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Transformation of Blood Monocytes to Multinucleated Giant Cells in vitro: Are there any Differences Between Malignant and Nonmalignant States ?

M. MUNZAROVÁ, D. ZEMANOVÁ

Department of Immunology and Tissue Cultures, Masaryk Memorial Cancer Institute, Brno

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Summary

Blood monocytes (BMs) from 139 subjects (70 malignant melanoma patients, 31 breast cancer patients, 38 healthy controls) were cultured for at least 7 days. The formation of multinucleated giant cells (MGCs), which was checked during the whole time of culture, was observed in all cases. By the seventh day MGCs represented 25-50 % and during the second and third month more than 90% of all cells. Lymphokines and/or concanavalin A stimulation (16-34 cases respectively) of BMs was performed as well. This stimulation greatly accelerated MGC formation. There were no differences either in spontaneous or in stimulated fusion between the different groups compared.

Key words

Cell fusion – Monocytes – Macrophages – Melanoma – Breast cancer – Multinucleated giant cells

Introduction

Macrophages (cells of myelo-monocyte-macrophage series) are a heterogeneous population of cells, unique in their genetically determined tendency to fuse (Ringertz and Savage 1976, Johnston 1988). In response to certain poorly characterized stimuli both immunological and nonimmunological, these cells may differentiate further into so called epithelioid cells and may then go to form multinucleated giant cells (MGCs) seen in granulomas of different origin (Chambers and Spector 1982, Epstein and Fukyama 1989). In clinical situations these conditions range from tuberculosis, leprosy and syphilis on the one hand to asbestosis and silicosis on the other. Transformation of blood monocytes (BMs) and macrophages to MGCs (in this study MGCs are defined as large cells having two or more nuclei) were proved to occur both *in vivo* (Mariano and Spector 1974, Smetana 1987) and *in vitro* (Chambers 1977, Postlethwaite *et al.* 1982, Schlesinger *et al.* 1984, Al-Sumdaie 1986, Nagasawa *et al.* 1987, Kreipe *et al.* 1988). In this case the path of differentiation depends in part on the substrate on which the cells are cultured (Mosser and Edelson 1984).

It has been reported that BMs from breast cancer patients when incubated for 6 days *in vitro*, form MGCs in almost all cases while this formation is only exceptional for BMs of healthy control subjects (Al-Sumidaye 1986, Al-Sumidaye *et al.* 1986). The presence of retroviruses in BMs of breast cancer patients was suggested to be the reason for this situation (Al-Sumidaie *et al.* 1986, Al-Sumidaie *et al.* 1988). The aim of the present study was to determine whether or not BMs from malignant melanoma patients form MGCs *in vitro* and whether there are differences in this respect between melanoma patients, breast cancer patients and normal healthy controls. Spontaneous MGC formation was a matter of special attention. In several cases this formation was induced (or better accelerated) by lymphokines derived from autologous lymphocytes stimulated by concanavalin A (Con A) and/or by Con A alone.

Material and methods

Altogether, 139 cases were examined in the present study.

Patients:

Seventy malignant melanoma patients, mostly of stage I and II of the disease from whom blood was taken before the beginning any therapy.

Thirty-one breast cancer patients at different stages of the disease. Blood was taken either before the beginning radio- and/or chemotherapy or at least two years after the end of therapy, usually when progression of the disease was observed.

Controls:
Thirty-eight control subjects were represented by healthy volunteers (24) and by persons with innocent and quite benign conditions, e.g. *hernia inguinalis* (14).

BM separation and culture conditions:
Fresh, heparinized venous blood (5-10 ml) was subjected to density gradient centrifugation with lymphoprep TM (Nyegaard Co A/S, Oslo, Norway). The mononuclear cells of the interphase were seeded on a glass coverslip put into 16 mm-diameter culture wells, in a density of 3-5 x 10^6/ml D-MEM (Dulbecco s modification of Eagle s medium with glutamine without sodium bicarbonate (Flow Laboratories-Irvine – KA 128NB, Scotland) supplemented with 12 % foetal calf serum and antibiotics. BMs were separated from other mononuclear cells by adherence for 2 hours, nonadherent cells were removed by washing. Adherent cells were held in culture overnight with subsequent washing, the monolayer on coverslip contained then more than 95 % of BMs as determined by positive staining for CD-14/antibody anti CD-14 -- Immunotech Marseille Cedex – against CD 14 on human monocytes and macrophages and MEM 18 (Bažil *et al.* 1986, Hořejší *et al.* 1986), antibody against a monocyte antigen of 53 kDa, both these antibodies detect probably the same epitope. Cells were cultured at 37 °C in a humidified atmosphere containing 5 % CO_2. The medium was changed each third day.

The spontaneous formation of syncytial cell groupings and MGCs
(bi- and trinucleated, foreign body giant cells, Langhans giant cells and Touton giant cells) was checked with an inverted microscope during the whole time of culture in all cases.

In vitro stimulation of BMs:
In 16 cases (proportionally from all three groups compared) nonadherent cells, predominantly lymphocytes, removed from adherent cells after 2 h by washing, were seeded in a density of 3-5 x 10^6 cells/ml D-MEM in 16 mm-diameter culture wells and stimulated over 48 hours by addition of 6 μg Con A (Pharmacia, Sweden). Cell-free lymphokine-rich supernatant was then given enriched by fresh D-MEM (2 : 1) to autologous 48 hours BM cultures for the next 48 hours.

In 34 cases (proportionally from all three groups compared) Con A itself at the concentration of 6 μg/ml was added to 48 hours BM cultures for the next 24 and/or 48 hours.

Cell culturing was finished at different intervals, in cases of stimulated fusions either immediately after stimulation and/or 24 hours later, in cases of spontaneous fusion usually between the 7th and 9th day. In six cases cell cultures were observed during 3 months (at the end of the third month all cultured cells died). Glass coverslips were then stained with May-Grünwald-Giemsa stain.

Results

1. Spontaneous formation of MGCs (139 samples):

From the second day of cultivation a fusing tendency of BMs was evident : great grouping of cells was invariably observed. During the third and fourth day cell grouping syncytial in character were found in all samples (Fig. 1) and in some of them (25 %) isolated evident foreign body giant cells and Langhans giant cells were found. During consecutive days the number of MGCs of different types and size increased. By the seventh day such cells were present in all samples and represented approximately 25-50 % of all cells with great individual differences. A greater density of MGCs was frequently found near the border of the glass. During the second and third month of cultivation multinucleated cells represented invariably more than 90% of all cells (confirmed in 6 samples – Fig. 2).

2. Stimulation of BMs by lymphokine-rich supernatant (16 samples):

In all cell samples during the third and fourth day of cultivation (48 hours' culture of BMs in D-MEM and 48 hours' culture in lymphokine-rich supernatant) the formation of MGCs was observed. At the end of the fourth day such cells, predominantly of the Langhans type, represented 80-90 % of stimulated BMs on the average (Fig. 3).

3. Stimulation of BMs by Con A (34 samples):

a) 48 hours' culture in D-MEM + 24 hours' culture in Con A enriched medium + 24 hours culture in D-MEM without Con A: At the end of Con A stimulation, foreign body giant cells and Langhans giant cells were formed (Fig. 4). At the end of the fourth day of culture the picture was almost identical to that of BMs stimulated by a lymphokine-rich supernatant (Fig. 3), most cells were represented by Langhans giant cells.

b) 48 hours' culture in D-MEM + 48 hours' culture in Con A enriched medium: Accelerated MGC formation was seen during the third day and at the end of the fourth day of culture great masses of nuclei with cytoplasmatic "baloons" were seen in all cases. Almost all cells were enfolded into these masses (Fig. 5).

Comparison of results in melanoma, breast cancer patients and control subjects:

There were no differences either in spontaneous fusion or in stimulated fusion between patients and the controls and no differences were found in either of these respects between melanoma and breast cancer patients. Variances in MGC formation were found in all groups compared.

Discussion

Our results proved that monocyte-macrophages in short-term tissue cultures fuse and form MGCs. This formation is accelerated in the presence of Con A. Spontaneous formation of MGCs from monocytes is well known (Schlesinger *et al.* 1984) and it is also known, at least for hamster macrophages, that lectins possess the ability to cause agglutination of cells and many other properties postulated to be necessary to cause fusion of macrophages (Chambers 1977).

From this point of view the distinction between Con A "induced" and lymphokine "induced" cell fusion in our study is dubious, since Con A was used for lymphokine production and it was also present in the lymphokine enriched supernatant. We suppose therefore that other authors, who used similar procedures with the supernatant as we (Kreipe *et al.* 1988), cannot define that giant cells were generated by lymphokine stimulation, the action of Con A present in their supernatants cannot be neglected. From our study we can conclude that, in the case of stimulation of BMs, the intensity of cell fusion is predominantly dependent on the concentration of Con A in the medium and on the time of its contact with monocytes.

We did not find any difference in MGC formation between patients with malignant disease and the controls and no differences were found in this respect between melanoma and breast cancer patients.On the contrary, Al-Sumidaie *et al.* (1986 and 1988) reported that BMs from most breast cancer patients form MGCs and that this formation is only exceptional in control healthy subjects. Recent findings of Kahl *et al.* (1991) are, however, quite in agreement with ours. This author found no clear-cut difference in MGC formation between breast cancer patients, patients with benign breast tumour and normal controls and he observed the same fact as we did, i.e. marked subject-to-subject variation in MGC formation in all the groups compared. (Moreover he did not confirm the finding of Al-Sumidaie *et al.* (1988) with respect to the presence of a retrovirus in BMs of breast cancer patients).

In spite of our results, we suppose that investigation of cell fusion between normal cells and of progeny of these hybrids is a very neglected area in cancer research. MGCs are not effete cells, which was generally accepted earlier, but are metabolically active and able to undergo mitosis (Mariano and Spector 1974, Papadimitriou and Van Bruggen 1986, Nickerson and Sordillo 1987).

We ourselves observed mitotic figures in MGCs derived from cultured monocytes and chromosomal analysis of these mitoses is under investigation.

If we consider fusions occurring naturally between cells in the living organism (a fact which was proved - Mariano and Spector 1974, Smetana 1987, Johnston 1988, Epstein and Fukuyama 1989), we must expect abnormal divisions of fused cells, subsequent fusions of daughter cells, chromosome segregations, breaks and rearrangement with possible activation of oncogenes, etc. – possibilities which themselves might switch on processes leading to transformation of resulting hybrids and their progeny (Munzarová and Kovařík 1987, Munzarová *et al.* 1992). We can imagine that monocytes–macrophages might also fuse with cells of other differentiation programs (e.g. melanocytes – Munzarová and Kovařík 1987, Munzarová *et al.* 1989).

Resulting hybrids inherit features of both parents, those of macrophages might be dangerous for patients (localized tissue degradation, production of growth factors - including angiogenetic ones - locomotion behaviour, homing to and proliferation in tissues naturally rich in macrophages, etc.). We suppose therefore that having fusion and postfusion processes in mind, we might open quite new horizons in tumour research.

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Dr. M. Munzarová, Masaryk Memorial Cancer Institute, CS-656 53 Brno, Žlutý kopec 7.

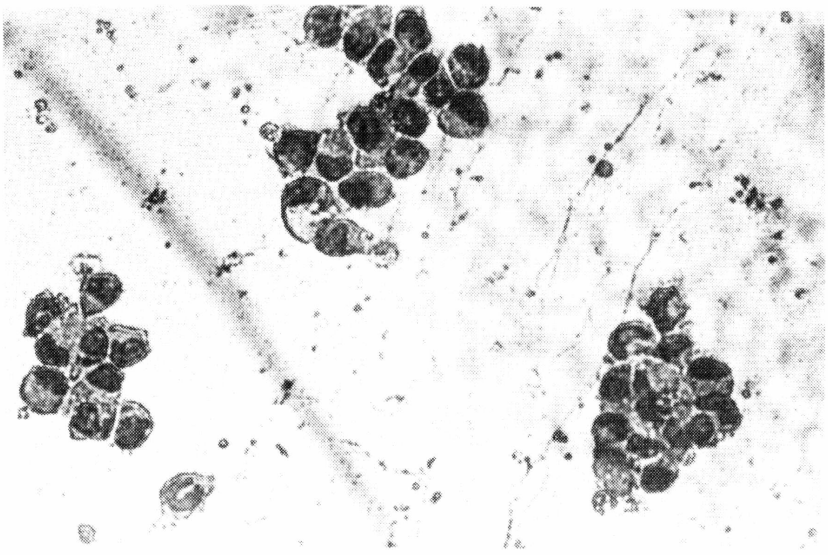

Fig. 1
Syncytial cell groupings: BMs the fourth day in culture. (May-Grünwald-Giemsa, x 40)

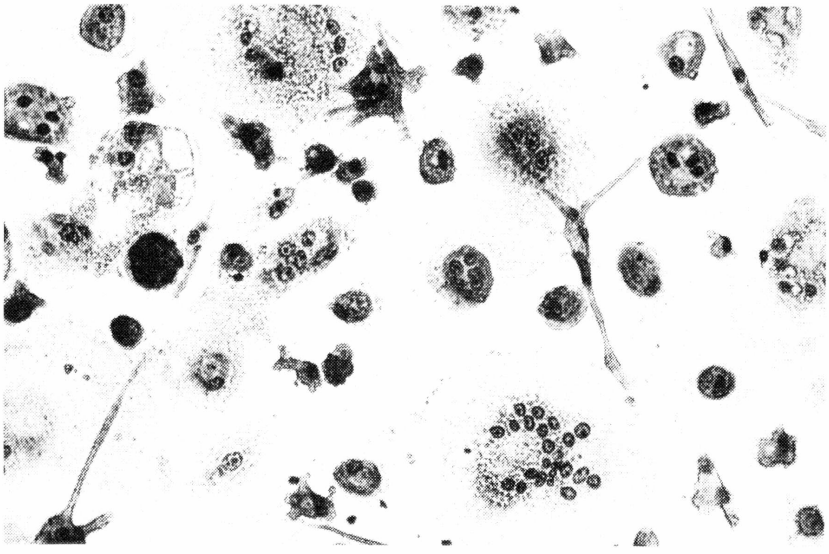

Fig. 2
Different forms of MGCs: BMs-eight weeks in culture. (May-Grünwald-Giemsa, x 16)

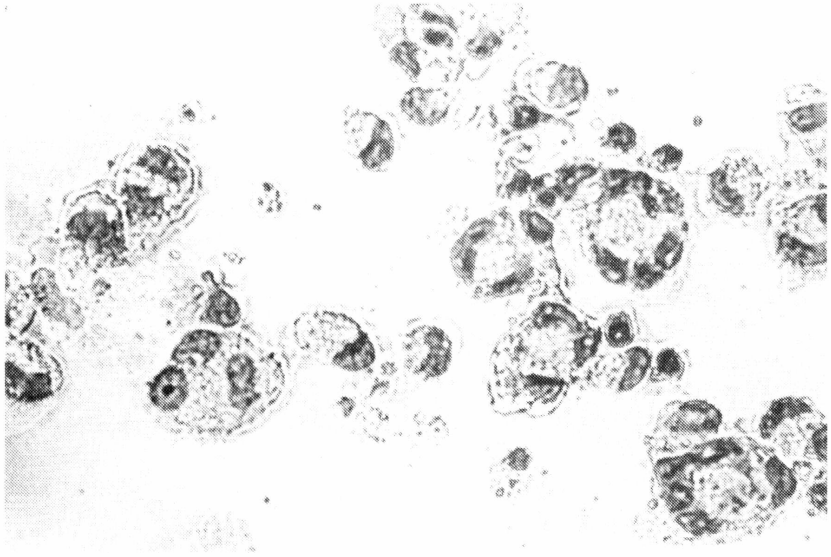

Fig. 3
MGCs – predominantly Langhans giant cells: BMs – the fourth day in culture, after 48 hours' stimulation with lymphokines. (May-Grünwald-Giemsa, x 40)

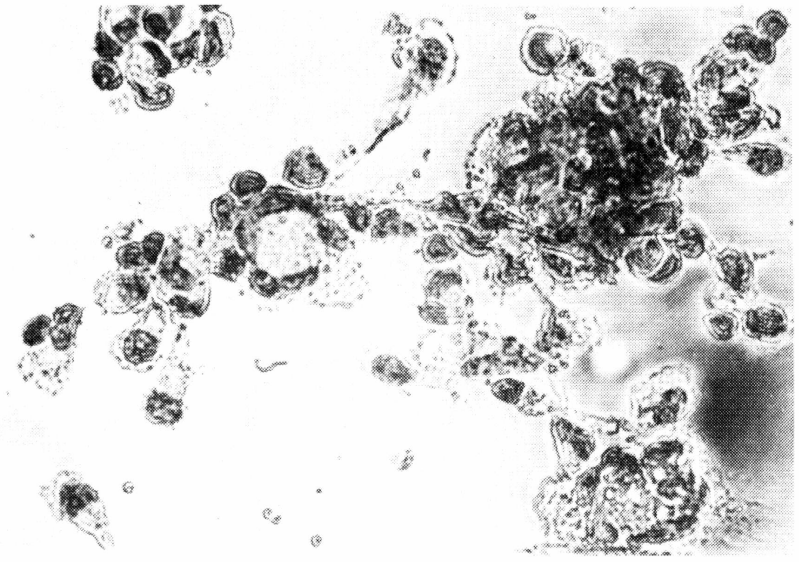

Fig. 4
Cell groupings and MGCs: BMs – three days in culture, after 24 hours' stimulation with Con A. (May-Grünwald-Giemsa, x 40)

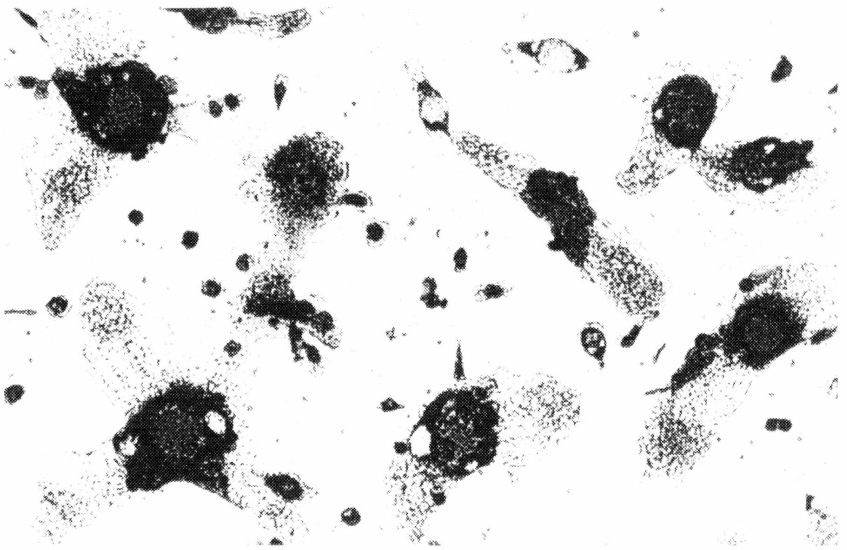

Fig. 5
Accelerated fusion of MGCs – great masses of nuclei with cytoplasmic "baloons": BMs – four days in culture, after 48 hours' stimulation with Con A. (May-Grünwald-Giemsa, x 16)