DIFFERENTIAL CELL-MEDIATED IMMUNE RESPONSE TO *S. MUTANS* IN CHILDREN WITH LOW AND HIGH DENTAL CARIES

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ABSTRACT

Role of cell-mediated immune response (CMI) in dental caries was studied in 171 subjects, comprising of 86 children with low caries (LC), 31 with high caries (HC), and 54 age matched controls.

[^{3}H] thymidine mediated lymphoblast transformation test (LTT) using mutans streptococci antigen as stimulant was used to study the stimulation index (SI) of in vitro cultured lymphocytes from these children. The analysis revealed low stimulation index in high caries children whereas low caries children exhibited high stimulation index normally ranging between 2 to 6. The findings indicated that low caries children had strong CMI response as compared to high caries children. Although, the findings are based on limited number of samples, it certainly lays emphasis on protective or regulatory role of CMI in different phases of dental caries.

Key words: *Cell-mediated immunity (CMI), Lymphoblast transformation test (LTT), Caries, S. mutans, Stimulation index (SI).*

Mutans streptococci are considered as the most important etiologic agent in dental caries(1,2). With intimate and prolonged contact with oral tissues, the organism evokes a variety of immunologic responses through local secretory and systemic humoral antibodies and also through local and systemic cell mediated immunity (CMI) (3-6). While a great deal has been studied in relation to humoral responses(6), the studies on CMI in dental caries are limited(3). In normal children, CMI to *S. mutans* is less detectable(3-5) bu if plaques are allowed to accumulate, a significant level of lymphocyte transformation and macrophage migration inhibition can be elicited(4). It has also been emphasized that T-lymphocytes from dental caries patients have greater potential to proliferate on stimulation with streptococcal antigen(7,8). Even in rhesus monkeys cellular immunity to *S. mutans* has been shown to play a protective role against dental caries(9).

These limited studies have been essentially originated from the developed world and virtually no study on the role of CMI in dental caries has been conducted in the Indian continent. This particularly becomes important when oral microflora may not be all the same or may be superadded with other organisms in different combinations, thereby disturbing the pattern of immune responses. In addition, no study seems to be insight where comparative cellular immune responses in low' and high' caries children

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are demonstrated emphatically. The present study was therefore, undertaken to study the cellular immune responses in low and high caries children in Indian context. The study also intended to ascertain whether the lymphoblast transformation test could be ideally used to monitor CMI responses in children with dental caries.

Material and Methods

Study Groups

A total of 171 children, aged between 10 to 15 years were included in the study. They belonged to three categories: (*i*) 86 low caries children with carious teeth less than 3; (*ii*) 31 high caries children with carious teeth more than 3; and (*iii*) 54 age matched controls with no known evidence of caries.

[^{3}H] Thymidine mediated LTT

Specific antigen: Streptococcus mutans (Strain NCTC 10449) serotype C was grown anaerobically on BHI for 48 h. The sonicated antigen was prepared by the method of Lehtonen *et al.* Briefly, the colonies were scrapped into PBS, (pH 7.4) containing 0.5% formaldehyde and kept overnight at 4°C. After three washings with PBS the cells were sonicated at 50w for 2 minutes. The sonicated suspension was ultracentrifuged and supernatant was collected. Protein estimation was done by the method of Lowry. Sonicated *S. mutans* antigen (stimulant) was used at the optimal concentration of 10 µg/ml (standardized in a separate experiment) and PHA (mitogen) (Difco), was used at the optimal concentration of 5 µg/ml.

Lymphocyte preparation: Peripheral blood (5 ml) was collected from each of the children in heparinized tubes. The interphase layer of mononuclear cells was separated by density gradient centrifugation on lymphoprep^{Tm} (Nycomed, As, Oslo, Norway)(8). The cells were washed with RPMI-1640 (Flow Laboratories), supplemented with L-glutamine (2 m-mole/ml), penicillin (100 U/ml) and streptomycin (100 µg/ml). Viable lymphocyte population in the suspension was always confirmed by light microscopy.

In-vitro lymphocyte culture and [^{3}H] thymidine uptake: Separated lymphocytes were washed three times with RPMI-1640 for 10 minutes by centrifugation at 1000 rpm in cold. Washed lymphocytes were adjusted to a concentration of 1×10^6 cells/ml in RPMI-1640 containing 20% autologous human plasma. Lymphocytes (100 µl cells/well) were suspended in flat bottom microtiter plate (Linbro, Flow Laboratories, UK). Fifty µl of each of PHA (5 µg/ml) and *S. mutans* antigen (10 µg/ml) were put in triplicate with the cells. Control wells had only cells without any stimulant. The plate was incubated at 37°C with 10% CO_2, for 48 h, in case of PHA and 4 days in case of *S. mutans* antigen.

[^{3}H] thymidine of specific activity 6.0 ci/m mol and concentration of 5.0 mci/ml in 2% aqueous ethanol was obtained from Sigma (St Louis, USA). Thymidine was diluted to 9.5 uci/50 µl of RPMI 1640, and was added to the cultures and incubated for 16-18 h in same conditions as above for 16-18 h. Cells were harvested on automated harvester and counted as counts per minutes in beta scintillation counter (LKB, Sweden).

Interpretation of the result: In order to evaluate the results the mean count of the antigen stimulated culture was divided by that of the control culture; the value thus obtained was recorded as the stimulation index (SI). The stimulation index of PHA stimulated culture was also recorded similarly.

Statistical analysis: The significance of the results between low caries, high caries

and controls were analysed by analysis of variance. Multiple range test was done to identify the significant pairs of groups.

Results

The results of analysis of variance and the multiple range test showed that mean SI in low caries children was significantly higher than that of high caries; although 3 high caries children had high SI and 12 low caries children had low SI as shown in *Fig. 1*. There was less significant difference in the SI values if high and low caries were combined and then the results compared with controls. SI of 2 was taken as insignificant stimulation throughout the study.

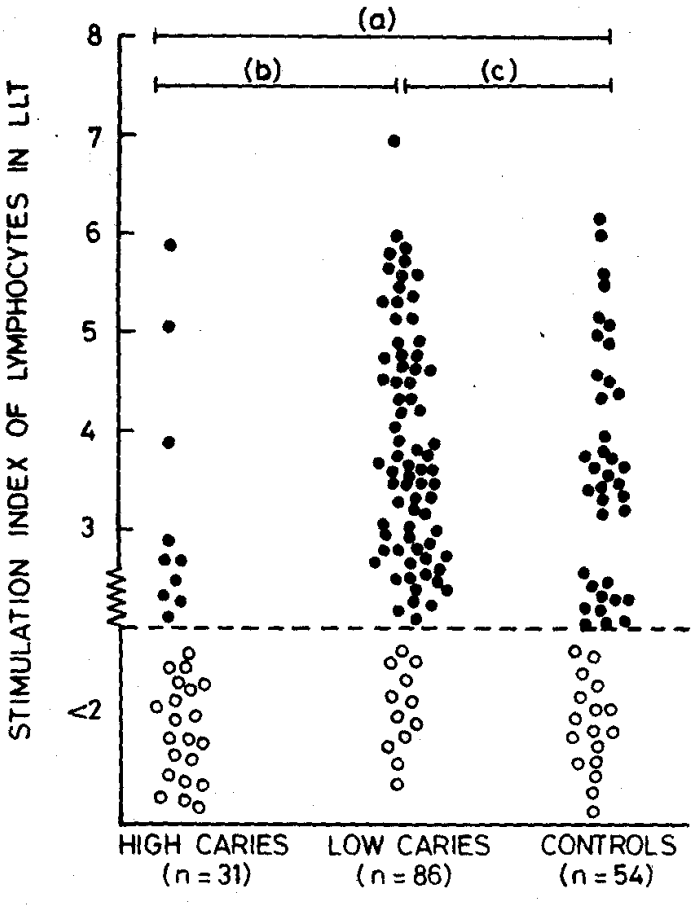

Fig. 1. Stimulation indices in lymphoblast transformation test (LTT) (a) High caries vs *Controls, (b) High caries* vs *Low caries, (c) Low caries* vs *Controls.*

High Caries vs *Controls*

Observation of high caries and controls revealed that stimulation index was lowered in high caries as compared to controls; out of 31 high caries individuals, 21 (79%) showed SI of 2, while out of 54 controls, only 17 (31%) showed SI 2 (*Fig 1a*). In case of controls, 37 children showed a good CMI response (ranging between 3 to 6) as shown in *Fig. 1.* Only 3 patients among the high caries group revealed high stimulation of 4, 5 and 6, respectively and 7 patients showed low stimulation response (SI 3) but not the insignificant stimulation index. It was, however, significant to note that only 3 high caries patients had very high stimulation index similar to many low caries and controls *Fig. 1.* This figure would have been further on higher side, had the total number of samples of low and high caries the same. Analysis of variance and multiple range test also showed statistically significant correlation between high caries and controls.

Low Caries vs Controls

Low caries susceptible showed high or very high stimulation index indicating a strong cell mediated immune response (*Fig. 1*). Only 12 children (14%) had low stimulation of 2 and rest 74 (86%) showed high stimulation index ranging between 3 to 7. However, the stimulation of lymphocytes in case of 4 controls was more or less similar to low caries in the SI range of 5 and 6 (*Fig. 1*) which could be attributed to the fact that some of the control subjects might have been recently infected but did not show any clinical symptoms of caries.

Low Caries vs High Caries

High caries children showed poor immune response as only three children expressed high stimulation index while 28 children showed very low stimulation index

(*Fig. 1*). On the contrary, in low caries children the stimulation was low only in 12 cases as compared to 74 children which showed significantly high immune response (*Fig. 1*). PHA stimulated lymphocytes cultures of caries and controls showed very high stimulation throughout the study as expected.

Discussion

The fact that T-lymphocytes from dental caries patients have greater potential to proliferate on stimulation with streptococcal antigen(8) formed the basis of the present study. While a great emphasis has been laid on the role of humoral immunity, there are only sporadic reports on the involvement of cell-mediated immune responses in dental caries excepting a few recent work on immunization with macromolecular components(6,7,10,11) in protective immunity where CMI has been used as a parameter. Unlike previous studies(3-7), the present study emphasized more on comparative cellular immune responses in high caries *vs* low caries children rather than simply comparing the CMI responses with controls. As evident from the results, high caries children had low stimulation and low caries children had high stimulation index; indicating thereby that the recent infection or low caries act as stimulatory factor leading to significant immune proliferation. Lower immune response in case of high caries as seen in our study, has also been reported previously(5). This could be attributed either to the pre-existing immune deficiency leading to the development of severe caries or due to prolonged antigenic stimulation by co-existing microflora of the oral cavity that may have possibly resulted in the inhibition of immune response(13,14). Ivanyi and Lehner(13) showed that in patients with severe periodontitis, lymphocyte transformation was significantly depressed as compared to mild or moderate disease, as also supported from experimental studies on rhesus monkeys(9). It was paradoxical, however, that the mean stimulation index of low and high caries when clubbed together, was almost similar to controls. It was therefore, important to attach significance to low and high caries while undertaking such studies. It is possible that some low caries patients may have partial resistance to infection. An immunological basis for this has been suggested by the findings that relatively caries-free' subjects have increased serum antibody titer and their T-lymphocytes proliferate quickly on stimulation(9). This observation, has been supported by the studies of Lehner *et al.*(8) in which helper factor activity was released by just 1-10 μg of streptococcal antigen I/II from lymphocytes of *caries resistant* subjects, compared with 1000 μg required to release similar helper factor activity from lymphocytes of *caries prone* subjects. Such low dose helper activity of lymphocytes are shown to be associated predominantly with HLA-DR W6, 1, 2, 3, cross reactive groups, whereas the high dose activity are shown to be associated with HLA-DR4-related gene product(8).

The findings of the present study, though based on limited number of samples, may be useful in establishing immunological basis of differentiating low caries children with those of high caries, and probably in predicting as to whether an initial caries will advance to low or high caries in course of time. More elaborate studies to identify major immunodominant subunit antigens of *S. mutans* will open new vistas for improvized diagnostics and immunization studies in caries at cellular and subcellular levels.

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