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Atypical lymphocytes and cellular cannibalism in chronic periapical lesions: A first insight with possible implications

Abhishek Singh Nayyar, Ketki P. Kalele, Kaustubh P. Patil

ABSTRACT

Aims: Atypical lymphocyte refers to an unusual structure of lymphocytes that are a part of the cell mediated immune system of the body. Also referred to as reactive lymphocytes, atypical lymphocytes have larger than normal size, attributed to antigen stimulation. Cellular Cannibalism is defined as a large cell enclosing a slightly smaller one within its cytoplasm. Previously, this feature was noted only in cases of malignant tumors. The objectives of this study were to determine the proportion of atypical lymphocytes in chronic periapical lesions; to determine the proportionate cellular cannibalism in these lesions; and to correlate the proportion of the atypical cells and cannibalistic cells with the clinical behavior of the lesions. Methods and Material: Hematoxylin and eosin stained 30 slides of chronic periapical granulomas and 20 slides of cysts reported in the year 2014–15 and the clinical proformas of the patients were retrieved. These slides were evaluated by three experts to determine the presence of atypical lymphocytes and cellular cannibalism under high power magnification (400X). Results: Out of the 30 slides of chronic periapical granulomas, about 12 slides (40%) revealed presence of atypical lymphocytes. Four out of the 20 slides (20%) of chronic periapical cysts examined histopathologically showed presence of atypical lymphocytes. Cannibalistic cells were present in 12 out of the 30 slides of chronic periapical granulomas (40%). None of the cysts, however, revealed cannibalistic cells (0%). Conclusions: In the present study, an observation on the unique cellular composition in histopathological sections of chronic periapical lesions has been highlighted. The question arises that whether presence of such atypical cells in these lesions denotes an aggressive clinical behavior as seen in malignant tumors and should be given a due consideration in deciding the treatment protocols in such patients.

Keywords: Atypical lymphocytes, Cellular cannibalism, Chronic periapical lesions

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INTRODUCTION

Lymphocytes are often termed to be isomorphic, having a monotonous light microscopic appearance. Morphological aspects of lymphocytes in tissue sections thereby are not routinely taken notice of as their morphology seems to vary only in case of certain lymphoid and hematological malignancies [1] apart from certain viral infections [2]. However, in chronic inflammatory conditions including dental periapical infections, there is an antigenic stimulus which leads to proliferation of lymphocytes [3]. Atypical lymphocyte refers to an unusual structure of lymphocytes that are a part of the cell mediated immune system of the body. Also, referred to as reactive lymphocytes, atypical lymphocytes have larger than normal size sometimes with a diameter of more than 30 microns. The large size of the lymphocytes is attributed to antigen stimulation. The nucleus of a reactive lymphocyte can be round, elliptical, indented, cleft or folded. Cytoplasm is often abundant and can be basophilic with vacuoles [4]. Atypical lymphocytes vary in morphologic details as well as surface marker characteristics showing that they comprise a heterogeneous mixture of cell types. This is seen as a result of a polyclonal immune response to varied antigenic stimuli [5]. These lymphocytes get easily identified by their abnormally increased sizes and presence of active DNA synthesis and with a high glycogen content [6] due to a similar reason giving them a characteristic staining property [1, 2, 7, 8]. They may actually look like a cross between a plasma cell and a lymphocyte and so called a lymphocytoid plasma cell or, a plasmacytoid lymphocyte [9]. Such atypical lymphocytes have been observed in cases of chronic periapical lesions. In these cases, at many places in the histopathological sections of the periapical lesions, there have been evidences of cells larger in size than normal lymphocytes and plasma cells and having atypical appearances with two exactly similar nuclei in a single cell as well as certain cells mimicking a typical "Owl-eye" appearance. These cells routinely raise suspicion regarding the nature of the pathosis. The blood investigations of these patients, however, in our study revealed absolutely normal counts. Alongwith these, the other rare feature which is recently coming under light is "Cellular Cannibalism" which is defined as a large cell enclosing a slightly smaller one within its cytoplasm. Previously, this feature was noted only in cases of malignant tumors [10]. Recently, Sarode et al. has described this phenomenon in oral squamous cell carcinoma [10, 11] as well as in benign lesions such as central and peripheral giant cell granulomas [12]. Cannibalism is said to influence biologic behavior in these lesions [10–12].

AIMS AND OBJECTIVES

The objectives of this study were to determine the proportion of atypical lymphocytes and characteristic cellular cannibals in chronic periapical granulomas and cysts and to correlate the proportion of the atypical cells and cannibalistic cells with clinical behavior of these lesions.

MATERIALS AND METHODS

This was a descriptive, observational study conducted in the Department of Oral Medicine and Radiology and Oral Pathology and Microbiology. Hematoxylin and eosin stained 30 slides of chronic periapical granulomas and 20 slides of cysts reported in the year 2014–15 and the clinical proformas of the patients were retrieved from the files of the Department of Oral Medicine and Radiology and Oral Pathology and Microbiology. These slides were evaluated by three experts from the specialization of Oral Pathology and Microbiology to determine the presence of atypical lymphocytes and cellular cannibalism under high power magnification (400X). Only those cases (slides) showing marked plasma lymphocytic infiltrates and clear-cut cannibalistic cells were chosen for evaluation. Quantification of atypical lymphocyte was done by selecting four hot spots showing maximum density of plasma lymphocytic infiltrates. Quantification of cannibalistic cells, however, was done by thorough screening of the histopathological sections.

RESULTS

Out of the 30 slides of chronic periapical granulomas, about 12 slides (40%) revealed presence of atypical lymphocytes (Figure 1). Out of these 12 slides, 7 slides showed marked populations of atypical lymphocytes i.e., atypical cells >5 per high power field (HPF) and the remaining showed a relatively less number of atypical cells i.e., about 2 per 10 HPF. In case of slides of chronic periapical cysts, however, only 4 out of the 20 slides (20%) examined histopathologically showed presence of atypical lymphocytes. Also, number of atypical lymphocytes in cysts did not exceed 3–4 per 10 HPF. Again, notably, only those tissue sections which contained marked plasma cell infiltrations showed this kind of cell population. An interesting feature of cellular cannibalism was noted in tissues with atypical cells (Figure 2). Cannibalistic cells were present in 12 out of the 30 slides of chronic periapical granulomas (40%). None of the cysts, however, revealed cannibalistic cells (0%). Also, the total number of cannibalistic cells was found to be directly proportional to the number of atypical lymphocytes and the amount of plasma cell infiltration (Figure 3).

DISCUSSION

The present study was an observational study conducted in the Department of Oral Medicine and Radiology and Oral Pathology and Microbiology with objectives of this study being to determine the proportion of atypical lymphocytes in chronic periapical granulomas and cysts; to determine the proportionate cellular cannibalism in these periapical lesions; and to correlate the proportion of the atypical cells and cannibalistic cells with the clinical behavior of these lesions. It was observed that out of the 30 slides of chronic periapical granulomas examined, those 12 slides in which atypical cells and cellular cannibalism was evident, the size of the granuloma was found to be relatively larger as was evident radiographically which they had acquired in a short span of time (less than a week) as observed on going through their case histories while in the rest of the cases, the size of the pathosis was comparatively smaller and did not show much change in the dimensions from the time it had occurred, thus, indicating aggressive behavior of the lesions having atypical cells and cellular cannibalism. Plump fibroblasts as well as budding capillaries were also observed in these sections confirming their biologically aggressive behavior. No such correlation was seen in cases of periapical cysts. The reason for the presence of such atypical cells in the tissue sections could be understood by a deeper understanding of the pathogenesis of these chronic periapical pathoses. Immunological reactions which are provoked by bacterial antigens from the necrotic pulp of the teeth are the basis for the pathogenesis of these lesions. Both humoral and cell-mediated immune responses are said to play a role in the formation of these lesions [13].

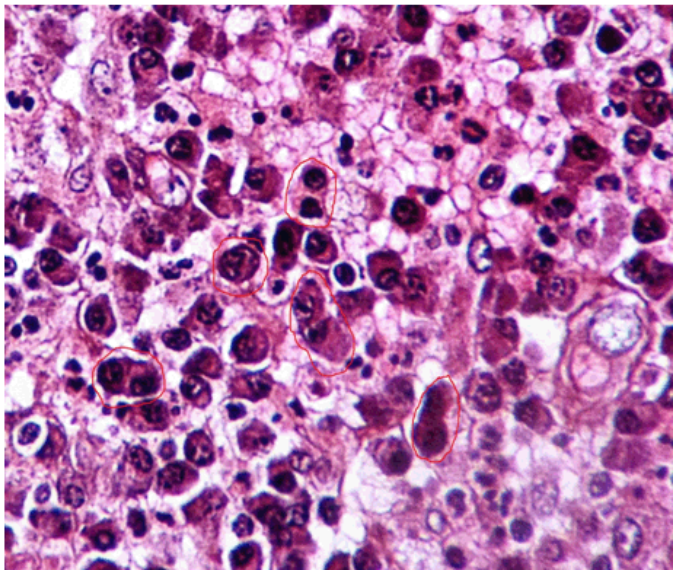

Figure 1: Revealing atypical bilobed cells with two nuclei and nucleoli closely mimicking a typical ‘‘Owl eye” appearance in mixed inflammatory cell infiltrates.

It has been hypothesized that antigen-activated B cells differentiate into centroblasts (rapidly proliferating large cells) which then differentiate into centrocytes (non-dividing, smaller, notched cell). Antigen-selected centrocytes eventually differentiate into memory B cells or plasma cells. The antigens that cause B cell activation and differentiation are defined as T cell independent (TI), and T cell dependent (TD). TI antigens, which are able to elicit responses, include bacterial cell wall components such as polysaccharides. These cells are specifically responsive to bacteria such as those of Hemophilus family of the beta subgroup, Streptococcus family and Neisseria meningitides [14, 15]. These TI challenges from the antigenic stimulus elicit long-lived antibody responses

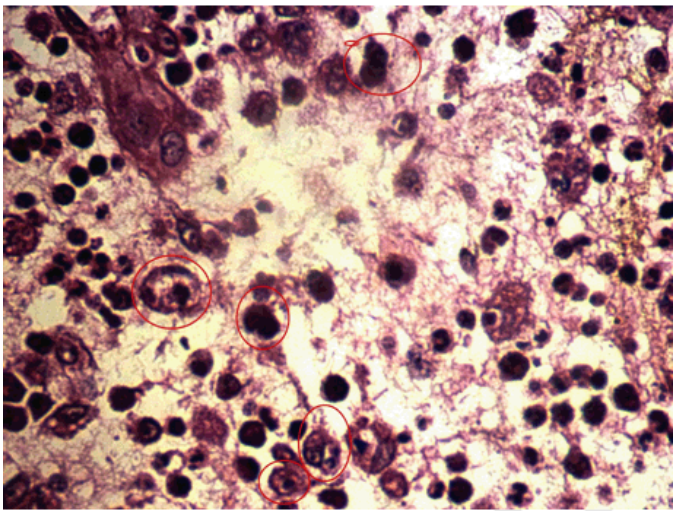

Figure 2: Characteristic cellular cannibalism in a background of intense lymphoplasmacytic reaction.

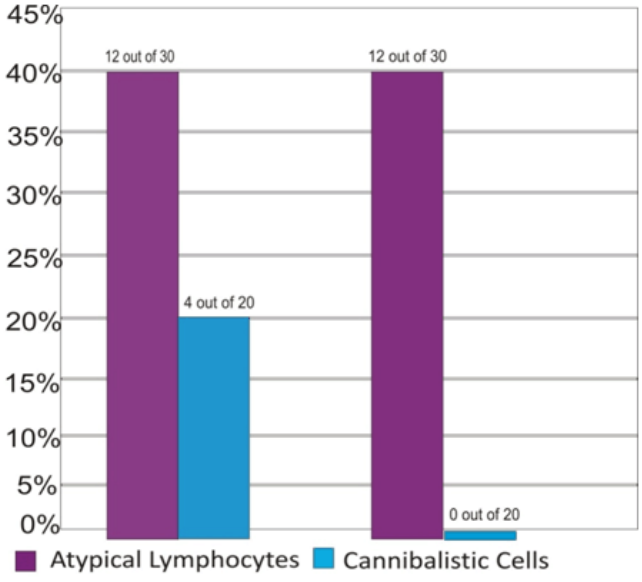

Figure 3: The relative percentages and numbers of atypical lymphocytes and cannibalistic cells observed in chronic periapical granuloma (first bar) and cysts (second bar) studied.

that continually create plasmablasts which become visible in tissue sections and are responsible for the formation of natural antibodies against the pathogen thereby leading to clearance of the pathogen. This role is in addition to the early response described by Martin et al. for B cells, where rapid proliferation and differentiation creates a 'wave' of plasmablasts in the first few days following infection. As also, most of the commensal bacteria from the mucosa are able to elicit these reactions. This is also confirmed from the studies which state that there is a seeding of these proliferating B cells and Plasma cells from the germinal centers in bone marrow [14].

The above mentioned findings were also confirmed in the study done by Yang et al. that bacterial lipopolysaccharide, which basically is a phosphatidylcholine compound, is highly antigenic and that this antigen is known to stimulate the migration of the B lymphocytes from different sites with such cells being recognized as immigrant cells. This antigenic substance causes proliferation of native as well as immigrant B cells and their differentiation into antibody producing plasma cells. It is proven in this study too that when antigen provoked immigrant lymphocytes differentiate into antibody producing plasma cells, they divide at least once into two identical daughter cells that is by the process of mitosis [15]. Such incompletely dividing plasma cells were seen in the tissue sections of our slides of periapical lesions which suggests strong antigenic stimulus. This also explains the strong association of atypical cells and predominance of plasma cells in tissue sections. Thus, from above discussion, it is known that lymphocytes have to pass through various functional milestones after antigenic stimulus, thereby providing an array of morphologies through various stages. Thereby, it can be concluded that these lymphocytes can appear unfamiliar and atypical in tissue sections due to their incomplete division or larger size during proliferation as mentioned above. These features are typically pronounced when there is a strong and complex bacterial antigenic stimulus. Another feature associated with the presence of these cells was the correlation of on an average, larger size, of the chronic periapical granulomas. Thus, the question arises that whether presence of such cells from the tissue sections denotes an aggressive tendency and should be treated with an altogether different line of treatment. Bacteria associated with such kind of antigenic stimulation should be isolated and cultured and put to antibiotic susceptibility testing so that combination of antibiotics could be administered to the patients, accordingly. The ubiquity of atypical lymphocytes suggests their important role in the mediation of immune responses [5]. The heterogeneity of these cells actually suggests that they are reactive in nature with varied morphologies.

The studies conducted also confirm that these cells are actually proliferating [8] with variable and varied antigenic stimuli leading to polyclonal proliferative lymphoid immune responses [5, 16]. Also, another feature noted was the presence of cellular cannibalism in the sections of periapical lesions. One of the major reasons which was put forth by Sarode et al. for the presence of cannibalistic cells was the lack of nutritional supply in malignant tumors and thereby for their survival, tumor cells were assumed to engulf other tumor cells and the leucocytes as well [10, 11]. The existence of unique cell-in-cell structures in clinical cancer specimens, in which lymphocytes penetrated both malignant and normal epithelial cells, has been reported [17]. Cell-in-cell structures have been known to exist for over 100 years and have recently aroused great interest due to its potential pathophysiological significance [18, 19]. Cell-in-cell structures can occur either homotypically or heterotypically. Heterotypic cell-in-cell structures, formed by the invasion of lymphocytes into tumor cells of epithelial origin, were found to occur more prevalently than homotypic structures both in vitro and in vivo [17]. The fates of the invading cells and host cells in cell-in-cell structures are diverse [20–22]. Most often the invading lymphocytes die by entosis or emperitosis [23]. Entosis is a lysosome-dependent cell death and involves the activation of the Rho/Rock signal pathway [24]. Emperitosis is a caspase 3-dependent apoptotic process [19]. Only immune cells with cytotoxicity undergo emperitosis. Non-cytotoxic cells, like B cells, undergo entosis. With the prevalence of heterotypic cell-in-cell structure formation between virus-loaded immune cells and tissue cells during chronic infection, in-cell infection of non-susceptible epithelial cells with broader potency and higher efficiency might be more inclined to form the viral latency [17].

The cell-in-cell pathway not only mediates the infection of epithelial cells but also provides virus-bearing tropic cells inside epithelial cells with a shelter to escape from immune attack. A few reports have described the presence of virions in oropharyngeal or nasopharyngeal epithelium [25, 26] and the phenomena of latent or replicating EBV in intro-epithelial B cells [27]. In addition, heterotypic cell-in-cell structure formation between B cells and epithelial cells might also induce the chromosome instability and formation of aneuploidy, which has been reported during the formation of homotypic cell-in-cell structures [28]. However, the exact mechanisms are unclear. These hypotheses can even be applied to chronic periapical granulomas, as it is known that during growth of these lesions, there is a reduction in the vascularity, eventually, leading to the death of centrally placed cells, a process known as avascular necrosis. Thus, cellular cannibalism might probably be the natural survival attempt by these cells. Since cyst did not suffer from such lack of blood supply, cannibalism was not seen in the sections from cystic lesions. Studies have denoted that cellular cannibalism generally denotes aggressive tendency of both benign and malignant tumors, however, no such study till date has been carried-out on periapical pathologies and to the best of our knowledge,

this has been the first of the studies to report such kind of features in periapical pathologies in the English medical literature. In recent years, the cell-in-cell structure has aroused broad concerns as increasing evidence has supported its roles in development [29, 30], homeostasis [31], aging [32, 33], malignant transformation [34] and tumorigenesis [28] across a wide range of species, exhibiting pathophysiological significance.

More recently, the cell-in-cell structure formation is demonstrated to be able to promote clonal selection and human tumor evolution mediated by cell competition, a mechanism evolved to eliminate less fit cells from a heterogeneous cell population [35, 36]. Further investigations on its molecular mechanisms will undoubtedly provide new clues and targets for the prevention and treatment of viral and other varied infections as well as in carcinogenesis. The chronic periapical granulomas thus reflect a complex interaction of both the immunologic and non-immunologic mechanisms and are the most common pathologies routinely encountered [37]. Thus, further studies are required in this direction so that these histopathological features can direct us regarding the biological behavior of these lesions and thereby lead to proper treatment protocols.

CONCLUSION

Chronic periapical pathologies are the most routinely encountered lesions in the dental practice. However, not much importance is given on their diagnostic and prognostic aspects. In the present study, we have quoted our observations on the unique cellular composition that was seen in histopathological sections which were then correlated with the clinical and radiological parameters of these lesions. The question arises whether presence of atypical cells from the tissue sections denotes an aggressive clinical behavior as seen in malignant tumors and should be given a due consideration in deciding the treatment protocols for such cases to provide an optimum patient care.

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Author Contributions

Abhishek Singh Nayyar – Substantial contributions to conception and design, Acquisition of data, Analysis and interpretation of data, Drafting the article, Revising it critically for important intellectual content, Final approval of the version to be published
Ketki P. Kalele – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published
Kaustubh P. Patil – Analysis and interpretation of data, Revising it critically for important intellectual content, Final approval of the version to be published

Guarantor

The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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