REVISTA ESPAÑOLA DE FISIOLOGIA, 42, (3), 301-308, 1986

Evidence for Common Antigens on Human Non-Adherent Synoviocytes (Type A) and Peripheral Monocytes

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(Received on September 2, 1985)

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The characterization of a homogeneous non-adherent synoviocyte (Type A) cell population (≥ 95 %) from non-rheumatoid patients by culturing the cells in the presence of forty percent foetal calf serum is reported. These cells were able to phagocyte latex beads, iron particles, fluoresceinated zymosan and yeast. Furthermore, non-adherent synoviocytes were capable of being infected by the obligate intracellular parasite of peripheral monocytes *Leishmania donovani*. Indirect immunofluorescence experiments with specific anti-human monocyte (OKM1) antibody and specific antisynoviocyte serum, showed the presence of common surface structures between synoviocytes A cells and peripheral monocytes. Fifty five percent of the synoviocytes were also positive for HLA Dr antiserum. Analysis by two dimensional gel electrophoresis showed that peripheral monocytes and synoviocytes secreted identical polypeptides *in vitro*. These results strongly suggest a relationship between synoviocytes A and mononuclear phagocyte system.

Key words: Synoviocytes, Rheumatoid arthritis.

The synovial lining cells are necessary for the normal function of the diarthrodial joint. The exchange of nutrients and elimination of residues, the secretion of proteins and proteoglycans and the continuous filtering of the synovial fluid are considered to be their main functions. Morphological studies of these cells, have distinguished two types of cells: Type A or macrophage-like with evidence of pinocytosis and phagocytosis of latex beads Type B, a large non-phagocytic cell with a high rate of protein synthesis (2, 6, 14, 28). These synovial lining cells appear to be involved in the pathological alterations of the joint, one of the most important being Rheumatoid Arthritis (RA).

It is widely accepted that the damage of the articular joints is directly associat-

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ed with a local chronic inflammatory reaction (18, 30). The synovial membrane in RA is characterized by proliferation of the synovial lining cells, an infiltration of the sublining layers by mononuclear cells and the enrichment in polymorphonuclear cells of the synovial fluid (2, 19).

The synovial lining cells appear to be involved in the inflammatory process though different actions: the liberation of lysosomal chemotactic factors (1, 17) and degrading products such as collagenase and other proteases (13). The induction of cell mediated immunity and/or the production of autosensitization (8, 10) as well as the relationship with the class of sublinig infiltrates is not yet well defined (19). Therefore, it seems of relevance to characterize the components of the synovial lining which may play an important role in the chronic inflammation present in RA. Type A, the major component of this cell population, is a good candidate to be studied considering the importance of macrophages on the immune response. Hitherto, most of the characterization studies have been carried out from a morphological point of view. Although these cells seem to be originated in the bone marrow in animals (7), the pertainance of these cells to the human mononuclear phagocyte system has not been unequivocally demonstrated. This paper reports the characterization of a homogeneous population of synoviocytes A from a mixture of both cellular types present in the synovial lining. These cells are characterized as phagocyte lineage cells which present surface polypeptides in common with other components of the phagocyte system (peripheral monocytes), as well as a similar protein secretion pattern.

Materials and Methods

Materials. — Culture medium 199, trypsin and foetal calf-serum were purchased from Flow Laboratories (Irvine, Ayrshire, U.K.). Tc glutamine was from Difco, Methionine was from Merck. $Na^{125}I$ and ^{35}S-Methionine were from Amersham. Monoclonal antibodies anti-monocyte OKM1 and anti T lymphocyte OKT3 were from Ortho Diagnostics Systems, Inc. (Raritan, N. J., USA). Goat FITC anti-mouse IgG was from Miles Co. Ltd. (Slough, England SL24LY). Mouse anti-human Dr serum was from BRL. Autoradiography paper X-Omat S was obtained from Kodak.

Cells. — Culture of synovial cells were prepared with synovial samples from non-rheumatoid patients, dispersed by incubation with trypsin (6). Cells were cultured in 35 mm culture dishes (Costar, Mass., USA) in 199 medium containing 40 % heat inactivated foetal calf serum and gentamycin (80 μg/ml).

After seven days of culture at 37°C in a 5 % CO_2 atmosphere the cells were harvested and washed twice with phosphate saline buffer (Pi/NaCl) before use. Peripheral monocytes were obtained from blood and purified as described by TERRITO (27).

Phagocytosis experiments. — Synoviocytes (10^5 in ml of 199 medium) were incubated with a suspension of 0.81 μm latex beads (Difco) for 15 min at 4°C. In the case of iron particles (10 μm) or zymosan (Sigma) fluoresceinated by us, synoviocytes were incubated at 37°C for 3 hours. They were washed twice in Hank's medium and subsequently mounted on a glass slide to be visualized in a phase contrast microscope. The phagocytosis of live organisms was carried out with yeast and with the obligate intracellular parasite of the mononuclear phagocyte system the protozoan *Leishmania donovani.* Synoviocytes (10^5) were incubated with 10^6/ml yeast or parasites at 37°C for 4 hours. The cells were washed twice and mounted on glass slides. The yeasts were

visualized directly in the cells by phase contrast microscopy. *Leishmania* amastigotes, were visualized after staining with Giemsa.

Anti-synoviocyte serum. — New Zealand rabbits were injected twice intravenously with 2×10^6 cells/injection. One week after the second injection, the rabbits were bled from an ear vein, the serum was recovered and heat-inactivated. Anti-synoviocyte serum was absorbed with freshly obtained human lymphocytes according to the method described by GOODING and EDIDIN (12).

Determination fo IgG Fc and C_{3b} receptors. — The presence of C_{3b} receptors on the surface of synoviocytes was determined using FITC conjugated *Salmonella typhii* reacted with fresh human serum as a source of complement (9). The receptor for IgG Fc was detected by rosette formation of sensitized red blood cells with IgG rabbit anti-ox serum (EA) according to MORETTA *et al* (22).

Lymphoblast transformation test. — The cells were challenged either with Concanavalin A (Con A) or Phytohemagglutinin (PHA) and the incorporation of ^{3}H-thymidine was measured as described (15).

Immunofluorescence. — Synovial cells (10^6) in 200 µl of Hank's were incubated with 5 µl of specific antibody (OKM1, OKT3, anti-human Dr and anti-synoviocyte) for 30 min at 4°C with gentle shaking. Cells were washed twice with 1 ml Hank's and suspended in a final volume of 100 µl to which 7 µl of the corresponding FITC antiserum were added. After 30 minutes incubation at 4°C, cells were washed three times with cold Hank's, mounted on glass slides and analyzed by microscopy.

Frozen sections (6-10 µm thick) were cut out in cryostat at –30°C and mounted on glass slides. The slides were incubated with monoclonal antibody anti-monocyte OKM1 and with specific anti-synoviocyte serum. The corresponding controls were treated (in parallel) in each case, as described by PELTIER *et al.* (25).

Polyacrylamide gel electrophoresis. — Two dimensional electrophoresis was carried out according to O'FARREL (24) as slightly modified by BRAVO (3). A 15 % acrylamide gel was used in the second dimension. Approximately 100,000 trichloroacetic precipitated c.p.m. were routinely applied per gel. Fluorography was carried out as described (20).

Protein synthesis. — 10^5 cells were seeded in 100 µl of MEM containing 500 µg/ml of glutamine and 1 mg/ml of methionine, 20 µCi of ^{35}S methionine was added to the medium and incubated at 37°C in a 5 % CO_2 atmosphere for 24 hours. The cells were disrupted in lysis buffer (9.8 M urea, 2 % w/v). Nonidet (P-40, 2 % Anpholytes pH 7-9, 100 mM ditiothreitol) and kept at –20°C. The trichloroacetic precipitable radioactivity was determined.

Results

Functional characteristics of non-adherent synovial cells. — When grown in the presence of 40 % FCS the synovial cells appeared after seven days in culture as an homogeneous population of round mononuclear cells. Considering that synovial cells have been indentified in animals as members of the monocyte lineage (7), it was decided to further investigate this possibility in humans. For this purpose their ability to phagocytose different particles as well as live cells was assayed (table I). More than 90 % of the non-adherent synoviocytes obtained were able to phagocytose latex beads, iron, zymosan particles and yeast. Furthermore,

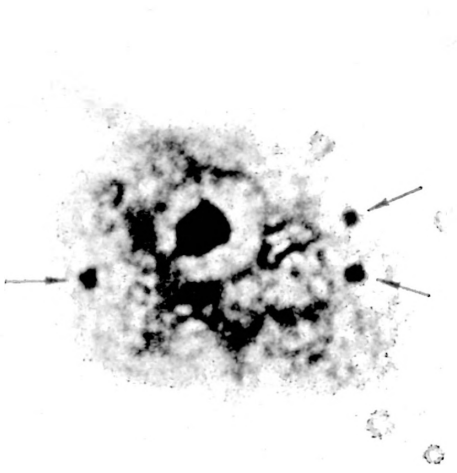

Fig. 1. Leishmania donovani *reproduction in the synoviocyte cytoplasm.*
The Giemsa stain a synoviocyte that has been infected by the parasite. The black points in the cell cytoplasm, indicated by the arrows, are *Leishmania* amastigotes. Amplification 12,500.

similar ingestion efficiency was obtained with the obligate intracellular parasite *Leishmania donovani* (table I). The presence of protozoan amastigotes inside the cell cytoplasm (fig. 1) is a clear evidence of the monocyte nature of these cells (4). These results seem to exclude the possibility that the data shown in table I could be accounted for unspecific adhesion of particles or cells to the synoviocyte membrane. Another proof for the homogeneity of the synovicyte cell population came from lectin stimulation activity. Synoviocytes were unable to be stimulated by either Con A or PHA which are known to be mitogenic for T lymphocytes or a mixture of T and B lymphocytes, therefore excluding the presence of these types of cells in the preparation. Because of a contamination by B lymphocytes can be reasonably excluded.

Common surface antigens between synoviocyte and monocyte phagocyte cells.

To further analyze the relationship between monocytes and synoviocytes the presence of monocyte surface structures such as C_{3b} and F_c receptors, HLA-Dr and Mo1 antigens, was studid. Conversely, the presence of synoviocyte antigens on monocytes by using an anti-synoviocyte serum was also studied.

The presence of C_{3b} and IgG Fc receptors was studied by their attachment to either gram negative bacteria which had fixed complement or sensitized red blood cells. After seven days of culture neither Fc nor complement receptors were detected on the synoviocyte surface (data not shown) as opposed to monocytes (11). This apparent contradiction could be ex-

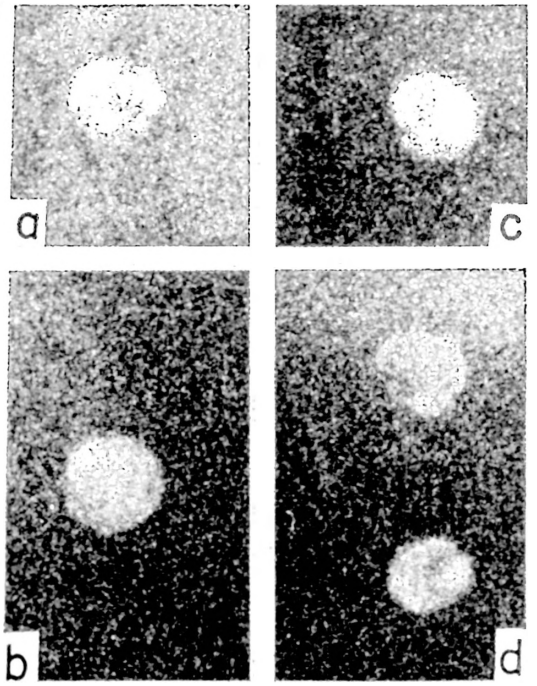

Fig. 2. *Immunofluorescence of synoviocytes and monocytes with specific antisera.*
a) Synoviocyte with lymphocyte-absorbed anti-synoviocyte serum. b) Peripheral monocyte with anti-synoviocyte serum. c) Synoviocyte with monoclonal antibody (OKM1) anti-human monocyte. d) Synoviocyte with antiserum anti-human HLA-Dr. Amplification 2,500.

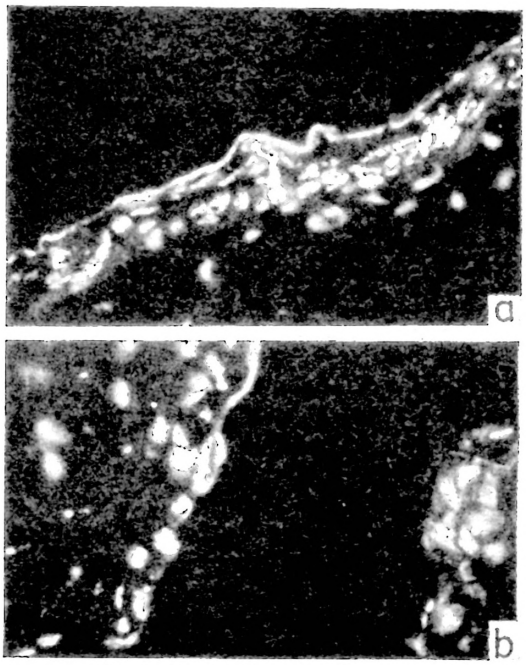

Fig. 3. *Immunofluorescence of synovial lining cells.*
(a) Indirect immunofluorescence of a section of non rheumatoid synovium with specific serum anti-synoviocyte. (b) Immunofluorescence reaction with monoclonal antibody (OKM1).

plained by the fact after three days of culture the C_{3b} and F_c receptors of monocytes start to decline (16).

Non-adherent synoviocytes expressed HLA-Dr antigen (55-60 %) as determined by indirect immunofluorescence experiments (fig. 2d). This figure is similar to that obtained with macrophages (29) and slightly lower than that found for peripheral monocytes (21). The monoclonal antibody OKM1 specific for monocytes was also able to react with synoviocytes in culture (fig. 2b) and in frozen tissue sections (fig. 3b).

This cross-reactivity between synoviocytes and monocytes was also confirmed by using anti-synoviocyte antiserum previously absorbed with human lymphocytes (fig. 2a and fig. 3). The synovial cells obtained belong exclusively to the lining cells of the synovium since the antiserum raised against cultured cells reacts only with the two external layers of cells in frozen tissue sections (fig. 3a). Control experiments with the monoclonal antibody OKT3 confirmed the absence of T lymphocytes in our synoviocyte preparation (data not shown).

Protein secretion. — Monocytes in culture are well known to secrete different molecules to the medium (21, 23, 26). In order to compare the secretion pattern of monocytes and non-adherent synoviocytes, protein synthesis experiments using ^{35}S labelled methionine were carried out. The secreted molecules were then analysed by two dimensional gel electrophoresis, giving both types of cells an almost identical pattern. A major group of proteins composed of nine (fig. 4a and b) spots of 64,000 molecular weight accounting for 80 % of the labelled secreted protein was found as well as another

Table I. *Phagocytosis by synoviocytes of different particles and live cells.*
Mean of three experiments. The viability of the cells was higher than 90 %.

	Percentage of phagocyting cells				
Cell type	Latex	Iron	Zymosan	Yeast	*L. donovani*
Lymphocytes	10 ± 1	12 ± 2	8 ± 1	13 ± 2	14 ± 1
Synoviocytes	90 ± 3	90 ± 2	95 ± 2	90 ± 5	90 ± 3

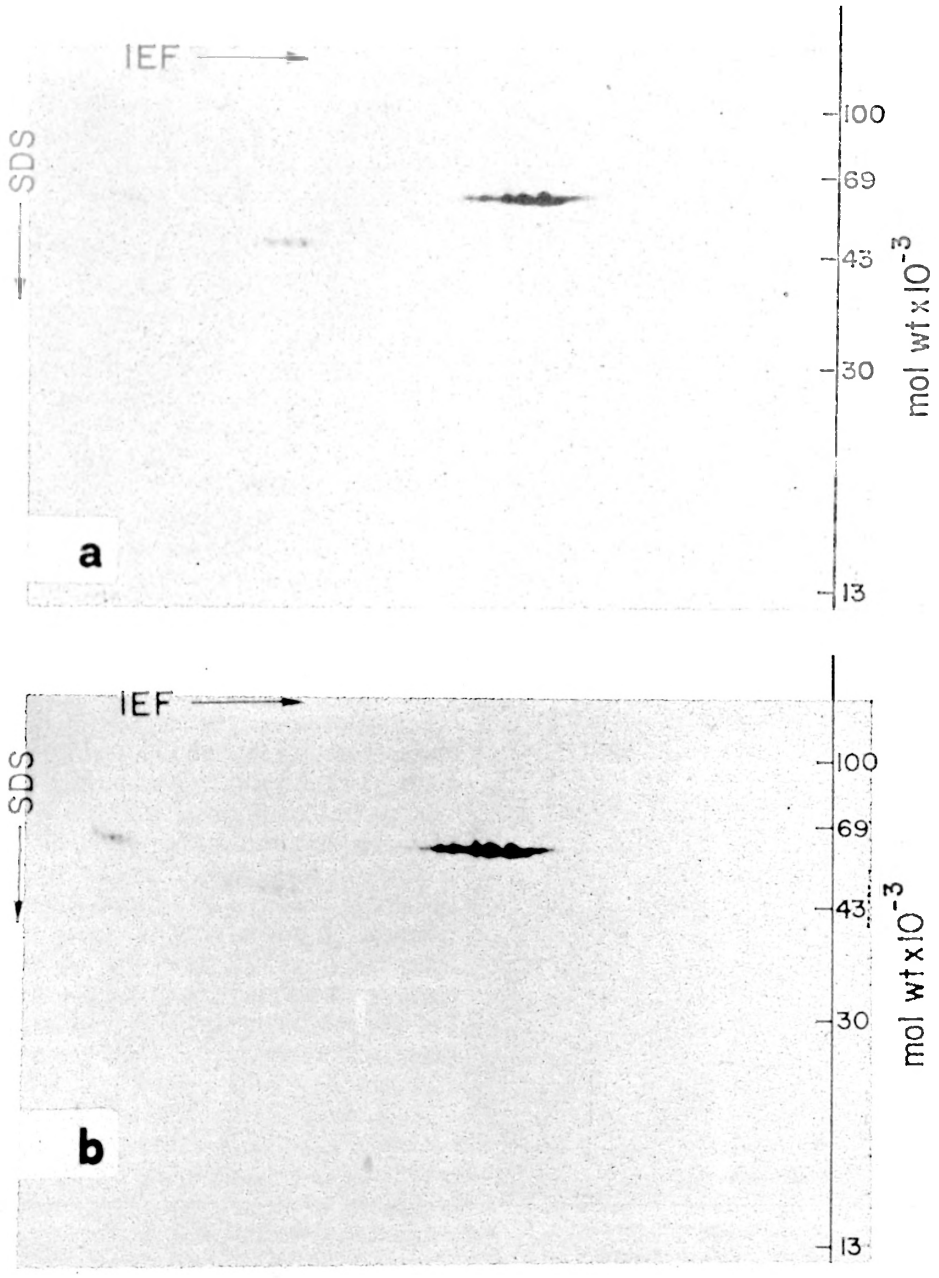

Fig. 4. *Autoradiography of two dimensional gel electrophoresis of ^{35}S-labelled proteins synthesized by the cells.*
a) Secreted protein by synoviocytes. b) Secreted proteins by peripheral monocytes.

group of 60,000 composed of five spots and a third family of spots with approximate molecular weight of 50,000.

Discussion

The major component of the synovial lining cells, the non-adherent synoviocyte A, has been obtained as a homogeneous population by stripping the cells from the synovial tissue and culturing them in a difined medium with a high concentration of FCS. The onset of fibroblast like cells in the cultures regresses and finally disappears. This may be due to an inhibitory effect of non-adherent synoviocyte secreted factors as described (5). The culture of synoviocytes appears to be free of lymphocyte contamination since immunofluorescence experiments with a monoclonal antibody specific for T populations were negative and the cells were unreactive with the mitogens PHA and Con A. Furthermore, the antiserum raised against cultured synoviocytes gave positive reaction only with the two external layers of cells when assayed in frozen tissue sections, ruling out the possibility that phagocyte cells other than synoviocytes were the type of cells obtained from the synovial tissue.

The results presented above showed the presence of three different types of common molecules between synoviocytes and peripheral monocytes: First, well known surface antigens such as Mo1 and HLA-Dr; second, still uncharacterized antigens as those recognized by the specific anti-synoviocyte serum; finally, three different families of secreted proteins with molecular weights of 64,000, 55,000 and 50,000 respectively. These results strongly suggest that synoviocytes belong to the monocyte lineage. This conclusion was confirmed by the fact that synoviocytes were able to phagocytose different particles and cells as well as being infected by the obligate intracellular parasite of monocytes *L. donovani.*

Further molecular and functional characterization of these cells will be helpful in the understanding of the role played by the synoviocytes in inflammatory processes such as Rheumatoid Arthritis.

Acnowledgements

This work has been partially carried out at Dr. Bravo's laboratory at the European Molecular Biology, laboratory at Heidelberg. We are grateful to Drs. Muñoz, Enjuanes and Bernabeu for helpful discussion of the manuscript and to Mrs. R. Díaz, C. Langa, A. Partearroyo and M. J. Vilela for their technical assistance.

Resumen

Se caracteriza una población homogénea de sinoviocitos no adherentes (Tipo A) procedentes de sinoviales de pacientes no reumatoides. Las células cultivadas en 40 % de suero fetal bovino, fagocitan partículas de látex, hierro, zimosan fluoresceinado y levaduras y se infectan por el parásito intracelular estricto de monocitos *Leishmania donovani.* Experimentos de inmunofluorescencia indirecta con anticuerpos anti-monocito (OKM1) y anti-suero específico anti-sinoviocito, muestran la presencia de estructuras comunes en la superficie celular entre células sinoviales y monocitos periféricos. Los sinoviocitos son positivos para el antisuero anti HLA-Dr. Mediante electroforesis bidimensional se muestra que, tanto los sinoviocitos como los monocitos periféricos, secretan idénticos polipéptidos *in vitro.* Estos resultados sugieren una relación entre los sinoviocitos A y el Sistema Mononuclear Fagocítico.

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