Sensitization with *Fusobacterium nucleatum* Targets Antibody-Dependent Cellular Cytotoxicity to Mammalian Cells

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Incubation of mammalian tumor cells with either soluble or insoluble fractions (10 to 100 µg/ml) of *Fusobacterium nucleatum* sensitizes them to the destructive activity of antibody-dependent cellular cytotoxicity (ADCC) effector cells in the presence of anti-*F. nucleatum* antisera. All three types of ADCC effector cells are capable of destroying *F. nucleatum*-sensitized target cells with varying degrees of effectiveness (lymphocytes >> monocytes > neutrophils). Hyperimmune rabbit anti-*F. nucleatum* antisera were active at a dilution as high as 1/100,000. Our studies indicated that *F. nucleatum* must be bound to the target cells since if either the effector cells are treated with *F. nucleatum* or *F. nucleatum* is directly to an ADCC reaction, there is no significant effect on cytotoxicity. The kinetics of *F. nucleatum*-targeted ADCC are identical to those of classical ADCC, suggesting a similar mechanism. The specificity of *F. nucleatum*-targeted ADCC was demonstrated by cold target inhibition studies and by showing that other antibacterial antisera were incapable of mediating the activity.

Antibody-dependent cellular cytotoxicity (ADCC) is an immune hypersensitivity mechanism mediated by effector cells which possess Fc receptors for recognizing antibodies bound to target tissues and cells (9). In a previous report we described a model, developed with lipoteichoic acid (LTA) and anti-LTA antibody, which we termed "targeted ADCC" (7). In this model the ADCC effector cells destroy host tissue by recognizing anti-LTA antibody complexed with LTA moieties bound to the host cell surface. Since LTA binds spontaneously to susceptible cells, host tissue damage via targeted ADCC might be expected to occur in areas of locally high concentrations of gram-positive microorganisms to which the ubiquitous anti-LTA antibody also has access. We feel that the application of this model can be expanded to explain, in part, the etiology of periodontal disease.

Periodontally involved gingival crevices are populated with a diverse array of microorganisms whose proportions and quantities change as the severity of the disease progresses and the pocket depth increases (17, 20). The binding characteristics and relatively high affinity of many of these microorganisms for mammalian tissues have been documented (3, 21, 22). Since the gingival tissues and sulcus have been shown to contain ADCC effector cells (18), the presence of the many ubiquitous antimicrobial antibodies completes all of the requirements of the ADCC targeting model. One such microorganism, *Fusobacterium nucleatum*, is frequently isolated from diseased oral tissues, including tissues having periodontal disease (16, 23). In this report we describe the nature and specificity of *F. nucleatum*-targeted ADCC.

MATERIALS AND METHODS

PBMC isolation. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood from healthy donors of both sexes (ages, 18 to 45 years), as previously described by Boyum (1). Donors were apprised of the study, and consent was obtained consistent with the policies of The University of Michigan and the National

Institutes of Health. The isolated PBMC were suspended in RPMI 1640 culture medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, 2 mM glutamine, and 50 µg of gentamicin (Schering Corp., Bloomfield, N.J.) per ml. Viability was determined by trypan blue exclusion and always exceeded 95% in freshly isolated cell preparations.

Monocyte depletion and enrichment. Monocytes were removed from PBMC suspensions by adherence to gelatin-fibronectin-coated plastic surfaces (4). Briefly, type T-25 tissue culture flasks were incubated for 2 h at 37°C with a 2% sterile solution of gelatin in distilled water. The flasks were aspirated to remove the gelatin and allowed to dry; 10 ml of human plasma was placed in each flask, which was then incubated for 1 h at 37°C. After washing with Mg²⁺- and Ca²⁺-free phosphate-buffered saline (PBS) (0.05 M PO₄, 0.15 M NaCl, pH 7.4), 15 ml of the mononuclear cell suspension (2 × 10⁶ to 4 × 10⁶ cells per ml) in RPMI 1640 medium containing 20% fetal bovine serum was then added to each flask and incubated for 40 min at 37°C. Nonadherent cells were removed by gentle washing with RPMI 1640 medium. The adherent cells were removed by adding 10 ml of a 1:1 mixture of 10 mM EDTA in Ca²⁺- and Mg²⁺-free PBS and RPMI 1640 medium containing 20% fetal bovine serum. Following incubation at room temperature for 15 min, gentle tapping of the flask resulted in maximum detachment of adherent cells. The cells were washed in PBS; this was followed by counting and nonspecific esterase staining (10) to determine purity.

Polymorphonuclear leukocyte isolation. Polymorphonuclear leukocytes were isolated from the erythrocyte-granulocyte pellet following Ficoll-Hypaque density isolation of mononuclear cells. After the upper layers were aspirated, 0.5 ml of plasma, 0.5 ml of PBS, and 1.0 ml of dextran (4.5%) were added to each pellet and incubated at room temperature for 30 min. The upper, leukocyte-rich layer was then removed and washed once in PBS. The contaminating erythrocytes in the pellet were lysed with cold ammonium chloride (0.85%), and this was followed by three

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TABLE 1. Influence of *F. nucleatum* treatment on CEM target cells^a

<i>F. nucleatum</i> concn (μg/ml)	% of radioactivity released in <i>F. nucleatum</i> supernatant	% of radioactivity released in <i>F. nucleatum</i> pellet
0	1.2	1.2
0.001	1.2	1.2
0.01	1.2	1.3
0.10	1.3	1.2
1	1.3	1.3
10	1.3	1.2
100	1.3	1.3

^a ⁵¹Cr-labeled CEM target cells (10⁴ cells per well) were incubated at 37°C in the presence of various concentrations of the *F. nucleatum* preparations. The amount of label released into the medium after 4 h was counted and compared with the total amount of label in 10⁴ cells. The results are the means of three experiments.

washes in PBS and suspension in RPMI 1640 medium. This procedure yielded fewer than 5% contaminating (mononuclear) cells.

Preparation of target cells. Human T-lymphoblastoid cell line CEM was used as the target cell line in this study. Antisera to these cells were prepared by subcutaneous hyperimmunization of rabbits with suspensions of the specific cells, without the use of adjuvant. The tumor targets were serially passaged in RPMI 1640 medium containing 10% fetal bovine serum and used in the cytotoxicity assays no more than 48 h after the last passage. The cells were washed twice in RPMI 1640 medium and tested for cell viability by trypan blue exclusion, and the concentration was adjusted to 5 × 10⁶ cells per ml. To 0.8-ml portions of RPMI 1640 medium containing 5 × 10⁶ viable tumor cells, 100 μCi of ⁵¹Cr as sodium chromate (New England Nuclear Corp., Boston, Mass.) was added. The cells were incubated at 37°C for 1 h in a humidified atmosphere containing 5% CO₂ with intermittent shaking. After incubation, the cells were washed three times in RPMI 1640 medium and adjusted to a concentration of 5 × 10⁵ cells per ml.

Culture and processing of *F. nucleatum*. *F. nucleatum* strain Per-1, isolated from the subgingival plaque of a patient with chronic periodontitis by Salam A. Syed (The University of Michigan, Ann Arbor), was cultured in Schaedler broth (Oxoid USA Inc., Columbia, Md.) under anaerobic conditions and processed as reported previously (8). The bacteria were centrifuged, washed, and suspended in water. The intact cells were disrupted by sonication, and the suspensions were centrifuged at 12,000 × *g*. The resulting pellet material (PEL fraction) was washed in water. Both the PEL fraction and the supernatant fraction (SUP fraction) were lyophilized and stored at -20°C. Antisera to strain Per-1 were prepared in rabbits immunized with whole *F. nucleatum* ultrasonic extract in complete Freund adjuvant.

Antigen coating of target cells. In studies involving *F. nucleatum*-sensitized target cells, ⁵¹Cr-labeled target cells (2 × 10⁶ cells per ml) were incubated with varying concentrations of either the PEL fraction or the SUP fraction (1 ng/ml to 100 μg/ml) for 1 h at 37°C. The radiolabeled, *F. nucleatum*-sensitized target cells were washed twice in RPMI 1640 medium and resuspended at a concentration of 2 × 10⁵ cells per ml. Microscopic examination of the washed target cell suspensions indicated that greater than 99% of the unbound *F. nucleatum* PEL fraction was removed by the washing steps. Control studies indicated that the amount of

free *F. nucleatum* PEL fraction had no effect on the results of the experiments.

ADCC assay system. The ADCC activity of effector cells was determined by a modification of the original method of Perlmann and Perlmann (7, 12). Briefly, in the wells of V-bottom tissue culture plates (type 3897; Costar, Cambridge, Mass.) 50-μl portions of varying concentrations of viable effector cells were added in triplicate to 50-μl portions of complete medium containing 10,000 ⁵¹Cr-labeled target cells and 100 μl of a dilution of rabbit anti-target serum. The plates were centrifuged at 40 × *g* for 2 min at room temperature and then incubated for 4 h at 37°C in 5% CO₂. Following incubation the plates were centrifuged at 40 × *g* for 10 min; 100 μl of supernatant was removed from each well and counted in a gamma counter. The percentage of ADCC activity was calculated as follows: percent cytotoxicity = (experimental release - spontaneous release)/(total release - spontaneous release) × 100, where spontaneous release represents counts released in control wells containing effector and labeled target cells and media instead of antibody and total release represents counts obtained in sample containing 10⁴ ⁵¹Cr-labeled target cells. Because the maximum releasable counts (total label obtained by either lysing a portion of labeled target cells or using unlysed target cells) were the same, the latter method was employed to obtain total release.

Statistical analysis. The statistical significance of differences in mean values of various treatment effects was determined by a paired *t* test. All of the data presented are representative of at least three separate experiments. Within each experiment reported below, all of the cultures were performed in triplicate, with the standard error of the mean not exceeding 10%.

RESULTS

Lack of direct cytotoxicity of *F. nucleatum* preparation. Direct incubation with certain bacterial species has been shown to be cytotoxic to mammalian cells (15). In order to distinguish direct cytotoxicity from that mediated by ADCC or natural killer (NK) effector mechanisms 10⁴ ⁵¹Cr-labeled CEM target cells were incubated with increasing concentrations of either the SUP fraction or the PEL fraction (0 to 100 μg/ml) at 37°C for 4 h. As shown in Table 1, there was no significant (*P* > 0.05) release of ⁵¹Cr by the treated cell categories compared with the spontaneous release control category (0 μg/ml). Thus, within the *F. nucleatum* concentration range employed in this study, the *F. nucleatum* preparations were not directly cytotoxic to the CEM target cells.

Determination of the optimal concentration of the *F. nucleatum* PEL or SUP fraction to sensitize target cells to ADCC sensitivity. ⁵¹Cr-labeled CEM target cells previously sensitized with different concentrations of either the SUP fraction or the PEL fraction (0 to 100 μg/ml) were incubated with mononuclear effector cells (PBMC) at an effector-to-target ratio (E:T ratio) of 50:1 and with heat-inactivated hyperimmune rabbit anti-*F. nucleatum* antibody (1/1,000 dilution, 56°C, 30 min) in the standard 4-h ADCC assay described in Materials and Methods. As shown in Fig. 1, *F. nucleatum*-mediated ADCC was demonstrable with as little as 10 μg of *F. nucleatum* per ml, but was maximal at a concentration of 100 μg/ml. In the absence of antibody (NK control), the release of ⁵¹Cr label did not significantly (*P* > 0.05) exceed the spontaneous release by the control. In Fig. 2 through 8, the cytotoxicity values shown represent the difference between the total label released (effector cells plus

target cells plus antibody) and the label released by *F. nucleatum*-sensitized CEM cells plus effector cells without antibody. The SUP and PEL fractions worked equally well in these studies; thus, only the results obtained with the PEL fraction are shown in Table 2 and Fig. 2 through 8. In all experiments, >10% above the value obtained with effector cells plus target cells without antibody was found to represent significant cytotoxicity ($P < 0.05$), since in the absence of antibodies, target cell-effector cell control cultures released approximately 5 to 10% of the total label of the target cells. The release of ^{51}Cr from target cells incubated with anti-*F. nucleatum* antibodies alone was generally less than or equal to the spontaneous release (target cells alone).

Comparison of the abilities of various ADCC effector subpopulations to mediate ADCC against *F. nucleatum*-sensitized target cells. ADCC activity may be mediated by any or all of three distinct effector cell populations (i.e., lymphocytes, monocytes, and neutrophils). We assessed the ability of each population to mediate *F. nucleatum*-targeted ADCC. Purified effector cells were incubated with PEL fraction-sensitized CEM target cells (100 $\mu\text{g}/\text{ml}$) at E:T ratios of 100:1, 50:1, 25:1, and 12:1. Anti-*F. nucleatum* antibody was added at a 1/1,000 dilution, and the assay mixture was incubated for 4 h. At all E:T ratios, the lymphocyte preparations expressed the most cytotoxic activity, attaining over 50% cytotoxicity at an E:T ratio of 50:1 (Fig. 2). The monocyte preparations showed almost 10% cytotoxicity at a 50:1 ratio (100:1 was not attainable because of the limited cell numbers isolated). Even at the highest E:T ratio tested (100:1), neutrophil cytotoxicity was barely detectable. The antibody sensitivity of this assay was determined by titrating the antibody while keeping the E:T ratio at 50:1. As Fig. 3 shows, the antisera could be diluted to at least 1/100,000 while still retaining cytotoxic activity in the ADCC assay.

Killing kinetics for unsensitized and *F. nucleatum*-sensitized CEM target cells. As an aid in determining whether *F. nucleatum*-mediated ADCC utilizes the same mechanism as classical ADCC does (with unsensitized target cells), the kinetics of the lytic mechanism were determined. ^{51}Cr -labeled PEL fraction-sensitized CEM cells (100 $\mu\text{g}/\text{ml}$) and

FIG. 2. Comparison of effector cell activity against *F. nucleatum*-sensitized CEM target cells. CEM cells sensitized with the *F. nucleatum* PEL fraction (100 $\mu\text{g}/\text{ml}$) were incubated with anti-*F. nucleatum* antibody (1/1,000) and either lymphocytes (■), monocytes (▨), or neutrophils (■). Cytotoxicity was assessed after 4 h at 37°C.

unsensitized target cells were incubated with purified PBMC effector cells (E:T ratio, 50:1) and rabbit anti-*F. nucleatum* and anti-CEM, respectively, for 1 to 19 h. As shown in Fig. 4, the CEM-anti-CEM and *F. nucleatum*-sensitized CEM-anti-*F. nucleatum* combinations demonstrated similar kinetics. Significant cytotoxicity could be measured after 1 h of incubation (20 to 30%), and cytotoxicity increased progressively until 19 h. Lysis occurring after 24 h was difficult to measure accurately because the spontaneous release of label increased significantly after that period of time (data not shown).

Treatment of the effector cells with *F. nucleatum* does not block their ability to mediate ADCC. The experiments described above did not prove conclusively that *F. nucleatum* sensitization of the target cells was actually a prerequisite to *F. nucleatum*-targeted ADCC. Thus, the effects of *F. nucleatum* treatment on the effector population and the presence of free *F. nucleatum* on the ADCC mechanism were also determined. PBMC effector cells were sensitized

FIG. 1. Concentration dependency of *F. nucleatum* SUP and PEL fraction sensitization of CEM cells. CEM cells sensitized with either the SUP fraction or the PEL fraction were incubated with PBMC effector cells. Cytotoxicity was calculated after 4 h at 37°C. Symbols: ▨, *F. nucleatum* SUP fraction + antibody + PBMC; ▨, *F. nucleatum* PEL fraction + antibody + PBMC; ▨, *F. nucleatum* SUP fraction + antibody (no PBMC); ▨, *F. nucleatum* PEL fraction + antibody (no PBMC). FN, *F. nucleatum*.

FIG. 3. Comparison of anti-*F. nucleatum* antibody dilution and ADCC effector cell type. CEM cells sensitized with the *F. nucleatum* PEL fraction (100 $\mu\text{g}/\text{ml}$) were incubated with dilutions of anti-*F. nucleatum* antibody and either lymphocytes (■), neutrophils (■), or monocytes (▨). The E:T ratio for lymphocytes and neutrophils was 100:1, and the E:T ratio for monocytes was 50:1. Cytotoxicity was assessed after 4 h at 37°C.

FIG. 4. Kinetics of ADCC lytic activity. CEM cells (●) and *F. nucleatum* PEL fraction (100 μ g/ml)-sensitized CEM cells (○) were incubated with PBMC effector cells (E:T ratio, 50:1) plus anti-CEM antibody and anti-*F. nucleatum* antibody, respectively. The cytotoxicity of each target-antibody combination was assessed at intervals between 0 and 19 h at 37°C.

with the PEL fraction (100 μ g/ml) by using the protocol for sensitizing target cells. In the presence of anti-CEM antibodies (Fig. 5) *F. nucleatum*-treated effector cells were still capable of lysing CEM target cells. Compared with the untreated effector cells a small reduction in lytic activity (approximately 10%) was measured, but was not found to be significant ($P > 0.05$). As expected, in the presence of anti-*F. nucleatum* antibody (1/1,000), there was no detectable lysis of the CEM target cells by either untreated or *F. nucleatum*-treated PBMC effectors. *F. nucleatum*-treated effector cells were next tested against *F. nucleatum*-treated CEM target cells (Fig. 6). Compared with untreated effector cells, which could mediate significant (50%) lytic damage against *F. nucleatum*-treated CEM target cells, *F. nucleatum*-treated effector cells (in the presence of anti-CEM antibodies) were only 50% as effective. When anti-*F. nucleatum* was substi-

FIG. 5. Effect of *F. nucleatum* effector cell treatment on ADCC activity against untreated CEM target cells. CEM cells were incubated with either anti-CEM antibody or anti-*F. nucleatum* antibody (ANTI-FN) (1/1,000). The effector cell population (E:T ratio, 50:1) consisted of either PBMC effector cells (▨) or *F. nucleatum*-sensitized (100 μ g/ml) PBMC effector cells (■). Cytotoxicity was measured after 4 h at 37°C.

FIG. 6. Effect of *F. nucleatum* effector cell treatment on ADCC activity against *F. nucleatum*-treated CEM target cells. CEM cells sensitized with *F. nucleatum* PEL fraction (100 μ g/ml) were incubated with either anti-CEM antibody or anti-*F. nucleatum* antibody (ANTI-FN) (1/1,000). The effector cell population (E:T ratio, 50:1) consisted of either PBMC effector cells (▨) or *F. nucleatum*-sensitized (100 μ g/ml) PBMC effector cells (■). Cytotoxicity was measured after 4 h at 37°C.

tuted for anti-CEM, the mixture of untreated effector cells and *F. nucleatum*-treated target cells gave about 25% cytotoxicity. Lytic activity was almost totally lost when the effector cells were also treated with the PEL fraction. Since *F. nucleatum* treatment did not reduce the viability of the effector cell population (as determined by trypan blue dye exclusion), even in the presence of anti-*F. nucleatum* antibodies, we attribute reduction of lytic activity to steric factors which prevented close juxtaposition of effector and target cells. Finally, the effect of adding free *F. nucleatum* to the CEM-anti-CEM ADCC reaction was assessed. As shown in Fig. 7, addition of *F. nucleatum* reduced ADCC by about one-third at the highest concentration tested (100 μ g/ml). Again, we attribute the cause of this inhibition to steric factors since *F. nucleatum* treatment is not cytotoxic to either target or effector cells and only minimal inhibition is noted unless both types of cells are treated.

FIG. 7. Effect of free *F. nucleatum* added to the CEM-anti-CEM ADCC assay. CEM target cells were incubated with anti-CEM antibody (1/1,000) and PBMC effector cells (E:T ratio, 50:1). To the cultures we added either medium (□), the *F. nucleatum* SUP fraction (▨), or the *F. nucleatum* PEL fraction (■). Cytotoxicity was assessed after 4 h at 37°C. FN, *F. nucleatum*.

TABLE 2. Inhibitory effect of unsensitized, non-⁵¹Cr-labeled target cells on anti-CEM and anti-*F. nucleatum* ADCC^a

Prepn	Cold target ^b	% Cytotoxicity				
		No cold target	Cold-to-hot target ratio			
			4:1	16:1	32:1	64:1
CEM + anti-CEM		1.15				
CEM + anti-CEM + effector	CEM	61.8	55.6 (10.0) ^c	25.6 (58.6)	17.3 (72.0)	14.3 (76.9)
<i>F. nucleatum</i> -sensitized CEM + anti- <i>F. nucleatum</i>		0				
<i>F. nucleatum</i> -sensitized CEM + anti- <i>F. nucleatum</i> + effector	CEM	33.0	35.2 (-6.7)	32.1 (2.7)	34.3 (-3.9)	33.3 (-1.0)
<i>F. nucleatum</i> -sensitized CEM + anti- <i>F. nucleatum</i> + effector	<i>F. nucleatum</i> -sensitized CEM	33.0	25.7 (22.1)	9.5 (71.2)	12.9 (60.9)	7.3 (77.8)

^a The standard assay conditions described in Materials and Methods were used. PBMC were employed as effector cells at an E:T ratio of 50:1.

^b Unlabeled target cells were added at various ratios to ⁵¹Cr-labeled target cells. The E:T ratio was always based on the labeled target cells.

^c The numbers in parentheses are percentages of inhibition. Inhibition of ⁵¹Cr release by cold targets was based on the categories containing only labeled (no cold) target cells.

Cold target inhibition studies demonstrate that the effector cells specifically recognize the *F. nucleatum*-sensitized target cells. Unlabeled (cold) *F. nucleatum*-sensitized CEM cells or CEM target cells were added to labeled (hot) target cells at ratios of cold cells to hot cells of 4:1 to 64:1. If the effector cell population cannot distinguish between the cold and hot target cells, then addition of cold target cells should inhibit ⁵¹Cr release. As shown in Table 2, addition of cold CEM target cells to the CEM-anti-CEM assay or cold *F. nucleatum*-sensitized CEM cells to the *F. nucleatum*-sensitized CEM-anti-*F. nucleatum* ADCC assay resulted in approximately 77% inhibition of label release at the highest ratio of cold to hot cells tested. However, when cold CEM target cells were added to the *F. nucleatum*-sensitized CEM-anti-*F. nucleatum* ADCC mixture, there was no inhibition of label release.

Sensitization of target cells with *F. nucleatum* results in *F. nucleatum*-specific ADCC. As a final test of specificity, hyperimmune rabbit antisera made against *Bacteroides gingivalis*, *Streptococcus sanguis*, *Treponema vincentii*, *Treponema denticola*, and *Actinomyces viscosus* were used in place of anti-*F. nucleatum*. Each antiserum had a titer of

at least 1/100,000, as measured by a micro enzyme-linked immunosorbent assay and was tested at a dilution of 1/1,000 in the ADCC assay. As shown in Fig. 8, while anti-*F. nucleatum* and anti-CEM produced cytotoxicity values of 44 and 29%, respectively, the other antisera had no effect on the target cells.

DISCUSSION

An etiologic role for the immune system in the pathologic aspects of periodontal disease has been intimated by many investigators (reviewed in reference 11). However, although sensitized lymphocytes, antibacterial antibodies in sera, and crevicular fluid have been demonstrated, the manner of focusing and delivery of the damage to the tissue has been a matter of conjecture. We previously reported a putative mechanism which we termed "targeted ADCC" (7). Purified LTA was shown to be capable of directing the lytic activity of ADCC effector cells to target cells by its ability to bind spontaneously to the target cells and, subsequently, to bind anti-LTA antibody, thereby attracting Fc receptor bearing leukocytes. We propose that such a mechanism might be a general feature in any microbial infection and habitation in which the microbe or its components have the capability to adhere spontaneously to host mucosal tissue. More specifically, we propose that this may be a significant mechanism in the etiology of periodontal disease.

The tissue-binding properties of *F. nucleatum*, which is frequently isolated from diseased oral tissues, has been extensively investigated (3, 23). Oral *F. nucleatum* strains attach to human and sheep erythrocytes in a Ca²⁺-dependent reaction involving a lectin like activity on the surface of the bacteria binding to a galactose-containing moiety on the erythrocyte surface. The ability to bind to a tissue is the first step in colonization of the tissue and in mediation of either nonspecific or immunologic damage.

In our studies we utilized two preparations of *F. nucleatum* derived from ultrasonication of the microorganism. These preparations, the SUP fraction (soluble) and the PEL fraction (particulate), mediated the targeted ADCC mechanism equally well. Neither fraction expressed any significant cytotoxicity against the target cells, and both were capable of sensitizing the target cells at relatively low concentrations (10 to 100 µg/ml; these are probably concentrations attainable within the gingival sulcus. In the present study we did not employ purified cellular components which, in our previous study with LTA, were shown to sensitize

FIG. 8. Demonstration of antibody specificity in ADCC against *F. nucleatum*-sensitized CEM cells. *F. nucleatum* PEL fraction-sensitized CEM target cells were incubated with PBMC effector cells (E:T ratio, 50:1) and antiserum (1/1,000) against *F. nucleatum* (FN), CEM cells, *B. gingivalis* (BG), *S. sanguis* (SS), *T. vincentii* (TV), *T. denticola* (TD), or *A. viscosus* (AV). Cytotoxicity was assessed after 4 h at 37°C.

target cells at concentrations as low as 1 ng/ml. Additional studies with purified components are again in progress. The soluble bacterial components have significance to periodontal disease since they can penetrate through the junctional epithelium, which is permeable to substances with molecular weights up to approximately 700,000 and may mediate immunologic damage in the deeper tissues (14).

There are three major leukocyte types which have been shown to be capable of mediating ADCC. These are large granular lymphocytes, monocytes, and neutrophils (13). The common feature of these cells is the fact that they possess an Fc receptor which recognizes the antibody molecule bound to the target. Each effector type also appears to possess a degree of target preference (6); that is, the mononuclear effectors appear to prefer tumor and fibroblast targets, while the neutrophils appear to prefer erythrocyte and bacterial targets. In our previous report, we showed that LTA sensitization of target cells made the tumor cell targets susceptible to neutrophilic attack, as well as to lymphocytes and monocytes. This was a surprising result, since the tumor cell target employed was itself resistant to neutrophil attack. Since previous investigators have demonstrated the presence of neutrophils in the gingival crevicular fluid capable of mediating ADCC (18) and that the early cellular infiltration of inflamed gingival tissue is rich in neutrophils (reviewed in reference 14), one might predict that gram-positive colonization of the gingival crevice would predispose the tissues to susceptibility to neutrophil-mediated ADCC damage. As the disease progresses, ADCC damage mediated by the mononuclear cells would then predominate. In contrast to LTA, none of the *F. nucleatum* preparations was capable of significant neutrophil-mediated ADCC. As a result, this mechanism is probably invoked by different microbe-effector cell combinations depending on the stage of disease.

Kinetic studies of lysis of target cells by effectors suggest that sensitization of the target cells does not alter the lytic mechanism. Studies are under way in this laboratory to characterize the lytic mechanism itself to confirm that it is not altered after sensitization. Since pretreatment of effector cells with preparations such as lipopolysaccharide and *Corynebacterium parvum* (reviewed in reference 5) has been shown to enhance NK activity, we wished to rule out the possibility that *F. nucleatum* sensitization was actually enhancing NK activity in the effector population, resulting in enhanced killing of the target cells. In addition, we wished to determine the effect of sensitization of the effector cells, since certain bacteria have been shown to bind specifically to leukocyte subpopulations (2). Such binding might be expected to specifically activate the cells or suppress their activity by hindering other receptor sites on the cells. We found no enhancement of the control ADCC system (CEM-anti-CEM); rather, we found an inhibition of about 10% when the effector cells were treated with the standard sensitizing concentration of *F. nucleatum* (100 µg/ml). When the effector cells were *F. nucleatum* cells treated in the *F. nucleatum*-sensitized CEM-anti-*F. nucleatum* system, there was total inhibition of the ADCC mechanism, which we attribute to steric factors that prevent the target and effector cells from coming in close juxtaposition to one another. Adding free *F. nucleatum* to the control system (CEM-anti-CEM) blocked ADCC by about 30%; again, this was attributable to steric factors.

The specificity of the system was documented by the cold target inhibition experiments and the demonstration of a lack of effect when other antimicrobial antisera were substituted for the anti-*F. nucleatum*.

Targeted ADCC is a potential mechanism in the etiology of periodontal disease that distinguishes health from disease perhaps only on the basis of the quality and quantity of the crevicular flora. We and many other investigators have reported the presence of antibodies to numerous members of the oral flora in serum and gingival crevicular fluid. Since the ADCC effector populations do not appear to require prior activation, expression of tissue damage appears to require only the accumulation of plaque in the gingival sulcus. Since expression of the ADCC mechanism is influenced by the immunoglobulin subclasses (19) and modulatory influences of certain biologically active substances (5), there are still factors that might predispose individuals to, or insulate them from developing, periodontal disease via this pathway.

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