

# Quantitative analysis of cellular composition of human periapical granuloma

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The cellular composition of 33 solid and cystic periapical granulomas was quantitated by a differential morphometric technique. Macrophages were the predominant inflammatory cell, followed in descending numerical order by lymphocytes, plasma cells, and neutrophils. Inflammatory cells comprised slightly more than half the formed elements; the others were connective tissue elements. There were no significant qualitative or quantitative differences in the inflammatory components of lesions differing in morphology (solid vs cystic), symptomatology (pain vs no pain), duration (less than one year vs more than one year), and treatment (endodontic intervention vs no intervention). The human periapical granuloma thus reflects a complex of immunologic and nonimmunologic inflammatory reactions.

In humans, the periapical granuloma is a focal accumulation of inflammatory cells and reactive elements attached to a tooth root. The structural components are macrophages, lymphocytes, plasma cells, neutrophils, and fibrovascular elements delimited by an outer fibrous pseudocapsule. Periapical granulomas are invariably preceded by pulpal inflammation. Clinically, the lesions are usually painless, chronic, and expansile.

The specific etiologic factors involved in the induction, maintenance, and resolution of periapical granulomas have not been delineated completely. Presumably, the lesion develops in response to an undefined irritant introduced into

the periapical tissues through the root canal. Causative agents may be of bacterial origin (structural proteins, cell wall fragments, metabolites), of host origin (caries-altered dental proteins, inflammation products), or both.

Despite decades of descriptive histopathologic studies, definitive data dealing with the numerical distribution of the inflammatory and reactive cellular elements in periapical granulomas are lacking. Differential quantitative analysis of the lesional components offers an objective means of determining the proportions of cells comprising the lesion. Quantitation of the granuloma cells provides numerical data that could be interpreted statistically and per-

mits comparisons between lesions from different patients. Such analyses also yield fundamental morphologic information, an important prerequisite to an understanding of the biologic nature of the granulomatous response.<sup>1</sup> Accordingly, the present study was undertaken to characterize and quantify the inflammatory and reactive fractions in human periapical granulomas.

## MATERIALS AND METHODS

The study material was comprised of 25 periapical granulomas and eight periapical cysts removed during apicoectomy. The average age of the patients was 37.2 years; the average duration of the lesions estimated

by history was 2.5 years. Fewer than one third had caused pain.

The specimens were fixed immediately in formol alcohol (1 ml 37% formaldehyde + 99 ml 95% ethanol) or prewashed in tissue culture medium RPMI-1640, supplemented with 1% fetal calf serum and gentamicin for 48 hours at 4 C before fixation. After fixation they were processed through graded alcohols and xylenes at 4 C and embedded in 56 C paraffin. Sections were cut at 3 to 5 μ and stained with hematoxylin and eosin.

Differential cell counts were made with a Weibel reticle according to the modified differential morphometric counting method for enumerating lymphocytes, plasma cells, neutrophils, macrophages, connective tissue (collagen and fibroblasts), epithelium, and vascular spaces.<sup>2</sup> Subgroup quantitative comparisons were made between painful and nonpainful lesions; lesions of up to one year's duration and more than one year's duration; lesions from patients with previous endodontic treatment and from patients without treatment; and cysts and granulomas. Student's *t*-test for significant differences was used to compare normalized data from each subgroup.

**RESULTS**

A total of 97,744 cells were counted in the 33 lesions. Macrophages were the predominant inflammatory cells, followed by lymphocytes, plasma cells, and neutrophils. Macrophages constituted 24%, lymphocytes 16%, plasma cells 7%, neutrophils 4%, fibroblasts 40%, vascular elements 6%, and epithelial cells 5% of the cell total. Inflammatory cells made up approximately 52% of the formed elements in the lesion

(Fig 1). When considered solely in terms of inflammatory cells (Fig 1), the percentage distribution was macrophages, 46.55% (range 17% to 69%); lymphocytes, 31.97% (range 12% to 62%); plasma cells, 12.94% (range 3% to 34%); and neutrophils, 8.11% (range 0.7% to 25%).

The most common numerical sequence, macrophage > lymphocyte > plasma cell > neutrophil (group 1), was found in 76% of the granulomas and 63% of the cysts (Table 1). Lymphocyte > macrophage > plasma cells > neutrophil (group 2) was the sequence in 8% of the granulomas and 25% of the cysts. Macrophage > plasma cells > lymphocyte > neutrophil (group 3) occurred in only 8% of granulomas and 13% of cysts. Only the differences in mean lymphocyte percentage among groups 1, 2, and 3 and in mean macrophage percentage between groups 1 and 2 were statistically significant (Tables 2, 3).

Seven of the 33 patients had pain before surgical treatment was instituted (Fig 2). The inflammatory cell composition in the periapical lesions from the pain group averaged 45% macrophages, 36% lymphocytes, 12% plasma cells, and 7% neutrophils. The means for the reactive cell components were 46% fibroblasts, 2% epithelial cells, and 7% vascular spaces. The corresponding figures for the 26 patients in the nonpain group

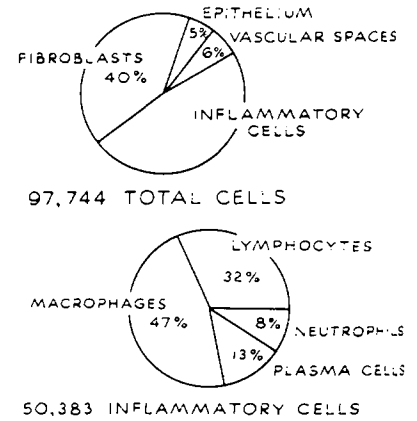


Fig 1—Top, analysis of selected reactive components of periapical biopsy specimens. Bottom, relative composition of inflammatory infiltrates from 33 human periapical lesions.

were 48% macrophages, 31% lymphocytes, 13% plasma cells, and 8% neutrophils. Reactive cell means were nearly identical to those of the pain group.

Lesion duration was documentable in 20 of the 33 patients (Fig 2). In eight patients with lesions of less than one year's duration, the means for the enumerated lesional components were 50% macrophages, 33% lymphocytes, 12% plasma cells, 5% neutrophils, 38% fibroblasts, 3% epithelial cells, and 6% vascular spaces. The respective means for the 12 lesions of more than one year's duration were 47%, 35%, 12%, 7%, 42%, 5%, and 6%.

As with the differentiation of lesions on the basis of duration, there

**Table 1 • Relative quantitative distribution of inflammatory cell patterns in human periapical biopsy specimens.**

Group	Pattern	Total cases	Cysts	Granulomas
1	M* > L > P > N	24/33 (73%)	5/8 (63%)	19/25 (76%)
2	L > M > P > N	4/33 (12%)	2/8 (25%)	2/25 (8%)
3	M > P > L > N	3/33 (9%)	1/8 (13%)	2/25 (8%)
4	P > M > L > N	1/33 (3%)	0/8 (0%)	1/25 (4%)
5	L > P > M > N	1/33 (3%)	0/8 (0%)	1/25 (4%)

\*M, Macrophage; L, lymphocyte; P, plasma cell; N, neutrophil.

**Table 2 • Differential analysis of cell distribution in subgroups based on inflammatory cell patterns.**

Group	Plasma cell	Lymphocyte	Macrophage	Neutrophil	N
Total	12.94 ± 1.31%	31.97 ± 2.09%	46.52 ± 2.06%	8.11 ± 1.22%	33
1	12.34 ± 1.09	30.30 ± 1.29	50.69 ± 1.85	7.55 ± 1.12	24
2	10.25 ± 4.92	49.00 ± 6.36	34.50 ± 2.50	6.20 ± 4.29	4
3	19.67 ± 3.18	17.66 ± 3.85	43.33 ± 3.76	20.30 ± 3.71	3
4	34%	23%	33%	10%	1
5	20%	62%	17%	1%	1

**Table 3 • Tests of significance using Student's t-test.**

Group Compared	t	df	P
Total vs 1			
PC*	0.328	55	> .05
L	0.611	55	> .05
M	1.436	55	> .05
N	0.320	55	> .05
1 vs 2			
PC	0.605	26	> .05
L†	4.408	26	< .001
M†	3.409	26	< .005
N	0.402	26	> .05
2 vs 3			
PC	1.261	05	> .05
L†	3.275	05	< .025
M	1.717	05	> .05
N	2.020	05	> .05

\*PC, plasma cell; L, leukocyte; M, macrophage; N, neutrophil.  
†Significant difference.

were no statistically meaningful quantitative differences in the various inflammatory and reactive cell elements between specimens from patients with and without endodontic treatment (Fig 3, 4). The periapical cysts contained nearly five times the number of epithelial cells found in the periapical granulomas (Fig 4). In all other cellular parameters the cysts and granulomas were quantitatively similar (Fig 4, 5).

**DISCUSSION**

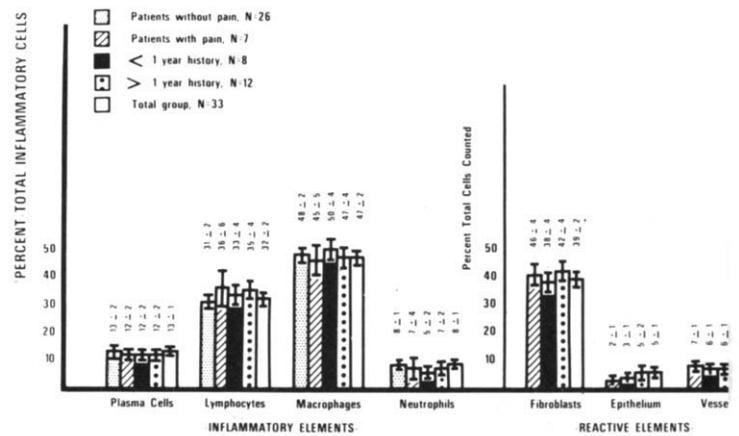
The modified morphometric method of cell quantitation was used to perform differential cell counts in 33 periapical granulomas and cysts. The results were combined to devel-

op a quantitative pattern of the inflammatory and reactive elements contained therein. Although ranges for some cell constituents exceeded 30%, there was considerable homogeneity in the relative cellular composition. Seventy-three percent of the lesions followed the pattern macrophages > lymphocytes > plasma cells > neutrophils. Despite wide variations in age of lesion, age of patient, and treatment received, the impressive degree of uniformity of cell content suggests a common reaction. Some of the variations in cell frequency probably stemmed from differences in the life span of the various inflammatory cells, differing intensities of stimuli that triggered

the cell responses, and the influence of one inflammatory cell on another.

There were no significant differences in the inflammatory cell composition of the periapical lesions in patients who had received endodontic treatment before surgery and those who had not. Endodontic treatment per se did not alter the quality or type of inflammatory response in the periapical tissues despite placement of cytotoxic chemicals in the root canals. Periapical granulomatous disease is thus not iatrogenic in primary causation.

There were no striking differences in inflammatory cell composition between the granulomas and cysts.



*Fig 2—Analysis of lesional components from selected clinical subpopulations.*

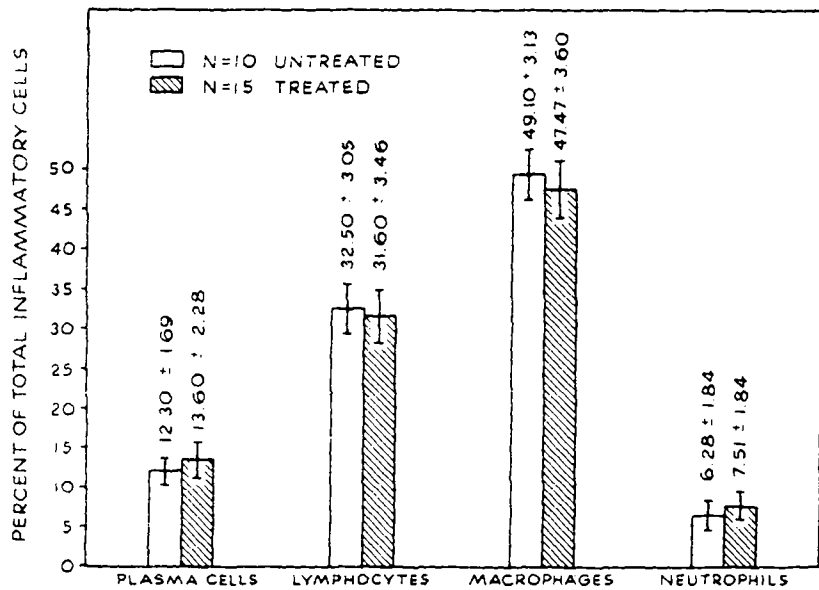


Fig 3—Inflammatory cell infiltrates in periapical biopsies: patients with and without prior endodontic treatment.

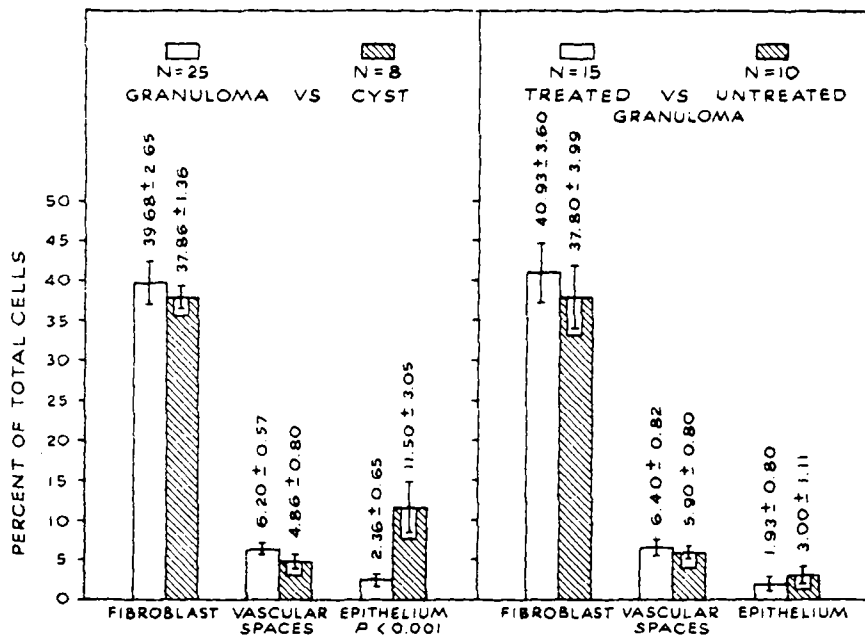


Fig 4—Quantitative analysis of selected tissue components in biopsy specimens from endodontically involved teeth.

The cell types and distribution were essentially the same when analyzed either as relative proportions of cells (subgroup analysis) or as overall cellular composition. The inflammatory response in granulomas and cysts seems to be identical despite the notable and significant increase in number of epithelial cells in the cystic lesions. The cause of differentiation into solid or cystic periapical lesions is not deducible from altera-

tions in the inflammatory cell populations.

The composition of human periapical granulomas where macrophages accounted for almost half and lymphocytes for one third of the inflammatory cells, is consistent with granulomatous inflammation. Macrophages and other mononuclear phagocytes (monocytes, giant cells, epithelioid cells) are the hallmarks of granulomatous inflammation, a spe-

cific form of chronic inflammation.<sup>3,4</sup> Compared with granulomatous inflammation, banal chronic inflammation lacks organization and is a diffuse, heterogeneous cell collection usually dominated by mononuclear cells other than macrophages.<sup>3,6,9</sup>

Chronicity is an integral feature of granulomatous inflammation; the average age of the periapical granulomas in this study was 2.5 years. The granuloma persists as long as the provocation exists.<sup>6,7</sup> The same etiologic agents that cause chronic inflammation can produce granulomas. These are frequently poorly soluble particulate nondegradable compounds or complexes that remain localized in tissue.<sup>7,10-12</sup> Some, like mycobacterial products, are good immunogens and induce delayed hypersensitivity and antibody formation in addition to granulomas.<sup>5,6</sup> Others, such as streptococcal cell wall fragments, produce granulomas with no measurable immune response.<sup>12</sup> Some are toxic to macrophages and hemolyze red blood cells<sup>13</sup>; others are nontoxic and seem to accumulate only in macrophage lysosomes without otherwise damaging the cell.<sup>10</sup> There is also a nonabsolute relationship between the ability of an irritant to activate macrophages and to cause granuloma formation. Thus, endotoxin, a potent *in vitro* activator of macrophages,<sup>7,14</sup> a phlogiston, and an immunogen,<sup>15</sup> does not precipitate granuloma formation.<sup>6,15</sup> Conversely, substances as streptococcal cell wall fragments, mycobacteria, and colloidal carrageenan, activate macrophages and stimulate granuloma formation.<sup>5,11</sup> Bacterial products, oils, metals, antigen-antibody complexes, and lymphokines are capable of instigating granulomatous inflammation, de-

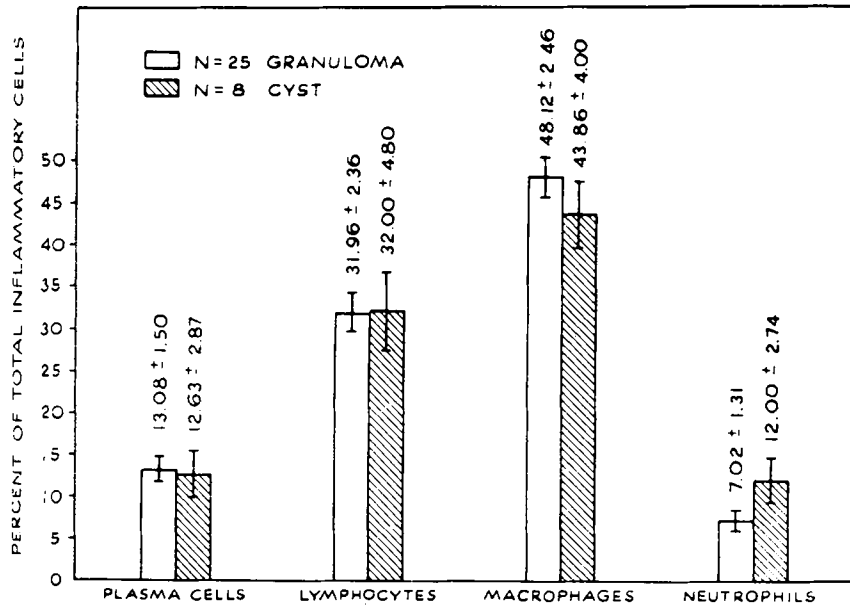


Fig 5.—Analysis of inflammatory cell infiltrates in periapical cysts and granulomas.

spite wide differences in chemical composition.

Lymphocytes, which comprised 32% of the inflammatory cells in the granulomas and cysts evaluated in this study, are always present in chronic inflammation. Lymphocytes have been separated functionally into B and T lymphocytes. B lymphocytes participate in humoral immune reactions by producing and secreting immunoglobulins and are identified primarily by immunoglobulins in their cell membranes.<sup>16-19</sup> Although they are noted most for antibody production, they also secrete lymphokines on appropriate stimulation.<sup>20</sup> T lymphocytes are so named because of their sojourn in the thymus before entering peripheral lymphoid structures as mature cells. T lymphocytes are the antigen-sensitive effector cells of the cell-mediated immune system responsible for the induction of graft rejection, delayed hypersensitivity, and contact hypersensitivity. The presence of a significant proportion of

lymphocytes in the periapical granuloma suggests a role for the immune system in the evolution of this lesion.

Plasma cells, the antibody synthesizing cells of the humoral immune system, comprised approximately 13% of all inflammatory cells in the periapical lesions. Plasma cells may be the effector cells between granulomatous inflammation and humoral immunity. Most evidence of a relationship between granulomas and antibodies is indirect. Antibodies influence granulomatous reactivity,<sup>21-24</sup> but do not enhance granuloma formation when transferred passively.<sup>25,26</sup> Specific hyperimmune serum and active sensitization reduce the granulomatous inflammatory response.<sup>21-24,27</sup> Serum antibodies are associated with amelioration of human schistosomiasis<sup>24</sup> and diminution of experimental granuloma formation.<sup>21</sup> The humoral immune system may exert a moderating influence on chronic granulomatous inflammatory disease.

The presence of neutrophils in the periapical lesions at an average level of 8% of the cell content denoted a persistent low-grade neutrophilic response. Endotoxins present in root canals with necrotic pulps<sup>28</sup> are known to be chemotactic for neutrophils.<sup>29</sup> Complement components, bacterial products, macrophage biosynthetic and collagen degradative products, and lymphokines in periapical lesions may also chemically attract neutrophils.<sup>14,30</sup> Neutrophils could contribute to the periapical tissue destruction through release of elastase, collagenase, and cathepsin.<sup>31</sup> They do not seem to be immunologically involved in these lesions.

Persistence of neutrophils in relatively small numbers is not characteristic of any particular disease. They have been described in granulomatous reactions associated with cell-mediated immunity,<sup>32</sup> antigen-induced granulomas,<sup>32,33</sup> and in some delayed hypersensitivity reactions.<sup>1</sup> They tend to persist in granulomas induced by antigens but not by inert substances as carbon.<sup>32</sup> Neutrophils comprise approximately 60% to 70% of the circulating white cells in peripheral blood and are the first cells at the site of injury. They continue to reenter the site of a recurring irritation, probably in proportion to the strength of the irritant.

## SUMMARY AND CONCLUSIONS

The human periapical granuloma is a relatively homogeneous lesion. Like other granulomas, it is comprised predominantly of macrophages (mononuclear phagocytic cells). The substantial contribution of lymphocytes, the effector cells of the immune system, to the cell content of the lesion suggests a potential

for immune-mediated reaction. The presence of plasma cells, the effector cells of the humoral immune system in numbers ranging from 3% to 34%, indicates possible local antibody synthesis. Together with a low-grade persistent lesional neutrophilia, these features are characteristic of an immunologic and nonimmunologic inflammatory response to bacteria or bacterial components productive of granuloma formation. The cellular composition of the periapical granuloma had no relation to symptom, treatment, morphology, or duration.

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