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SCIENTIFIC ARTICLES

Interleukin-1β Production in Periradicular Lesions in a Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome Model Compared with a Noninfected Host

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This study elucidates the role of interleukin-1 (IL-1) in developing periradicular lesions in immunocompetent and immunocompromised (human immunodeficiency virus/acquired immune deficiency syndrome) hosts. Eight cats were immunosuppressed with steroids before infection with feline immunodeficiency virus (FIV). Eight uninoculated cats served as controls. Periradicular lesions were induced around the canine teeth. At 1 and 4 wk periradicular exudate was sampled via the root canals. IL-1β levels were measured with ELISA. Data were analyzed using the Mann-Whitney U test and the Wilcoxon signed rank test. Statistically significant differences existed in cytokine levels between the FIV and non-FIV groups ($p < 0.001$). Cytokines were below detectable levels in the FIV group. A significant decrease in IL-1β levels at 4 wk compared with 1 wk occured in the non-FIV group ($p < 0.05$). In conclusion decreased IL-1β production was obtained in the FIV group. In the non-FIV group decreases in IL-1β levels were encountered at the chronic stage of the periradicular lesion compared with the acute stage.

A developing periradicular lesion represents a dynamic interplay between the immune system and infecting bacteria. Egress of bacteria and bacterial byproducts (antigens) from the root canal system attract phagocytic macrophages into the periradicular tissues. These macrophages remove the antigens from the periradicular region and present them to specific T- or B-lymphocytes. The interaction between the various immunocompetent cells determines ultimately the structural changes in the periradicular bone. Macrophages also play a very important role in the activation of osteoclasts and inhibition of complete bone repair being a major source of the proinflammatory cytokine interleukin-1 (IL-1) (1). Specifically at the periradicular level IL-1 is a potent mediator of bone resorption through osteoclast activation (2), and therefore plays an important role in the pathogenesis of periradicular lesions. Quantification of this cytokine over time can help understand its action on the disease process and bone remodeling. Osteoclast activation also involves stimulation of the hemopoietic stem cells, which are partially controlled by the T-cells through cytokines.

The production of appropriate cytokines is essential for the development of protective immunity. If inappropriate cytokines are produced, destructive or progressive disease results. Bacterial components, especially lipopolysaccharides, are capable of stimulating low levels of osteoclastic activity. However they are very potent activators of proinflammatory cytokine production, which stimulates bone resorption and inhibits reparative bone formation (2). Imbalances in cytokine production have been identified with retroviruses (3), which could explain the downregulation in immune function seen in human immunodeficiency virus (HIV) infection. There are conflicting reports, however, on the imbalances of cytokine production in peripheral blood of the HIV-infected patient. IL-1 may be elevated (4), though other results have been unclear (5).

$CD4^+$ T-lymphocytes and mononuclear phagocytes (tissue macrophages and blood monocytes) are primary targets of HIV. However mononuclear phagocytes are more resistant to virus-induced apoptosis, providing a reservoir of ongoing vital replication (6). T-cells exert a regulatory influence over macrophage-induced activities. Subsequently infection of the T-cells can alter a variety of the macrophages immunological functions, such as cytokine production (6), chemotaxis, accessory cell function, and microbicidal activity (7).

The present study was designed to elucidate the role of IL-1β in the development of periradicular disease states in the immunocompromised and the nonimmunocompromised host. For this purpose a feline model was used to characterize the molecular biological responses to these disease states.

MATERIALS AND METHODS

The development of an immunocompromised host model in cats with feline immunodeficiency virus (FIV), described elsewhere (8), enabled the comparative assessment of periradicular disease processes in cats with FIV versus immunocompetent, seronegative controls. Briefly, eight adult male cats were immunosuppressed by the use of steroids (methylprednisolone acetate; The Upjohn Company, Kalamazoo, MI) and infected with FIV (infectious clone FIV-PPR, provided by personnel at the College of Veterinary Medicine, Texas A&M University, College Station, TX). Complete blood counts were performed biweekly for 2 months, and then monthly for the following 8 months. Four enzyme-linked immunosorbent assay (ELISA) tests were performed for the detection of FIV antigen in the laboratory samples, and results were correlated with the blood counts. Eight age- and sex-matched littermates, not given the virus, were monitored and tested at equivalent periods of time and served as controls to the FIV animal model. In addition the eight control animals allowed the evaluation of the normal progression of periradicular disease processes, which are not fully understood.

For experimental manipulation animals were sedated by intramuscular injections of 0.5 ml of Ketaset/PromAce (ketamine HCl/acepromazine maleate; Fort Dodge Laboratories, Inc., Fort Dodge, IA) at a 10:1 ratio (100 mg/ml/10 mg/ml vials). Intraoral anesthesia was achieved by maxillary and mandibular infiltration of 1 ml of 2% lidocaine containing 1:100,000 epinephrine (Astra USA, Inc., Westborough, MA).

Maxillary and mandibular canine teeth were allocated randomly to either a 1-wk infection or a 4-wk infection group. To achieve infection two-thirds of the crowns of the canine teeth were removed with a high-speed fissure bur and the canals were exposed. Plaque was inoculated into the canals with a new size 25 K-file, a uniform size cotton pellet was placed in the access, and cavities were sealed with Ketac-Silver (Aplicap, ESPE, Seefeld, Germany) to ensure an anaerobic bacterial population.

Samples of periradicular exudate were obtained via the root canals at 1- and 4-wk postbacterial inoculation. For this teeth were isolated with a rubber dam and the field disinfected with 70% alcohol. The Ketac-Silver and cotton pellets placed in the chamber were removed with the use of a high-speed turbine. Care was taken to remove only the bulk of the material with the high speed and when close to the pellet, the remaining material was removed with hand instruments and a broach was used to retrieve the cotton pellet. Sterile standardized paper points (Henry Schein, Inc., Melville, NY) were inserted into the canal space to collect the sample and were used continuously until the pulp space was dry (9). Samples were placed in a microfuge tube containing 600 μl of phosphate-buffered saline and stored over crushed ice until all samples were collected. Tubes were transported to the laboratory, vortexed for 10 s, centrifuged at 4°C, 8000 rpm for 10 min, and the supernatant was collected and stored for 1 wk at −20°C.

IL-1β levels were measured by ELISA using human IL-1β ELISA system (Quantikine HS, R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's instruction. This assay allows for the detection of less than 0.1 pg/ml of IL-1β. Microplates were read with the SpectraMax 250 and computer software (SOFTmaxPRO, version 1.2.0, Palo Alto, CA). Standard Lowry assay was also performed on the same samples for correction of cytokine levels to milligrams of total protein. For this a standard curve was generated and samples could be read comparatively using the Lowry High Sensitivity software program on the DU Spectrophotometer (Beckman DU64, Palo Alto, CA). The concentrations obtained were then divided by 100 μl, which was the amount of sample used for this test, providing the number needed to divide the results of the ELISA to obtain the amount of cytokine present in the sample with respect to the amount of total protein. Data were analyzed statistically using the Mann-Whitney U test and the Wilcoxon signed rank test.

TABLE 1 Levels of IL-1β in pg/mg of total protein for the FIV group

Catalog No.	IL-1 Week 1	IL-1 Week 4
2	0	0
5	0	0
8	0	0
10	0	0
13	0	0
16	0	0
Mean	0	0

TABLE 2 Levels of IL-1β in pg/mg of total protein for the control group

Catalog No.	IL-1 Week 1	IL-1 Week 4
3	2.846	1.659
4	3.839	2.803
6	4.881	1.851
9	7.629	2.430
12	25.753	10.035
14	43.988	0
15	1.498	1.015
Mean	12.919	2.8276

RESULTS

Summary data for both FIV and non-FIV groups are presented in Tables 1 and 2. Two cats from the FIV group died before the experimental procedures were performed. Samples of one cat from the non-FIV group were contaminated and therefore not evaluated.

The mean level of IL-1β in the non-FIV group at week 1 was 12.919 pg/mg of total protein, and at week 4 it was 2.8276 pg/mg of total protein. There was a statistically significant difference in IL-1β levels at week 4 compared with week 1 (Wilcoxon signed rank tests; $p < 0.05$). Specifically higher levels were observed at the 1-wk period corresponding to the active stage of the periradicular lesion. However a wide range of IL-1β was detected in the non-FIV group within each time frame.

IL-1β was below detectable levels in the FIV group, both at the 1-wk and the 4-wk evaluation periods (Table 1). There was a statistically significant difference in IL-1β cytokine levels between the FIV group and the non-FIV group (Mann-Whitney; $p < 0.001$).

DISCUSSION

Bacteria and their byproducts within the pulp space are capable of initiating the host immune response in periradicular tissues. Mediators of the inflammatory process provide protective functions, like tissue repair and host defense. However this activity may also result in a destructive process. This process involves osteoclastic activating functions, and the different levels of mediators

detected in periradicular samples may reflect the bone resorptive activity of the lesion.

Cytokines produce multiple actions on a wide range of different cells and tissues (10). They act in concert with the immune system in the regulation of T-cell growth and differentiation, B-cell growth and differentiation, macrophage activation, modulation of class I and class II histocompatibility antigen expression, selected antibody production, and apoptosis. Several cytokines identified at sites of bone repair are thought to exert a direct or indirect effect, with others thought to regulate mitosis and differentiation.

IL-1β was detected in 13 of the 14 samples of the non-FIV group, and as in previous studies there was a wide distribution in the concentration of the samples (9). Our knowledge of cytokine levels over time in active and chronic periradicular lesions is limited. There is a tendency of increased cytokine levels in symptomatic lesions (11) and where cells have been stimulated by pathogens (12). Matsumoto et al. (13) showed expression of IL-1 was localized further from the bone surface over time, decreasing its expression during the chronic stage. This would agree with the increased cytokine levels observed in the active stage of the disease reported in the non-FIV group of this study (1 wk), with a decreased quantity in the more chronic stage of the disease (4 wk). Variations of cytokine levels in the non-FIV group within the same time frames may be due to different bacterial populations within the periradicular samples, which may stimulate different cytokine levels (14) because pathogens were not controlled or evaluated in this study.

In contrast, in the FIV group, IL-1β was below detectable levels at both time frames observed. IL-1β is produced by a variety of cell types, such as macrophages, B-cells, osteoclasts, polymorphonuclear cells, and fibroblasts. However the most important sources of this cytokine are macrophages and monocytes at inflammatory sites (15). Therefore, since T-cells activate macrophages, it can be hypothesized that the reason for not being able to detect IL-1β in the FIV group is the lower number of T-cells ($CD4^+$ cells) in the periradicular area of this group (8). This correlates with the fact that cytokine production can be used to assess T-cell function (16). The decreased levels of IL-1β would suggest depressed T-cell function and concentration, which is what occurs in the infectious process of HIV.

The mechanism of entry of HIV into mononuclear phagocytes seems to require viral tropism of an appropriate HIV strain and an interaction between the $CD4^+$ cellular receptor with noncovalent virions (gp120 and gp41 envelope proteins). It is thought that the binding of the $CD4^+$ monocyte triggers a conformational change in gp120 that exposes determinants and allows the sole entry of macrophage tropic strains of the virus (6). These strains of virus seem to be noncytopathic to the macrophage and predominantly involved in viral replication within the cell itself. Consequently alterations may also exist in the production of IL-1β from the macrophage proper. Moreover the various macrophage phenotypes are altered differently by HIV (17), with their corresponding differences in dysregulation of cytokine production. Hence much lower cytokine levels may be present and therefore undetected in this study. Nevertheless increased levels of cytokines have been demonstrated in blood from cats with FIV, where peak expressions are observed with periods of depressed immune responses (18). Other studies, on the contrary, have shown decreased IL-1β levels in lipopolysaccharide-stimulated blood of acquired immune deficiency syndrome patients (19).

An additional explanation for the decreased levels of IL-1β observed in this study is that decreased exudate was encountered when sampling from the FIV group compared with the control group. However the degree of dilution was the same for both groups, which was necessary to have enough sample to run several tests and to keep samples as uniform as possible. Thus the protein in the FIV sample would have been diluted to a higher extent than the non-FIV group, therefore making the protein in the sample below detectable levels with the techniques used in this experiment. If this model were to be repeated a more concentrated sample of exudate would be necessary to obtain better readings.

Cytokine expression in peripheral blood is altered by HIV infection (6). Current studies in nonimmunocompromised hosts have focused on cytokine expression from $CD4^+$ cells, but little information is provided on $CD8^+$ cells, which would be the major cell population involved in the FIV-infected host, as was the case at the periradicular level of the FIV group in this study. Furthermore progression of the periradicular lesion does not seem altered in the immunocompromised host (8, 20). Hence, nonspecific mechanisms must play a role in the development of the lesion. Given the large number of cytokines and the extreme complexity of their interactions with one another and cells of the immune system, it is difficult to decipher the clinical significance of the alterations of cytokine production reported in HIV infection.

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