Induction of Activated Lymphocyte Killing by Bacteria Associated with Periodontal Disease

R.A. Lindemann, K.T. Miyasaki and L.E. Wolinsky

J DENT RES 1988 67: 846
DOI: 10.1177/00220345880670051001

The online version of this article can be found at:
http://jdr.sagepub.com/content/67/5/846

Published by:

SAGE
http://www.sagepublications.com

On behalf of:
International and American Associations for Dental Research

Additional services and information for Journal of Dental Research can be found at:

Email Alerts: http://jdr.sagepub.com/cgi/alerts
Subscriptions: http://jdr.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav
Citations: http://jdr.sagepub.com/content/67/5/846.refs.html

>> Version of Record - May 1, 1988

What is This?
Induction of Activated Lymphocyte Killing by Bacteria Associated with Periodontal Disease

R. A. LINDEMANN, K. T. MIYASAKI1, and L. E. WOLINSKY2

Section of Oral Diagnosis, Oral Medicine, Oral Pathology, Section of Periodontics1, Section of Oral Biology2, and the Dental Research Institute, UCLA School of Dentistry, Center for the Health Sciences, Los Angeles, California 90024

Complex interactions occur among host defense cells during bacterial infection. Bacteria and bacterial products may enhance or inhibit the effector and regulatory activity of human lymphocytes. Accordingly, we tested the ability of human periodontal pathogens to activate peripheral blood lymphocytes using standard 31-chromium-release assays to measure lymphocyte-mediated cytosis. Human adherent-cell depleted peripheral blood lymphocytes (PBL) with the addition of glutaraldehyde-fixed bacteria at a 5:1 bacteria:lymphocyte ratio were incubated at 37°C for 24 hr in RPMI 1640 medium. Six of eight bacteria tested significantly augmented lymphocyte killing of the natural killer (NK) cell-sensitive human erythroleukemia cell line K562. E. corrodens, representing activating bacteria, was also able to induce the killing of NK-resistant targets (M14, Raji), comparable with induction by interleukin-2. Lipopolysaccharides extracted from A. actinomyctecitomans strains, when incubated with PBL, were able to enhance cytotoxicity without the presence of whole bacteria. A majority of cytotoxicity was mediated by NK cells bearing Leu-11 and NKH-1 markers.


Introduction.

Considerable interest has been focused on the ability of human lymphocytes to be activated by lipopolysaccharides. Enhancement of the cytotoxic potential of peripheral blood lymphocytes by inter-leukin-2 has resulted in the development of cells called lymphokine-activated killers (LAK) (Grimm et al., 1982). In addition to lymphokines, activated killer cells have been generated from mixed lymphocyte culture (seeley and Golub, 1978; D’Amore and Golub, 1982), in the presence of lectins (Bradley and Bonavida, 1982), and from the stimulation of viruses (Perlman et al., 1982).

Bacteria and bacterial products have also been shown to potentiate lymphocyte killing (Herberman et al., 1979; Wolfe et al., 1976). Recently, it was demonstrated that strains of Salmonella, which were fixed with glutaraldehyde, enhanced lymphocyte cytotoxicity against natural killer (NK)-sensitive target cells (Tarkkanen et al., 1986a). The cell-type responding to the inductive effects of glutaraldehyde-fixed Salmonella was found to be CD16+ (Leu 11) and Leu 19+ (Tarkkanen et al., 1986b). Phillips and Lanier (1986) have proposed that NK cells bearing these markers mediate a majority of LAK activity against NK-resistant cell lines and fresh tumors.

The role of NK cells in oral diseases has not been established. Tsoumis et al. (1985) demonstrated a significant positive correlation between peripheral blood NK cytotoxicity and the degree of inflammatory periodontal disease. An increase in antibody-dependent cellular cytotoxicity, mediated by NK cells, was noted in the early stages of the chronic mucosal disease, recurrent aphthous ulceration (Greenspan et al., 1981). Lindemann et al. (1987) demonstrated that oral epithelial cells infected with herpes simplex virus 1 were lysed by NK cells. NK cells may have other roles in oral disease in addition to direct cytotoxic involvement. Abruzzo and Rowley (1983) have suggested that NK cells down-regulate antibody responses by eliminating antigen-presenting cells while displaying antigen. Recently, NK cells were demonstrated in human gingival tissue in association with B-cells (Wynn et al., 1986). The authors postulated that this association was in vivo evidence of the regulatory function of NK cells.

Previous reports have indicated that lymphocytes have some antibacterial activity, especially in the presence of antibody (Lowell et al., 1979, 1980; Kleinman and Hunt, 1982; Nencioni et al., 1983). Antibody-dependent cellular cytotoxicity, mediated by Fc-receptor-bearing NK cells, is a well-described mechanism of host immune defense. However, there is a paucity of evidence for the direct killing of bacteria by NK cells. Although high levels of cytotoxicity were achieved against target cells after stimulation with glutaraldehyde-fixed Salmonella strains, Tarkkanen et al. (1986a) could not show significant bactericidal activity from the same lymphocytes against the same strains of live bacteria.

Current concepts on the pathogenesis of destructive periodontitis support the hypothesis that specific microbial groups are present during active periods of the disease. Among the Gram-negative bacteria identified from sampling studies, several are thought to be of particular importance. Among these are Actinobacillus actinomycetemcomitans, Bacteroides gingivalis, Eikenella corrodens, Capnocytophaga spaguiena, and Treponema vincentii (Socransky et al., 1979; Tanne et al., 1981; Moore et al., 1982a,b; Sgale et al., 1982). We were interested in the reciprocal effects of these periodontopathogens and the NK subset of peripheral blood lymphocytes. The purposes of the study were: (1) to determine the potential of a battery of common pathogenic periodontal bacteria to activate peripheral blood lymphocytes and (2) to determine the responsive cell and its target spectrum. The hypothesis tested was that NK cells were activated by contact with periodontopathogens.

Materials and methods.

Isolation of lymphocytes.—Human peripheral blood was drawn from healthy volunteers; lymphocytes were obtained by Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) density gradient centrifugation, and adherent cells were depleted by plastic adherence (subsequently referred to as PBL). PBL were re-suspended in RPMI 1640 medium. The percentage of monocytes remaining, calculated by universal rosetting reagent, described elsewhere (Karavodin and Golub, 1983), was 1-3%. Thoracic duct lymph fluid, removed from one additional subject during surgery, was washed several times, after which lymphocytes were prepared in a manner similar to that used for PBL. All lymphocytes were assayed immediately.

Separation of lymphocyte subpopulations. — A panning technique (D’Amore and Golub, 1985) was used to isolate lymphocytes in conjunction with the monoclonal antibodies Leu-11b (Becton Dickinson, Inc., Mountain View, CA) and NKH-1A (Coulter Immunochemistry, Hialeah, FL). Adherent cells were...

Received for publication June 23, 1987
Accepted for publication December 15, 1987
first removed by plastic adherence for one hr. The nonadherent cells were then treated with both monoclonal antibodies (1 μL per 1 x 10⁶ cells) and incubated on ice for 30 min. After the cells were washed twice, 3 x 10⁶ of the antibody-treated cells were placed on tissue culture plates previously coated with an affinity-purified goat anti-mouse IgM antibody (Tago, Inc., Burlingame, CA). After a two-hour incubation at 4°C, the nonadherent cells were removed, and the adherent cells were then gently detached by a rubber policeman.

_Bacteria._—The bacteria used are described in Table 1. All bacteria, except B. gingivalis and T. vincentii N-9, were grown at 37°C overnight under an atmosphere of 5% CO₂:95% air (B. gingivalis under 80% N₂:10% CO₂:10% H₂) on plates consisting of trypticase soy agar (BBL Microbiology Systems, Cockeysville, MD) supplemented with 0.1% yeast extract (DIFCO Laboratories, Detroit, MI), 0.001% menadione (Sigma Chemical Company, St. Louis, MO), 0.05% equine hemin III (Sigma), and IsoVitalex (BBL). The oral spirochete, T. vincentii N-9, was grown at 37°C for two days under an anaerobic atmosphere consisting of 85% N₂:10% H₂:5% CO₂ in Spiorelate medium (BBL) containing 10% trace hemolyzed rabbit serum (Pel-Freez, Inc., Rogers, AR). Bacteria were collected, washed twice, and suspended in Dulbecco’s phosphate-buffered saline (DPBS), pH 7.4 (Whittaker M.A. Bioproducts, Walkersville, MD).

Glutaraldehyde fixation of bacteria.—Washed bacteria were suspended in DPBS to an optical density of 0.3 at 540 nm. Glutaraldehyde (Kodak Chemical Corp., Rochester, NY), 25% v/v, was added slowly to achieve a final concentration of 0.25%. The suspension was incubated overnight at 20°C, and subsequently stored at 4°C. Prior to use, bacteria were washed four times and re-suspended in DPBS at 2 x 10⁸ cells/mL.

Activation of cytotoxicity by bacteria.—PBL were incubated for 24 hr at 37°C with the glutaraldehyde-fixed bacteria at a 5:1 bacteria-to-lymphocyte ratio in serum-free RPMI medium. The lymphocytes were then washed twice and re-suspended in RPMI 1640 with 10% heat-inactivated fetal calf serum (FCS) prior to cytotoxicity assays. Viability, tested by trypan blue exclusion, was >85%. In one experiment, the effect of varying bacteria-to-lymphocyte ratios on cytotoxicity was determined. Additionally, the kinetics of activation over time was measured by allowing the bacteria and lymphocytes to remain in RPMI 1640 medium with 10% AB serum. Cytotoxicity was calculated at one-, four-, and seven-day intervals for one week. As a positive control for activation, 100 u/mL of human recombinant interleukin-2 (rIL-2) (Amgen, Thousand Oaks, CA) was added to PBL at 2 x 10⁶ cells per mL and incubated for the appropriate time period, after which lymphocytes were tested against the target cells.

**Lipopolysaccharide (LPS) stimulation._—LPS—prepared by phenol-water extraction (Westphal and Jann, 1965), from _A. actinomycetemcomitans_ 67, ATCC 29522, ATCC 29523 (provided by Dr. C. Hoover, University of California-San Francisco), and Y4 strain (provided by Dr. A. Nowotny, University of Pennsylvania)—was incubated with lymphocytes at various concentrations for 24 hr. Cytotoxicity was measured in standard ⁵¹chromium-release assays (Trinchieri and Perussia, 1984).

_Target cells._—The NK-sensitive human erythroleukemic cell line, K562 (Trinchieri and Perussia, 1984), and two NK-resistant cell lines [M14 (melanoma) (Zielieski and Golub, 1976) and Raji (Burkitt’s lymphoma) (Ramsdell and Golub, 1987)] were used as targets in the cytotoxicity assays. Target cells (5 x 10⁶ in 1 mL RPMI with 10% FCS) were labeled with 250 μCi ⁵¹chromium (⁵¹Cr) for one hr at 37°C.

_Cytotoxicity assay._—K562, M14, or Raji target cells (5 x 10⁶) were mixed with effector cells (@ 50:1, 25:1, and 12.5:1 ratios) in round-bottomed microtiter plates containing 200 μL of RPMI 1640 with 10% FCS. The plates were centrifuged at 65 g for four min for initiation of cell-to-cell contact and then incubated for four hr at 37°C in a humidified incubator with 5% CO₂. At the end of the assay, plates were centrifuged at 150 g for eight min, and 100 μL of supernatant was harvested from each well and counted for ⁵¹Cr released from target cells. Each assay was performed in quadruplicate. Cytotoxicity was defined as % specific ⁵¹Cr released or [(experimental release spontaneous release) / (maximal release spontaneous release)] x 100%. Experimental release was defined as the counts of ⁵¹Cr released from target cells caused by effector cells as measured by counts per minute. Maximal release was the ⁵¹Cr released from target cells induced by 2% Nonidet P-40 detergent. Spontaneous release was the counts of ⁵¹Cr from target cells incubated alone.

_Statistics._—Significance of cytotoxicity assays was determined by initial conversion of the three effector-to-target ratio values into lytic units (LU) (Pross et al., 1981). LU are defined as that number of cells required to cause a specified amount of target lysis (in this case 30%), and is usually expressed as LU/10⁶. This method allows for a more accurate comparison between lymphocyte donors. The paired t test (two-tailed) was then applied to determine significance of LU 30% values.

**Results.**

_Bacterial activation of PBL and thoracic duct lymphocytes._—PBL incubated for 24 hr with bacteria were washed and tested against K562 in ⁵¹Cr-release assays. In Table 2, data from five subjects demonstrate that cytotoxicity was significantly increased after incubation with six of the eight bacteria tested. Only _A. actinomycetemcomitans_ Y4 and _T. vincentii_ had no inductive effect on PBL cytotoxicity.

Lymphocytes isolated from the thoracic duct from an additional subject (Table 2) were modestly activated by the panel of bacteria tested. _A. actinomycetemcomitans_ Y4 had no effect on the lymphocytes, but _T.vincentii_ slightly enhanced cytotoxicity.

_E. corrodens_ and _A. actinomycetemcomitans_ Y4—bacteria causing a significant effect and no effect, respectively, on induction of cytotoxicity against K562—were then tested against NK-resistant cell lines, M14 and Raji. In Fig. 1, a representative experiment demonstrates that PBL incubated for 24 hr with _E. corrodens_ was able to kill both M14 and Raji, comparable with rIL-2 activated lymphocytes. _A. actinomycetemcomitans_ Y4 continued to have little effect on enhancing PBL killing of resistant targets. _A. actinomycetemcomitans_ strains 67, 29523, _H. aphrophilus_, and _C. spuitigena_ were also able.
TABLE 2
CYTOTOXICITY MEANS AND STANDARD ERROR OF THE MEANS (SEM) OF PERIPHERAL BLOOD LYMPHOCYTES (PBL) FROM FIVE SUBJECTS AND THORACIC DUCT LYMPHOCYTES (TDL) FROM ONE ADDITIONAL SUBJECT ACTIVATED BY 24-HOUR INCUBATION WITH BACTERIA MEASURED AGAINST KS62 IN LYTIC UNITS (LU)

<table>
<thead>
<tr>
<th>Activating Agent</th>
<th>PBL LU SEM</th>
<th>TDL LU SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (untreated PBL)</td>
<td>34.43 (11.65)</td>
<td>2.14 (0.08)</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. actinomycetemcomitans #29523</td>
<td>48.45* (12.56)</td>
<td>4.51* (0.10)</td>
</tr>
<tr>
<td>A. actinomycetemcomitans #Y4</td>
<td>33.76 (07.34)</td>
<td>2.95 (0.15)</td>
</tr>
<tr>
<td>A. actinomycetemcomitans #67</td>
<td>54.43* (18.41)</td>
<td>3.77* (0.19)</td>
</tr>
<tr>
<td>B. ginvialis</td>
<td>63.78* (11.97)</td>
<td>Not Tested</td>
</tr>
<tr>
<td>E. corrodens</td>
<td>69.00* (19.68)</td>
<td>6.71* (0.13)</td>
</tr>
<tr>
<td>H. aphrophilus</td>
<td>56.23* (14.91)</td>
<td>6.95* (0.14)</td>
</tr>
<tr>
<td>C. sputigena</td>
<td>61.07* (17.21)</td>
<td>4.33* (0.09)</td>
</tr>
<tr>
<td>T. vincentii</td>
<td>35.12 (11.46)</td>
<td>3.59* (0.07)</td>
</tr>
</tbody>
</table>

*Significant at p<0.05 level, Student’s t test.
†LU Number of cells required to cause 30% target lysis. Expressed as LU per 1×10⁶ PBL.

![Fig. 1](#) Lymphocytes incubated with E. corrodens for 24 hr were able to kill NK-resistant targets M14 and Raji comparable with IL-2 (100 u/mL)-induced cytotoxicity. Results are reported from a representative experiment.

![Fig. 2](#) The effect of E. corrodens bacterial:lymphocyte ratio on development of cytotoxicity against NK-sensitive (KS62) and NK-resistant (M14) targets (24-hour incubation). Results are reported from a representative experiment. Bacteria-to-lymphocyte ratios of 5:1 and above were significantly higher than control values (p<0.05, Student’s t test).

10:1, cytotoxicity plateaued. With M14 as a target, a significant increase in cytotoxicity was recorded at 5:1, with a maximal effect also exerted at 10:1.

**Kinetics of induction.** —PBL were cultured for seven days with A. actinomycetemcomitans 67, B. ginvialis, and E. corrodens at a 10:1 bacteria-to-lymphocyte ratio. Cytotoxicity assays were performed on days 1, 4, and 7. Fig. 3 demonstrates that activation over this time period differed between bacterial-induced and IL-2 induced cytotoxicity. Bacterial-induced cytotoxicity peaked at day 1, while the effects of IL-2 were sustained over the seven-day incubation period.

**Phenotype of the effector cell.** —PBL were separated into Leu-11b, NKH-1A positive and negative populations, and E. corrodens bacteria (10:1 bacteria-to-lymphocyte ratio) were added in culture. Effector cells from the Leu-11b, NKH-1A population were responsible for significantly higher cytotoxicity than the Leu-11b, NKH-1A population after 24 hr against KS62 (Table 3) in all subjects tested. Other bacteria were similarly tested, and the same results were found (data not shown).

**Effect of extracted LPS on PBL.** —LPS from all A. actinomycetemcomitans strains tested augmented cytotoxicity over an untreated PBL control in a representative experiment (Table 4). Concentrations of 12.5 μg/mL were required for ATCC 29522 and 29523 to induce significant lymphocyte cytotoxicity. Significantly augmented cytotoxicity was measured at 6.25 μg/mL for strain #67 and 3.12 μg/mL for strain Y4.
TABLE 3

<table>
<thead>
<tr>
<th>PBL Subpopulation</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated PBL</td>
<td>59.53</td>
<td>11.15</td>
<td>8.64</td>
</tr>
<tr>
<td>± 11.16  ± 1.69  ± 0.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu-11b,NKH-1A+</td>
<td>78.40†</td>
<td>18.40†</td>
<td>13.22†</td>
</tr>
<tr>
<td>± 2.39  ± 2.00  ± 1.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leu-11b,NKH-1A-</td>
<td>34.33</td>
<td>2.77</td>
<td>2.83</td>
</tr>
<tr>
<td>± 3.49  ± 2.42  ± 0.48</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*LU—Number of cells required to cause a 30% target lysis. Expressed as LU per 1 X 10^6 PBL.†p<0.05.

TABLE 4

<table>
<thead>
<tr>
<th>LPS conc. µg/mL</th>
<th>25</th>
<th>12.5</th>
<th>6.25</th>
<th>3.12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control PBL</td>
<td>9.20 (3.61)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.a. 67</td>
<td>37.42*</td>
<td>30.41*</td>
<td>21.19*</td>
<td>16.88</td>
</tr>
<tr>
<td>(3.17)</td>
<td>(2.75)</td>
<td>(2.03)</td>
<td>(3.18)</td>
<td></td>
</tr>
<tr>
<td>A.a. 29522</td>
<td>32.74*</td>
<td>19.74</td>
<td>13.42</td>
<td>8.46</td>
</tr>
<tr>
<td>(3.06)</td>
<td>(1.00)</td>
<td>(1.75)</td>
<td>(1.31)</td>
<td></td>
</tr>
<tr>
<td>A.a. 29523</td>
<td>15.53*</td>
<td>14.01*</td>
<td>12.73</td>
<td>9.48</td>
</tr>
<tr>
<td>(1.70)</td>
<td>(0.41)</td>
<td>(1.74)</td>
<td>(1.89)</td>
<td></td>
</tr>
<tr>
<td>A.a. Y4</td>
<td>19.46*</td>
<td>21.88*</td>
<td>18.18*</td>
<td>20.61*</td>
</tr>
<tr>
<td>(4.37)</td>
<td>(1.21)</td>
<td>(0.26)</td>
<td>(3.32)</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05, paired t test.

Discussion.

We have shown that several Gram-negative periodontal pathogens cause the rapid enhancement of peripheral blood lymphocyte cytotoxicity. This increased cytotoxicity occurs after 24 hr of incubation with glutaraldehyde-fixed bacteria at bacterio-to-lymphocyte ratios of 5:1, with a maximal effect at 10:1. These data support the findings of Tarkkanen et al. (1986a), who demonstrated activated killing of K562 targets after incubation with Sal monella bacteria. Those authors later showed that the spectrum of killing included NK-resistant cell lines, comparable with rIL-2-induced killing (Tarkkanen et al., 1986b). We have demonstrated that a representative enhancing bacterium, E. corrodens, also activates PBL to lyse NK-sensitive target cells (K562) as well as NK-resistant lines (M14, Raji) comparable with rIL-2 activation. Additionally, the bacteria able to stimulate PBL were also able to induce thoracic duct lymphocytes (TDL) to kill K562 targets at significantly higher cytotoxicity values than untreated TDL. Lower NK activity was observed from fresh TDL than from PBL, probably due to the low percentage (~4%) of circulating Leu-11b+, NKH-1+ cells in thoracic duct lymph, as determined by application of a universal rosetting reagent (Karavodin and Golub, 1983) (data not shown).

The cell responding to the effects of the bacteria with the highest cytotoxicity was characterized as being associated with the Leu-11b+, NKH-1+ population. As additional evidence, lymphocyte cytotoxicity generated from thoracic duct lymph was low, indicating minimal activation. Thoracic duct lymph was utilized because it was shown, by universal rosetting reagent, to be an NK-depleted population. This substantiates the finding that the NK cell is preferentially activated by bacteria. The data support the initial findings of Tarkkanen et al. (1986a), who showed that primarily NK cells and, secondarily, non-NK fractions of PBL were able to be activated. However, in a subsequent study, the authors (Tarkkanen et al., 1986b) reported that all bacterial stimulation of NK activity was mediated by Leu-19+ (NKH-1A) cells without contribution by other fractions of PBL. Although most Leu-19+ cells co-express the Leu-11b marker (Lanier et al., 1986), the discrepancy in their report was presumably due to a small percentage of Leu-11b-, Leu-19+ high-density cells mediating cytotoxicity after bacterial activation. The low cytotoxicity remaining in our Leu-11b-, NKH-1A+ population could be due to contamination of NK cells remaining after panning. These data support our hypothesis that NK cells are activated by the periodontopathogens tested.

Our observations contradict those of Tarkkanen et al. (1986a), who showed that activated lymphocyte killing was inhibited by lipopolysaccharide (LPS) from enteric bacteria. Clearly, LPS from four A. actinomycetemcomitans strains had an immunostimulatory effect on NK cells. However, there are differences between enteric and oral bacteria that could be responsible for the apparent contradiction. The LPS from the oral bacteria C. sputigena and E. corrodens lacks 3-deoxy-D-manno-octulosonic acid (KDO), whereas that from A. actinomycetemcomitans is relatively low in KDO (Hammond and Stevens, 1982; Kiley and Holt, 1980). Sodium dodecyl sulfate polyacrylamide gel electrophoresis shows that the O antigen chain-lengths of A. actinomycetemcomitans, C. sputigena, and E. corrodens also exhibit far less heterogeneity than those from enteric bacteria (Hoover and Fisher, 1985; Jonak-Urbanczyk et al., 1986). The inability of two bacteria, A. actinomycetemcomitans Y4 and T. vincentii, to augment cytotoxicity may reflect differences in their surface LPS or alterations due to fixation. LPS extracted from the Y4 strain activated lymphocytes, supporting the possibility that its surface-bound LPS was altered to become non-immunogenic.

The ability of bacteria to induce lymphocyte activation demonstrates that defense cells are capable of recognizing pathogenic bacteria and responding, perhaps by the release of specific lymphokines. Results from the kinetics-of-activation experiment revealed that bacteria-induced and IL-2-induced cytotoxicity profiles were dissimilar, which suggests that bacteria may activate NK cells by a different pathway than IL-2. LPS derived from Gram-negative periodontopathogens, as demonstrated by the data, is a likely candidate for this “activator”. Kang and Lee (1987) have recently demonstrated by electron microscopy that LPS from E. coli is incorporated into NK cells; however, the mechanism whereby LPS activates NK cells is unknown. Kang et al. (1986) speculated in an earlier paper that LPS may induce NK interferon production, which has a subsequent autocrine effect to increase cytotoxicity.

The exact method whereby periodontopathogens activate NK cells and the significance of this mechanism for defense cell regulation and pathogenesis of periodontal disease are currently under study. In the periodontium, the function of NK cells is not fully understood. It is becoming apparent that NK cells have more roles in addition to anti-tumor effects. This investigation has demonstrated that a new-found NK function is recognition and response to pathogenic periodontal bacteria. Whether the primary role of the NK cell in this response is one of a cytotoxic effector or an immune regulator remains to be tested.

Acknowledgments.

We thank Drs. Sidney H. Golub and Hungyi Shau for stimulating discussion and critical review of the manuscript, and Irene Petravic for the illustrations.
REFERENCES


