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Increased Susceptibility of RAG-2 SCID Mice to Dissemination of Endodontic Infections

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Specific immunity has been implicated in the pathogenesis of periapical lesions, although the extent to which these mechanisms are actually involved in either protection or destruction of the pulp-periapex complex is yet to be established. To investigate this question we compared periapical-lesion pathogenesis in RAG-2 severe combined immunodeficient (SCID) mice with immunocompetent control mice following surgical pulp exposure. In order to equalize the bacterial challenge, an infection protocol using *Prevotella intermedia*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, and *Streptococcus intermedius* was devised. The results demonstrated that after infection, the proportion of the root canal flora represented by the four pathogens was almost identical in both groups (39.9 and 42.2% for RAG-2 and immunocompetent control mice, respectively). The effects of abrogation of T- and B-cell mechanisms on periapical pathogenesis were then assessed. Approximately one-third of the RAG-2 mice developed endodontic abscesses, while no immunocompetent controls had abscesses, results which indicated regional dissemination of the infection. A similar incidence of abscesses was found in two additional experiments. Abscessed RAG-2 teeth had significantly larger periapical lesions than did nonabscessed RAG-2 teeth ($P \leq 0.05$) and exposed immunocompetent controls ($P \leq 0.01$), whereas nonabscessed RAG-2 teeth were not significantly different from those of exposed immunocompetent controls in periapical-lesion size. We conclude that B- and T-cell-mediated immunity protects the host from the dissemination of endodontic infections and that RAG-2 mice are more susceptible to infection-induced pulp-periapex destruction.

Specific immune mechanisms are implicated in periapical pathogenesis, based largely upon the presence of T and B lymphocytes (7, 19, 20, 25, 31, 32), immunoglobulins (4, 5, 17, 24, 39), immune complexes (16, 37, 38), complement components (16, 44), and cytokines (3, 22, 23, 34, 35, 41, 42) in periapical lesions. Although these associations are informative, researchers have been unable to determine whether these mechanisms protect against pulpal infection and periapical pathology, actually contribute to their development, or are merely epiphenomenal.

A more direct approach to these questions involves the use of naturally occurring and genetically engineered mutations that result in defects of the specific immune system in animals. To date, only a few studies have made use of these models to investigate the pathogenesis of periapical disease, and these have yielded conflicting results (33, 40). In the present study we have developed a model of induced pulpal infection by using a defined pathogenic bacterial inoculum in RAG-2 severe combined immunodeficient (SCID) mice. RAG-2 animals are genetic knockouts for the recombination activation 2 gene which are unable to join V, D, and J segments to generate functional immunoglobulin or T-cell receptors. They consequently lack mature T and B cells and are incapable of mounting either specific antibody or cell-mediated responses, although cells involved in nonspecific immunity, including neutrophils and monocytes, are intact and fully functional (30). Unlike conventional SCID beige mice, which generate VDJ recombinants and functional lymphocytes with increasing age, RAG-2 animals do not exhibit a "leaky" phenotype and are therefore an appropriate model in which to determine the role of B- and T-cell-mediated mechanisms in periapical disease.

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MATERIALS AND METHODS

Animals. Breeding pairs of RAG-2 mice produced by targeted gene disruption were kindly provided by Frederick Alt, Children's Hospital Medical Center, Boston, Mass. Animals were bred and maintained in isolators in the Forsyth Dental Center Animal Facility under pathogen-free conditions. Immunologically intact wild-type mice (Taconic, Germantown, N.Y.) with the same background as the RAG-2 animals (F_1 between strains C57BL/6 and 129/SvEvTac) served as controls and were bred and maintained separately under identical conditions.

Pulp exposures. RAG-2 and control mice aged 10 to 12 weeks and weighing 20 to 25 g were anesthetized by the intramuscular injection of ketamine (80 mg/kg of body weight) and xylazine (10 mg/kg) in sterile phosphate-buffered saline (PBS) and mounted on a jaw retraction board. The pulps of all four first molars were exposed with a surgical microscope (model MC-M92; Seiler, St. Louis, Mo.), a portable variable-speed electric handpiece (Osada Electric, Los Angeles, Calif.), and a sterile size 1/4 round bur. The pulp chambers were opened until the entrances of the canals could be visualized and probed with a no. 10 endodontic file.

Infection with endodontic pathogens. Tryptic soy broth with yeast (TSBY) agar plates of four common endodontic pathogens, *Prevotella intermedia* ATCC 25611, *Fusobacterium nucleatum* ATCC 25586, *Peptostreptococcus micros* ATCC 33270, and *Streptococcus intermedius* ATCC 27335, were grown for 7 days under anaerobic conditions (80% N_2, 10% H_2, and 10% CO_2), harvested, and transferred to vials containing prereduced anaerobically sterilized Ringer's solution (PRAS) under the influx of nitrogen. The final concentration of each organism was determined spectrophotometrically, and the four pathogens were mixed to yield 10^9 cells of each pathogen/ml in 0.01 g of methylcellulose/ml.

In order to create "ecological space" for introduced organisms, prior to infection both groups of animals were placed on an antibiotic regimen of trimethoprim (0.125 ml)-sulfamethoxazole (4 ml) (Sulfatrim) in the drinking water for 3 days/week for 2 weeks (2). The animals were then caged together for 1 week to allow exchange of residual oral flora. At the time of pulp exposure (day 0), animals were infected with 100 μl of the inoculum mixture in the exposed pulp and oral cavity, a procedure which was repeated on days 7 and 14.

Bacterial sampling and quantification. Swab samples were obtained from the oral cavities prior to pulp exposure (day −1). Swabs were placed into PRAS, serially diluted, plated on TSBY agar plates, and incubated in an anaerobic chamber in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2. Abscesses were sampled by needle aspiration of pus after surface disinfection with 2% tincture of iodine and 70% isopropyl alcohol. Pus specimens were transferred to vials

with PRAS, serially diluted, plated on TSBY agar plates, and incubated anaerobically as described above. The root canal flora was sampled at sacrifice on day 35. The maxillae were isolated, the tissue surface was disinfected with 2% tincture of iodine, gingival tissues were removed, and the apical third of the mesial root of one of the maxillary first molars was extracted with a surgical microscope. Unexposed teeth served as negative sterility controls. Root tips were homogenized in 2 ml of PRAS by using a sterile mortar and pestle. Log_{10} dilutions of samples were plated on TSBY plates and were incubated in an anaerobic chamber. After 1 week of growth, plates were photographed for counting of CFU.

Pathogens were quantified by a colony lift method, as described previously (13). In brief, digoxigenin-labeled whole chromosomal DNA probes were prepared for each of the four introduced pathogens. Colonies cultured from the root tips were lifted onto Nytran filters and treated with a lysing and denaturing solution (0.5 M NaOH–1.5 M NaCl) and with proteinase K (100 μg/ml in 0.5% sodium dodecyl sulfate) to remove cellular proteins. Chromosomal DNA was fixed to the filters by baking for 2 h at 80°C. The filters were cut into quadrants, and each quadrant was prehybridized for 2 h at 42°C in 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, 25 mM sodium phosphate (pH 6.5), and 0.5 mg of freshly denatured herring sperm DNA/ml. Each filter quadrant was then hybridized with one of the four probes at 52°C overnight. Positive reactions were detected with an antidigoxigenin antibody conjugated to alkaline phosphatase and revealed by bromo-chloroindolyl phosphate and nitroblue tetrazolium.

ELISA. Serum samples were obtained at sacrifice on day 35 by cardiac puncture. Serum from control animals not subjected to pulp exposure was also evaluated to assess the presence of "natural" antibodies. For determination of specific antibodies against pathogens, plates were coated with formalin-killed microorganisms in PBS (optical density at 580 nm [OD_{580}], 0.3) and incubated for 3 h at room temperature. After at least two days at 4°C, the plates were washed three times with buffer II (0.9% NaCl, 0.05% Tween 20) and incubated with diluted serum (1/300) in buffer III (PBS with 0.05% Tween 20 and 0.02% NaN_3) for 2 h at room temperature. The optimal serum dilution of 1/300 was determined after testing a range of dilutions of serum (1/100 to 1/2,700). The plates were washed three times with buffer II, and bound immunoglobulin G was detected by reaction with goat anti-mouse immunoglobulin G conjugated to alkaline phosphatase (1/500) (Sigma, St. Louis, Mo.) for 12 h at room temperature. Conversion of substrate (*p*-nitrophenylphosphate [1 mg/ml]; Sigma) was determined at OD_{405} by an enzyme-linked immunosorbent assay (ELISA) reader (BIO-TEK Instruments, Inc., Winooski, Vt.).

Quantification of pulp and periapex destruction. Mandibles were dissected free of soft tissue, fixed in 5% neutral formalin, and decalcified in EDTA (10% [wt/vol] in 0.1 M Tris, pH 6.96). Six-micrometer paraffin sections were cut and stained with hematoxylin and eosin. The sections were encoded and evaluated by two independent blinded observers. Sections which included the crown and distal root of the mandibular first molar and which exhibited a patent root canal apex representing the central portion of the pulp and root canal were selected for analysis. A minimum of three sections per tooth were evaluated histomorphometrically with an Optimas Bioscan image analysis system. The largest values of periapical-lesion size, in square millimeters, from replicate sections of left and right lower first molars were averaged to obtain summary measures of tissue destruction for each animal. In order to assess the relationship between abscess development and periapex destruction, a separate analysis was carried out with the tooth as the unit.

Statistical analysis. All comparisons between groups were made with a two-tailed Wilcoxon rank sum test.

RESULTS

Equalization of bacterial challenge. In previous studies, we observed that the endogenous flora of RAG-2 animals was able to induce periapical bone destruction, albeit with relatively low pathogenicity. However, since these animals have been periodically exposed to antibiotics and are maintained under pathogen-free conditions, they have very few gram-negative microorganisms resident in their oral cavities compared to wild-type controls (36a). In this situation, it is difficult to separate the effects of immunodeficiency from those secondary to a difference in the pathogenicity of the oral flora. An infection protocol was therefore devised to equalize the bacterial challenge in RAG-2 and immunocompetent control animals by using a combination of four commonly isolated human root canal pathogens, *P. intermedia*, *F. nucleatum*, *P. micros*, and *S. intermedius*. Both groups ($n = 15$) were first placed on an antibiotic regimen to create ecological space for introduced organisms (2). Following pulp exposure (day 0), pathogens were introduced into the exposed pulp and oral cavity. Microbiological samples taken from the oral cavities prior to infection were negative for all 4 organisms (day −1), as assessed by using DNA probes specific for each bacterium (data not shown). The apical one-third of maxillary molar roots were isolated aseptically on day 35, and the microbiota infecting the root canals was analyzed. Twelve animals in the RAG-2 group and 14 animals in the control group were available for analysis. Nonexposed second molars served as sterility controls and yielded negative cultures. As shown in Table 1, the total numbers of cultivable anaerobic CFU present in the apical third of the mesial root of the first maxillary molars were similar in the two groups, with 381.6 ± 265.4 and 311.9 ± 265.0 CFU/apical third of the maxillary first molar mesial root (mean ± standard deviation) isolated from RAG-2 and control animals, respectively. Of interest, the proportion of the root canal flora represented by the four pathogens was almost identical in both groups: 39.9% for RAG-2 and 42.2% for controls (Table 1). There were no significant differences in the distribution of the four pathogens in the two groups. These findings demonstrate that the infection regimen was successful in equalizing the bacterial challenge in the two groups.

TABLE 1. Distribution of introduced pathogens in RAG-2 mice and exposed control mice

Pathogen	Mouse strain			
	RAG-2		Control	
	% of total CFU[a]	Prevalence[b]	% of total CFU[c]	Prevalence[d]
F. nucleatum	6.0 ± 14.2	6	3.2 ± 4.4	8
P. intermedia	5.0 ± 7.7	6	8.2 ± 12.4	9
S. intermedius	23.4 ± 22.3	11	20.4 ± 21.3	9
P. micros	5.4 ± 12.7	3	10.5 ± 19.0	9
Total	39.9 ± 19.2	12	42.2 ± 38.3	12

[a] Total CFU, 381.6 ± 265.4 (anaerobic colonies from the apical third of the mesial root of the maxillary first molar). Values are means ± standard deviations.
[b] $n = 12$.
[c] Total CFU, 311.9 ± 265.0.
[d] $n = 14$.

Development of periapical abscesses. The effect of the abrogation of the specific immune response on periapical pathogenesis was then assessed. Twenty RAG-2 and 13 C57BL/6-129 immunocompetent controls were subjected to pulp exposure and the standardized infection regimen. Notably, of the 20 RAG-2 animals, 7 developed grossly evident orofacial abscesses of endodontic origin (one abscess per animal) (Fig. 1), while none of the 13 control animals exhibited abscesses. Abscesses developed between days 12 and 28 and persisted to the end of the experiment (day 35). All animals within the RAG-2 group lost weight and exhibited malaise and lymphadenopathy. Splenomegaly was evident in abscessed RAG-2 animals, indicating systemic effects of the root canal infection. One abscessed animal died before the end of the experiment. Similar incidences of abscesses were found in two additional experiments, with 5 of 13 mice exhibiting abscesses in an experiment preceding that reported in this paper and 6 of 26 mice exhibiting abscesses in a later experiment.

Five of the seven abscesses could be sampled microbiologically, revealing the presence of a mixture of *P. intermedia*, *S. intermedius*, and *P. micros* in three of the abscesses and *P. intermedia* and *S. intermedius* in a fourth abscess, while one abscess lacked any of the introduced pathogens (Table 2). Interestingly, the latter was the last abscess to develop, on day

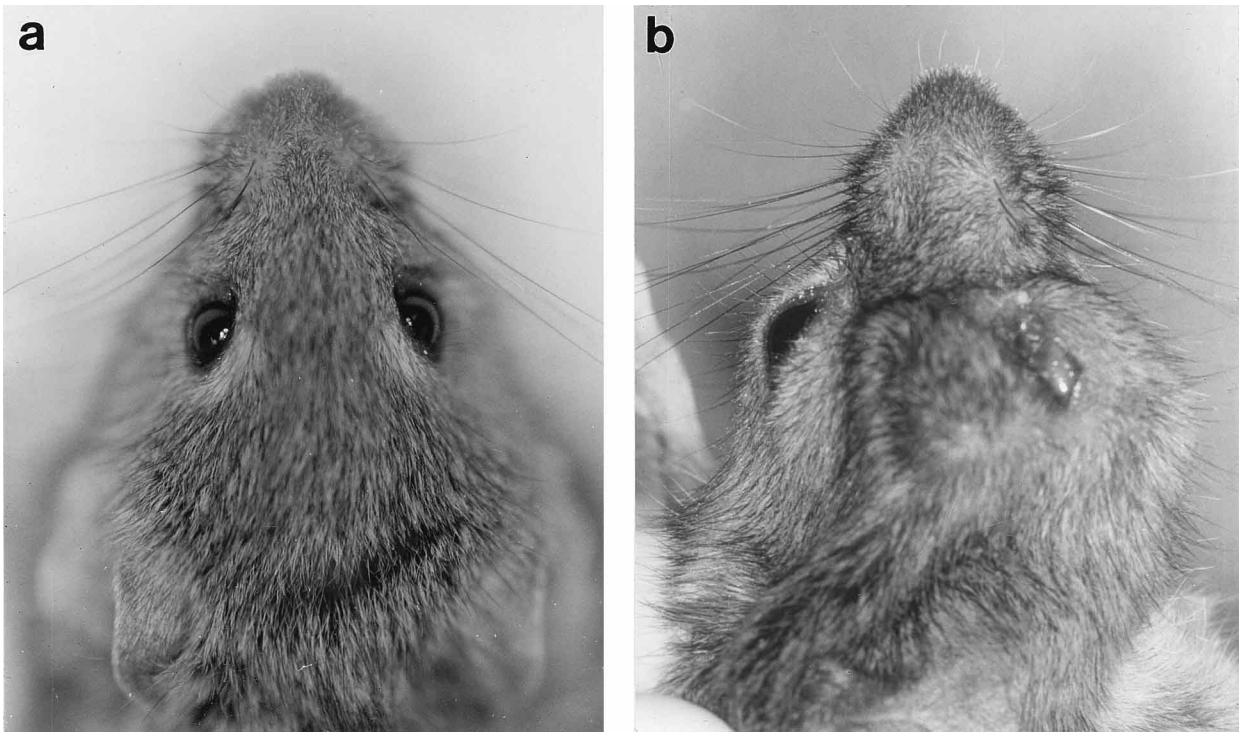

FIG. 1. (a) Nonabscessed RAG-2 mouse. (b) Clinical manifestation of an orofacial abscess of endodontic origin in a RAG-2 mouse.

28 after pulp exposure. Taken together, these findings indicate that T- and B-cell immune responses are protective against dissemination of endodontic infections from the root canal system.

Kinetics of bone destruction. In order to study the kinetics of development of periapical lesions, half of the animals in each group were sacrificed 21 days after exposure, with the remainder sacrificed on day 35. The amount of periapical tissue destruction was assessed by histomorphometry (Fig. 2). As shown in Fig. 3, the median area of lesions in the RAG-2 mice was larger than in control animals at both time points ($P \leq 0.01$ on day 21 and $P \leq 0.05$ on day 35). Lesions in the RAG-2 animals reached maximum size faster than those in control animals. This was reflected in the finding that lesion size in the RAG-2 group did not change significantly between days 21 and 35, whereas there was a considerable increase ($P \leq 0.05$) in the area of the lesions in the control group (Fig. 3).

The relationship between abscess development and increased tissue destruction was substantiated by comparisons between the abscessed teeth and the remaining teeth within the RAG-2 group. As shown in Fig. 4, when abscessed teeth were factored separately in the analysis, they were found to have larger lesions than did nonabscessed RAG-2 teeth ($P \leq 0.05$) and control teeth ($P \leq 0.01$). In addition, nonabscessed teeth in the RAG-2 group had no significant difference in periapical lesion size compared to the controls. Therefore, it appears that the immunodeficient status of the RAG-2 mice resulted in dissemination of the endodontic infection, leading to a concomitant increased tissue destruction at the periapex.

Antibody response to pathogens. ELISAs were carried out to assess the presence of specific antibodies against the four pathogens. The results demonstrated a statistically significant response against all pathogens in the sera from exposed immunocompetent controls compared to sera from nonexposed, noninfected immunocompetent controls (Fig. 5). As expected, RAG-2 mice possessed absolutely no antibody against any organism, confirming their immunodeficient status. The presence of modest antibody levels against *F. nucleatum*, *S. intermedius*, and *P. micros* in normal serum from noninfected, nonexposed control animals indicates natural or cross-reactive antibodies. These data demonstrate that the introduced pathogens elicited a vigorous antibody response in exposed controls, which may represent a mechanism which interferes with bacterial dissemination.

DISCUSSION

The specific immune response has been implicated in the pathogenesis of periapical and periodontal diseases, although it remains unclear to what extent these mechanisms play a significant role in tissue protection or destruction. In order to begin to answer this question, we developed a murine periapical-lesion model in RAG-2 knockout SCID mice which lack both T- and B-cell-mediated functions. RAG-2 and immunocompetent controls were subjected to an infection protocol which equalized the pathogenic oral flora so that observed differences in periapical pathogenesis would be ascribable to their immunological status and not to differences in the bac-

TABLE 2. Isolation of pathogens from sampled abscesses

Abscess	Pathogen			
	F. nucleatum	*P. intermedia*	*S. intermedius*	*P. micros*
1	−	+	+	+
2	−	+	+	+
3	−	+	+	+
4	−	+	+	−
5	−	−	−	−

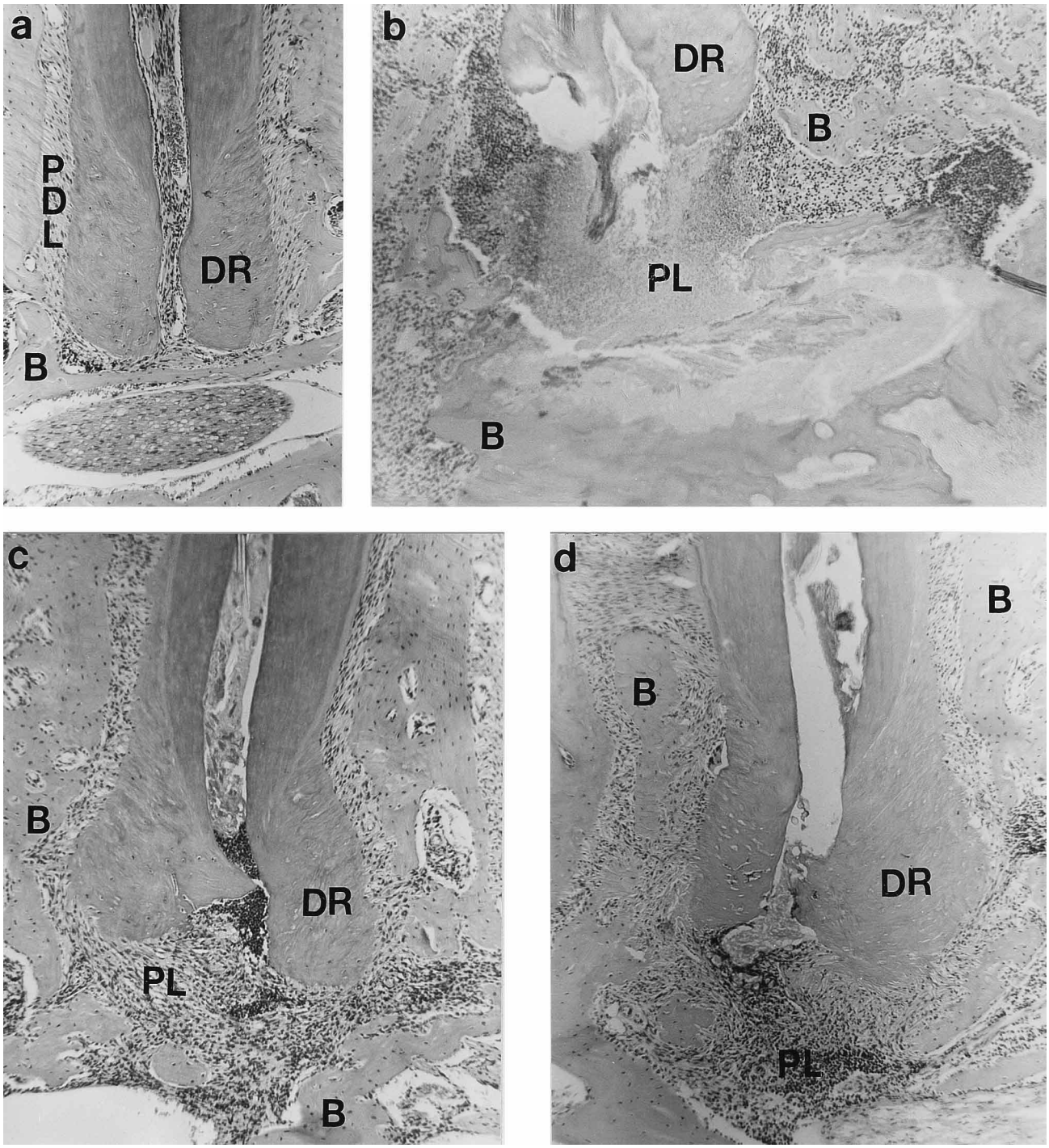

FIG. 2. (a) Photomicrograph of the periapex of a nonexposed tooth from an immunocompetent control mouse. (b) Photomicrograph of periapical lesion associated with an abscessed tooth in a RAG-2 mouse 35 days after pulp exposure and infection of the root canal system. (c) Photomicrograph of periapical lesion associated with a tooth from an immunocompetent control mouse 35 days after pulp exposure. (d) Photomicrograph of periapical lesion associated with a nonabscessed tooth in a RAG-2 mouse 35 days after pulp exposure. Hematoxylin-eosin stain; magnification, ×200. DR, distal root of mandibular first molar; PDL, periodontal ligament; B, bone; PL, periapical lesion.

terial challenge. Our findings demonstrate an increased susceptibility of systemically compromised SCID animals to the dissemination of endodontic infections, as well as to increased local periapical bone destruction. To our knowledge, this is the first study which clearly shows that immune mechanisms mediated by B and T cells serve to limit infections to the confines of the root canal system and prevent sepsis of endodontic origin.

Acute periapical abscesses result when large numbers of bacteria escape through the apex and elicit a severe inflammatory response. Grossly evident abscesses developed in approximately one-third of the RAG-2 mice, while none of the im-

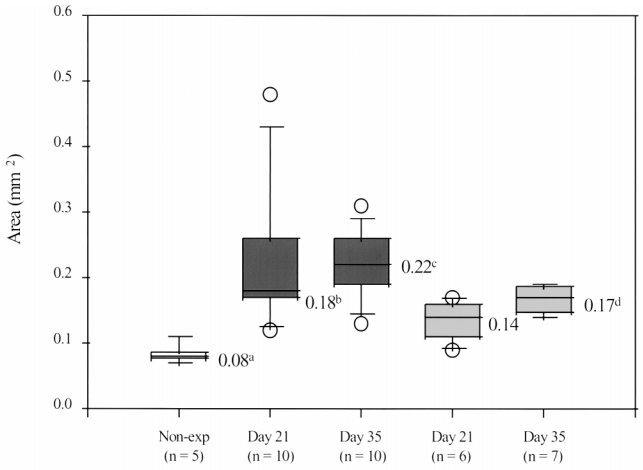

FIG. 3. Kinetics of periapical-lesion development in nonexposed (Non-exp) immunocompetent control mice, exposed RAG-2 mice (darkly shaded bars), and exposed immunocompetent control mice (lightly shaded bars). The bars encompass the 25th to 75th percentiles, the horizontal lines represent the medians (values indicated), the brackets extend to adjacent values, and the circles represent outliers in the data. a, significantly different from the exposed RAG-2 group at days 21 and 35 ($P \leq 0.01$) and the exposed immunocompetent control group at days 21 and 35 ($P \leq 0.05$ and $P \leq 0.01$, respectively); b, significantly different from the exposed immunocompetent control group at day 21 ($P \leq 0.01$); c, significantly different from the exposed immunocompetent control group at day 35 ($P \leq 0.05$); d, significantly different from the exposed immunocompetent control group at day 21 ($P \leq 0.05$).

munocompetent animals became abscessed. The development of abscesses was found to be reproducible in two other experiments and was never observed in non-RAG-2 animals. Most microbiological samples obtained from abscesses contained the inoculated pathogens, although in one case none of the four pathogens were identified. Thus, although this inoculum clearly contributed to dissemination of the endodontic infections in RAG-2 mice, in certain cases organisms from the endogenous oral flora may also emerge and cause sepsis. Interestingly, microbiological studies of pus samples from human acute dentoalveolar abscesses revealed that *Prevotella* spp. and *Peptostreptococcus* spp. are common isolates (21), a finding that correlates well with our results. Conversely, two-thirds of the RAG-2 animals failed to form abscesses, at least within the time frame of the experiment. It is possible that the strains utilized, which were originally isolated from humans, are not optimally adapted to the murine system. Organisms isolated from abscesses, or which have been selected by passage in mice, are being assessed for their ability to cause disseminated infections.

Athymic *nu*/*nu* models have previously been explored for effects on oral infections, including periapical disease, but have yielded conflicting results. *nu*/*nu* rats were reported to have similar periapical bone destruction, as did immunologically intact animals (40), whereas in another study *nu*/*nu* mice had significantly less periapical resorption than controls (33). It is not clear whether immunodeficient and immunocompetent animals were subjected to an equivalent microbial challenge in these experiments. Nevertheless, the lack of T-cell function per se, including delayed type hypersensitivity and T-cell-dependent antibody responses, may not predispose animals to the spread of root canal infections. Alternatively, the intact T-cell-independent antibody responses in *nu*/*nu* animals may be protective.

The generation of an antibody response in immunocompetent controls both confirms the establishment of pathogens within the root canal system and suggests that antibodies or other specific immune mechanisms may serve to prevent microbial dissemination and lesion progression. Such a protective effect of specific antibodies was suggested by Dahlen et al. (10), who showed that preimmunization of monkeys resulted in a more distinct limitation of the periapical resorptive process in immunized animals, as determined radiographically, although no significant difference in lesion size was observed. In nonimmunized animals, the inflammatory infiltrate extended into the trabecular system of bone, resembling osteitis or osteomyelitis.

Although not strictly comparable, periodontal disease models have also been used to investigate the role of T- and B-cell mechanisms in modulating oral infections. Baker et al. (2), employing infection with *Porphyromonas gingivalis*, reported that beige SCID animals developed less alveolar bone loss than did two other strains of immunocompetent mice, although these differences were quite small (<10%). A protective effect of immunization was reported in some periodontal disease studies (6, 9, 12, 14, 18, 26), although in others increased tissue destruction was observed (8, 11, 36). It is possible that the specific immune system may play a less critical role in localizing periodontal versus pulpal infections, given that the epithelial barrier of the periodontal pocket serves to separate pathogens from host tissues in the periodontium, whereas direct parenteral contact occurs following root canal infection. Indeed, as noted above, when dissemination of the root canal infection did not occur, there was no significant difference in the size of periapical lesions between the RAG-2 and control mice. Taken together, these data suggest that specific immune responses may help to protect against systemic spread of infections from the root canal but may otherwise have relatively minor modulatory effects on localized periapical bone destruction.

Animal models of periapical and periodontal disease using immunosuppressive agents have also shown an increased amount of bone loss in immunosuppressed animals (1, 15, 28,

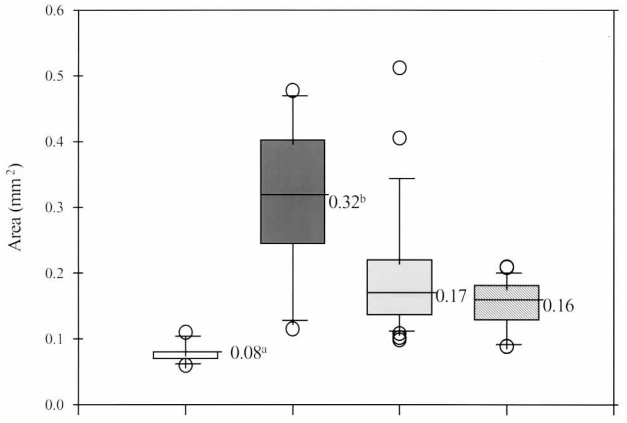

FIG. 4. Comparison of areas of periapical lesions in teeth from nonexposed immunocompetent control mice ($n = 7$) (open bar), abscessed teeth from exposed RAG-2 mice ($n = 6$) (darkly shaded bar), nonabscessed teeth from exposed RAG-2 mice ($n = 30$) (lightly shaded bar), and teeth from exposed immunocompetent control mice ($n = 25$) (hatched bar). The bars encompass the 25th to 75th percentiles, the horizontal lines represent the medians (values indicated), the brackets extend to adjacent values, and the circles represent outliers in the data. a, significantly different from abscessed teeth from exposed RAG-2 mice ($P \leq 0.05$), nonabscessed teeth from exposed RAG-2 mice ($P \leq 0.01$), and teeth from exposed immunocompetent control mice ($P \leq 0.01$). b, significantly different from nonabscessed teeth from exposed RAG-2 mice ($P \leq 0.05$) and teeth from exposed immunocompetent control mice ($P \leq 0.01$).

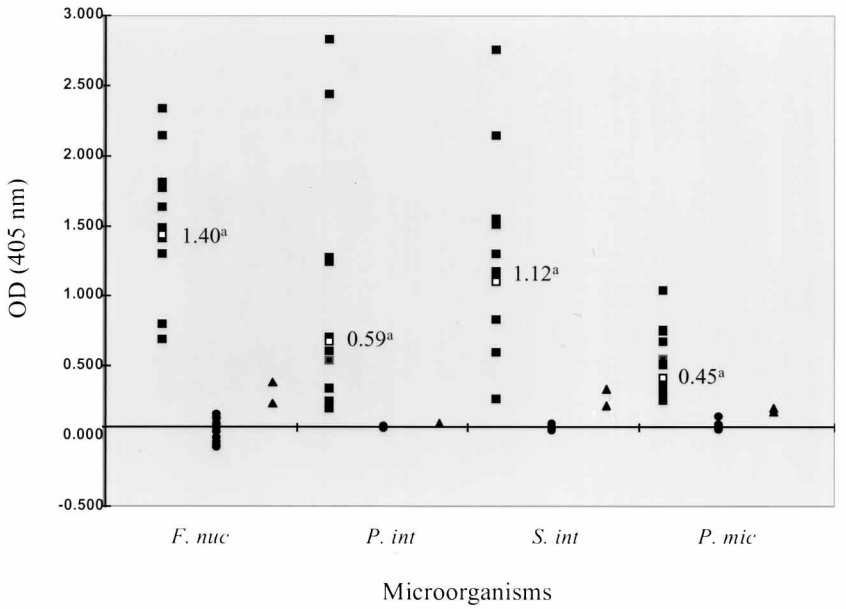

FIG. 5. ELISA for specific serum antibodies against *F. nucleatum* (*F. nuc*), *P. intermedia* (*P. int*), *S. intermedius* (*S. int*) and *P. micros* (*P. mic*). Squares represent values for sera from exposed immunocompetent control mice ($n = 13$), circles represent values for sera from exposed RAG-2 mice ($n = 18$), and triangles represent values from sera from nonexposed control mice ($n = 2$). a, significantly different from nonexposed controls ($P \leq 0.05$).

29, 43, 45). Kawashima et al. (15) demonstrated that immunosuppression induced by cyclophosphamide resulted in increased periapical bone destruction in rats. Although those authors did not investigate the development of septicemia, bacterial colonies were found not only in the pulp but also in the periapical area, suggesting some degree of dissemination. In two periodontal disease studies, septicemia developed in cyclophosphamide-treated animals (27, 28). In general, the risk of sepsis has been linked to depressed neutrophil counts, but it is clear that lymphocytes are also affected, given the nonspecific nature of chemotherapeutic regimens. Hence, the model described here may be useful not only in exploring the function of different immune mechanisms in the pathogenesis of periapical disease but also in assessing therapeutics in those clinical situations in which infections of oral origin may lead to sepsis.

In summary, our studies have shown that animals deficient in both T and B cells have increased susceptibility to the dissemination of endodontic infections. Although it is likely that this is the result of a deficiency in specific T- and/or B-cell functions (i.e., secondary to antigen-induced cell activation), it is possible that one or more nonspecific activation pathways and functions of these cells are also important in protection.

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REFERENCES

1. **Aoyama, Y., N. Yoshinari, T. Nogushi, and Y. Kameyama.** 1989. Effect of methotrexate-induced neutropenia on the periodontal lesion in rats (abstract 1229). J. Dent. Res. **68:**1020.
2. **Baker, P. J., R. T. Evans, and D. C. Roopenian.** 1994. Oral infection with Porphyromonas gingivalis and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. Arch. Oral Biol. **39:**1035–1040.
3. **Barkhordar, R. A., M. Z. Hussain, and C. Hayashi.** 1992. Detection of interleukin-1 beta in human periapical lesions. Oral Surg. Oral Med. Oral Pathol. **73:**334–336.
4. **Baumgartner, J. C., and W. A. Falkler, Jr.** 1991. Reactivity of IgG from explant cultures of periapical lesions with implicated microorganisms. J. Endod. **17:**207–212.
5. **Baumgartner, J. C., and W. A. Falkler, Jr.** 1991. Biosynthesis of IgG in periapical lesion explant cultures. J. Endod. **17:**143–146.
6. **Behling, U. H., C. Sallay, F. Sanavi, P. H. Pham, and A. Nowotny.** 1981. Humoral immunity and reduced periodontal bone loss in *Eikenella corrodens*-monoassociated rats. Infect. Immun. **33:**801–805.
7. **Bergenholtz, G., U. Lekholm, B. Liljenberg, and J. Lindhe.** 1983. Morphometric analysis of chronic inflammatory periapical lesions in root-filled teeth. Oral Surg. Oral Med. Oral Pathol. **55:**295–301.
8. **Burckhardt, J. J., R. Gaegauf-Zollinger, R. Schmid, and B. Guggenheim.** 1981. Alveolar bone loss in rats after immunization with *Actinomyces viscosus*. Infect. Immun. **31:**971–977.
9. **Crawford, J. M., M. A. Taubman, and D. J. Smith.** 1978. The effects of local immunization with periodontopathic microorganisms on periodontal bone loss in gnotobiotic rats. J. Periodontal Res. **13:**445–459.
10. **Dahlen, G., L. Fabricius, G. Heyden, S. E. Holm, and A. J. Moller.** 1982. Apical periodontitis induced by selected bacterial strains in root canals of immunized and nonimmunized monkeys. Scand. J. Dent. Res. **90:**207–216.
11. **Ebersole, J. L., M. Brunsvold, B. Steffensen, R. Wood, and S. C. Holt.** 1991. Effects of immunization with *Porphyromonas gingivalis* and *Prevotella intermedia* on progression of ligature-induced periodontitis in the nonhuman primate *Macaca fascicularis*. Infect. Immun. **59:**3351–3359.
12. **Evans, R. T., B. Klausen, and R. J. Genco.** 1992. Immunization with fimbrial protein and peptide protects against Porphyromonas gingivalis-induced periodontal tissue destruction. Adv. Exp. Med. Biol. **327:**255–262.
13. **Gunaratnam, M., G. L. Smith, S. S. Socransky, C. M. Smith, and A. D. Haffajee.** 1992. Enumeration of subgingival species on primary isolation plates using colony lifts. Oral Microbiol. Immunol. **7:**14–18.
14. **Holt, S. C., M. Brunsvold, A. Jones, R. Wood, and J. L. Ebersole.** 1995. Cell envelope and cell wall immunization of Macaca fascicularis: effect on the progression of ligature-induced periodontitis. Oral Microbiol. Immunol. **10:** 321–333.
15. **Kawashima, N., T. Okiji, T. Kosaka, and H. Suda.** 1993. Effects of cyclophosphamide on the development of experimentally-induced periapical lesions in the rat. Jpn. J. Const. Dent. **36:**1388–1396.
16. **Kettering, J. D., and M. Torabinejad.** 1984. Concentrations of immune complexes, IgG, IgM, IgE, and C3 in patients with acute apical abscesses. J. Endod. **10:**417–421.
17. **Kettering, J. D., M. Torabinejad, and S. L. Jones.** 1991. Specificity of antibodies present in human periapical lesions. J. Endod. **17:**213–216.

18. **Klausen, B., R. T. Evans, N. S. Ramamurthy, L. M. Golub, C. Sfintescu, J. Y. Lee, G. Bedi, J. J. Zambon, and R. J. Genco.** 1991. Periodontal bone level and gingival proteinase activity in gnotobiotic rats immunized with Bacteroides gingivalis. Oral Microbiol. Immunol. **6:**193–201.
19. **Kontiainen, S., H. Ranta, and I. Lautenschlager.** 1986. Cells infiltrating human periapical inflammatory lesions. J. Oral Pathol. **15:**544–546.
20. **Kopp, W., and R. Schwarting.** 1989. Differentiation of T lymphocyte subpopulations, macrophages, and HLA-DR-restricted cells of apical granulation tissue. J. Endod. **15:**72–75.
21. **Lewis, M. A., T. W. MacFarlane, and D. A. McGowan.** 1986. Quantitative bacteriology of acute dento-alveolar abscesses. J. Med. Microbiol. **21:**101–104.
22. **Lim, G. C., M. Torabinejad, J. Kettering, T. A. Linkhardt, and R. D. Finkelman.** 1994. Interleukin-1 beta in symptomatic and asymptomatic human periradicular lesions. J. Endod. **20:**225–227.
23. **Matsuo, T., S. Ebisu, T. Nakanishi, K. Yonemura, Y. Harada, and H. Okada.** 1994. Interleukin-1 alpha and interleukin-1 beta periapical exudates of infected root canals: correlations with the clinical findings of the involved teeth. J. Endod. **20:**432–435.
24. **Matsuo, T., T. Nakanishi, and S. Ebisu.** 1995. Immunoglobulins in periapical exudates of infected root canals: correlations with the clinical findings of the involved teeth. Endod. Dent. Traumatol. **11:**95–99.
25. **Nilsen, R., A. C. Johannessen, N. Skaug, and R. Matre.** 1984. In situ characterization of mononuclear cells in human dental periapical inflammatory lesions using monoclonal antibodies. Oral Surg. Oral Med. Oral Pathol. **58:**160–165.
26. **Persson, G. R., D. Engel, C. Whitney, R. Darveau, A. Weinberg, M. Brunsvold, and R. C. Page.** 1994. Immunization against *Porphyromonas gingivalis* inhibits progression of experimental periodontitis in nonhuman primates. Infect. Immun. **62:**1026–1031.
27. **Sallay, K., M. Listgarten, F. Sanavi, I. Ring, and A. Nowotny.** 1984. Bacterial invasion of oral tissues of immunosuppressed rats. Infect. Immun. **43:**1091–1093.
28. **Sallay, K., F. Sanavi, I. Ring, P. Pham, U. H. Behling, and A. Nowotny.** 1982. Alveolar bone destruction in the immunosuppressed rat. J. Periodontal Res. **17:**263–274.
29. **Samejima, Y., S. Ebisu, and H. Okada.** 1990. Effect of infection with Eikenella corrodens on the progression of ligature-induced periodontitis in rats. J. Periodontal Res. **25:**308–315.
30. **Shinkai, Y., G. Rathbun, K. P. Lam, E. M. Oltz, V. Stewart, M. Mendelsohn, J. Charron, M. Datta, F. Young, and A. M. Stall, et al.** 1992. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell **68:**855–867.
31. **Stern, M. H., S. Dreizen, B. F. Mackler, and B. M. Levy.** 1981. Antibody-producing cells in human periapical granulomas and cysts. J. Endod. **7:**447–452.
32. **Stern, M. H., S. Dreizen, B. F. Mackler, A. G. Selbst, and B. M. Levy.** 1981. Quantitative analysis of cellular composition of human periapical granuloma. J. Endod. **7:**117–122.
33. **Tani, N., K. Kuchiba, T. Osada, Y. Watanabe, and T. Umemoto.** 1995. Effect of T-cell deficiency on the formation of periapical lesions in mice: histological comparison between periapical lesion formation in BALB/c and BALB/c nu/nu mice. J. Endod. **21:**195–199.
34. **Tani-Ishii, N., C. Y. Wang, and P. Stashenko.** 1994. Bone-resorptive cytokine production in inflamed pulp and periapical lesions (abstract 144). J. Dent. Res. **73:**119.
35. **Tani-Ishii, N., C. Y. Wang, and P. Stashenko.** 1995. Immunolocalization of bone-resorptive cytokines in rat pulp and periapical lesions following surgical pulp exposure. Oral Microbiol. Immunol. **10:**213–219.
36. **Taubman, M. A., H. Yoshie, J. R. Wetherell, Jr., J. L. Ebersole, and D. J. Smith.** 1983. Immune response and periodontal bone loss in germfree rats immunized and infected with Actinobacillus actinomycetemcomitans. J. Periodontal Res. **18:**393–401.
36a. **Teles, R.** Unpublished observations.
37. **Torabinejad, M., W. C. Eby, and I. J. Naidorf.** 1985. Inflammatory and immunological aspects of the pathogenesis of human periapical lesions. J. Endod. **11:**479–488.
38. **Torbinejad, M., and J. D. Kettering.** 1979. Detection of immune complexes in human dental periapical lesions by anticomplement immunofluorescence technique. Oral Surg. Oral Med. Oral Pathol. **48:**256–261.
39. **Torres, J. O., M. Torabinejad, R. A. Matiz, and E. G. Mantilla.** 1994. Presence of secretory IgA in human periapical lesions. J. Endod. **20:**87–89.
40. **Wallstrom, J. B., M. Torabinejad, J. Kettering, and P. McMillan.** 1993. Role of T cells in the pathogenesis of periapical lesions. A preliminary report. Oral Surg. Oral Med. Oral Pathol. **76:**213–218.
41. **Wang, C. Y., and P. Stashenko.** 1993. Characterization of bone-resorbing activity in human periapical lesions. J. Endod. **19:**107–111.
42. **Wang, C. Y., and P. Stashenko.** 1993. Bone resorptive cytokine gene expression in developing periapical lesions (abstract 876). J. Dent. Res. **72:**213.
43. **Yamasaki, M., M. Kumazawa, T. Kohsaka, and H. Nakamura.** 1994. Effect of methotrexate-induced neutropenia on rat periapical lesion. Oral Surg. Oral Med. Oral Pathol. **77:**655–661.
44. **Yanagisawa, S.** 1980. Pathologic study of periapical lesions. 1. Periapical granulomas: clinical, histopathologic and immunohistopathologic studies. J. Oral Pathol. **9:**288–300.
45. **Yoshinari, N., Y. Kameyama, Y. Aoyama, H. Nishiyama, and T. Noguchi.** 1994. Effect of long-term methotrexate-induced neutropenia on experimental periodontal lesion in rats. J. Periodontal Res. **29:**393–400.

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