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IMMUNE COMPONENTS IN HUMAN DENTAL PERIAPICAL LESIONS

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Summary—Immunofluorescence techniques were used to investigate periapical lesions for the presence of components indicative of hypersensitivity reactions mediated by humoral factors. In periapical granulomas, IgG, IgA, IgM and IgE cells represented 70, 14, 4 and 10 per cent, respectively, of the immunoglobulin-containing cells (ICC) observed. Intact and apparently degranulating mast cells were seen in the vicinity of the IgE ICC. Thus, components required for anaphylactic-type hypersensitivity were present in these lesions. The connective tissue of unwashed periapical granulomas stained intensely for IgG but less so for IgA and complement (C_3). Prewashing failed to eliminate this staining which suggested that a portion of these components were bound to tissue. Quick-frozen sections showed enhanced C_3 fluorescence both of connective tissue and of structures resembling small blood vessels. The potential therefore also exists for antigen–antibody complex-type hypersensitivity in this type of lesion. Radicular cysts showed a different pattern of fluorescence in that both IgG and IgA represented 45 per cent of the ICC, whereas IgM and IgE ICC each represented 5 per cent of the ICC. Only anti-IgG tissue fluorescence was noteworthy in cystic tissue. The one periapical scar examined revealed no cellular or connective tissue staining. Thus, periapical tissues can contain the components necessary for host immunopathologic responses. These responses may participate in the development of periapical pathosis.

INTRODUCTION

Two of the most common forms of periapical pathosis, granulomas and cysts, are characterized by the infiltration of chronic inflammatory cells. These cells consist of lymphocytes, macrophages and plasma cells (Bhaskar, 1969; Morse, Lasater and White, 1975). It has recently been reported that cells resembling plasma cells, containing IgG, IgA or IgM immunoglobulins, were present in periapical lesions (Kuntz *et al.*, 1977). In addition, these immunoglobulins were observed extracellularly and complement (C_3) was detected in 50 per cent of the lesions which were fast frozen (Kuntz *et al.*, 1977).

A preliminary report (Naidorf, 1975) demonstrated qualitatively IgA, IgG and IgM immunoglobulins in periapical granulomas by using a freeze–thaw extraction procedure. Studies using an immunofluorescent antibody technique on tissue from cysts showed the presence of many IgA and fewer IgG and IgM staining cells (Toller and Holborow, 1969). Immunoglobulins G, A and M are invariably present in cyst fluids examined immunochemically (Skaug, 1973).

We have previously shown (Pulver, Taubman and Smith, 1977) that normal tooth pulp is devoid of immunoglobulin-containing cells (ICC), whereas inflamed pulp contains various ICC. Our present purpose was to examine periapical lesions for the presence of various humoral immune components which might provide evidence for immunologic reactions in such lesions.

MATERIALS AND METHODS

Samples

Thirteen periapical tissue samples were obtained from randomly-chosen, freshly-extracted permanent teeth. Ten specimens were classified histologically as periapical granulomas. Microscopically these lesions consisted of connective tissue, as well as an infiltration of plasma cells, lymphocytes and neutrophils. Two of the specimens were classified as radicular cysts. Microscopically these lesions consisted of a cyst cavity lined with epithelium and a connective tissue wall containing plasma cells and lymphocytes. One lesion containing dense fibrous connective tissue and very few inflammatory cells was diagnosed as a periapical scar. These diagnoses were confirmed by Dr. Gerald Shklar (Harvard University, Department of Oral Medicine and Oral Pathology).

Washing, fixation and sectioning of the tissue

Immediately after surgical removal of the teeth, the periapical lesions were removed with a curette from the apices and processed. The specimens were either fixed in 10 per cent neutral buffered formalin according to the method of Eidelman and Berschauer (1969) (fixed and unwashed), or prewashed (Brandtzaeg and Kraus, 1965) and subsequently fixed (Pulver *et al.*, 1977). Some tissue sections were immediately frozen at the time of surgery and then sectioned on the cryostat (quick frozen).

Two periapical granuloma tissue samples were divided into three segments so that one segment of each was fixed and unwashed, prewashed or quick frozen. The prewashing was performed to remove any diffusible globulins and demonstrate tissue-bound proteins. Samples were quick frozen to compare complement (C_3) fluorescence with that seen when the tissue was processed by the formalin fixation technique.

Method of tissue staining

Sections of tissue were stained with the following mono specific fluorescein-conjugated goat antiserums: anti-human IgG, anti-human IgA, anti-human IgE, anti-human IgM, anti-human C_3 and anti-human C_4 (Pulver *et al.*, 1977). Adjacent sections were stained with haematoxylin and eosin, Methyl-green thionine (Roque, Jafrey and Coulter, 1964) and Toluidine blue (Zachrisson, 1971). The monospecificity of all antiserums was confirmed by means of gel-diffusion analyses (Öuchterlony, 1958). In addition, specificity was confirmed by reacting the conjugates with human small intestine. The fluoresceinated IgE and C_3 antiserums were also tested for specificity by blocking with unlabelled antiserum of the appropriate specificity (Nairn, 1964). Normal goat IgG (NIgG) was fluorescein-conjugated according to the method of Beutner, Sepulveda and Barnett (1968) and was used as a determination of the extent of non-specific fluorescence. FITC-conjugated antiserums IgG, IgA, IgM were adsorbed on solid immuno-adsorbants prepared as described by Cuatrecasas, Wilchek and Anfinsen (1968) and Axen, Porath and Ernbach (1967), and used as control reagents. We have described these procedures previously (Pulver *et al.*, 1977).

Microscopy

Sections were examined with a Leitz Ortholux microscope equipped with a Pleom vertical illuminator and filters. Photomicrographs were taken of selected sections using 35 mm colour reversal film (High Speed Ektachrome, ASA 160) and an exposure time of 45–60 s. The presence or absence of immunoglobulin-containing cells (ICC) or tissue fluorescence was recorded using previous criteria (Pulver *et al.*, 1977).

RESULTS

Periapical granuloma

The number of immunoglobulin-containing cells and the intensity of connective tissue fluorescence in 10 periapical granuloma tissue specimens are reported in Table 1. IgG and IgA Ig-containing cells were observed in each lesion examined. IgM and IgE Ig-containing cells were also found in all lesions. However, in 3 out of 10 lesions the number of IgM ICC was less than 1 cell/10 high power fields (HPF) and in 2 out of 10 lesions the number of IgE ICC was less than 5 cells/10 HPF. The fluoresceinated IgG antiserum demonstrated the greatest number of immunoglobulin-containing cells of all the fluoresceinated antiserums examined. More than 70 per cent of the ICC contained IgG and these cells appeared as contiguous masses rather than isolated ICC (Fig. 1). Cells resembling both plasma cells and lymphocytes were seen. IgA ICC were the second most numerous cells observed in the periapical granulomas, constituting more than 14 per cent of the total ICC observed. Although these numbers are low compared to the IgG ICC, it should be emphasized that the number of IgA ICC observed in periapical granulomas was more than twice the number of IgG ICC (the predominant cell type) seen in inflamed human dental pulps (Pulver *et al.*, 1977). IgA ICC in periapical granulomas were mainly isolated single cells (Fig. 2). Some IgM ICC (approximately 4 per cent of all ICC) were seen. An interesting feature of the granuloma was that about 10 per cent of the ICC contained IgE. Blocking with the unlabelled IgE antiserum prior to exposure to the fluoresceinated antiserum eliminated this staining entirely. IgE ICC were observed as individual cells (Fig. 3). Staining of adjacent tissue sections with Toluidine blue revealed numerous mast cells; numerous plasma cells were also observed. Mast

Table 1. Human periapical granuloma reacted with fluoresceinated antiserums

Antiserum	Number of HPF observed	Number of ICC/10 HPF ± SE (n = 10)	Fluorescence connective tissue (unwashed)
IgG	415	218.0 ± 20.8	+ + + +
Ads. IgG*	114	0	−
IgA	322	44.0 ± 8.1	+ +
Ads. IgA*	89	0	−
IgM	308	13.0 ± 3.2	+
Ads. IgM*	96	0	
IgE	301	31.0 ± 4.3	−
C3	283	0.7 ± 0.1	‡
C4	48	0	−
IgE Block†	164	0	−
C3 Block‡	149	0	−
NIgG	368	0.6 ± 0.1	±

* Antiserum after adsorption with homologous Ig bound to Sepharose 2B.

† Sections were initially covered with the specific unlabelled antiserum followed by exposure to the corresponding labelled antiserum.

‡ + + fluorescence sc in 5 out of 10 lesions observed, others essentially negative.

Abbreviations: NIgG, conjugated normal goat IgG; HPF, high power fields (× 400); ICC, immunoglobulin-containing cells; n, number of tissue samples examined; + + + +, + + +, + +, +, ± and − indicate fluorescence intensity ranging from maximum to negative.

Table 2. Human periapical granuloma reacted with fluoresceinated antiserums: effects of prewashing or no prior fixation

Antiserum	Number of HPF observed U	P	NF	Number of ICC/10 HPF ± SE (n = 2) U	P	NF (n = 2)	Fluorescence connective tissue U	P	NF
IgG	60	62	54	154.8 ± 16.5	147.0 ± 11.4	135.2 ± 2.7	+ + + +	+ +	+ + + +
IgA	57	67	61	20.0 ± 3.8	23.0 ± 4.1	26.0 ± 7.3	+ +	+	+ +
IgM	57	58	43	4.0 ± 0.4	5.0 ± 0.3	4.0 ± 0.1	±	−	±
IgE	74	62	57	13.2 ± 1.4	15.0 ± 1.1	13.5 ± 1.5	−	−	−
C3	61	57	56	0.2 ± 0.2	0.5 ± 0.2	0.1 ± 0.1	±	±	+ +
NIgG	68	71	54	0.7 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	±	−	±

Abbreviations: U, unwashed and fixed; P, prewashed and fixed; NF, no fixation, immediately frozen; NIgG, conjugated normal goat IgG; HPF, high power fields (×400); ICC, immunoglobulin containing cells, n, number of tissue samples examined; + + + +, + + +, + +, +, ± and − indicate fluorescence intensity ranging from maximum to negative.

cells appeared to be both intact as well as degranulating. Formalin fixation may have influenced this observation. However, intact mast cells are observed in periapical tissues after formalin fixation (Eda and Langeland, 1970). Other sections with IgE ICC alternatively stained with Toluidine Blue (at pH 1) also contained in the same field mast cells that appeared to be degranulating.

When the unwashed periapical granulomas were stained with the fluoresceinated anti-IgG serum, brilliant connective tissue fluorescence was noted (Fig. 1). Only moderate fluorescence was observed with the fluoresceinated anti-IgA serum (Fig. 2). In all cases, the antiserums directed to IgG, IgA and IgM after adsorption with homologous Ig showed virtually no fluorescence at either the cellular or connective tissue level. The conjugated normal goat IgG serum control showed only the slightest amount of cellular and connective tissue fluorescence. Complement (C_3) tissue fluorescence of at least + + magnitude was seen in 5 out 10 lesions examined. Blocking with unlabelled C_3 prior to exposure to the fluoresceinated antiserum completely eliminated this fluorescence.

There was a striking similarity in the number of ICC found regardless of how the tissue was processed (Table 2). Prewashing diminished connective tissue fluorescence but, contrary to the staining of human dental pulp tissue (Pulver *et al.*, 1977), this staining with the IgG antiserum was not completely eliminated. In the case of the labelled IgM and NIgG antiserums, connective tissue fluorescence was abolished by prewashing the tissue.

In 8 out of 9 quick-frozen tissues observed, complement (C_3) fluorescence was slightly enhanced. Prewashing did not reduce the C_3 connective tissue fluorescence, indicating that the C_3 may have been tissue bound (Fig. 4). Staining of only structures resembling vessels was more common (Fig. 5).

Radicular cyst

The most striking feature of the cyst (Table 3), in contrast to the granuloma, was the presence of a relatively large number of immunoglobulin-containing cells fluorescing with the labelled IgA antiserum (45 per cent of the total ICC). IgG ICC were equally prominent, also constituting 45 per cent of the total ICC. IgM and IgE ICC each comprised about 5 per cent of the total ICC. In fact, the mean number of total Ig-specific ICC/10 HPF observed in the radicular cyst was somewhat higher than the number observed in the granuloma (343 ICC/10 HPF vs 306 ICC/10 HPF, respectively).

Immunofluorescence at the connective tissue level did not parallel the ICC pattern (Table 3). Only anti-IgG tissue fluorescence was remarkable, but this fluorescence was less pronounced than in unwashed connective tissue from granulomas. Staining of the cyst tissue with the anti-IgA serum showed barely as much connective tissue fluorescence as the granuloma, although each section of cyst tissue had more than three times the number of IgA ICC than did the granuloma sections.

Periapical scar

Staining with each of the labelled antiserums revealed no cellular or connective tissue fluorescence. As this specimen lacked inflammatory cells, it could be regarded as a control for the specificity of the tissue staining. It is clear from this scar tissue and the normal pulp tissue examined (Pulver *et al.*, 1977) that, in the absence of inflammation, there was no fluorescence with any of the specific antiserums.

DISCUSSION

Cells containing immunoglobulin G, A, M or E and C_3-staining connective tissue were observed in

Table 3. Human radicular cyst reacted with fluoresceinated antiserums

Antiserum	Number of HPF observed	Number of ICC/10 HPF ± SE (n = 2)	Fluorescence connective tissue (unwashed)
IgG	59	155.0 ± 15.0	+ + +
Ads. IgG*	40	0	−
IgA	47	154.0 ± 28.6	+
Ads. IgA*	37	0	−
IgM	68	18.0 ± 12.5	±
Ads. IgM*	13	0	−
IgE	47	16.0 ± 0.7	−
C3	41	1.0 ± 0.4	±
C4	13	0	−
IgE Block†	22	0	−
C3 Block†	17	0	−
NIgG	68	0.7 ± 0	±

* Antiserum after adsorption with homologous Ig bound to Sepharose 2B.

† Sections were initially covered with the specific unlabelled antiserum followed by exposure to the corresponding labelled antiserum.

Abbreviations: NIgG, conjugated normal goat IgG, HPF, high power fields (×400); ICC, immunoglobulin containing cells; n, number of tissue samples examined; + + + +, + + +, + +, +, ± and − indicate fluorescence intensity ranging from maximum to negative.

periapical granulomas and radicular cysts, suggesting that several types of immunologic reaction potentially participate in the pathogenesis of these lesions.

Periapical granuloma

The results indicate that IgG is the predominant class of immunoglobulin in periapical granulomas. The relatively low level of IgM Ig-containing cells may be explained by the chronicity of this lesion. The IgG and C_3 tissue fluorescence was not completely eliminated by prewashing, so that at least some of these proteins were bound to the tissue. The presence of C_3, despite the absence of C_4, may have been due to insensitivity of the C_4 antiserum in the tissue or, more likely, indicates the quantitative predominance of C_3 as opposed to C_4 (Ruddy, Gigli and Austen, 1972). The immunoglobulin and complement suggests that the components necessary for immune antigen–antibody complexes may be present and that they may subsequently play a role in periapical pathosis. The antigens involved in these reactions presumably derive from bacteria. When these antigens, at certain concentrations, gain entrance to the pulp or periapical tissues, antigen–antibody complexes may form. If the complexes are large enough, they will be deposited in the tissue or, if they have an affinity for some tissue component, they will localize on that component (Genco *et al.*, 1974). The complexes may co-activate complement, resulting in generation of factors leading to increased vascular permeability and a leukotactic response. These events are seen in early periapical lesions (Seltzer and Bender, 1975) and they may exert an initial protective effect. Later, complexes ingested by leukocytes can lead to the release of tissue-destroying lysosomal enzymes from the leukocytes. The studies of Shindell (1961), Zerlotti (1969) and Bhaskar (1969) support the concept that, once the presence of microorganisms or microbial products initiate a host inflammatory response, the process of inflammation may be responsible for subsequent sequelae seen in periapical lesions in regions remote from the initial bacterial exposure.

Anaphylactic hypersensitivity reactions may represent another immunologic phenomenon active in periapical granulomas as both IgE ICC and mast cells, possibly undergoing degranulation, were observed in this lesion. IgE ICC were also found in inflamed human dental pulp (Pulver *et al.*, 1977) and in human radicular cysts (Table 3). It may be further suggested that the following features of anaphylactic hypersensitivity could contribute to the extension of pulpal inflammation leading to a periapical lesion. IgE antibodies on mast cells combining with antigen can lead to alteration in the Fc portion of the antibody. This, in turn, may trigger energy-dependent enzyme reactions leading to the release of vaso-active substances such as histamine, SRS-A and bradykinin This then may result in vasodilation, increased capillary permeability, leukocytic chemotaxis and, in the case of the bradykinin, pain (Elliott, Horton and Lewis, 1960).

Although the finding of IgE ICC in mucosal pathology may at times be inconclusive (Brandzaeg and Baklien, 1976), we report IgE ICC in periapical granulomas with some confidence for the following reasons: (1) The tendency of eosinophilic granulocytes to be non-specifically stained by fluorescein-conjugated proteins is accounted for in our system in that eosinophils could be easily discerned in dark field illumination. These observations were confirmed by staining with Giemsa stain to avoid inclusion of eosinophils in the ICC count. (2) The fluoresceinated anti-human IgE serum had a relatively low molar F:P ratio (3.0) and was prepared according to the method of Cebra and Goldstein (1965). (3) This antiserum was adsorbed with insoluble immuno-adsorbents. (4) The anti-IgE conjugate produced no non-specific staining when tested on normal tooth pulp (Pulver *et al.*, 1977) and on periapical scar tissue. (5) Staining of cells by fluoresceinated anti-IgE reagent was completely abolished by prior blocking with an unconjugated IgE antiserum obtained from a different commercial source. (6) Virtually no non-specific staining of periapical lesion tissue was observed after reaction with conjugated normal goat IgG.

Radicular cyst

The immune components were qualitatively identical to those in periapical granulomas. Therefore, the immunologic reactions could also be similar. An exceptional feature is the larger number of IgA-containing cells compared with the granulomas which is in accord with the observation by Toller and Holborow (1969) that IgA plasma cells were preponderant in cyst walls. However, the potential role of IgA in periapical pathosis is less clear than IgG or IgE, as IgA is not involved in complement fixation or in anaphylaxis. Some clues to the significance of the IgA predominance in this lesion are found by examining IgA in other systems. IgA-synthesizing plasma cells are the predominant Ig-containing cells in the lamina propria of the intestinal tract (Crabbé, Carbonara and Heremans, 1965) and bronchial and nasal mucosa (Martinez-Tello, Braun and Blanc, 1968; Brandtzaeg, Fjellenger and Gjeruldsen, 1967), presumably representing an immune response to multiple antigenic stimuli, chiefly microorganisms. However, the intestinal, bronchial and nasal mucosal IgA secreted in response to challenge is secretory in nature, whereas only a small portion of the cyst fluid IgA carries the "secretory piece" (Thompson and Asquith, 1970). Immunoglobulin and specific antibody-producing cells accumulate in chronic inflammatory foci produced by local injection of antigen or non-specific agents (Jasin and Ziff, 1969), which could account for the mechanism of inflammation in granulomas. Inflammation in relation to the cyst is more difficult to explain in these terms. Bacterial infection, for the most part, can be ruled out in many cysts (Toller and Holborow, 1969). Explanations of the lymphoid cell reactions in their walls may either lie in an antigenic stimulus of another sort, e.g. cyst wall epithelium, or in the presence of residual bacterial products which may attract lymphocytes and induce predominantly IgA immunoglobulin production (Toller and Holborow, 1969) or generate lymphokines.

No significant correlation exists between the levels of IgG and IgA in cyst fluid and the levels of these immunoglobulins in autologous serum (Thompson and Asquith, 1970). Our finding of numerous IgA and IgG ICC in cyst walls also supports the concept that

the immunoglobulins in cyst fluid are locally derived. On the other hand, it is unlikely that all cyst fluid immunoglobulins are of local origin because Skaug (1974) identified non-immunoglobulin proteins of plasma in cyst fluid in appreciable amounts and serum-derived immunoglobulins are also probably present as a result of inflammatory exudation. Likewise, rheumatoid synovial fluid is comparable with cyst fluid in that both contain increased levels of IgA, IgG and IgM (Sliwinski and Zaifler, 1970; Hrncir *et al.*, 1972). Our findings of only traces of tissue-bound C_3 in periapical cyst tissue and the reported low C_3 concentrations in cyst fluid (Mergenhagen, 1972) are also analogous to low C_3 values in rheumatoid synovial fluid (Bianco *et al.*, 1971). Consumption of C_3 during the formation of possible antigen–antibody complexes in the cyst is conceivable and could be responsible for the low C_3. Recently, rheumatoid factor-containing plasma cells have been demonstrated in dental periapical lesions (Malmström and Natvig, 1975). Therefore, while certain immunologic reactions may be taking place in the cyst wall, other reactions may simultaneously be occurring in the cyst fluid itself.

It is apparent that the lesions such as periapical granulomas and cysts have the components necessary for the development of a host immunopathologic response. How this response is activated or what its role is in the perpetuation or resolution of endodontic infections remains to be answered.

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Plate 1 overleaf

Plate 1.

Fig. 1. Unwashed periapical granuloma reacted with fluoresceinated IgG antiserum. An extensive number of immunoglobulin-containing cells are present in addition to tissue fluorescence × 750

Fig. 2. Unwashed periapical granuloma reacted with fluoresceinated IgA antiserum. Several immunoglobulin-containing cells are evident × 750

Fig. 3. Unwashed periapical granuloma reacted with fluoresceinated IgE antiserum. Several immunoglobulin-containing cells are present × 750

Fig. 4. Periapical granuloma fast frozen and reacted with fluoresceinated C_3 antiserum. There is fluorescence of a structure resembling a blood vessel to the left as well as fluorescence of the connective tissue stroma × 750

Fig. 5. Periapical granuloma fast frozen and reacted with fluoresceinated C_3 antiserum. Intense fluorescence of structures resembling blood vessels can be seen. × 750

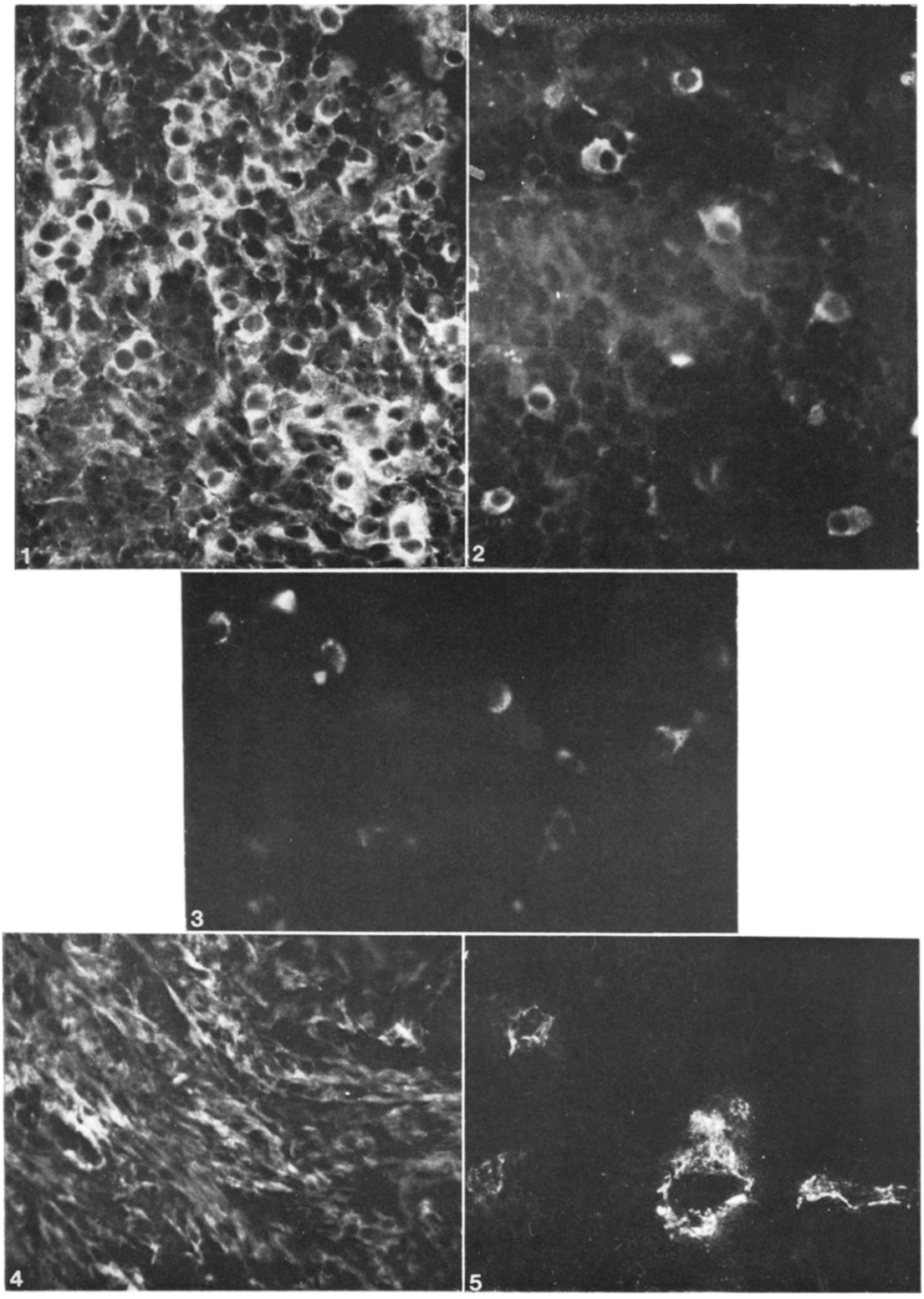

Plate 1