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Accelerated alveolar bone loss in male HLA-B27 transgenic rats: adult onset

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Objective and background: HLA-B27 transgenic (TG) rats exhibit severe colitis, arthritis and other inflammatory lesions. Previous studies in female TG rats indicate that they develop severe alveolar bone loss (ABL). Lack of data on male TG rats has left open the question of possible hormonal/sex dependence for the observed ABL. The purpose of the present study was to assess the natural history of ABL in male HLA-B27 rats, compared to age- and sex-matched wild-type Fischer 344 (WT) rats.

Materials and methods: Fourteen WT and 11 TG male rats, aged 7–8 weeks, were used. Sacrifice times occurred at 10, 22 and 35 weeks. Animal heads were defleshed and treated to remove organic material, and skulls were stained to locate the cemento–enamel junction. ABL was measured as exposed molar root surface area (mm^2) on the right maxilla and right mandible. Blinded measurements were performed using a computer-assisted image analysis system.

Results: ABL for the entire TG group was significantly different from the WT group ($p < 0.05$). There was no significant difference in ABL between WT and TG rats at 10 weeks of age. At 22 and 35 weeks of age TG rats experienced 23% and 37% greater ABL than WT rats, respectively; these differences were statistically significant ($p < 0.015$). For both TG and WT animals, ABL was significantly different between the three age groups.

Conclusions: These results, consistent with previous findings in female TG rats, suggest that the accelerated ABL found in TG rats is an adult-onset, age-dependent, and sex-independent process.

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HLA-B27 transgenic rats were originally developed for the study of human HLA-B27-associated diseases. These diseases encompass a broad range of severe multi-system inflammatory conditions. When HLA-B27 and human beta 2-microglobulin genes were introduced into rats, they developed arthritis, chronic colitis, and other inflammatory lesions, consistent with the range of conditions associated with HLA-B27 in humans (1).

There is mounting evidence that a host with hyper-inflammatory conditions, such as rheumatoid arthritis and inflammatory bowel disease, may also be susceptible to periodontal attachment loss and alveolar bone loss (2–4). Because of such associations between arthritis or inflammatory bowel disease and periodontitis, HLA-B27 transgenic rats were recently studied in this laboratory with respect to alveolar bone loss. Results indicated that HLA-B27 transgenic rats experience marked susceptibility to alveolar bone loss compared to wild-type Fischer 344 controls (5, 6).

However, all previous alveolar bone loss studies in HLA-B27 rats were performed using female animals (5, 6). Female sex hormone variations have been established as a significant factor in the development of bone loss, whether systemic or localized (7–9). The lack of information on the natural history of alveolar bone loss in male HLA-B27 rats has left open the question of possible hormonal dependence for the observed accelerated alveolar bone loss in female animals. The purpose of the present study was to assess

the natural history of alveolar bone loss in male HLA-B27 rats compared to the indicated wild-type control animals, male Fischer 344 rats (5, 6).

Materials and methods

Experimental animals, experimental design

Eleven HLA-B27 transgenic (TG) and 14 wild-type Fischer 344 (WT) male rats, 7–8 weeks-old, were obtained from Taconic (Germantown, NY, USA). Animals were group housed (three to four per cage) under conventional conditions, given standard laboratory rat chow and water ad libitum, and kept on a 12- h light cycle. Animals were monitored daily. At the age of 10, 22, and 35 weeks, animals were sacrificed by CO_2 inhalation, decapitated and heads were immediately frozen. Of the 11 TG animals, three, four, and four were sacrificed at 10, 22, and 35 weeks of age, respectively. The corresponding number of sacrificed WT rats was three, six, and five animals. The study protocol was approved by the Institutional Laboratory Animal Care and Use Committee of Loma Linda University, where all live animal experimentation took place.

Alveolar bone loss (ABL) measurements

A slight modification of a method previously described in detail was used (5). Animal heads were manually defleshed and then underwent short, successive treatments with sodium hypochlorite, followed by hydrogen peroxide to remove organic debris. The jaws were dyed in 1% methylene blue to locate the cemento–enamel junction. The stained jaws were appropriately oriented under a stereomicroscope and digital images were obtained of the lingual aspect of the right mandible, and buccal and palatal aspects of the right maxilla. Calibration measurements were made by recording images of a millimeter ruler under the same magnification and orientation. A computer-assisted image analysis system (Scion Image, Scion Corporation, Frederick, MD, USA) was utilized to plot points on the images and calculate the exposed molar root surface area, in mm^2. The two maxillary measurements (buccal and palatal) were averaged to calculate mean maxillary bone loss, while animal ABL was calculated as the sum of the mean maxillary and the mandibular bone loss. All measurements were performed after sacrifice of the last (35 week old) group of rats, and the sole operator performing the measurements was blinded regarding age and type of animal.

To determine reproducibility, three TG and three WT animals were randomly chosen. The maxillas and mandibles were repositioned, photographed and reanalyzed 8, 9 and 10 weeks after the original measurements were made. Therefore, a total of three sets of replicates plus the original measurements were used to determine reproducibility. The average coefficient of variation (standard deviation/mean) was determined to be 2.6% for all six animals, 1.6% for TG animals and 3.7% for WT animals.

ABL rate (mm^2/month) was defined as: $(ABL_2 - ABL_1)/(t_2 - t_1)$, where ABL_2 and ABL_1 represent ABL values for age t_2 and t_1 (in months), respectively.

Data management and statistical analysis

Animal ABL data were analyzed by non-parametric tests (Mann–Whitney U and Kruskal–Wallis). Significance level for rejection of the null hypothesis was set at $\alpha = 0.05$.

Results

Detailed descriptive statistics for ABL results are provided in Table 1. ABL was 9.7 ± 2.4 mm^2 for the entire group of TG rats ($n = 11$) and 7.8 ± 0.9 mm^2 for the WT group ($n = 14$). The difference between the two groups was statistically significant ($p < 0.05$, Mann–Whitney). At 10 weeks of age, there was no difference in ABL between TG and WT rats ($p = 0.12$, Mann–Whitney). Within the 22 and 35 weeks of age groups, ABL was significantly greater in TG compared to WT animals ($p < 0.015$). Results remained significant when analyzed separately for each jaw (maxilla or mandible; data not shown). The greater ABL in 22 and 35-week-old, male TG rats compared to age- and sex-matched WT rats was readily evident by gross examination (Figs 1 and 2).

ABL was significantly different between the three age groups for both WT ($p = 0.0035$, Kruskal–Wallis) and TG ($p = 0.016$, Kruskal–Wallis) animals. Figure 3 presents the ABL data (mean ± SD) in a graphic format to illustrate the temporal aspect.

The rate of ABL between ages 10 weeks (2.3 months) and 35 weeks (8 months) was 0.89 mm^2/month for TG and 0.40 mm^2/month for WT rats. Between 22 weeks (5 months) and 35 weeks of age, the corresponding ABL rates were 0.83 and 0.35 mm^2/month, while between 10 weeks and 22 weeks the corresponding ABL rates were 0.97 and 0.45 mm^2/month.

Table 1. Alveolar bone loss (mean ± SD; in mm^2)

Age	Male transgenic (TG)	Male wild-type (WT)
10 weeks	6.86 ± 0.13 ($n = 3$) (6.93; 6.71–6.93)[a]	6.48 ± 0.32 ($n = 3$) (6.50; 6.17–6.79)
22 weeks	9.49 ± 1.31* ($n = 4$) (9.27; 8.13–11.26)	7.70 ± 0.32 ($n = 6$) (7.78; 7.18–8.02)
35 weeks	11.97 ± 1.59† ($n = 4$) (11.70; 10.34–14.14)	8.75 ± 0.47 ($n = 5$) (8.76; 8.23–9.46)

[a]Values indicate median and range.
*$p = 0.011$ from age-matched WT animals (Mann–Whitney *U*-test).
†$p = 0.014$ from age-matched WT animals (Mann–Whitney *U*-test).

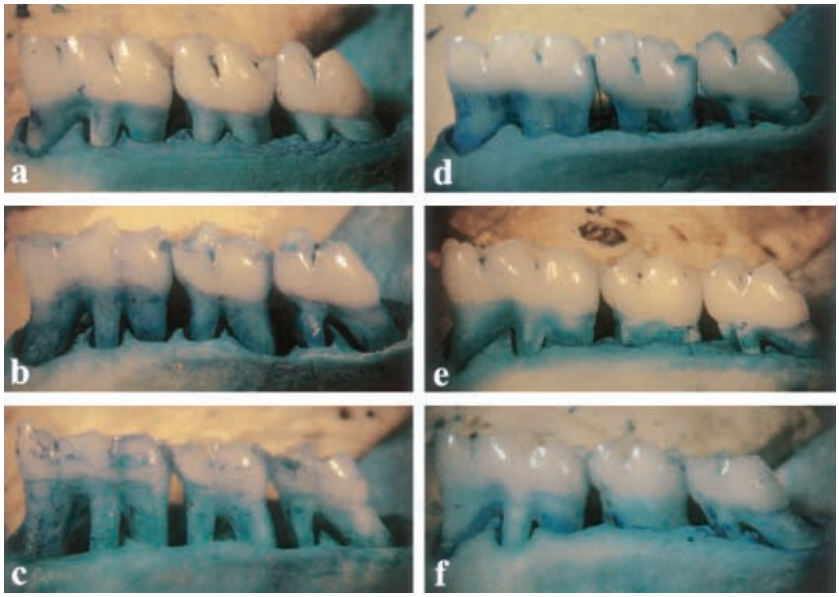

Fig. 1. Alveolar bone loss (mandibular lingual aspect) in HLA-B27 transgenic (TG) and wild-type Fischer 344 (WT) rats. Representative TG (a–c) and WT (d–f) animals aged 10 (a, d), 22 (b, e), and 35 (c, f) weeks. The depicted animals, same as in Fig. 2, were chosen to best mirror the mean alveolar bone loss value of their respective group.

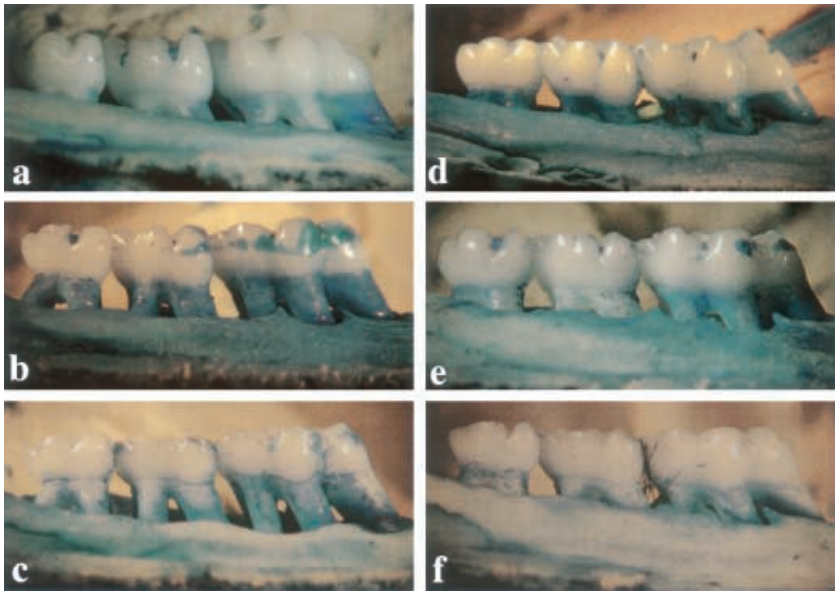

Fig. 2. Alveolar bone loss (maxillary palatal aspect) in HLA-B27 transgenic (TG) and wild-type Fischer 344 (WT) rats. Representative TG (a–c) and WT (d–f) animals aged 10 (a, d), 22 (b, e), and 35 (c, f) weeks. The depicted animals, same as in Fig. 1, were chosen to best mirror the mean alveolar bone loss value of their respective group.

Discussion

The present study sought to examine the naturally occurring ABL in male HLA-B27 transgenic (TG) and wild-type Fischer 344 (WT) rats housed under conventional conditions. The results indicate that male TG rats exhibit significantly different ABL. At 2.3 months (10 weeks) of age, a time corresponding to young adulthood, there was no difference in ABL between TG and WT rats. However, by 5 months (22 weeks) of age TG animals had experienced significantly elevated ABL (23% increase) compared to WT animals. At 8 months (35 weeks) of age, male TG rats exhibited approximately 37% greater ABL than WT age-, and sex-matched rats. The morphometric method of bone loss analysis employed in the present study does not permit assessment of intrabony defects. Therefore, the severity of bone loss present in HLA-B27 rats may have been underestimated. The present results reported in male animals are consistent with previous findings in female animals (5, 6). The rate of ABL between months 2.3 and 5 for the male TG animals was double the corresponding ABL rate for the male WT animals. In a previous study, the ABL rate for female TG rats between months 2.6 and 6 was double the corresponding rate for female WT rats (6). Collectively, these findings indicate that the accelerated ABL manifested in HLA-B27 rats is an adult-onset, age-dependent, sex-independent condition that does not precede the appearance of gastrointestinal complications, which usually first appear around 10 weeks of age.

The potential mechanisms underlying the development of accelerated ABL in HLA-B27 rats have been recently discussed (6). Humans with inflammatory bowel disease develop osteopenia/osteoporosis (10, 11), a condition associated with ABL (9, 12, 13). Development of osteopenia/osteoporosis has also been described in rheumatoid arthritis (14), another systemic condition associated with ABL (4). The HLA-B27 rats develop arthritis, spondyloarthropathy and chronic colitis. Our working hypothesis is that HLA-B27 rats also develop osteopenia/osteoporosis, which then leads to accelerated ABL. Therefore, the relevance of the HLA-B27 rat model to the human condition may reside in the reported association of periodontal bone loss with Crohn's disease cases (2, 15) and arthritis (4).

In the absence of commensal gut flora, HLA-B27 rats develop neither joint nor gastrointestinal inflammatory lesions (16, 17). The role of commensal gut or oral flora in the development of ABL in this model has not been

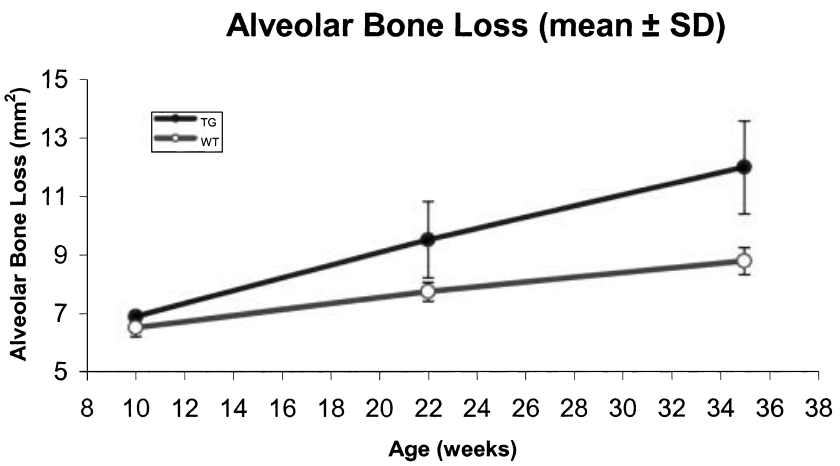

Fig. 3. Alveolar bone loss (mean ± SD) in male HLA-B27 transgenic (TG) and wild-type Fischer 344 (WT) rats.

investigated. However, it should be noted that the oral environment of these animals was never manipulated, in contrast to what is required for the induction of alveolar bone loss in other rodent models (18, 19). The alveolar bone loss observed in the control animals is consistent with the age-dependent alveolar bone loss reported in rodents (18, 19).

HLA-B27 transgenic rats are currently being used as an animal model to study the pathogenesis and treatment of several conditions, e.g. arthritis, colitis, psoriasis and alopecia. The results of the present study indicate that the severe alveolar bone loss spontaneously occurring in male HLA-B27 transgenic rats is an adult-onset, age-dependent condition. Therefore, both male and female HLA-B27 transgenic rats could be a very informative model for the study of alveolar bone loss pathogenesis and the study of common immunoregulatory dysfunctions that link inflammatory conditions with periodontal bone loss.

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