Received: 7/9/2005 **Accepted: 10/24/2005**

Short Communication

Immunohistochemical Assessment of Natural Killer cells in normal and inflamed Dental Pulps

SB. Mousavi DMD, MSD, A. Talebi A TCP**, S. Kianoosh DDS, MSc*

ABSTRACT

Introduction: Immunohistological studies have shown the pulpal responses to bacteria and their product's inflammation, but presence of natural killer (NK) cells were unclear. This study detected NK cells in normal and inflamed pulps.

Methods: From the third molars, 15 normal and 15 inflamed pulps were extirpated out and studied by indirect immune peroxides technique and microscope for NK cells.

Results: We did not find NK cells in normal coronal pulps, but they were detected in one third of inflamed coronal pulps.

Conclusion: NK cells are absent in normal coronal pulps and present in the intensively inflamed pulps. They may play a role in the pathogenesis of pulp lesion.

Key Words: Immunohistological, Inflamed pulp, Natural killer cells, Antibody, Inflammation

JRMS 2006; 11(2): 119-121

Infection stimulates inflammatory processes in dental pulp. Normal pulp is an immunocompetent tissue with responsibility to noxious stimuli including bacteria and their products in dental caries, trauma and operative dental procedures [1]. Inflammatory cells and their products especially cytokines, are significantly higher in symptomatic inflamed pulps than in normal pulps [2-7]. Ransuchberyer and Mousavi have shown IL-2 concentration in inflamed pulps nearly three times higher than in normal pulps [3, 8].

Early pulpal responses to bacteria or to diffusion of bacterial products include the influx of polymorphonuclear leukocytes and monocytes. As infection progresses, cellular infiltration becomes more intense with T-helper, T-cytotoxic cells /suppressors, B cells, plasma cells and immunoglobulins[1-5], and non-specific components including polymorphoneuclear cells (PMNs), monocytes, complement components and NK cells appear [1,6,7]. We identified and quantified NK cells in normal and inflamed pulps [9].

Materials and Methods

In an experimental study in Isfahan dental school, thirty coronal pulp samples were extirpated from third extracted molars. Each tooth was examined, using cold test, hot gutta-percha test, electric pulp test on sound enamel, and the percussion test. Normal groups (n=15) had no caries or response to vitality test within normal limits and nor clinical signs and symptoms. Inflamed group (n=15) were abnormally sensitive to vitality tests. None of both groups had periapical lesion in radiography.

Preparation of pulpal specimens

Extracted teeth were immediately placed into dry ice -76˚C, transported to Torabinejad's laboratory at Isfahan dental school, and grooved longitudinally along the roots and coronal surface

* Associate Professor, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran
** Assistant professor, Department of Pathology, Isfahan University of Medical Sciences, Isfahan, Iran
Correspondence to: Dr. Sayed Behrouz Mousavi, Email: sbmousavi2@yahoo.com

by a carbide # 170 fissure bur, using high speed with air water coolant. The grooves were tracked open by an elevator; coronal pulps were removed and immediately transferred to dry ice at -76° C at AI-Zahra pathology clinic, Isfahan. Pulpal samples were embedded in tissue-tek II, OCT compound, immediately immersed in liquid nitrogen, labeled and stored at -70`c until cryosectioning, and sectioned 4 μm in thickness using cryostat. Three sections per slide were picked up with Elmer's glue pretreated slides, and after 2 hours air-drying were fixed in cold acetone (99%) for 5 seconds and stored at -20' C.

H&E staining

Eight slides (each containing 3 sections of pulpal tissue) were randomly selected and stained with H&E, using the same technique for frozen and formalin fixed section, but less time needed for the first.

IHC staining

Eight slides(each containing 3 sections of pulpal tissue) were randomly selected and stained, using indirect immune peroxides procedures as described in the Dako company USA catalogue (2003), which utilizes primary mouse mono-clonal antibodies (Anti-Natural killer cells=CD_{56} clone: T_{199}) with sequential incubation of two peroxidase-Labled secondary antisera. After 20 minutes incubation with primary monoclonal antisera and with peroxidase-conjugated secondary antisera, the sections were washed and incubated with substrate chromogen (Diaminobenzidine tablets) for 10 minutes, washed in distilled water and counterstained with Maier's hematoxylin for 5 minutes after being decolorized via 10 dips in ammonium water(0.2%). The slides were cover slipped using liquid glycerol gelatin as a mounting medium. Positive controls (4 slides) were stained with monoclonal anti-body (Anti-CD56) at the same time as the pulpal tissue sections. Negative control (4 slides) were stained with the substitution of Tries buffer (0.05 M, pH 7.6 ± 0.2) [2,10]. In normal coronal pulp (15 teeth), 1041 sections as 240 slides (each slide containing 3 pulpal sections) were stained with IHC and H&E technique (120 for each one). In inflamed coronal pulp(15 teeth), 655 sections as 219 slides(each slide containing 3 pulpal specimens) were stained with IHC and H&E technique(113 for IHC and 106 for H&E). The data were analyzed by T-test.

Results

Light microscopy showed no NK cells in normal coronal pulp. NK cells were observed in 37 sections (of five teeth) of inflamed coronal pulp (Figures 1, 2). Almost in all of these sections, inflammatory reactions were intense, and NK cells had focal pattern. NK cell/surface ratio (cellular density) in all carious teeth was 42.11 ± 17.19, and in five carious teeth was 117.05 ± 107.31(P-value<0.0 1).

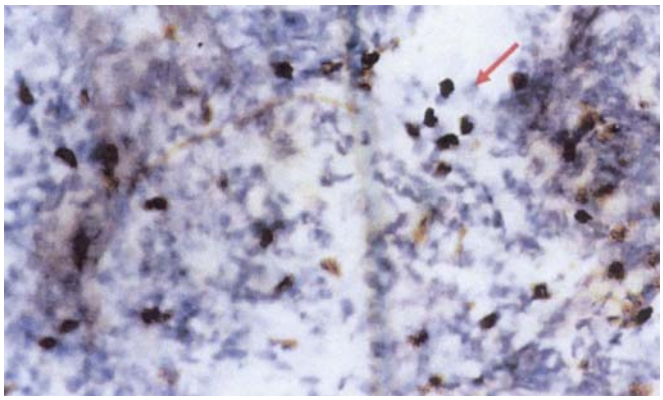

Figure 1. NK cells in IHC staining of inflamed coronal pulp (arrow) (400)

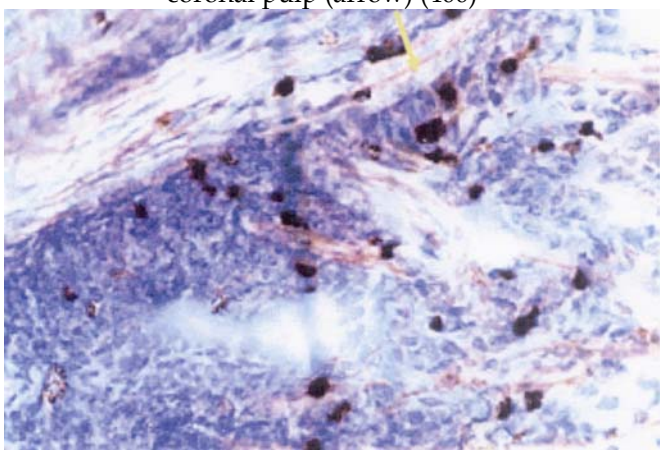

Figure 2. H&E and IHC staining of inflamed pulp, intensive inflammatory reactions, and NK cells (arrow) (400)

Discussion

Microscopic study showed no NK cells in normal coronal pulps but they were present in the intensively inflamed pulps. Some investigators have shown a correlation between viral infections specially Herpes-zoster and spontaneous pulpal degeneration and pulpal necrosis [11-13]. Also, HIV and hepatitis B virus have been observed in patients' dental pulps [14, 15]. Thus, the presence of NK cells in inflamed pulps may indicate the role of viruses and intracellular bacteria in the pathogenesis of pulpal lesions.

This study shows focal pattern of NK cells distribution in the inflamed coronal pulp due to one of the following probable reasons:

Firstly; different concentrations of NK cells chemotatic factors (like IL-2, IL-15, and IL-18) may be present in different zones of the inflamed pulps.

Secondly; there might be different concentration of the pulps of the pathogens in various regions of the example around the vessels of nerves.

Further investigations need to be carried out using functional antibodies on the formalin-fixed specimen, using decalcification method for pulpal preparation for coronal and radicular pulps examination at the same time. Further studies would be necessary to clarify the role of NK cells, viruses and intracellular bacteria in the immunopathology of dental pulp.

References

1. Orstavik D, Pitt Ford TR. Essential Endodontology: Prevention and treatment of apical periodontitin. First Edition, Blackwell science 1998: 42-67.

2. Hahn CL, Falker W A JR, Siegel MA. A study of T cells and B cells in pulpal pathosis. J Endodon 1989: 15-20.

3. Rauschenberger CR, Bailey JC, Cootauco Cj. Detection of human IL-2 in normal and inflamed dental pulps. J of Endodon 1997; 23: 366-70.

4. Honjo H, Tsubakimoto K, Utsumi N, Tsutsui M. Localization of plasma protein in the human dental pulp. J Dent Res 1970; 49: 888.

5. Pulver WH, Taubman MA, Smith OJ. Immune components in normal and inflamed dental pulp of human. Arch Oral Biol 1977; 22: 103-11.

6. Pekovic DD, Finery ED. Identification of bacteria in Immunopathologic mechanisms of human dental pulp. Oral Surg 1984; 57: 625-61.

7. Speer ML, Madonia N, Heuer MA. Quantitative evaluation of the immunocompetence of dental pulp. J Endodon 1977; 3: 418-23.

8. S.B Mousavi, A. Rezaie, R. ShekarAmiz. A Comparison of IL2 in normal and systematic pulps. Medical J of Iran 2003; 17(2): 137-140.

9. Walton R, Torabinejad M. Principles and practice of endodontics. 2nd edition Saunders 1991 P: 58.

10. Falini B, Taylor CR. New developments in Immunoperoxidase Techniques and their applications. Arch pathol Med 1983; 107: 105-17.

11. Goon WWY, Jacobsen PL. Prodromal odontalgia and Multiple devitalized teeth caused by a herpes zoster infection of the trigeminal nerve, report of a case. J Am Dent Assoc 1988; 116: 500.

12. Gregory WB, Brooks LE, Penick EC. Herpes zoster associated with pulpless teeth. J Endodon 1975; 1: 32.

13. Lopes MA, de Souza Fj, Filbo JJJR, de Almeida Op. Herpes zoster infection as a diffemtial diagnosis of acute pulpitis. J Endodon 1998; 24: 143.

14. Glick M, Trope M, Pliskin ME. Detection of HIV in dental pulp of a patient with AIDS. J Am Dent Assoc 1989; 119(5): 649-50.

15. Levy lA. Human Immunodeficiency virus and the pathogenesis of AIDS. JAMA 1989: 261: 2997.