Journal of Immunological Methods, 66 (1984) 235–244
Elsevier

JIM 02919

Isolation of Follicular Dendritic Cells from Human Tonsils and Adenoids. I. Procedure and Morphological Characterization

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(Received 6 April 1983, accepted 30 August 1983)

Follicular dendritic cells have been isolated from human tonsils and adenoids and characterized at the ultrastructural level. Follicles were dissected and digested with different hydrolytic enzymes. The cells were separated by sedimentation at unit gravity. By this procedure we obtained follicular dendritic cells enveloping lymphocytes with their cytoplasmic extensions in a way analogous to that described for isolated thymic nurse cells. The ultrastructural features of isolated follicular dendritic cells are similar to those observed in situ. Prolonged enzymatic action caused loss of the enveloped lymphocytes.

Key words: *isolation of follicular dendritic cells – germinal centre cells*

Introduction

Immunological reactions are partly controlled by non-lymphoid cells. Among these, the follicular dendritic cells (FDC) described by White (1963) and Nossal et al. (1965) play an important role in the germinal centres. FDC, also called dendritic reticulum cells, are located, according to specific monoclonal antibody labelling, in the light and dark zones of tonsillar follicles and send cellular extensions into the mantle zone (Stein et al., 1982).

FDC retain immune complexes (Nossal et al., 1965; Herd and Ada, 1969; Heinen et al., 1983; Radoux et al., 1984), apparently linked to complement factors (Gajl-Peczalska et al., 1969; Papamichail et al., 1975). According to Tew et al. (1979), this retention of immune complexes modulates the level of antibody synthesis during the

Abbreviations: BSA, bovine serum albumin; DEM, Dulbecco's minimum medium; FDC, follicular dendritic cells; HSA, human serum albumin; MEM, Eagle's minimum medium; PBS, phosphate buffer solution; TGL, tyrode-glycosol solution.

maintenance phase of the humoral response. Other authors (Thorbecke et al., 1974; Klaus et al., 1980) propose that FDC play a major function in B memory cell formation. Kunkl and Klaus (1981) suggest that the retained complexes influence the affinity maturation of antibodies. According to Stein et al. (1982), FDC serve to trap B cells in follicles, independently of antibody or C3b-linked antigen retention. Thus the precise function as well as the origin of FDC remains unknown.

Various methods of isolating FDC have been described. Humphrey and Grennan (1982) and Klaus et al. (1980) isolated FDC from the spleen of mice previously treated with fluorescent immune complexes. The cells they obtained were, however, multinucleate and ultrastructural details were lacking. Tew and coworkers (1980) attempted to isolate FDC on continuous BSA gradients but reported cell damage after isolation. Steinman and Cohn (1973) apparently succeeded in purifying dendritic cells from mouse spleens, but in fact isolated another cell type which participates in different T cell reactions such as stimulation of the mixed leucocyte reaction (Steinman et al., 1983). Two reasons explain why few laboratories have succeeded in isolating FDC: FDC are scarce in number, and are intimately intermingled with lymphocytes. According to our own estimate in mouse lymph nodes, FDC represent about 2% of the total germinal centre cells. Their frequency markedly decreases in the mantle zone and they are absent from the interfollicular area (Heinen et al., 1980). To develop new ways of studying these cells we have isolated them from human tonsils and adenoids. We chose tonsils and adenoids from children since these contain large follicles which can be dissected (Tsunoda et al., 1978), and we treated them in the way described for isolation of thymic nurse cells (Wekerle and Ketelsen, 1980; Houben-Defresne et al., 1982).

Materials and Methods

Freshly dissected tonsils or adenoids from 3–10-year-old children were transported, at 4°C, in physiological solution (tyrode-glycosol solution, TGL) containing 0.4% human serum albumin (HSA) or 10% horse serum. Dissection of follicles in the same medium was performed under a biomicroscope.

Procedures for methods of digestion and sedimentation are described in Results. For initial digestion, collagenase (Worthington, 0.05%) was diluted in Dulbecco's minimum medium (DEM) containing 5% horse serum. For the final digestion, dispase (Boehringer, 0.05%) and deoxyribonuclease (Worthington, 0.004%) were added to 0.05% collagenase in phosphate buffer solution (0.1 M; PBS) at pH 7.2 containing 5% horse serum.

Cells were centrifuged at 1000 rpm for 10 min in a Janetzky T32c centrifuge. Living cells were observed with a phase contrast microscope (Wild).

Cell viability was tested by the trypan blue exclusion test. Trypan blue, dissolved in NaCl (4.5%), was added to the cell suspension at 0.1% final concentration.

Fixation of pellets of cells was in glutaraldehyde (2.5% in 0.1 M cacodylate buffer, pH 7.2 and 0.003 M $CaCl_2$) and in 2% osmium tetroxide. The pellets were embedded in Epon and sectioned with diamond knives on an LKB ultratome or a

Reichert OMU3 microtome. The sections were treated with lead citrate and uranyl acetate for contrast and observed in a Philips 301 microscope at 80 kV.

Fragments of tonsils or adenoids and isolated follicles were also fixed and prepared for optical or electron microscopy.

Results

Morphological aspects of FDC inside human tonsils or adenoids

FDC were mainly found inside the germinal centres of human tonsil or adenoid follicles. They contained 1 or 2 clear, indented nuclei (Fig. 1). The heterochromatin was disposed along the nuclear envelope against a thick and easily visible lamina densa (Fig. 2). The cytoplasm was subdivided into long and complex processes extending between the lymphocytes. The processes of different FDC were connected by desmosome-like junctions and constituted a network enveloping lymphocytes and dense material containing viruses and cell debris. The cytoplasmic organelles were mainly mitochondria, free ribosomes, Golgi apparatus and centrioles. Sometimes FDC bore a microcilium. Lysosomes and phagolysosomes were found infrequently.

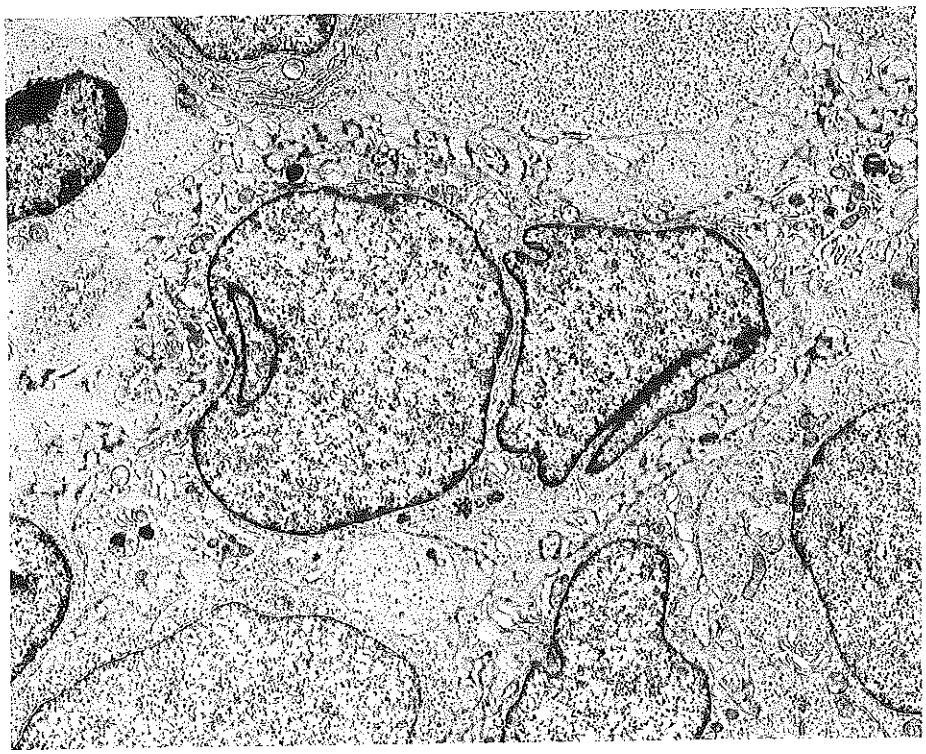

Fig. 1. Ultrastructure of a binucleated follicular dendritic cell in a germinal centre of a human tonsil. Nuclei are clear and indented; fine cytoplasmic extensions envelope surrounding lymphocytes. ×4750.

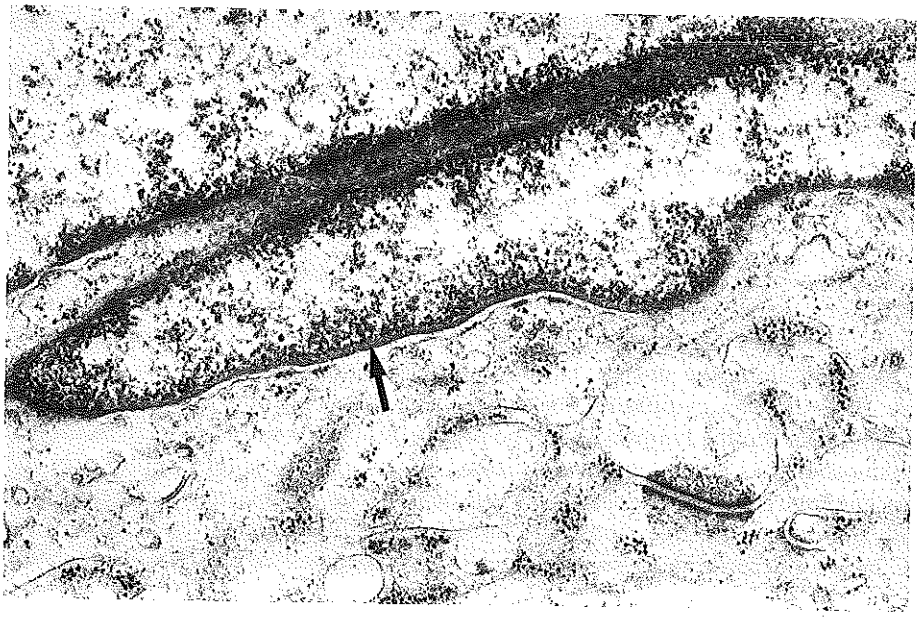

Fig. 2. Detail of Fig. 1. The lamina densa (arrow) is well developed in FDC and is bordered by a thin rim of heterochromatin. Desmosome-like junctions are characteristic of these cells. ×31,900.

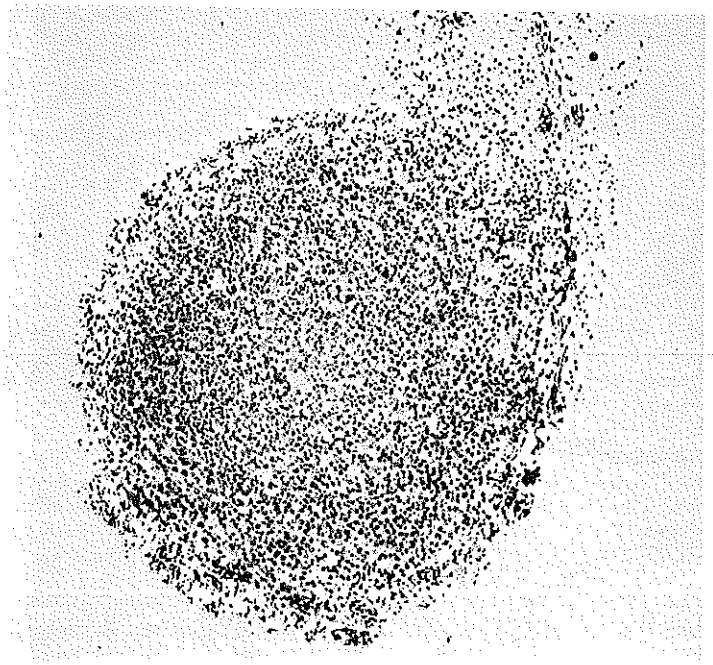

Fig. 3. Isolated lymph follicle obtained by dissection of a human tonsil; the germinal centre and a part of the mantle zone are visible. Semithin section. ×42.

Dissection of follicles

In order to isolate FDC, we first eliminated under the biomicroscope the majority of non-lymphoid tissues (epithelium, glands, fibrillar capsules) and separated the follicles from the other lymphoid zones (Fig. 3). Generally, dissection produced a cleavage at the exterior level of the mantle zone. The follicles were well-preserved and showed the presence of FDC. In certain cases when several follicles were contiguous we dissected them in clusters. Adenoids were very suitable for dissection as the follicles are generally located just beneath the epithelium. Dissection time was limited to 1 h.

Enzymatic digestion

We tested several enzymes at various concentrations, temperatures and durations, finally using the following schedule. The dissected follicles were rinsed twice in physiological solution (tyrode-glycosol solution containing 0.4% human serum albumin) in order to remove free cells and cellular debris. Four successive incubations with enzymes were performed. After each digestion, freed cells were collected by centrifugation while the non-digested fractions were incubated in the next enzyme solution. In each case the freed cells obtained were stored at 4°C until all the incubations were completed. The 4 enzyme incubations were as follows: first and

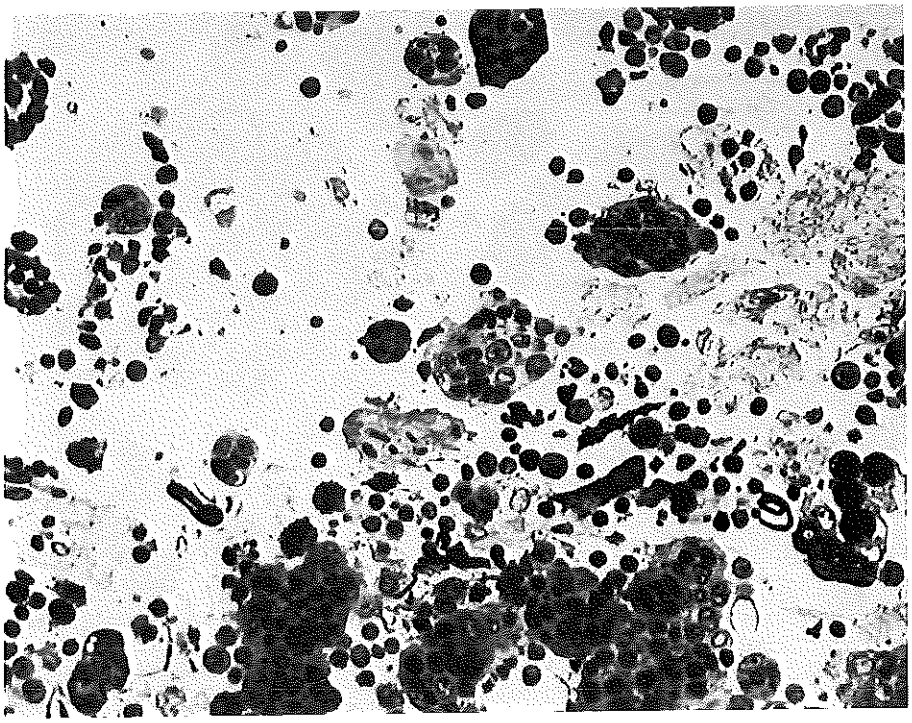

Fig. 4. Groups of cells containing lymphocytes in the serum fraction after sedimentation. Some cell debris and free cells accompany them. Semithin section. ×360.

second in DEM solution containing 0.05% collagenase, for 15 min at room temperature. Third and fourth in a mixture of enzymes (collagenase 0.05%, dispase 0.05% and deoxyribonuclease 0.004% in PBS) at 37°C for 15 min.

Following the last incubation, most of the fragments had been digested. The non-digested fraction containing mainly collagen bundles was discarded.

We obtained best cellular preservation when the enzyme digestion steps were performed in the presence of 5% decomplemented serum. We used human, horse or foetal calf serum. No clear difference was observed between these.

Sedimentation

The various resuspended pellets, obtained after collagenase and mixed enzyme digestion, were pooled in 2 ml of physiological salt solution containing 0.07 M phenol red and layered on 30% horse decomplemented serum in PBS. Two serum columns, 1 cm in diameter and 7 cm high were used; 1 ml of the cell suspension was deposited on each. Thirty minutes after sedimentation at unit gravity the supernatant solution (top fraction) was sucked off the serum solution and both fractions were centrifuged. Single cells remained in the top fraction whereas groups of cells

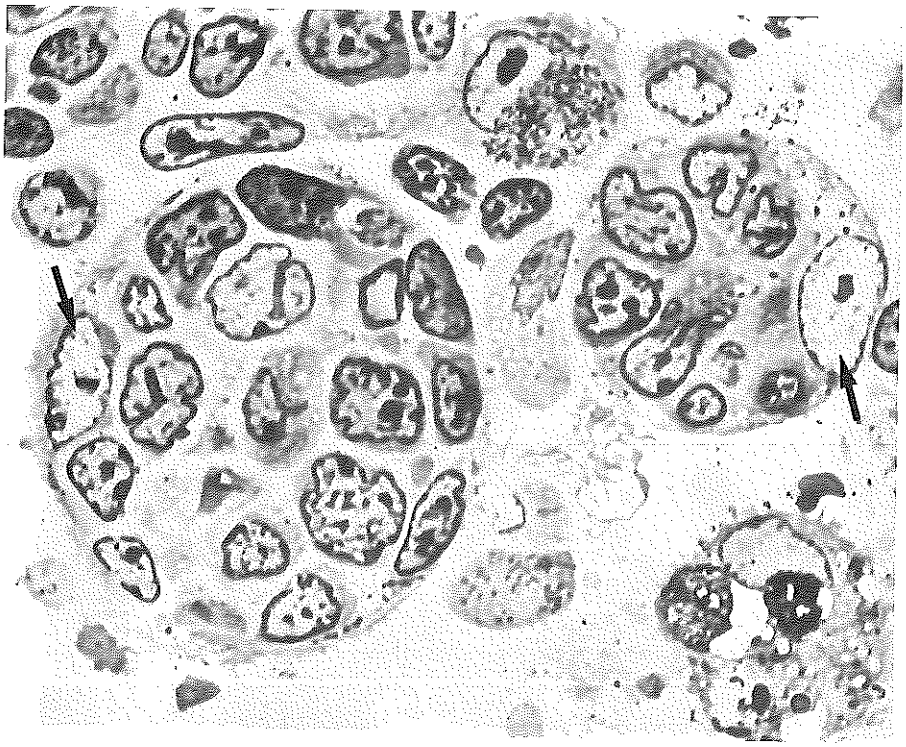

Fig. 5. Groups of cells in the serum fraction observed at high magnification by optical microscope in a semithin section. Cells with clear nuclei (arrows) surround lymphoid cells with their cellular extensions. ×1920.

sedimented in the serum fraction. Generally, these cell groups were mixed with free cells. We assessed the level of this contamination with a phase contrast microscope. When necessary we repeated the sedimentation on another 30% serum solution.

Cytological analysis of the isolated structures

At the optical level (phase contrast microscope and semithin sections) we found only free cells in the top sedimentation fraction. These were mainly lymphocytes or macrophages. At the lower sedimentation level we found free cells (lymphocytes, macrophages) and groups of cells most of which contained lymphocytes and were organized as spherical structures (Figs. 4 and 5). Besides these cell groups, some vessels and epithelial cells could be identified.

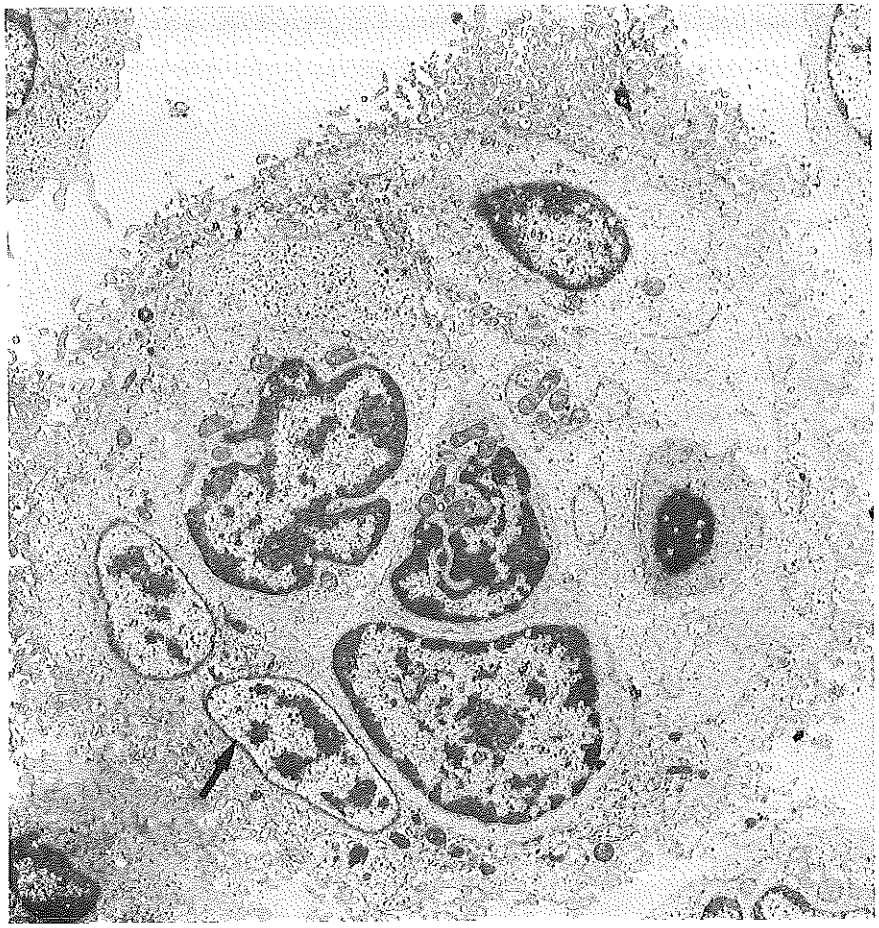

Fig. 6. Group of cells in the serum fraction observed ultrastructurally. Lymph cells are enveloped by cytoplasmic extensions bearing numerous microvilli. Sections of the nucleus of the cell surrounding the lymphocytes are seen in the lower part of the figure (arrow). ×5750.

At the ultrastructural level, the free cells in the lower sedimentation fraction were mainly lymphocytes, lymphoblasts and macrophages. Occasionally we observed free cells with the characteristic morphological features of FDC (clear indented nuclei, lamina densa, cytoplasmic extensions). The groups of cells, found in the lower fraction were composed of large cells enveloping lymphocytes and forming round clusters (Fig. 6). These large cells had clear indented nuclei with dispersed chromatin and a developed lamina densa (Fig. 7). Their cytoplasmic processes surrounded lymphocytes. Occasionally desmosome-like junctions were seen (Fig. 7). These large cells had the same morphological characteristics as FDC in normal tonsil tissue. Most of the groups of cells containing lymphocytes thus seem to be FDC enveloping lymphocytes.

We attempted to count the lymphocytes retained within the processes of the FDC: they varied from 3 to 70, with a mean value of about 15 lymphocytes. In the larger cell groups containing many lymphocytes, 2 or more FDC were present and

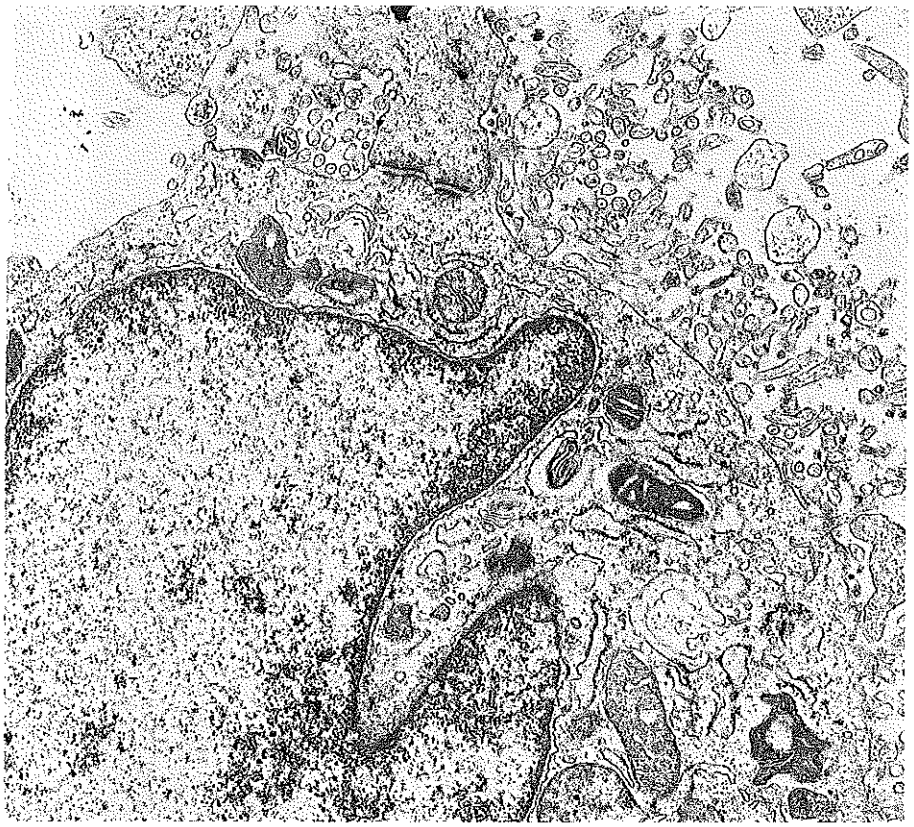

Fig. 7. High magnification of an isolated FDC present in the serum fraction. An indented nucleus with a thin rim of heterochromatin against a well developed lamina densa, cytoplasmic extensions and desmosome-like junctions. ×16,530.

inter-connected. Many lymphocytes also adhered to the external aspects of these clusters. At a rough estimate, in each experiment we obtained about 200,000 FDC groups mixed with lymphocytes, vessels, epithelial cells. The proportion of FDC groups to free cells or other structures varied from 1/100 to 1/500. Between 7 and 10% of the isolated cell groups were trypan blue positive.

The size of the isolated cell groups apparently depended on the concentrations of the enzymes used and the duration and temperature of the digestion. With increase in these factors, the size diminished and free FDC were more frequently found.

Confusion at the ultrastructural level between FDC and phagocytes was infrequent, since FDC contain no phagolysosomes. Groups of epithelial cells bore characteristic cytoplasmic structures (microfilaments or vacuoles) and their nuclei contained more heterochromatin than FDC. Epithelial cells were joined by larger and more numerous desmosomes than FDC.

Longer periods of enzyme action or higher concentrations of enzymes produced a loss of lymphocytes and degenerate FDC with chromatin condensation and cytoplasmic changes.

Discussion

Our description of the follicular dendritic cells of human tonsils or adenoids is similar to that of Sordat et al. (1969). FDC are characterized by a clear indented nucleus within a thin rim of heterochromatin and a well-developed lamina densa. Their cytoplasmic extensions envelope lymphocytes and are joined together by desmosomes. Tonsillar FDC resemble those found in other tissues or animals (Tew et al., 1979; Radoux et al., 1984).

Dissection of tonsils or adenoids from children provided follicles or groups of follicles free from most other structures. Children's tonsils contain large follicles and are not too heavily invaded by fibrous tissue. Digestion with enzymes is a conventional procedure for cell separation. We added 5% decomplemented serum (usually horse serum) in order to obtain better cell preservation. Sedimentation in 30% serum solution at $1 \times g$ grossly separated free cells from groups of cells, the latter usually sedimenting to the lower zone. Ultrastructurally, cells in these groups show the same morphological characteristics as FDC in situ.

The cells we describe here differ from those obtained by Humphrey and Grennan (1982), who state that the FDC they isolated coalesce to form multi-nucleate syncytia and that the envelopment of lymphocytes by these cells is an artefact. Tew and coworkers (1980) did not report sufficient details of their isolated cells to allow comparison with our observations.

FDC enveloping lymphocytes usually have a rounded appearance similar to that of nurse cells prepared from the thymus (Wekerle and Ketelsen, 1980; Houben-Defresne et al., 1982). Both cell types are intimately associated with lymphocytes, and the spherical cell groups we obtained may represent functional microenvironmental entities in germinal centres and thus provide a useful basis for studying B cell maturation.

Thus the procedure described here dissociates lymphoid follicles and separates FDC from other cells. Although not pure preparations of FDC, they are useful for studying this cell type inasmuch as about 90% of them are viable. Moreover, isolated FDC apparently preserve their surface receptors (data not shown).

The FDC we isolated did not resist long incubation in physiological solution. Our next aim is to determine optimal culture conditions for their maintenance in vitro.

Acknowledgements

We are grateful to the Fonds de la Recherche Scientifique Médicale for financial support. Tonsils and adenoids were kindly provided by Dr. Boniver (Bruyères' Hospital), Dr. Zicot (Merlot's Clinic) and Professor Melon (Bavière's Hospital).

References

Gajl-Peczalska, K.J., A.J. Fish, H.J. Meuwissen, D. Frommel and R.A. Good, 1969, J. Exp. Med. 130, 1367.

Heinen, E., C. Kinet-Denoël, D. Radoux and L.J. Simar, 1980, 7th Int. Conf. Lymphatic Tissues and Germinal Centers in Immune Reactions, Groningen (Nordic I.L., Tilburg) abstract no. 142.

Heinen, E., D. Radoux, C. Kinet-Denoël and L.J. Simar, 1983, J. Immunol. Methods 59, 361.

Herd, Z.L. and G.L. Ada, 1969, Aust. J. Exp. Biol. Med. Sci. 47, 63.

Houben-Defresne, M.-P., A. Varlet, G. Goffinet and J. Boniver, 1982, Leuk. Res. 6, 231.

Humphrey, J.H. and D. Grennan, 1982, Adv. Exp. Med. Biol. 149, 823.

Klaus, G.G.B., J.H. Humphrey, A. Kunkl and D.W. Dongworth, 1980, Immunol. Rev. 53, 1.

Kunkl, A. and G.G.B. Klaus, 1981, Immunology 43, 371.

Nossal, G.J.V., G.L. Ada, C.M. Austin and J. Peye, 1965, Immunology 9, 349.

Papamichail, M., C. Gutierrez, P. Embling, P. Johnson, E.J. Holborow and M.P. Pepys, 1975, Scand. J. Immunol. 4, 343.

Radoux, D., E. Heinen, C. Kinet-Denoël, E. Tihange and L.J. Simar, 1984, Cell Tissue Res. in press.

Sordat, B., R. Moser, H. Gerber and H. Cottier, 1969, Adv. Exp. Med. Biol. 5, 73.

Stein, H., J. Gerdes and D.Y. Mason, 1982, Clin. Haemat. 11, 531.

Steinman, R.M. and Z.A. Cohn, 1973, J. Exp. Med. 137, 1142.

Steinman, R.M., B. Gutchinov, M.D. Witmer and M.C. Nussenzweig, 1983, J. Exp. Med. 157, 613.

Tew, J.G., T.E. Mandel and G.A. Miller, 1979, Aust. J. Exp. Biol. Med. Sci. 57, 401.

Tew, J.G., R.P. Phipps and T.E. Mandel, 1980, Immunol. Rev. 53, 175.

Thorbecke, G.T., T.J. Romano and S.P. Lerma, 1974, Prog. Immunol. Stand II 3, 25.

Tsunoda, R., K. Terashima, K. Takahashi and M. Kojima, 1978, Acta Pathol. Jpn. 28, 53.

Wekerle, H. and U.-P. Ketelsen, 1980, Nature (London) 283, 402.

White, R.G., 1963, in: The Immunologically Competent Cell, eds. G.E.W. Wolstenholme and J. Knight, Ciba Foundation Study Group no. 16 (Churchill, London) p. 6.