76)	33 ± 12[c] (55)[c]
AP	85	54 ± 7 (34)	49 ± 11 (58)	12 ± 4 (17)
Normal	82	63 ± 7 (38)	33 ± 13 (51)	8 ± 6 (27)

[a] Mean ± standard error of the mean.
[b] Positive samples are those with antibody levels greater than 2 standard deviations above the mean baseline value of four adsorbed sera.
[c] Significantly greater than all other groups at $P < 0.01$.

significantly elevated serum IgG and IgA reaction ($P < 0.01$) to the microorganism when compared with other diseased or normal persons. The frequencies of antibody levels above background were generally similar among the groups, except for the IgA frequency in the ADP group, which was significantly elevated. A similar pattern of reactivity was noted with *B. intermedius* 8944 (17).

To determine whether the antibody levels of diseased patients to *B. gingivalis* and *B. intermedius* were mutually exclusive, individual reactions were compared (Fig. 2). A high percentage of ADP patients showed a combined response to both organisms (27 of 62), whereas LJP and AP patients reactions were primarily limited to one or the other of the microorganisms. Thus, a systemic response to both of these black-pigmented oral *Bacteroides* species was indicative of a more severe type of periodontal disease.

Serum antibody to other black-pigmented *Bacteroides* species. Examination of the antibody activity to other black-pigmented species, including *B. melaninogenicus*, *B. loescheii*, *B. denticola*, and *B. corporis*, revealed no significant trends in systemic antibody when related to categories of disease. Although no differences in antibody distribution were noted among the diseased groups, as was found with *B. gingivalis* and *B. intermedius*, extreme responders were observed. We thus explored the response patterns of the total population to determine the frequency and classification of the extreme responses. This approach was taken because the antibody levels indicated that with respect to these other *Bacteroides* species the distribution of responses in the patients was independent of disease classification, and thus the individuals could be assumed to be drawn from the same population distribution. Extreme responses to the organisms were observed only among diseased patients (Fig. 3). Extreme reactivities were those determined to be greater than 2 standard deviations from the mean reaction of the entire population being studied. All of the individuals showing an extreme reaction to these *Bacteroides* species were periodontal disease patients. Patients in different disease categories do not show elevated antibody levels to these *Bacteroides* species; however, the elevated levels were principally associated with individual disease patients.

Serum antibody to nonpigmented *Bacteroides* species. Population antibody levels were also examined in cross-sectional analyses to nonpigmenting *Bacteroides* species, including *B. oralis*, *B. gracilis*, and *B. buccae*. All individuals who manifested the high serum antibody levels to these organisms were diagnosed as periodontal disease patients (Fig. 4). Approximately 1 to 4% of the population exhibited the elevated antibody, most of whom (95%) were ADP and AP patients.

Although it has been demonstrated that the *Bacteroides* species contained some antigenic uniqueness, the possibility existed that the extreme antibody reactions to the different species were in a few patients showing broad cross-reactions among the organisms. To evaluate this hypothesis, extreme reactivities to each of the *Bacteroides* species were com-

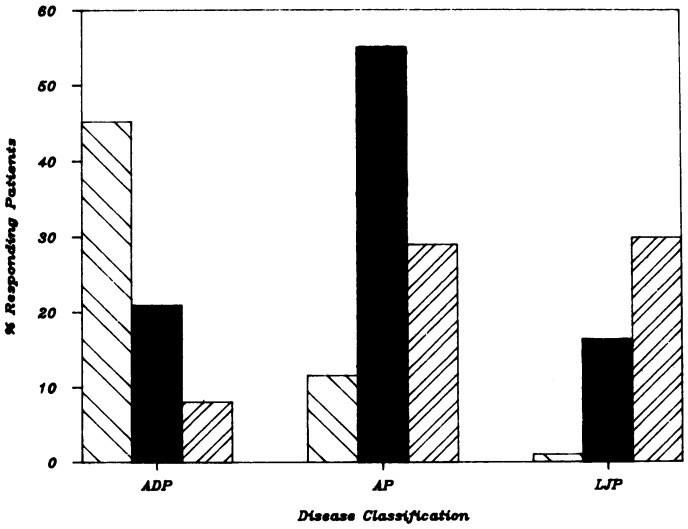

FIG. 2. Distribution of serum IgG antibody reactivity to *B. gingivalis* and *B. intermedius* in periodontal disease patients. The bars denote the percentage of patients exhibiting antibody levels greater than background to *B. gingivalis* (■), *B. intermedius* 581 (▨) or a combination to both (▧). The percentages of responding patients were those values greater than 2 standard deviations above the mean of four adsorbed sera.

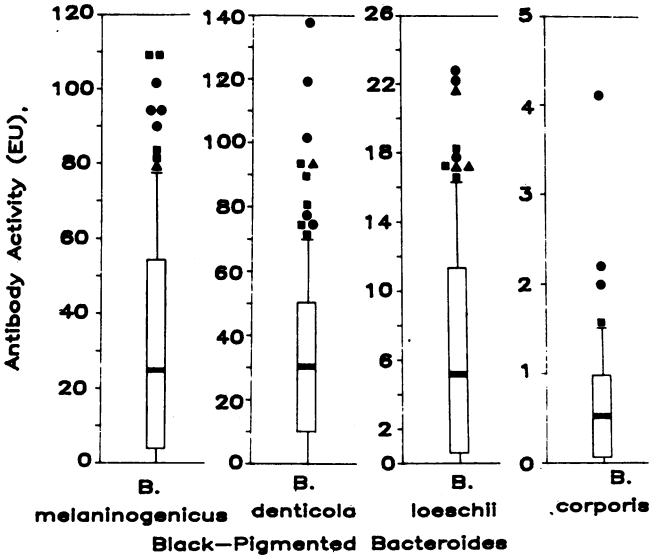

FIG. 3. Human serum IgG antibody levels to black-pigmented *Bacteroides* species. The open bars represent the antibody reactivity of 50% of the population, and the vertical brackets enclose the antibody reactivities of 95% of the population. The solid horizontal line denotes the mean antibody reactivity to the microorganism. The points denote ADP (●), AP (■), or LJP (▲) patients whose antibody levels were significantly elevated when compared with the entire population studied.

pared (Fig. 5). The results show that nearly two-thirds (27 of 38) of the disease patients with extreme reactions exhibited systemic antibody levels to only one of the species being investigated. The differences in the extreme reactions are found to be irrespective of disease category.

DISCUSSION

Cross-sectional studies have been divided on the relationship between serum antibodies to *B. gingivalis* and adult periodontitis (1, 18). Our findings agree with those of Mouton et al. (18), demonstrating a significantly higher level and frequency of IgG antibody to *B. gingivalis* in adult periodontitis patients. Although age differences existed between the groups due to the utilization of this parameter in assigning disease classifications, the elevated antibody to *B. gingivalis* was not associated with age within the disease categories. Additionally, since the age range of the normal group overlapped both the ADP and AP groups, the results supported a disease association for the antibody levels. We have also extended this information by demonstrating a similar relationship in ADP patients. Serum IgM and IgA antibodies to *B. gingivalis* were increased in periodontal disease patients versus normal individuals. Although there was no difference in the frequency of elevated IgM responses between the ADP and AP groups, the levels of IgM antibody to *B. gingivalis* were significantly increased in the ADP patients. These findings could result from a similar frequency of colonization of the periodontal disease patients with *B. gingivalis*; however, the more extensive disease associated with the ADP group (5) may result in a significantly greater antigenic challenge, which results in elevated levels of antibody. Also, there was a lack of serum IgE antibody to *B. gingivalis*. This is in contrast to significantly elevated levels of IgE antibody to *A. actinomycetemcomitans* in LJP patients (4). This result suggests that anaphylactic-type hypersensitivity reactions are not a prominent feature of the immune response in generalized types of periodontitis associated with this microorganism.

Further analyses defined a significantly increased serum antibody level to *B. intermedius* in ADP patients. A natural reactivity to this microorganism was shown in normal individuals and periodontal disease patients. Previous studies have shown that both oral and gastrointestinal *B. intermedius* isolates exhibit extensive antigenic cross-reactions (23). Thus, the natural response may be a result of gastrointestinal stimulation in most of the population. However, the significantly increased level of IgG and IgA antibody in the ADP patients presumably reflects a specific response to additional colonization of the oral cavity with the microorganism. Support for this contention is found in microbiological evidence indicating that *B. intermedius* is a frequent isolate from lesions of periodontitis patients (2). A substantial proportion of the ADP patients exhibited high serum responses to both *B. gingivalis* and *B. intermedius*, whereas AP and LJP patients showed reactivities limited to one of these microorganisms. Adsorption studies provided evidence that the human antibody to these *Bacteroides* species was specific and that a combined specific reaction to these microorganisms may indicate a more extensive disease syndrome. Additionally, an extended analysis of the antigenic composition has shown that in both rabbit and human systems, there exist unique antigenic determinants on the various *Bacteroides* species (J. L. Ebersole, unpublished data). The antigenic differences in the microorganisms are also demonstrable in that the elevations of responses in individual subjects are most frequently directed to a single species of *Bacteroides*. Although there existed significantly increased levels of antibody to *B. gingivalis* and *B. intermedius* that were associated with the clinical diagnoses, there was an heterogeneity in the presence and level of

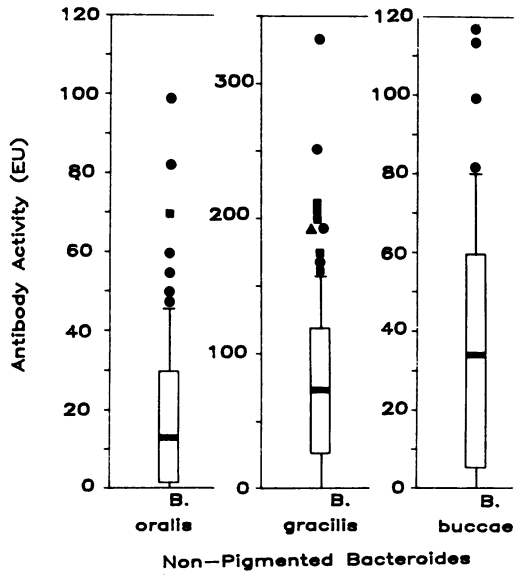

FIG. 4. Human serum IgG antibody levels to nonpigmented *Bacteroides* species. See legend to Fig. 3 for the description of the parameters.

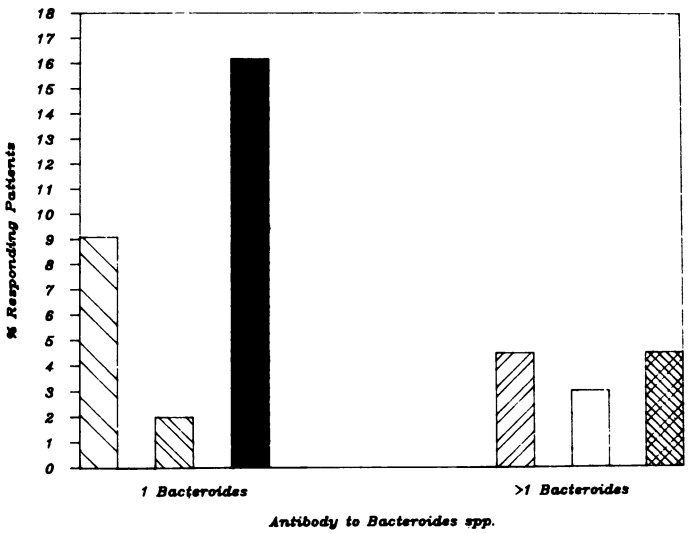

FIG. 5. Human serum IgG antibody levels to oral *Bacteroides* species. The bars represent the percentage of patients exhibiting antibody levels that were significantly elevated when compared with the entire population. The left portion of the figure depicts the distribution of patients showing responses to only one *Bacteroides* species as follows: (▨) response to *B. gingivalis*, (▨) response to *B. intermedius*, and (■) response to a *Bacteroides* species other than *B. gingivalis* or *B. intermedius*. The right portion of the figure depicts the distribution of patients showing responses to more than one *Bacteroides* species as follows: (▨) responses to *B. gingivalis* and at least one additional *Bacteroides* species, (□) responses to *B. intermedius* and at least one additional *Bacteroides* species and (▨) responses to at least two *Bacteroides* species excluding *B. gingivalis* and *B. intermedius*.

antibody reactivity within the disease groups. This finding may be a reflection of different disease syndromes with different etiologies that are at present being treated as a single entity. Alternatively, the results may represent different stages of the pathogenic process of a microorganism with a characteristic systemic response. To further study these differences, particularly in those patients lacking responses to *B. gingivalis* or *B. intermedius*, the antibody reactivities to other oral *Bacteroides* species were examined.

No trends in responses to the other *Bacteroides* species associated with any disease category were noted. In contrast, significant differences among the disease group antibody levels to *B. gingivalis* and *B. intermedius* were observed, which by definition indicates that the patient groups are associated with a different population with respect to their antibody responses. However, inspection of the antibody responses to the other *Bacteroides* species demonstrated that certain individuals exhibited serum antibody levels that were substantially greater than those of the general population. Also, all of these elevated responses were present in periodontal disease patients, irrespective of the category of disease. Thus, if the serum response to these microorganisms is reflective of specific bacterial colonization, selected periodontal disease patients (1 to 5% of the population) should show these other *Bacteroides* species in the subgingival flora, whereas the microorganisms would be infrequent in the normal population. Bacteriological studies have provided evidence that the principal source of these bacterial species has been periodontitis patients (33, 34, 37).

An analysis of the extreme reactions to these other *Bacteroides* species demonstrated that a percentage of disease patients showed multiple high antibody levels; however, nearly two-thirds of the responding patients showed extreme reactivities to only one of the *Bacteroides* species. Consequently, *B. gingivalis* and *B. intermedius* responses appear to be strongly associated with a major portion of generalized-type periodontal disease patients. However, other *Bacteroides* species have been shown to contain various factors that may be related to virulence, and these species may be pathogenic in a limited group of these individuals as demonstrated by extreme elevations in systemic antibody. A compilation of the elevated antibody levels of the ADP and AP patients to all of the *Bacteroides* species indicated that nearly 40% of ADP patients and 30% of AP patients presented no significant differences in antibody responses from the normal population. Recently, there has been a further definition of oral *Bacteroides* species including *B. oris* (8), *B. capillus* (10), and fusiform *Bacteroides* species (34) as well as other *Bacteroides* isolates of undetermined species. Therefore, examination of the systemic reactivity to these other *Bacteroides* species, in addition to numerous other suspected periodontopathic microorganisms, may determine the existence of specific humoral responses in the remaining patients. The relationship of these antibody responses to the development and maintenance of periodontal disease may help to elucidate the members of the subgingival microflora associated with disease activity in the different disease syndromes.

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