Effects of Salivary Immune Response to *Streptococcus mutans* on Caries Occurrence and Caries Development in Mice with Autoimmune Disease

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(Received 28 September and accepted 5 December 1994)

Key words: dental caries, autoimmune disease, mice, salivary immunoglobulin level

Abstract

MRL/l strain mice, which possess a lymphoproliferative gene inducing swelling of systemic lymph nodes, develop a SLE (systemic lupus erythematosus)-like syndrome at around 8 w of age. MRL/n mice, which carry 99.6 % of the genes of MRL/l mice, lack the gene for lymphoproliferation and exhibit only a slight degree of lymph node swelling late in life.

This study investigated whether the salivary immune response caused by *Streptococcus mutans* (*S. mutans*) infection prevented dental caries in MRL/l and MRL/n mice after 8 w of age.

A total of 10 MRL/l mice and 10 MRL/n mice were fed a commercial pellet diet without sucrose until 74 d of age, and then fed Diet 2000 containing 56 % sucrose *ad libitum* from 75 to 130 d of age.

On d 75, both strains of mice were inoculated with *S. mutans* JC-2 for 7 d. At 130 d of age, saliva samples were collected and caries scores were assessed.

The results obtained suggested that the salivary immune response was one of the most important factors regulating caries occurrence.

Introduction

Michalek and McGhee^[1] pointed out that the following factors must be considered when using an animal model in a caries-promotion study: (1) the caries-promoting diet, (2) the cariogenic microorganism, and (3) the susceptible host employed. As an animal model, inbred strains of mice have several advantages over rats in dental research, since genetical control can be maintained well. It is not difficult to induce caries lesions by infection of inbred mice with *S. mutans* serotype *c*^[2]. Kurihara et al.^[3] demonstrated that the H-2 region is genetically related to susceptibility to dental caries using several kinds of inbred mice with different haplotypes.

Several laboratories have demonstrated that salivary immunoglobulin A (IgA) and serum antibodies are important for preventing dental caries in human and animals^[4-6]. However, Krasse et al.^[7] found no consistent correlation between antibodies and dental caries prevalence among adults.

Murphy et al.^[8] produced MRL/Mp-lpr/lpr (MRL/l) mice, which possess a lymphoproliferative (*lpr*) gene inducing swelling of systemic lymph nodes, and develop a lupus-like syndrome characterized by antibodies against nucleic acids and immune-complex glomerulonephritis. Several studies have indicated that autoimmunity in MRL/lpr mice is caused by proliferation of a distinct subpopulation of T cells^[8,9].

These proliferating cells are thought to be helper T cells because they lack *Lyt-2*^[9], which is found on suppressor/cytotoxic T cells. However, these T cells support immunoglobulin synthesis by B cells *in vitro*^[10,11]. MRL/Mp-+/+ (MRL/n) mice, which carry 99.6 % of the same genes as MRL/l mice and lack the *lpr* gene, exhibit only a slight degree of lymph node swelling late in life^[8]. Therefore, for research on the immune response in MRL/l mice, MRL/n mice are thought to provide the best control.

The purpose of our previous study^[12] was to examine the relationship between salivary immunoglobulin

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lin levels and caries development in MRL/l mice using MRL/n mice as a control. Both strains of mice were inoculated with *S. mutans* at 22 d of age, when no signs of disease were evident, and MRL/l mice were recognized to have massive lymph node enlargement as an autoimmune disease at the time of the caries score assessment. On the other hand, MRL/n mice were healthy throughout the experimental period. The purpose of this study was to determine whether salivary immunoglobulin levels regulated caries occurrence in MRL/l mice, using MRL/n mice as a control. At 75 d of age of *S. mutans* inoculation, MRL/l mice already suffered from autoimmune disease and their immune function was thought to be defective. MRL/n mice remained healthy throughout the experimental period.

Materials and methods

Mice

MRL/l mice and MRL/n mice were obtained from Jackson Laboratory, U.S.A.

All the mice employed were bred in our colony under specific pathogen-free conditions, and were fed on Diet 2000, containing 56 % sucrose, *ad libitum* and sterilized water during both experimental periods.

Bacterial strains and conditions

S. mutans strain JC-2 (serotype *c*), which is resistant to streptomycin (1.0 mg/ml), was used. The strain was maintained on brain heart infusion (BHI) agar containing excess calcium carbonate at 4 °C. Bacteria grown to log phase in BHI broth for 18 h at 37 °C in an atmosphere of CO₂ (5%) in nitrogen (95%) were used.

Prior to the experiments, it was confirmed that no *S. mutans* was present in the flora using oral swabs and culturing on *Mitis Salivarius* (MS) and BHI agar plates.

Measurement of caries score

The mandibles were stained with murexide solution, and the molars were hemisectioned. The buccal, lingual, sulcal and proximal molar surfaces were subsequently scored for caries analysis according to the Keyes^[13] procedure modified for mice.

Measurement of immunoglobulin levels in saliva

Salivary levels of IgA against *S. mutans* JC-2 were measured by ELISA using 50 µl *S. mutans* of optical density 1 as the primary antibody, and serial dilutions of the saliva samples were added to the wells. Absorbance was read at 415 nm.

Experimental design

Ten MRL/l female mice and 10 MRL/n female mice were used. Until d 74, both inbred strains were fed a pellet diet without sucrose and sterilized water *ad libitum*. On d 75 they were inoculated with 50 µl of *S. mutans* JC-2 (1×10^9 cells/ml) for 7 d when MRL/l mice showed massive systemic enlargement of lymph nodes, and MRL/n mice were healthy. After 75 d of age, all mice were fed on Diet 2000, containing 56 % sucrose, *ad libitum* and sterilized water until 130 d of age. Saliva samples from MRL/l and MRL/n mice at 130 d of age were collected using capillary tubes by subcutaneous injection of pilocarpine (10mg/kg) as a stimulant and stored at -20 °C.

Results

Caries scores

The mean caries score and standard error in MRL/l and MRL/n mice were 230.8 ± 15.6 and 31.6 ± 11.9 , respectively showing a significant difference ($p < 0.01$) (Table 1).

Levels of salivary IgA against *S. mutans*

The mean levels of salivary IgA against *S. mutans* in MRL/l mice were significantly lower than those in MRL/n mice at 130 d of age after infection with *S. mutans* on d 75 (Table 2).

Discussion

Kurihara et al.^[3] have demonstrated that H-2 (murine Major Histocompatibility Complex) affects susceptibility to dental caries in mice. Meanwhile it has been reported that bacterial adhesion is inhibited by secretory IgA^[14]. Several laboratories have shown that salivary IgA is important for preventing dental caries in humans and animals. Some studies have demonstrated the protective effects of salivary IgA,^[4,6,15-17] whereas others support serum antibody-mediated control of caries lesions^[18,19]. Nevertheless, studies in humans have shown that oral immunization with *S. mutans* whole cells results in the induction of a salivary IgA response^[20,21].

Gregory et al.^[22] reported that caries-free subjects or individuals with low caries susceptibility exhibited significantly higher levels of naturally occurring salivary IgA and serum IgG, IgA, IgM against a *S. mutans* ribosomal preparation than subjects with high caries susceptibility.

Human MHC antigens may regulate the host immune response including the interaction between immunoglobulins and the HLA-DR antigen, since a human MHC class 2 antigen has been demonstrated to associate with helper T cell activity in the control of dental caries^[23]. Niiyama et al.^[24] reported that the immune response to dental caries was controlled by the class 2 gene(s) in the major histocompatibility complex of the rat (RT1). MRL/l mice develop a T-cell lymphoproliferative syndrome with a systemic lupus erythematosus (SLE)-like disorder, and the female mice develop the disorder earlier than the male mice. The disease is marked by massive lymph node enlargement, hypergammaglobulinemia characterized by antibodies against nucleic acids, and by immune-complex glomerulonephritis. The massive lymph node enlargement is due to abnormal T cells which have abnormal helper and suppressor/cytotoxic function. In response to the *lpr* mutant gene, under non-stimulated conditions, Ly 1+T cells develop, causing the polyclonal BCDF, a B-cell differentiation factor, to produce a high level of immunoglobulin and autoimmune antibodies^[8]. Therefore we investigated how MRL/l mice with polyclonal hyperimmunoglobulins are affected by dental caries development, and compared them with MRL/n mice, which have the same H-2 haplotype and normal serum immunoglobulin levels^[12].

In a previous study^[12] the salivary IgA level of MRL/l mice at 115 d of age inoculated with *S. mutans* was significantly higher than that of MRL/n female mice in the infection group. In particular, serum IgA levels in MRL/l mice were two to three times higher, and serum IgG levels were five to six times higher than those in MRL/n mice. Similar results for serum immunoglobulin levels were obtained by Murphy et al.^[8] and Andrews et al.^[25] The salivary IgA levels in the infected MRL/l mice on d 22 were significantly higher than those in the non-infected group at 115 d of age, although the caries scores of the MRL/l mice were not significantly different from those of MRL/n mice^[12]. These results suggested no regulation of dental caries development by salivary IgA and serum IgA and IgG in MRL/l mice. The immune response of MRL/l mice at the time of caries development might be weak, because polyclonal hyperimmunoglobulin levels by autoimmune disease developed around 8 w of age^[8], when dental caries had already occurred. The increase in immunoglobulin against *S. mutans* or dental lesions may have been responsible for the higher salivary IgA levels in infected MRL/l mice than those in non-infected MRL/l mice. Lehner et al.^[26] reported that serum IgG anti-*S. mutans* antibodies were negatively correlated with a previous history of caries in humans. Our results indicated that the high salivary immunoglobulin levels in MRL/l mice were not related to dental caries development compared with MRL/n mice. Salivary immunoglobulin may prevent dental caries if high levels are maintained at the incipient stage of cariogenesis.

Accordingly, in this study *S. mutans* was inoculated on d 75 for 7 d, when the abnormal immune response with hyperimmunoglobulinemia occurred in MRL/l mice, and the immune response was normal in MRL/n mice. The mean caries score on d 130 in MRL/l mice was more than seven times higher than that in MRL/n mice, the difference being significant ($p < 0.01$) (Table 1).

The mean caries score of MRL/n mice which were inoculated with *S. mutans* on d 75 was noticeably decreased compared with that after inoculation at 22 d of age. The reason for the decline in caries score in MRL/n mice was thought to be maturation of enamel calcification with age.

On the other hand, the mean caries score of MRL/l mice which were inoculated with *S. mutans* on d 75

was increased in comparison with that on d 22 (Table 1). One of the reasons for the increased caries score was thought to be the poor immunoglobulin response to *S. mutans* antigens at 75 d of age in MRL/l mice. Wofsy et al.^[27] indicated that the proliferating cells in MRL/l mice lack an important functional marker of helper T cells (L3T4), which participates in the response to MHA (major histocompatibility antigen)2, and Lyt-2, which participates in the response to MHA 1, and that these cells in MRL/l mice may respond poorly to antigens association with either MHA 2 or MHA 1 in the H-2 region which are found in the murine MHC (major histocompatibility complex) on chromosome 17.

One of the regulatory factors of dental caries might be immune function in response to the causative agent, which might be related to H-2 region on chromosome 17 in mice. In fact, salivary IgA against *S. mutans* in MRL/l mice was significantly lower ($p < 0.05$) than that in MRL/n mice at 130 d of age after infection with *S. mutans* on d 75. At over 8 w of age, the immune response to antigens associated with MHA 1 or MHA 2 might decline, despite the high serum immunoglobulin levels in MRL/l mice.

Taken together with the results of our previous study^[12], we conclude that salivary IgA does not work to prevent caries development after dental caries initiation in MRL/l mice, although salivary IgA against *S. mutans* is one of the important factors regulating the occurrence of dental caries.

Conclusions

Differences between effects on the salivary immune response to caries occurrence and caries development were demonstrated using MRL/l mice with autoimmune disease and MRL/n mice as controls.

From our previous study and this experiment, we conclude that salivary IgA does not work to regulate dental caries development, and that salivary IgA is one of the most important factors regulating the occurrence of dental caries.

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Table 1 Caries scores at 130 d of age in mice inoculated with *S. mutans* on d 75

Strains	n	Mean $\pm$ S.E.
MRL/l	10	230.8 $\pm$ 15.6
MRL/n	10	31.6 $\pm$ 11.9

** : $p < 0.01$

Table 2 Salivary IgA levels against *S. mutans* at 130 d of age in mice inoculated with *S. mutans* on d 75

Strains	n	Mean $\pm$ S.E. ($\times 10^{-3}$ mg/ml)
MRL/l	10	1.08 $\pm$ 0.95
MRL/n	10	2.58 $\pm$ 1.30

* : $p < 0.05$