Antibody-producing cells in human periapical granulomas and cysts

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Immunoglobulin-producing cells were quantitated in 20 human periapical granulomas and cysts. Results showed that 42% of the lymphoid cells comprising 19% of all inflammatory cells contained immunoglobulins. The majority of lymphocytes (81%) were not associated with immunoglobulin production. IgG was found in 74%, IgA in 20%, IgE in 4%, and IgM in 2% of the immunoglobulin-positive cells. Pain did not correlate with any measurable change in the distribution of immunoglobulin-positive cells or other inflammatory cells. Endodontic treatment of inflamed pulps did not alter the lesional distribution of immunoglobulin-positive cells compared with the distribution that prevailed in untreated patients. There were no significant differences in immunoglobulin distribution between the solid and cystic forms of the periapical lesions.

The human periapical granuloma is a relatively homogeneous lesion comprised predominantly of macrophages, lymphocytes, and plasma cells, thereby qualifying as an immune-type granuloma.[1] Immune granulomas have more lymphocytes[2] and plasma cells[3] than do nonimmune granulomas. As exemplified by the foreign body granuloma, nonimmune granulomas are relatively pure collections of macrophages and giant cells; plasma cells are rare.[3] The classes of immunoglobulins in human periapical granuloma and cyst lymphoid cells have been identified qualitatively.[4-7] Immunoglobulin G was the predominant class in approximately 70% of the immunoglobulin-containing cells; whereas, IgA, IgE, and IgM were present in approximately 14%, 10%, and 4%, respectively.[8] The ratio of immunoglobulin-containing cells to total inflammatory cells was not determined.

The immunologic characteristics of periapical granuloma lymphocytes are still virtually unknown. These cells may be immune effector cells, such as lymphokine-secreting T lymphocytes of the cellular immune system, or immunoglobulin-containing precursors of plasma cells of the humoral B lymphocyte system, or non-B non-T (null) lymphocytes. Accordingly, the objectives of the present study were to establish both the proportions of immunoglobulin-positive B lymphocytes and plasma cells according to immunoglobulin class, and the immunoglobulin distribution in lesions subgrouped on the basis of cyst or granuloma, painful or nonpainful, and endodontically treated or untreated.

MATERIALS AND METHODS

Twenty histologically confirmed periapical lesions composed of 15 granulomas and five cysts were analyzed in this study. The average patient age was 44 years (20 to 71 years); the average lesion age, 3.5 years (2 months to 11 years). Biopsy specimens were obtained during apicoectomy, and were either fixed immediately in a formol alcohol solution (1 ml of 37% formaldehyde in 99 ml of 95% ethanol) at 4 C, or prewashed (seven specimens) to remove intercellular and cytophilic immunoglobulins. Prewashed tissues were held in 60-ml tissue culture medium RPMI-1640 (Grand Island Biological Co.) supplemented with 1% fetal calf serum and gentamicin for 48 hours at 4 C before fixation. After fixation, tissues were dehydrated at 4 C through graded alcohols and xylene, and embedded in 53-66 C paraffin. Serial sections were cut at 3 to 5 μm, and representative slides stained with hematoxylin and eosin for cytologic evaluation, peroxidase-labeled antisera for immunoglobulin determination, and fluorescinated antisera for kappa and lambda light chain identification.

Secondary antisera for indirect methods (peroxidase-conjugated goat antirabbit IgG) were purchased from Miles Laboratories. Kappa and lambda light chain antisera were purchased from Behring Diagnostics. Tests to ensure monospecificity

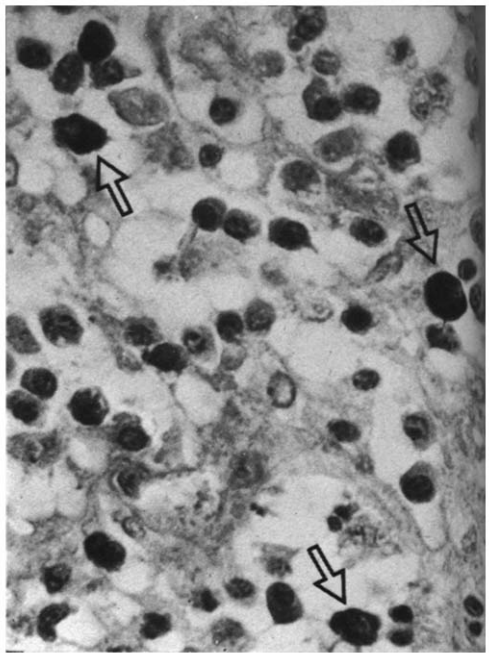

Fig 1—Human periapical granuloma stained by immunoperoxidase method for IgG. Plasma cells containing IgG appear black; whereas, nonimmunoglobulin-producing cells are gray. Arrows point to several positive IgG containing plasma cells in field. Lymphocytes and macrophages are also seen (orig mag ×400).

included immunoelectrophoresis, double diffusion in gel (Ouchterlony method), competitive inhibition with unlabeled antisera, adsorption with specific antigens, and performance testing with known positive controls (myeloma tissue). Nonspecific binding of secondary antisera used in the indirect technique was assessed by deleting the primary antisera. Endogenous peroxidase was evaluated by omitting all peroxidase conjugated immunologic reagents and by examining the slides for innate peroxidase activity.

Tissues were stained by a direct immunoperoxidase method for IgG and by indirect methods for IgM, IgA, and IgE.[9-11] The specific immunologic attachment was visualized as a dark brown reaction product resulting from the precipitation of diaminobenzidine tetrahydrochloride at the site of peroxidase localization. The tissues were then stained with Mayer's hematoxylin and coverslipped, using a permanent mounting medium.

Serial sections were stained individually with each monospecific antiserum; positive and negative lymphoid cells (lymphocytes and plasma cells) were quantitated by the modified morphometric method.[12] Data were recorded as number of positive immunoglobulin-producing cells per total number of lymphoid cells. Because of small cell size, no attempt was made to distinguish between surface and intracytoplasmic lymphocyte immunoglobulins. Other cells that exhibited surface or cytoplasmic immunoglobulins (macrophages or neutrophils) were excluded.

Selected sections were stained for kappa and lambda light chains in 11 of the 20 cases by standard immunofluorescent methods. A fluorescent microscope (Leitz Ortholux II) with phase optics was used to view lymphoid cells first by direct light and then by fluorescent light to determine the approximate ratio of fluorescent-positive kappa- or lambda-containing lymphoid cells to the total number of lymphoid cells.

Comparisons of the immunoglobulin distributions were made between several subgroups within the population: solid granulomas and cystic lesions; lesions from patients with a history of pain before apical surgery and lesions that were asymptomatic; lesions from patients who received endodontic treatment before apicoectomy and those from patients not previously treated; and prewashed and direct-fixed specimens were used to determine whether washing before fixing altered the cell counts.

RESULTS

Nearly 38,000 lymphoid cells in 80 slides from the 20 biopsy specimens were evaluated. Plasma cells were readily recognized by their full cytoplasmic staining and eccentric nuclei and typical chromatin pattern (Fig 1, 2). Round cells, approximately eight to ten microns in diameter, with a central nucleus containing peripherally clumped heterochromatin and little cytoplasm were recorded as lymphocytes. Immunoglobulin-positive staining lymphocytes showed a scant amount of deeply stained cytoplasm (Fig 3).

Forty-two percent of the lymphoid cells were immunoglobulin-positive (IgG + IgA + IgE + IgM). Only 19% of the total inflammatory cells stained for immunoglobulins (Fig 4). Approximately 6% of the positive cells were B lymphocytes; the remainder were plasma cells. Eighty-one percent of the lymphocytes did not stain positively for immunoglobulins. IgG and IgA were found in 100%, IgE in 95%, and IgM in 80% of the lesions studied.

The relative immunoglobulin distribution among the immunoglobulin-producing cells is shown in Figure 5. IgG was the predominant cell-associated immunoglobulin, followed in descending order by IgA, IgE, and IgM. Qualitative assessment indicated kappa and lambda light chains were present in approximately equal proportions. IgG was also the most common intercellular immunoglobulin and was detectable even after prewashing the tissues for up to 48 hours in tissue culture medium at 4 C. The intercellular staining was coarsely granular and occasionally created problems in interpretation. IgA was occasionally noted in the interstitial compartment; whereas, IgM and IgE were seldom present extracellularly.

Brown staining of a nonspecific, and presumably nonimmunologic, nature was seen in many sections. This was easily distinguishable from the dark brown specific peroxidase reaction product. The intensity of the diffuse brown background staining varied

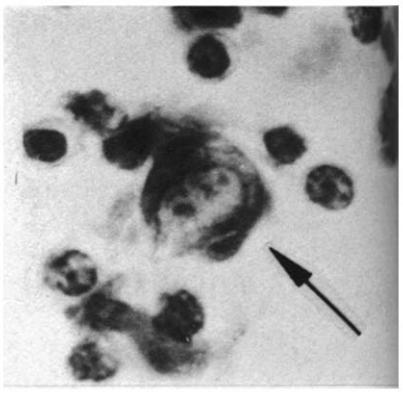

Fig 2—Human periapical granuloma stain immunoperoxidase method for IgG. High-p review shows plasma cell with eccentric nu containing peripherally clumped heterochro (open arrow) and "immunoblast" (closed a both cells contain IgG in their cytoplasm. S lymphocytes are seen that do not stain for I (orig mag ×1,000).

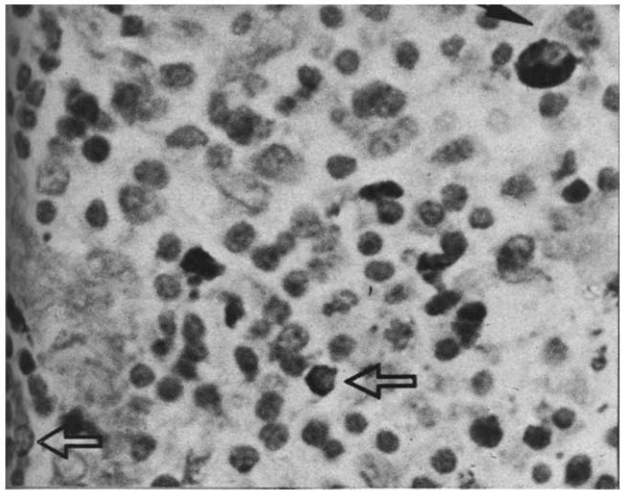

Fig 3—Human periapical granuloma stained for IgG. Typical lymphocytes with scant cytoplasm (open arrows) containing IgG are demonstrated. IgG-containing and non-IgG-containing plasma cell are also shown (closed arrow) (orig mag ×400).

from lesion to lesion. Despite the nonspecific staining of the intercellular structures, the immunoglobulin-positive cells were easily identifiable because of the stability of the stain and the excellent cellular detail. Endogenous tissue peroxidase activities (myeloperoxidase, hemoglobulin peroxidase, and macrophage peroxidase) were effectively destroyed by methanolic hydrogen peroxide incubation before immunologic staining.

There were no significant differences in either immunoglobulin production or distribution between granulomas and cysts (Table 1), lesions with and without a history of pain (Table 2), endodontically treated and untreated patients (Table 3), and washed and direct-fixed tissues (Table 4).

DISCUSSION

The role of the humoral immune system in the pathogenesis of periapical granulomas and cysts was evaluated by quantitating the immunoglobulin-producing and nonimmunoglobulin-producing lymphoid cells in the lesions. Cell quantitation was made by the modified morphometric sampling method[12]; immunoglobulin quantitation was made by peroxidase-labeled antibody methods. The peroxidase method, wherein horseradish peroxidase is chemically bound to immunoglobulins and serves as the cytochemical marker for the immunologic attachment, has certain distinct advantages over other methods: the positive cells are identifiable with the light microscope; the cytochemical marker is stable for years; and the cytologic detail that permits critical evaluation of the immunoglobulin-producing cells and their relation to other structures is excellent. The sensitivity of the peroxidase-label method is at least as great as that of fluorescent antibody methods.[9-11,13-16] In this laboratory the peroxidase method was also more informative because fading of the stain as occurs with fluorescein was not a problem, thus affording unlimited time to scrutinize the tissues. The peroxidase reaction with diaminobenzidine was readily visible in dilutions of antisera up to 600-fold, which far exceeds the dilutions used to stain the tissue sections. The immunoperoxidase technique was also much more sensitive than double diffusion in gel or immunoelectrophoresis.

Immunoglobulin G was the predominant immunoglobulin, encompassing 74% of the total antibody production in the lesions. It was also the major extracellular immunoglobulin appearing as brown granular and amorphous precipitates in the granuloma and cyst interstitium. Intercellular IgG staining was also seen after washing the tissues to remove cytophilic and intercellular unbound immunoglobulins[17] suggesting thereby that some of the IgG was bound or trapped in the tissues as previously reported.[8] IgA was the next most prevalent immunoglobulin; 20% of the immunoglobulin-producing cells stained for this class. Some intercellular IgA was also seen. IgE and IgM were made by 4% and 2% of the immunoglobulin-producing cells, respectively. These results are in relative agreement with the 70% IgG, 14% IgA, 10% IgE, and 4% IgM immunoglobulin-positive cells noted by Pulver and others.[8]

The majority of immunoglobulin-positive cells in the lesions were plasma cells. Plasma cells in periapical tissues have the capacity for local antibody synthesis,[18] and their presence in the lesions suggests a role for the humoral immune system. The plasma cells may make specific antibodies against antigens related to the etiology of the granuloma, or they may produce nonspecific antibodies in response to stimulation by T cells (helper function) or by other inducers of plasma cell activity, such as endotoxin. Local antibody production could be important in the pathogenesis of the disease. Specific antibody production might shield antigenic sites and prevent recognition by antigen-sensitive T lymphocytes, thus resulting in immunologic suppression of granulomatous reactivity.[19] In contrast, nonspecific antibody synthesis could theoretically perpetuate the granuloma formation.[20] The presence of antibody-producing plasma cells in periapical granulomas is extremely important because antibodies may be modulators of disease activity. Furthermore, their specificities constitute clues to the antigens, which may be involved in periapical lesion formation.

B lymphocytes, the other participant in humoral immune reactions, are identified primarily by surface immunoglobulins in their cell membranes.[21-23] The vast majority of the small lymphocytes (81%) seen in these lesions were non-B lymphocytes, as they did not exhibit immunoglobulins by the methods employed. It is possible that these lymphocytes, which lack demonstrable surface immunoglobulins, are thymic-derived T cells of the cellular arm of the immune system.

Immunoglobulin production and distribution between the various clini-

Table 1 • Immunoglobulin distribution in granulomas (15) and cysts (5).

Immunoglobulin-positive cells	Granuloma Mean ± SD*	Cyst Mean ± SD*	t†	P‡
% IgG + cells	29.2 ± 14.1	28.6 ± 7.9	0.095	>.05
% IgA + cells	9.5 ± 4.2	11.6 ± 7.1	0.735	>.05
% IgE + cells	1.9 ± 1.9	2.2 ± 1.6	0.307	>.05
% IgM + cells	0.7 ± 0.9	0.5 ± 0.3	0.641	>.05
Total % Ig+ cells	42.2 ± 14.6	42.9 ± 12.2	0.087	>.05

*SD = Standard deviation.
†t = Actual difference between means/standard error of difference.
‡P = Level of significance.

Table 2 • Immunoglobulin distribution in four patients with pain and 16 patients without pain.

Immunoglobulin-positive cells	Pain Mean ± SD*	No pain Mean ± SD	t†	P‡
% IgG + cells	26.3 ± 17.9	29.8 ± 11.6	0.461	>.05
% IgA + cells	10.5 ± 3.9	10.0 ± 5.3	0.167	>.05
% IgE + cells	1.5 ± 1.3	2.1 ± 1.9	0.591	>.05
% IgM + cells	0.4 ± 0.5	0.7 ± 0.8	0.652	>.05
Total % Ig+ Cells	38.6 ± 19.8	43.4 ± 12.5	0.566	>.05

*SD = Standard deviation.
†t = Actual difference between means/standard error of difference.
‡P = Level of significance.

Table 3 • Immunoglobulin distribution in 17 treated and three untreated biopsy patients.

Immunoglobulin-positive cells	Treated Mean ± SD*	Untreated Mean ± SD	t†	P‡
% IgG + cells	27.8 ± 12.9	36.7 ± 9.5	1.088	>.05
% IgA + cells	10.6 ± 4.9	6.3 ± 2.1	1.410	>.05
% IgE + cells	2.1 ± 1.9	1.5 ± 0.9	0.500	>.05
% IgM + cells	0.52 ± 0.5	1.5 ± 2.1	1.494	>.05
Total % Ig+ cells	41.0 ± 14.4	50.2 ± 5.2	1.028	>.05

SD = Standard deviation.
†t = Actual difference between means/standard error of difference.
‡P = Level of significance.

Table 4 • Comparison of immunoglobulin distribution in nine washed and 11 direct-fixed periapical specimens

Immunoglobulin	Washed Mean ± SD*	Direct-fixed Mean ± SD	t†	P‡
% IgG + cells	24.0 ± 9.30	33.0 ± 13.8	1.59	>.05
% IgA + cells	10.0 ± 4.40	10.0 ± 5.4	0.00	>.05
% IgE + cells	2.6 ± 2.10	1.5 ± 1.4	1.52	>.05
% IgM + cells	0.5 ± .70	0.7 ± 0.8	0.56	>.05
Total % Ig+ cells	37.22 ± 11.98	46.68 ± 14.21	1.51	>.05

*SD = Standard deviation.
†t = Actual difference between means/standard error of difference.
‡P = Level of significance.

cal and histological subgroups were not significantly different. In previous studies, cysts have been shown to contain immunoglobulins of each class[8,24-26] but there was disagreement as to immunoglobulin distribution. Some found that IgA was greater in cysts than in granulomas. The IgA was present in both immunoglobulin-producing cells[8] and in cyst fluid.[25] Conversely, another study found that IgG in cyst fluid comprised 80% of the total immunoglobulins with 16% IgA, and 4% IgM.[26] In the present study, there were essentially no differences in the distribution of antibody-producing cells (IgG >IgA >IgE >IgM) between cysts and granulomas; thus, the cystic form of the periapical lesion was indistinguishable from the solid form in cell content[1] and humoral immune responses.

Immunoglobulin production and distribution were the same in granulomas from endodontically treated and untreated patients. This observation is in accord with the previously reported inflammatory cell distribution in these groups.[1] These findings suggest that the commonly accepted methods of pulp removal, canal preparation, and canal filling do not alter the periapical cellular reactions and humoral immune responses.

Attempts to correlate pain with histologic alterations have not been successful. A previous report showed that there were no significant differences in inflammatory cell distribution between painful and nonpainful lesions[1]; in the present study, there were no meaningful differences in immunoglobulin distribution between the two groups. Pain, a highly subjective sensation, does not seem to be associated with a specific immune response.

The lack of any specific differences between the washed and direct-fixed specimens connotes that cell loss due to

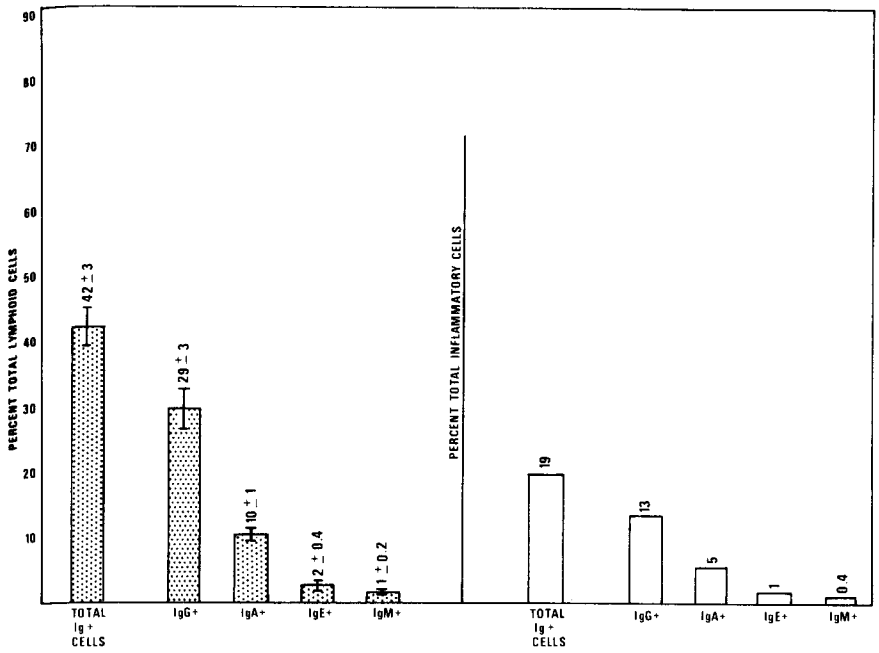

Fig 4—Distribution of immunoglobulin-positive cells in human periapical granulomas (15) and cysts (5). Numbers above stippled bars are mean ± standard error of mean; numbers above open bars are percent of total inflammatory cells.

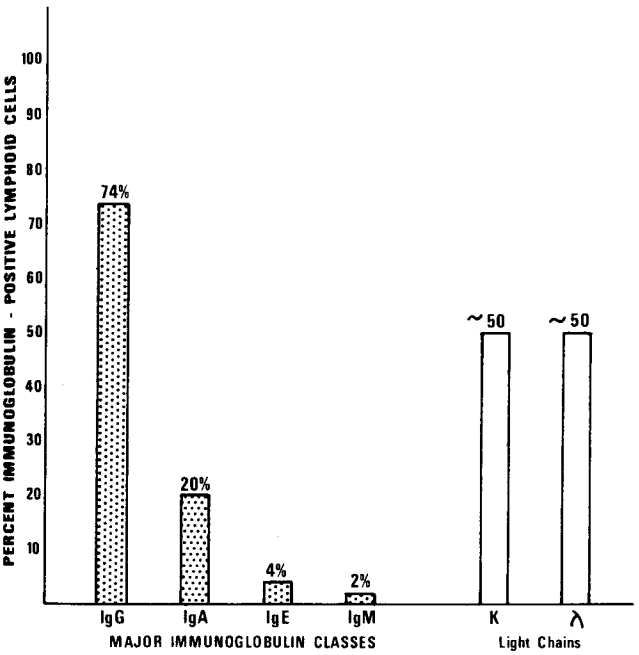

Fig 5—Relative distribution of immunoglobulin-producing cells in human periapical biopsies. Stippled bars are quantitative measures; open bars are qualitative assessments.

washing and membrane-bound cytophilic antibodies did not materially influence the results. The great majority of the immunoglobulin-producing cells were plasma cells with full cytoplasmic staining that would not be affected by washing. Intracellular staining was easily differentiated from intercellular staining by the immunoperoxidase method.

SUMMARY AND CONCLUSIONS

The solid or cystic periapical granuloma is a complex, immunologically influenced, granulomatous reaction. In the series examined in the present study, 74% of the antibody-producing lesional cells synthesized IgG, 20% IgA, 4% IgE, and 2% IgM. Eighty-one percent of the lymphocytes in the lesions did not contain cytoplasmic immunoglobulins, suggesting that most were either T cells or null cells, rather than B cells. There were no significant differences in immunoglobulin distribution between the clinical and histological subgroups, indicating that the humoral immune response was unidirectional rather than multidirectional.

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