Histol Histopathol (2000) 15: 1077-1085

http://www.ehu.es/histol-histopathol

Histology and Histopathology
Cellular and Molecular Biology

Dendritic cells and interleukin-2: cytochemical and ultrastructural study

M. Civallero[1], S. Barni[1], R. Nano[1] and E. Capelli[2]

[1]Department of Animal Biology, University of Pavia and Centre of Study for Histochemistry, CNR and
[2]Department of Genetics and Microbiology, University of Pavia, Pavia, Italy

Summary. The aim of the present study was to verify the effect of IL-2 on dendritic cell (DC) differentiation. Various cytokines have been indicated as factors inducing DC differentiation, but no data about the interleukin-2 (IL-2) effect on DC differentiation have been reported. Monocytes isolated from peripheral blood were treated *in vitro* with the following factors: IL-2, IL-4, GM-CSF and G-CSF alone or in combination. Morphological (also ultrastructural) and cytochemical observations were carried out starting from 3 to 21 days of treatment. The results indicate that the differentiation of cells showing dendritic pattern is related to the presence of IL-2. Moreover a synergic effect of IL-2 and GM-CSF was observed. The enzymatic features changed with the culture time: before the differentiation into DC, the stimulated cells expressed the typical pattern of monocytes. On the contrary, at advanced stage of differentiation, some enzyme activities changed and in terminally differentiated dendritic cells the reactions for peroxidase and serine esterase were negative.

Considering the morphological features, the ability to interact with lymphocytes and the enzymatic pattern observed, we suppose that IL-2 may act as a maturative factor rather than as a growth factor in the DC differentiation.

Key words: Blood monocyte, Dendritic cell, Ultrastructure, Cytochemistry

Introduction

Recently, it has been suggested that the dendritic cell system of antigen presentation is probably the main initiator and modulator in the first steps of the immune response (Grabble et al., 1995). Dendritic cells (DC) are efficient stimulators of B and T lymphocytes (Steinman, 1991). The T-cell antigen receptor (TCR) recognises antigenic peptides bound to the major histocompatibility complex (MHC) expressed on the surface of antigen-presenting cells (APC). These cells can differentiate both from myeloid precursors and from circulating monocytes (Young et al., 1995). Some authors assert that macrophages and dendritic cells are not able to exchange. On the contrary, it has been demonstrated that peripheral blood monocytes can differentiate into cells with dendritic features (Peters et al., 1996). The growth factors mainly involved in the dendritic differentiation seem to be the interleukin-4 (IL-4), the granulocyte-monocyte colony stimulating factor (GM-CSF), tumor necrosis factor (TNF) and the interferon-γ (INF-γ). Moreover, the presence of T lymphocytes in the cultures of dendritic cells seems to be an enhancing factor for the proliferation of dendritic cells (Banchereau et al., 1998) suggesting that factors released by T lymphocytes are necessary for the health of DC. On the other hand, the T cell growth factor interleukin-2 (IL-2) has been demonstrated to affect almost all immune functions. A previous observation on the presence of proliferating dendritic cells in peripheral blood mononucleated (PBMC) cultures maintained with IL-2 (Capelli et al., 1997) led us to suppose that this factor could be involved in DC differentiation. The aim of the present work was to study the effect of IL-2, alone or in combination with other factors, on proliferation and differentiation of human blood monocytes into DC *in vitro*.

Offprint requests to: Rosanna Nano, Professore Associato, Department of Animal Biology, University of Pavia, Piazza Botta 10, 27100 Pavia, Italy. Fax: +39 0382 506406. e-mail: nano@ipv36.unipv.it

Materials and methods

Monocyte cultures

Peripheral blood mononucleated cells (PBMC) from healthy donors were isolated by a density gradient (d= 1.077, Histopaque, Sigma, Poole, Dorset, UK). The cell suspension was seeded in 5 cm-diameter Petri dishes ($5x10^6$ cells/5ml/dish). After two hours the non adhering cells were gently harvested and discarded. The dishes were washed with culture medium (RPMI 1640), then complete medium containing 20% foetal calf serum, penicillin (100U/ml), streptomycin (1000g/ml), amphotericin B (0.0085%) and growth factors under study were added. The cells were incubated for 3, 6, 8

and 16 days at 37 °C in a 5% $C0_2$ humidified incubator.

Growth factors

Stock solutions of GM-CSF (10mg/ml, Eurobio Lab., Les Ulis Cedex, Paris, France), IL-2, (100ng/ml, Eurocetus, Holland), G-CSF (300mg/ml, Neupogen, Hoffman-La Roche, Basel, Switzerland) and IL-4 (100ng/ml, Reprotech, Inc, Rocky Hill, NY, USA) were prepared by dissolving the lyophilised factors in culture medium. The solutions obtained were subdivided into aliquots and stored at -20 °C until use. The IL-2 doses were chosen according to previous experiments in which we considered the proliferating index and vitality in relationship to the concentration use (Capelli et al., 1997). We used the following concentration: 100pg/ml IL-2. The doses of the GM-CSF, G-CSF and IL-4 were respectively: 0.5ng/ml, 30pg/ml and 10ng/ml. The doses were selected according to the data reported in the literature for the stimulation of phagocytic mononucleated subsets (Tanaka et al., 1991; Lutz et al., 1996; Reddy et al., 1997).

As control, PHA solution was used (1%, Gibco BRL Poistey, Renfrenshire, UK).

Electron microscopy

Cells were gently transferred into tubes containing an excess volume of a freshly prepared solution of 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4). After fixation (2 hours) at 4 °C, the cells were centrifuged, washed three times in 0.1M cacodylate buffer containing 1% sucrose and post-fixed for 1 hour at 4 °C in 1% OsO_4 diluted in cacodylate buffer. After several washings the cells were pre-embedded with 2% agar in buffer, dehydrated in a graded series of ethanol and embedded in Epon 812. Thin silver to gold sections were stained with saturated uranyl acetate in 50% acetone and with Reynold's lead citrate and were examined with a Zeiss EM 900 operating at 50 kV.

Cytochemical reactions

The following cytochemical reactions were performed: naphtol-AS-D-cloroacetate esterase [NCAE; EC 3.1.1.2] (Wachstein and Wolf, 1958); α-naphthylacetate esterase [NAE; EC 3.1.1.1] (Yam et al., 1978); α-naphthyl-butyrrate esterase [NBE; EC 3.1.1.6] (Higgy et al., 1977); acid phosphatase [AP; EC 3.1.3.2] (Li et al., 1970); alkaline phosphatase [ALP; E.C. 3.1.3.1] (Yam et al., 1978) peroxidase [PerOX; E.C. 1.11.1.7] (Burstone, 1958; Graham and Karnovsky, 1966); dihydrofolate reductase [DHFR; EC 1.5.1.3] (Gerzeli and De Piceis Polver, 1969); dipeptidyl(amino) peptidase IV [DAP IV; EC 4.4.14.5] (Loyda, 1977) and serine esterase BTL-dependent (SE) (Wagner et al., 1993). Control samples of the enzymatic reactions were performed by omitting the substrate; samples were then mounted in buffered glycerine and examined by light microscopy. Enzyme positivity was studied by qualitative evaluation (Chilosi et al., 1982). The percentage of positive cells was estimated on a range of 350-700 cells per sample.

Morphological stain

The cell smears obtained from different cultures were stained with May-Grunwald Giemsa (MGG) for the morphological identification of the cell types. The scoring was performed by inspecting a sample ranging from 350 to 700 cells on MGG-stained specimens.

Statistical analysis

The double-tailed Fisher's exact test was employed for each comparison made.

Results

We studied the growth response of human blood monocytes after stimulation with the following different factors: IL-2, GM-CSF, G-CSF and IL-4. The different growth factors were used alone or in combination, by treating circulating monocytes for a maximum of 16 days culture. Differences in cell proliferation were observed in the various conditions and in relation to the time of culture. In fact, at early culture time, the highest recovery of proliferating cells was detected in PHA, PHA plus IL-2 and IL-2 treated cultures with the duplication of the culture at 3rd day. At prolonged time (10-21 days), the cells treated with PHA or G-CSF underwent to degeneration. In G-CSF treated cells we observed rare survival clones after a 21 days incubation. At early culture time (3 to 8 days) we observed, in all conditions, the appearance of cells with dendritic morphology (DC) accompanied by large mononucleated cells like activated monocytes. Within the monocytoid population we considered the typical monocyte (m) (11-14 μm in diameter) with a peripheral folded nucleus, and enlarged cells (M) (30-35 μm in diameter) with roundish or irregular nucleus. The results of the scoring of the different cells (m, M, DC) are reported in table 1. The data obtained with the factors under study are compared with the data obtained in PHA-stimulated cultures.

In all conditions, the highest percentage of monocytoid cells (m+M) was observed in the 8-day cultures with a mean value of heighty percent of the total cells (range: 70-90%). The lowest percentage of monocytes was obtained in the GM-CSF- and G-CSF plus GM-CSF-treated cultures (72% and 70% respectively).

After a 16-day culture the percentage of monocyte cells was generally reduced. Only in the cultures treated with IL-4 no difference was observed between the 8- and 16-day cultures. On the contrary, in the PHA-treated cultures an increased percentage of monocytes was observed. Distinguishing the monocytoid cells into the two morphological types described above, we could

deduce from the analysis, that the M cells generally decreased or remained at the same value with the prolonged time in culture. Only after the treatment with IL-4, these cells showed a ten percent increase over the mean recovery.

The highest amount of dendritic cells after eight days culture was obtained after treatment with IL-2, GM-CSF, IL-2 plus G-CSF and G-CSF plus GM-CSF (20, 28, 20 and 26 percent of the total cell recovery respectively). Evaluating the variation in dendritic cell incidence with increased time in culture we observed that in the presence of IL-4 or PHA no increase in the percentage of dendritic cells was obtained. On the contrary, in the remaining treatment conditions, an increase in the percentage of cells with dendritic morphology was observed. Particularly significant ($p<0.0001$) was the increase in dendritic cells observed in the cultures treated with IL-2 plus GM-CSF and in the cultures treated with IL-2, GM-CSF plus IL-4 (seven and six times respectively).

The enzymatic characterization of the cells was performed by cytochemical reactions for the following enzyme activities: naphtol-AS-D-cloroacetate esterase (NCAE), α-naphthylacetate esterase (NAE), α-naphthylbutyrrate esterase (NBE), acid phosphatase (AP), alkaline phosphatase (ALP), peroxidase (PerOX), dihydrofolate reductase (DHFR), dipeptidyl(amino) peptidase IV (DAP IV) and serine esterase BTL-dependent (SE) as described in Materials and Methods. Fig. 1a shows cells with a dendritic subtype morphology after MGG staining and the different histochemical enzyme reactions (Fig. 1b-g).

These cells were observed in all conditions and the highest percentage was observed in the cultures treated with GM-CSF and IL-2 after 16 days of incubation.

The percentages of cells showing positivity for seven different enzymatic activities studied are reported in Fig. 2, where the data were derived from three independent experiments.

The NCAE, NAE, NBE and AP activities, which are common activities of monocytic lines, resulted in a diffuse granular staining over all the monocyte cells obtained. Most of the cells with dendritic morphology displayed a dot-like pattern of reactivity and a small percentage were negative or showed a scattered fine and granular pattern. Comparing the two times of observation (8 and 16 days) the dendritic cells showing a diffuse cytoplasmic reactivity increased with the prolonged time of culture. On the contrary, the frequency of dendritic cells showing a moderate granular pattern for PerOX activity did not display the same trend. In haematic cells PerOx activity was present in the granulocytic lines and positive reactions may occur in monocytes.

All cell types were positive for DHFR activity. This enzyme is particularly important in metabolic processes during proliferation of the cell. DAP IV is a post-proline hydrolytic enzyme that cleaves dipeptides from the N-terminal region of polypeptides and is expressed mainly on the activated T cells surface. SE is associated with the

Table 1. Percentage of small monocyte cells (m), enlarged monocytoid cells (M), total monocytoid cells (m+M), dendritic cells (DC) and large monocytoid cells plus DC (M+DC) in the cultures treated with different growth factors and in the PHA-treated cultures. Mean values (±SD) obtained from triplicate experiments.

GROWTH FACTORS	DAYS OF CULTURE	m	M	m+M	DC	DC+M
IL-2	8	35±1.5	45±0.8	80	20±1.5	65
	16	26±1.1	35±1.8	61	39±1.1	74
IL-4	8	39±2.3	52±2.1	91	9±1.2	61
	16	30±1.7	61±1.9	91	9±1.0	70
G-CSF	8	25±1.5	63±2.1	88	12±3.6	75
	16	27±2.5	48±1.7	75	25±1.9	73
GM-CSF	8	21±2.7	51±2.0	72	28±3.5	79
	16	20±3.1	43±1.6	63	37±2.6	80
IL-2+IL-4	8	58±2.2	26±1.7	84	16±8.5	42
	16	34±1.1	28±1.2	62	38±1.4	66
IL-2+G-CSF	8	48±1.7	32±2.1	80	20±2.9	53
	16	41±1.4	29±2.1	70	30±1.3	59
IL-2+GM-CSF	8	23±0.9	67±1.9	90	10±1.8	77
	16	14±2.8	15±1.6	29	71±2.0	86
IL-2+IL-4+GM-CSF	8	73±3.1	21±1.8	94	6±2.1	37
	16	41±2.1	23±1.9	64	36±2.4	59
IL-4+GM-CSF	8	38±1.9	49±1.3	87	13±1.6	62
	16	32±0.2	48±1.4	80	20±1.3	68
G-CSF+GM-CSF	8	40±1.4	30±1.7	70	30±1.6	60
	16	31±0.7	7±1.29	38	62±2.2	69
PHA	8	62±2.3	21±1.9	83	17±1.9	38
	16	74±3.3	18±1.9	92	8±1.65	26

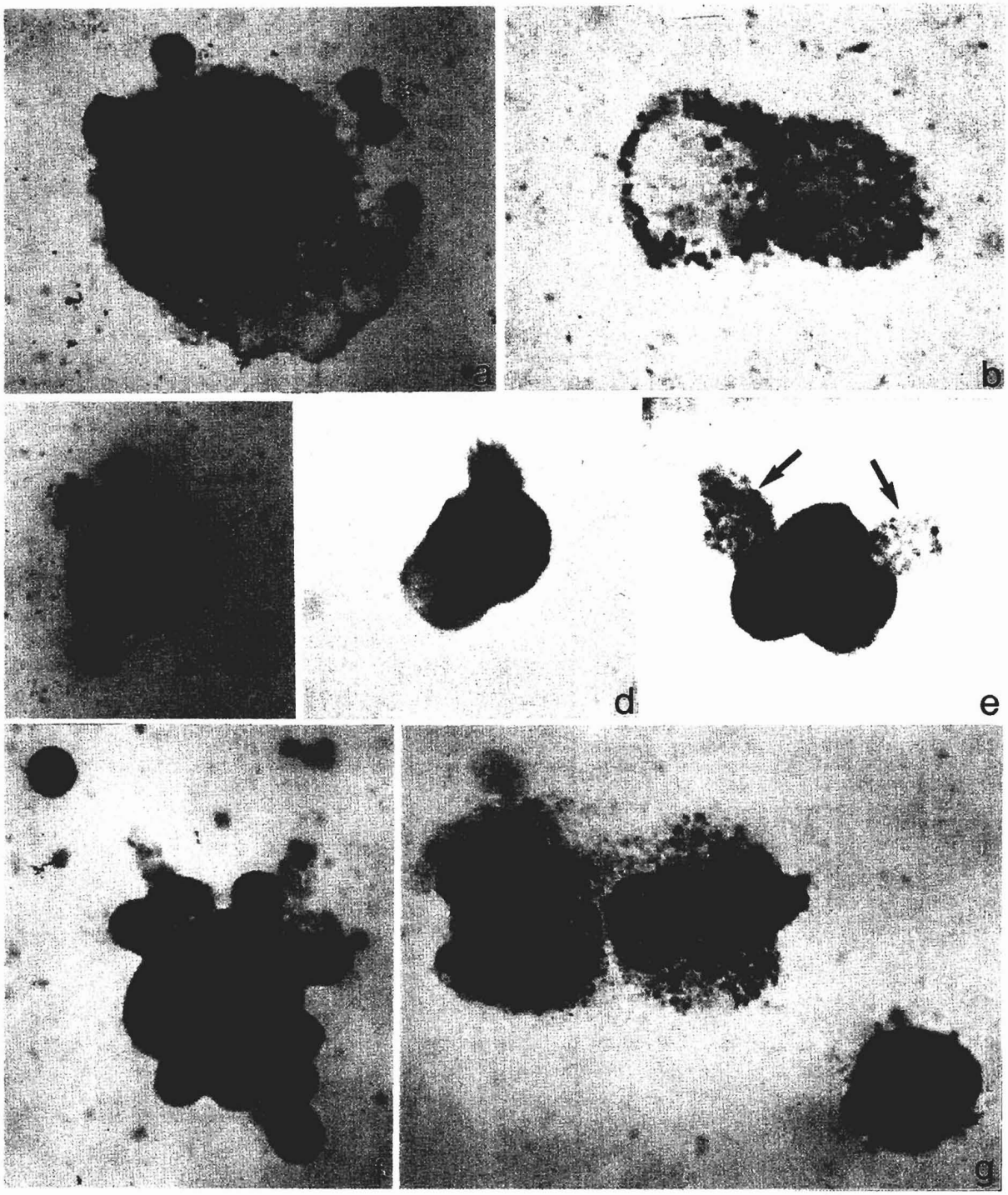
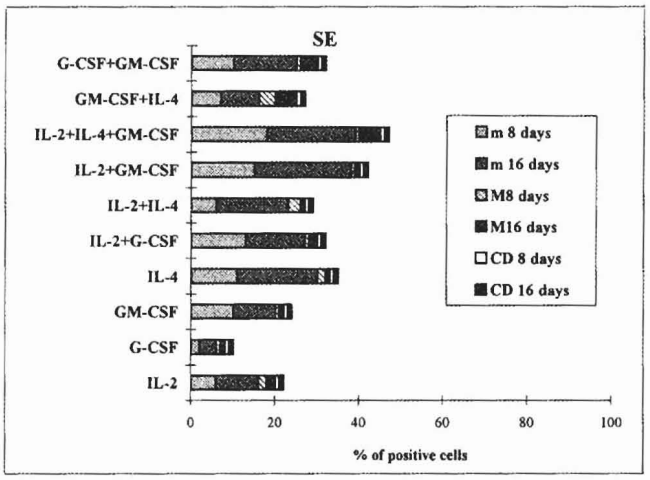
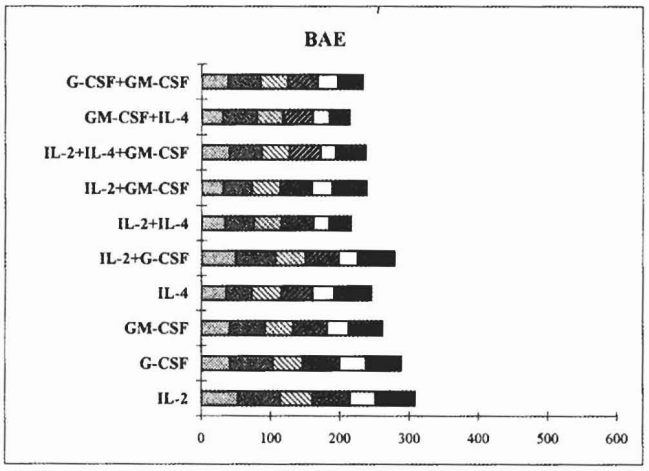
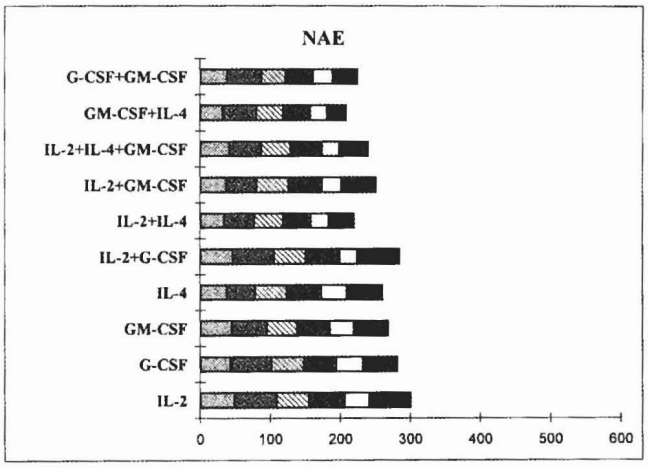
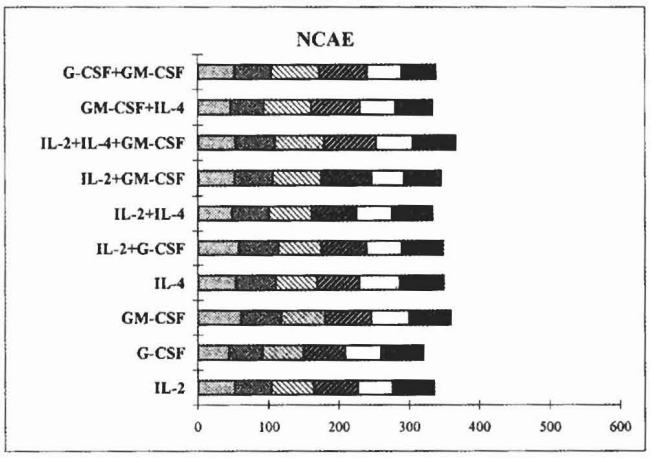


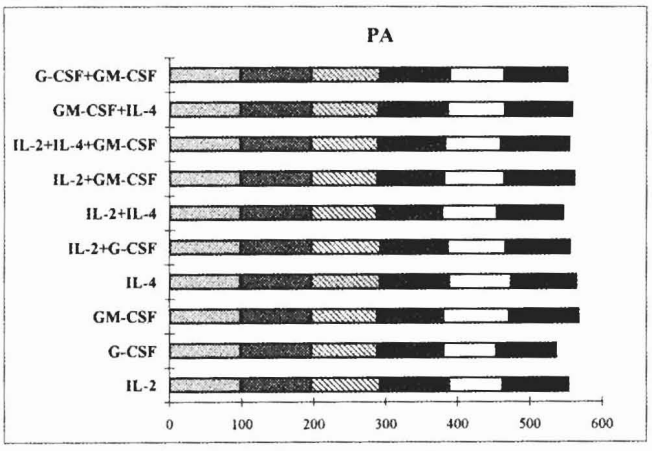
Fig. 1. a. Cell with dendritic morphology stained with May Grunwald Giemsa. Cultures treated with GM-CSF plus IL-2. A low nucleus/cytoplasm ratio and cytoplasmic protusions (black star) are shown. The cells are interacting with some lymphocytes (white star). **b.** A cell with dendritic morphology showing a positive and coarsely granular dihydrofolate reductase reaction. **c.** An enlarged monocytoid cell (arrow) with a diffuse staining product of butirrate esterase. Lymphocytes with few scattered granules are also present. **d.** An irregular monocytoid cell showing a moderate positive pattern for peroxidase activity. **e.** Cells with dendritic morphology showing diffuse and intense naftol-AS-D-cloroacetate esterase stain product. The arrows indicates scattered positive granules in cytoplasmatic tails. **f.** An enlarged monocytoid cell showing a strong acid phosphatase positivity that interact with lymphocytes positive for AP staining. **g.** Two cells with dendritic morphology and an enlarged monocytoid cell showing diffuse and finely granular acid phosphatase reaction over nucleus and cytoplasm. x 1,200

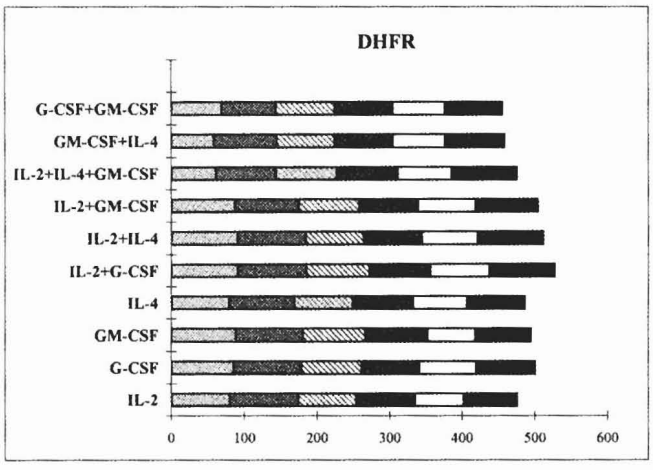












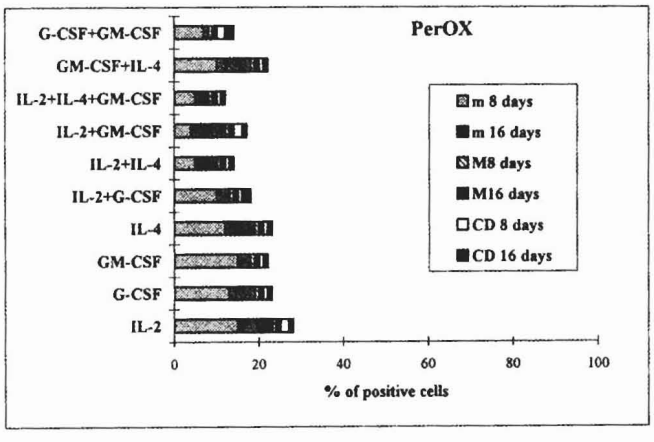


Fig. 2. Positivity for enzymatic reactions: naphtol AS-D-cloroacetate esterase (NCAE), α-naphtilacetate esterase (NAE), α-butirate esterase (BAE), acid and alcaline phosphatase (AP), peroxidase (PerOX), dihydrofolate reductase (DHFR), serine esterase BTL-dependent (SE). Percentage of small monocyte cells (m), enlarged monocyte cells (M) and dendritic cells (DC), treated with different growth factors for 8 and 16 days. Mean values obtained from three independent experiments.

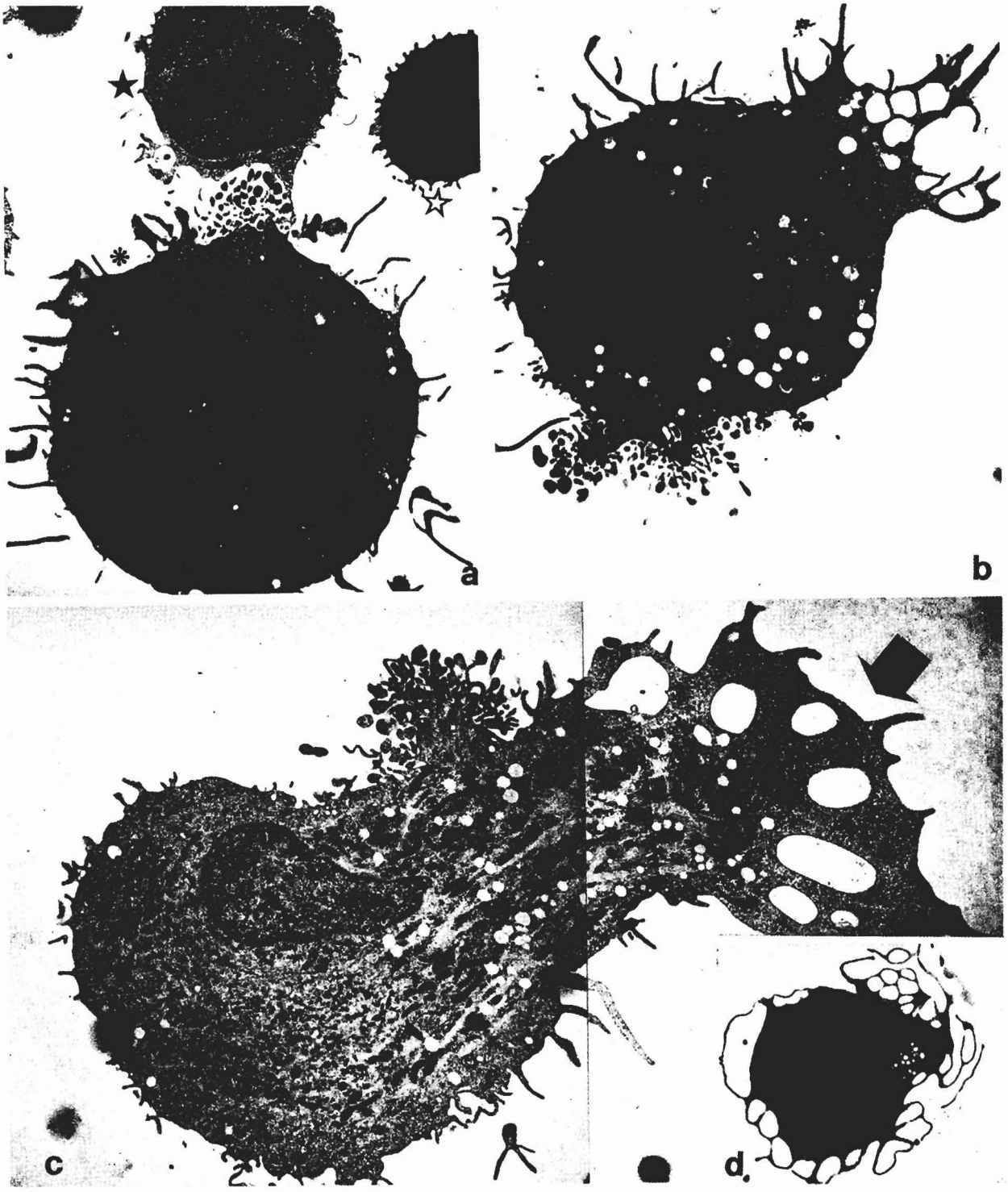


Fig. 3. Electron microscope morphology. **a.** A monocytoid cell (black star), an enlarged monocytoid cell (white star). A small lymphocyte is also detected (l). **b.** Profile of a dendritic cell showing the typical spiny processes. **c.** A large dendritic cell showing a cytoplasmatic weave (large arrow). Cultures treated with GM-CSF and IL-2. Note the high incidence of mitochondria, lysosomes and lipidi droplets. **d.** Apoptotic cell of probable dendritic derivation showing a network of spiny processes. a-c, x 3,000; d, x 7,000

cytotoxic activity of some T cell subsets. The ALP activity of blood cells is typically localised in mature cells of the granulocyte series and is poorly expressed in monocytoid line (Beckstead et al., 1981). All cell types resulted negative for these enzyme reactions at both times of observation (8 and 16 days).

At the ultrastructural level, some subcellular morphological parameters were studied in order to characterize the functional state of cells during their differentiation into the dendritic state.

The ultrastructure was examined in cells obtained from 16 days cultures. We observed, in all conditions, a high level of cellular heterogeneity. In the cultures maintained in presence of IL-2 we observed 1) monocytoid cells with a folded nucleus, 2) enlarged cells with a round shape, asymmetrical nucleus and abundant cytoplasm with heterogeneous lysosomes (Fig. 3a), and 3) irregularly shaped cells with cytoplasmic projections, nuclear invaginations, rich chondrioma and diffuse ribosomes (Fig. 3b). From the above described features we considered that these last cells belonged to the dendritic subset at terminally differentiation stage (mature dendritic cells) (Fig. 3c). These cells presented small, homogeneously shaped lysosomes, with variable electron density. In these cells, spherical droplets with a variable degree of osmiophility related to lipid vacuoles were also found.

Small lymphocytes interacting with dendritic cell were frequently observed. In particular, during the interactions, cytoplasmic projections from the large irregular cells surrounded the lymphocytes. An interesting finding was the observation of dendritic cells presenting a chromatin condensation typical of apoptotic degeneration (40%) (Fig. 3d).

Cells from cultures treated with GM-CSF presented mainly monocytoid feature and dendritic morphology.

The cells stimulated with IL-4 or G-CSF showed similar ultrastructural features: presence of monocytoid cells; a lot of enlarged round cells with abundant cytoplasm enriched with pleomorphic granules; and dendritic cells. In these sample we noticed many cells undergoing degenerative processes with disintegration of the cytoplasm and organelles (35%).

The cultures stimulated with IL-2 plus IL-4 or IL-2 plus G-CSF appeared ultrastructurally similar: cells with monocyte and dendritic features. Moreover, patterns of interaction between small lymphocytes and dendritic cells were observed. On the other hand, a distinctive finding of these cultures was the observation that dendritic cells showed small homogeneous lysosomes and presented multivesicular bodies.

In the cell samples obtained from cultures stimulated with IL-2 plus GM-CSF and IL-2 plus GM-CSF and IL-4, cells with monocyte features, many large round cells with pleomorphic granules, cells with dendritic pattern and many lymphocytes were present. Like the dendritic cells observed in the IL-2-treated samples, these cells showed homogeneous lysosomes, and the presence of apoptosis was remarkable (40-45%).

In the cultures maintained in the presence of IL-4 plus GM-CSF, cells with monocytoid and dendritic morphology were found together with many cells showing a degenerative pattern.

Discussion

In the present work we studied the effect of IL-2 on the differentiation of human blood monocytes into dendritic cells *in vitro*, following treatment with IL-2, alone or in combination with other growth factors (IL-4, G-CSF, GM-CSF). By ultrastructural analysis we concluded that full differentiation of DCs only occurs in the presence of IL-2 (Hart,1997). We previously reported the observation of well differentiated dendritic cells (Capelli et al., 1997) in peripheral blood cultures treated with IL-2. In the present study we observed a synergic action of IL-2 and other cytokines, such as IL-4, G-CSF and GM-CSF. In these conditions, in addition to well differentiated DC, we observed the highest frequency of interactions between dendritic cells and lymphocytes. This could be related to the functional activity of DCs as antigen-presenting cells. In fact, mature DC localised in the lymphonodes are always found in association with T lymphocytes (Wu et al., 1996; Imai et al., 1998). At the end of the maturation process DCs lose antigen-capturing ability and become immunostimulatory cells that trigger naive T cells recirculating through lymphoid organs (Ardavin et al., 1993; Thomas et al., 1993). The presence of activated lymphocytes in our cultures is probably the consequence of clonal expansion of contaminating leukocytes. It is reasonable to think that functional maturation of DCs depends on the presence of interleukin-2 and on the release of the same cytokine by stimulated T lymphocytes (Capelli et al., 1997). Other authors demonstrated that the presence of T lymphocytes is essential for the maturation of dendritic cells (Caux et al., 1994). In the cultures treated with IL-2 only, we did not observe an increase in proliferating cells but only changes in phenotypic features of DCs. In the cultures treated with IL-2 and GM-CSF or with IL-2, IL-4 and GM-CSF, we also observed increased proliferation. Some authors reported that GM-CSF and IL-4 are able to induce the differentiation of cells with dendritic morphology (Sallusto et al., 1994). We confirm this observation and moreover, our data indicate that DC show a functional maturation pattern only in the presence of IL-2. The enzymatic activities observed in DC obtained in our conditions, resulted lower than those observed in monocytes. In fact, the enzymatic features changed with the culture time: before the differentiation into DC, the stimulated cells expressed the typical enzymatic pattern of monocytes. At an advanced stage of differentiation, some enzyme activities change. In terminally differentiated dendritic cells the reactions for peroxidase and serine esterase are usually negative (Hu et al., 1993).

An interesting finding was the presence of apoptotic

cells derived from DC in the cultures showing the highest incidence of mature cells. This is in agreement with the predisposition to apoptosis of terminally differentiated DC (Santiago-Schwarz et al., 1997). Apoptotis is considered to be a control phenomenon in cell growth and differentiation. It is important to note that apoptotic events, which occur at terminal maturation, play a role in supporting DC lineage selection and homeostasis. Distinct apoptotic events could be associated with different phases of DC development in cultures (Santiago-Schwarz et al., 1997).

Ultrastructural study allows the detailed observation of some functional aspects of differentiated cells. In each culture condition we observed, in immature DC, large heterogeneous granules. The presence of this type of granule could be involved in phagocytic activity. Phagocytosis is a pivotal function of the monocyte/macrophage system. Langerhans cells that are DC precursors have been shown to be phagocytic. It has been demonstrated that phagocytic activity is present in DCs at early differentiation steps only (Reis et al., 1993). The presence of heterogeneous lysosomes and phagosomes is indicative of intense phagocytic activity of the cells. In the present study we observed that DC recovered from IL-2-treated cultures were characterized by homogeneous lysosomes in the cytoplasm, differing from DC in the cultures in presence of the other studied factors and without IL-2, that also presented heterogeneous granules. The presence of endosomes and homogeneous lysosomes is essential for antigen processing (Reis et al., 1993). It is well-known that mature dendritic cells of lymph nodes containing MHC class II proteins and antigenic peptides, have thin and homogeneous granules (Kleijmeer et al., 1995). DCs from the cultures maintained in the presence of IL-2 alone, or in combination with GM-CSF and/or IL-4, have mainly this type of granule. Many data refer to the ability of IL-4 and GM-CSF to support DC growth *in vitro*. Our data are in agreement with these findings. In fact from our previous observation and the present results the IL-2 induces an enhancement of DC growth and differentiation. For the presence in these cells of a lysosomal pattern similar to that observed in the mature DC of lymph nodes, we suppose that IL-2 may act as a maturative factor rather than as a growth factor.

References

Ardavin C., Wu L., Chung-Leung L. and Shortman K. (1993). Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. Nature 362, 761-763.

Banchereau J. and Steinman R.M. (1998). Dendritic cells and the control of immunity. Nature 392, 245-252.

Beckstead J.H., Halverson P.S., Ries C.A. and Bainton D.F. (1981). Enzyme histochemistry and immunohistochemistry on biopsy specimens of pathologic human bone marrow. Blood 57, 1088-1098.

Burstone M.S. (1958). Histochemical comparison of naphtol-AS substrates using frozen-dried tissue. J. Histochochem. Cytochem. 6, 87-89.

Capelli E., Nano R. and Barni S. (1997). Dendritic cell differentiation in a peripheral blood mononucleated cell culture treated with interleukin-2. Haematology 82, 634.

Caux C., Massacrierc C. and Vanbervliet B. (1994). Activation of human dendritic cells through CD40 cross-linking. J. Exp. Med. 180, 1263-1272.

Chilosi M., Pizzolo G., Janossy G., Bofill M. and Fiore-Donati L. (1982). Enzyme histochemical analysis on cryostat sections of human bone marrow. J. Clin. Pathol. 35, 1220-1226.

Gerzeli G. and De Picel Polver P. (1969). Un nuovo metodo citochimico per lo studio dell'attività tetraidrofolico reduttasi in Plasmodium borghei: analisi dell'effetto di inibitori specifici. Riv. Parassitol. 30, 19-26.

Grabble S., Beissert S., Schwarz T. and Granstein R.D. (1995). Dendritic cells as initiator of tumor immune responses: a possible strategy for tumor immunotherapy. Immunol. Today 16, 117-121.

Graham R.C. and Karnovsky M.J. (1966). The early stages of absorption of injected horseradish peroxidase in the tubules of mouse kidney: ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. 14, 291-302.

Hart D.N.J. (1997). Dendritic cells: unique leukocyte populations which control the primary immune response. Blood 90, 3245-3287.

Higgy K.E., Burns G.F. and Hayhoe F.G.J. (1977). Discrimination of B, T and null lymphocytes by esterase cytochemistry. Scand. J. Haematol. 18, 473-48.

Hu Zb.B. Ma W., Uphoff C.C., Metge K., Gignac S.M. and Drexler H.G. (1993). Myeloperoxidase: expression and modulation in a large panel of human leukemia-lymphoma cell lines. Blood 82, 1599-1607.

Imai Y., Yamakawa M. and Kasajima T. (1998). The lymphocyte-dendritic cell system. Histol. Histopathol. 13, 469-510.

Kleijmeer M.J., Osservoort M.A., Van Veen C.I.H., Van Hellemond J.J., Neefjes J.J., Kast W.M., Melief C.I.M. and Genze H.S. (1995). MHC class II compartments and the kinetics of antigen presentation in activated mouse spleen dendritic cells. J. Immunol. 154, 5715-5724.

Li C.Y., Yam L.T. and Lam K.W. (1970). Acid phosphatase isoenzyme in human leukocytes in normal and pathologic conditions. J. Histochem. Cytochem. 18, 473-481.

Loyda Z. (1977). Studies on glycil-proline naphthylamidase I. Histochemistry 54, 299- 309.

Lutz M.B., Abman L.U., Girolomoni G. and Ricciardi Castagnoli P. (1996). Different cytokines regulate antigen uptake and presentation of a precursor DC line. Eur. J. Immunol. 26, 586-594.

Peters J. H., Gieseler R., Thiele B. and Steinbach F. (1996). Dendritic cells: from ontogenic orphan to myelomonocytic descendant. Immunol. Today 17, 273-278.

Reddy A., Sapp M., Feldman M., Subklewe M. and Bhardway N. (1997). A monocyte conditioned medium is more effective than defined cytokines in mediating the terminal maturation of human DC. Blood 90, 3640-3646.

Reis E.. Sousa C., Stahl P.D. and Austyn J.M. (1993). Phagocytosis of antigens by Langerhans cells *in vitro*. J. Exp. Med. 178, 509-519.

Sallusto F. and Lanzavecchia L. (1994). Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by GM-CSF plus IL-4 and down regulation of TNF-α. J. Exp. Med. 179, 1109-1118.

Santiago-Schwarz F., Borrero M., Tucci J., Palaia T.and Carsons S.E . (1997). *In vitro* expansion of CD13+CD33+ dendritic cell precursors from multipotent progenitors is regulated by a discrete fas-mediated

apoptotic schedule. J. Leukoc. Biol. 62, 493-502.

Steinman R.M. (1991). The dendritic cell system and its role in immunogenicity. Annu. Rev. Immunol. 9, 271-296.

Tanaka T., Suda T., Suda J., Inoue T., Hirabayashi Y., Hirai H., Takaku F. and Miura Y. (1991). Stimulatory effects of granulocyte colony-stimulating factor on colony-forming units-spleen differentiation and pre-proliferation in mice. Blood 77 , 2597-2602.

Thomas R., Davis L.S. and Lipsky P.E . (1993). Comparative accessory cell function of human peripheral blood dendritic cells and monocytes. J. Immunol. 15, 6840-6852.

Wachstein M. and Wolf G. (1958). The histochemical demonstration of esterase activity in human blood and bone marrow smears. J. Histochem. Cytochem. 6, 457-458.

Wagner L., Sunder-Plassman G., Base W., Wieshlzer M., Sexl V., Lang G. and Worman C.P. (1993). *In vivo* and *in vitro* primed lymphocytes: correlation of cytochemically detected BTL. Specific lymphoid serine protease with cytotoxic activity. J. Immunol. Meth. 160, 173-180.

Wu L., Li C.L. and Shortman K. (1996). Thimic DC precursors: relationship to the T lymphocyte lineage and phenotype of the dendritic cell progeny. J. Exp. Med. 184, 903-911.

Yam L.T., Li C.Y. and Crisby W.H. (1978). Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. 55, 283-290.

Young J.W., Szalboles P. and Moore M.A.S. (1995). Identification of dendritic colony-forming units among human CD34+ bone marrow progenitors that are expanded by c-kit ligand and yield pure dendritic cell colonies in the presence of granulocyte/macrophage colony stimulating factor and tumor-necrosis factor-α. J. Exp. Med. 182, 1111-1120.

Accepted May 31, 2000