

IN VITRO DIFFERENTIATION OF HUMAN MONOCYTES
Monocytes Cultured on Glass are Cytotoxic to Tumor Cells but Monocytes
Cultured on Collagen are Not*

BY GILLA KAPLAN[†]

From the Department of Tumor Biology, Norwegian Cancer Society, Institute of Medical Biology, University of Tromsø, Tromsø, Norway

Monocytes from the peripheral blood of normal adult human donors have been repeatedly reported to have appreciable levels of cytotoxic activity against murine and human tumor cell lines (1-5). In vitro cultivation and various stimuli have been used to induce an enhanced cytotoxic activity in these cells (6-9). In a majority of these studies, the monocytes tested have been isolated and cultured by adherence to plastic or glass surfaces.

In a previous paper we demonstrated that the in vitro differentiation of human peripheral blood monocytes to macrophages is dependent on the conditions of monocyte cultures (10). Cultivation of monocytes on glass or microexudate-coated glass gave rise to cells resembling foreign body granuloma macrophages. In comparison, cultivation of monocytes on collagen matrices gave rise to cells resembling human resident tissue macrophages. The two cell populations differed in morphology, phagocytic activity, receptors, and surface antigen expression.

To evaluate the effect of the culture surface on the cytotoxic activity of human blood monocytes the killing of human target cell lines was tested. Monocytes were seeded on glass, microexudate-coated glass, and collagen matrices, and their interaction with various human tumor cell lines investigated.

Materials and Methods

Preparation of Mononuclear Leukocytes. Peripheral blood mononuclear leukocytes (MNL)¹ were isolated from venous blood of healthy adult human donors by the Bøyum method (11) and introduced into culture as described (10). In short, blood collected into EDTA-containing vacutainers was diluted with an equal volume of Dulbecco's Modified Eagle's Medium (DME) (Gibco, Glasgow, Scotland) and separated by centrifugation on lymphoprep (density 1.077 g/ml) (Nyegaard & Co., Oslo, Norway). The interphase cells were washed by centrifugation and suspended in DME containing 20% autologous serum, 100 µg/ml streptomycin, and 100 IU/ml of penicillin.

Preparation of Hydrated Collagen Matrices. Collagen extracted from rat tail tendons was a kind gift of Dr. Bjørn Obrunk, Dept. of Medical Chemistry, BMC, Uppsala, Sweden. Collagen solutions in 0.1 M acetic acid were gelled with 10 × DME medium and 0.142 M NaOH as described (12, 13). The collagen gels were kept hydrated at 37°C until use.

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[†] Dr. Kaplan's present address is the Dept. of Cellular Physiology and Immunology, The Rockefeller University, New York 10021.

¹ *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle medium; FCS, fetal calf serum; MNL, mononuclear leukocytes; NBCS, newborn calf serum; SEM, scanning electron microscopy.

Preparation of Microexudate-coated Surfaces. Rat fibroblasts (R22 CIF) were grown to confluence in DME with 20 % fetal calf serum (FCS) (Gibco) and lysed with 0.25 M NH_4OH as described (13). Microexudate-coated coverslips were sterilized and stored dry until use, as described (10).

Culture of Monocytes. 2×10^6 MNL in 1 ml DME with 20% autologous serum were added to 16-mm culture wells with collagen matrices or glass coverslips. After incubation at 37°C for 2 h in 5% CO_2 and 95% humidified air, the cultures were washed well with DME to remove nonadherent cells. 1 ml of DME with 20% autologous serum was added to each culture. After 7 d, 1 ml of the same medium or 1 ml of DME alone was added to the cultures. New medium was added every 4 d thereafter.

[^{14}C]Glucosamine Incorporation. Monocyte cultures were incubated for 20 h with DME with 0.4 $\mu\text{Ci/ml}$ of [^{14}C]glucosamine (58 mCi/mmol; Radiochemical Centre, Amersham, England), washed with DME, and incubated for 2 h with 1 ml DME. The coverslips (crushed) or collagen gels were washed in cold PBS, the proteins dissolved in 0.5 ml of 1 N NaOH, and all solid material removed by centrifugation. Duplicates of 50 μl of the NaOH protein solution were solubilized in 5 ml of Insta-gel II liquid scintillation fluid (Packard Instrument Company Inc., IL) and radioactivity counted (14).

Protein Determinations. Monocyte monolayers were washed in PBS and solubilized in 1 N NaOH. Protein solutions were diluted in distilled H_2O to give 0.1 N NaOH and the lysates stored frozen (-70°C) until use (15). The protein content of cell lysates was measured by the method of Lowry et al. (16) using bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) as standard. All analyses were performed in duplicate.

Human Cell Lines. The adherent cell line NHIK 3025, originating from a carcinoma of the human uterine cervix (17), and the nonadherent human myeloid leukaemia cell line K-562 (18), were obtained from Dr. J. Hammerström of the Dept. of Medicine, University of Trondheim, Norway. The cells were maintained in RPMI 1640 medium (Gibco) with 20% pooled human AB Rh-positive serum.

The adherent cell line NHIK 1485, isolated from a human malignant melanoma, and the adherent cell line T24 isolated from a human carcinoma of the urinary bladder were obtained from Dr. R. Oftebro of the Norwegian Radium Hospital, Oslo, Norway. These cells were maintained in DME medium with 10–20% fetal calf serum (FCS) (Gibco).

Adherent HeLa cells, derived from human cervical carcinoma cells, were maintained in DME with 10% newborn calf serum (NBCS) (Gibco). The adherent L929 fibroblast cell line of C_3H mouse origin, transformed in vitro by methylcholanthrene (19, 20) was cultured in DME with 10% NBCS.

Cytotoxicity Assays

[^{14}C]THYMIDINE RELEASE ASSAY. Tumor target cells were prelabeled with 2-[^{14}C]thymidine (59 mCi/mmol, The Radiochemical Centre, Amersham, England) in culture medium (0.5 $\mu\text{Ci/ml}$) for 24 h. The adherent cells were washed well and trypsinized off the plastic surface with 0.5 ml of a 0.05% trypsin solution (Sigma) in PBS containing 1 mM EDTA. K-562 cells were washed by centrifugation. Prelabeled and washed viable target cells were added to monocytes in 1 ml culture medium and incubated together for up to 5 d. Samples of the medium were removed at various time points and radioactivity released into the medium determined (21). Monocyte cultures were fixed at all time points for morphological observations.

^{51}Cr CHROMIUM RELEASE ASSAY. Target cells were prelabeled with 400 μCi of sodium-chromate- ^{51}Cr (141 mCi/mg, Institute for Kjeller, Norway) in 2.5 ml RPMI medium at 37°C for 1 h. The cells were washed, removed from the surface, and 4×10^4 cells were added to monocytes in 1 ml of culture medium. Samples of the medium were removed at various time points and radioactivity released into the medium determined. Monocyte cultures were fixed at 1 h and 70 h for morphological observation.

COLONY INHIBITION ASSAY. Colony inhibition assay was carried out according to the method described by Freedman et al. (Freedman, V. H., G. Kaplan, C. S. Copeland, and S. C. Silverstein, manuscript in preparation). 1×10^4 trypsinized and washed viable NHIK 3025 cells were added to monocyte cultures. At 24 h the NHIK 3025 cells were trypsinized off, counted, diluted, and reseeded on culture dishes in RPMI with 20% AB Rh⁺ human serum. The cells were incubated for 5 d and the resulting colonies counted.

Morphological Studies. Monocyte cultures on coverslips or collagen matrices were removed

from the culture wells, washed with PBS, and then fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer with 0.1 M sucrose, pH 7.3 at room temperature. For phase contrast microscopy the cultures were washed, mounted, and photographed with Kodak Panatomic-X black and white film. For scanning electron microscopy (SEM) the fixed cultures were dehydrated in alcohol, transferred to amyl-acetate, and critical-point-dried (Hitachi CPI, Tokyo) in carbon dioxide. The specimens were coated with gold (Polaron SEM Coating Unit E 5000) and examined with a high resolution Hitachi scanning electron microscope (HHS/2R) at 20 kV and a tilt angle of 30°. Pictures were taken on Kodak Plus-X film.

Results

Protein Content and Glucosamine Incorporation into Differentiating Monocytes. The culture conditions used in these experiments supported the long term survival and differentiation of monocytes, as described in an earlier study (10). Both glass- and collagen-differentiated monocytes became much larger with time in culture. This change in cell size was mirrored by the protein content of the cultures. In both types of monocyte cultures, after an initial decline, the adherent cell protein content per culture increased steadily (Fig. 1A). By 20–22 d in vitro the cultures contained about four times the original amount of protein. These results (for glass-differentiated monocytes) are in agreement with observations reported by Nakagawara et al. (22).

The rate of glucosamine incorporation into macrophages has been shown to correlate to their state of activation and differentiation (14, 23). In-vitro-differentiated monocytes were tested for incorporation of glucosamine. Both types of cultures showed an increase in the rate of glucosamine incorporation during the first week in vitro (Fig. 1B). Monocytes cultured on collagen maintained this level of incorporation. The incorporation of glucosamine into cells cultured on glass continued to rise to about twice that found in the collagen-differentiated cells. This suggested that monocytes cultured on glass were more activated than monocytes cultured on collagen.

Lysis of Target Cells by In-Vitro-differentiated Monocytes. Experiments were performed to determine whether the monocytes differentiated in vitro were capable of lysing a number of human cell lines, and one mouse cell line. As shown in Table I, glass-differentiated monocytes lysed all the cell lines tested. However, no thymidine release above background occurred when the target cells were coincubated with monocytes differentiated on collagen gels. The level of spontaneous thymidine release differed from target to target. In addition, the length of time of coincubation required to obtain target cell killing varied from cell line to cell line. This suggested that the spontaneous cytotoxic activity of monocytes, reported by many investigators, was induced by the cultivation on glass. Once activated the monocytes showed typical nonspecific activity against tumor cells, the kinetics of which were dependent on target cell properties.

Effector/Target Ratios. The cytotoxic activity of in-vitro-differentiated monocytes against NHIK 3025 cells was investigated in more detail. Effector/target cell ratios were varied, and cytolysis was half maximal when monocyte cultures were obtained after seeding $0.5-1 \times 10^6$ MNL on glass, and incubated with 2×10^4 target cells (Fig. 2). Specific release of radioactivity was optimal when monocyte cultures were obtained by seeding 2×10^6 MNL. Results were similar when 4×10^4 target cells were added to monocyte cultures (Table II). The inability of collagen-differentiated monocytes to kill NHIK 3025 target cells was preserved over the whole range of monocyte/target cell ratios tested (Fig. 2).

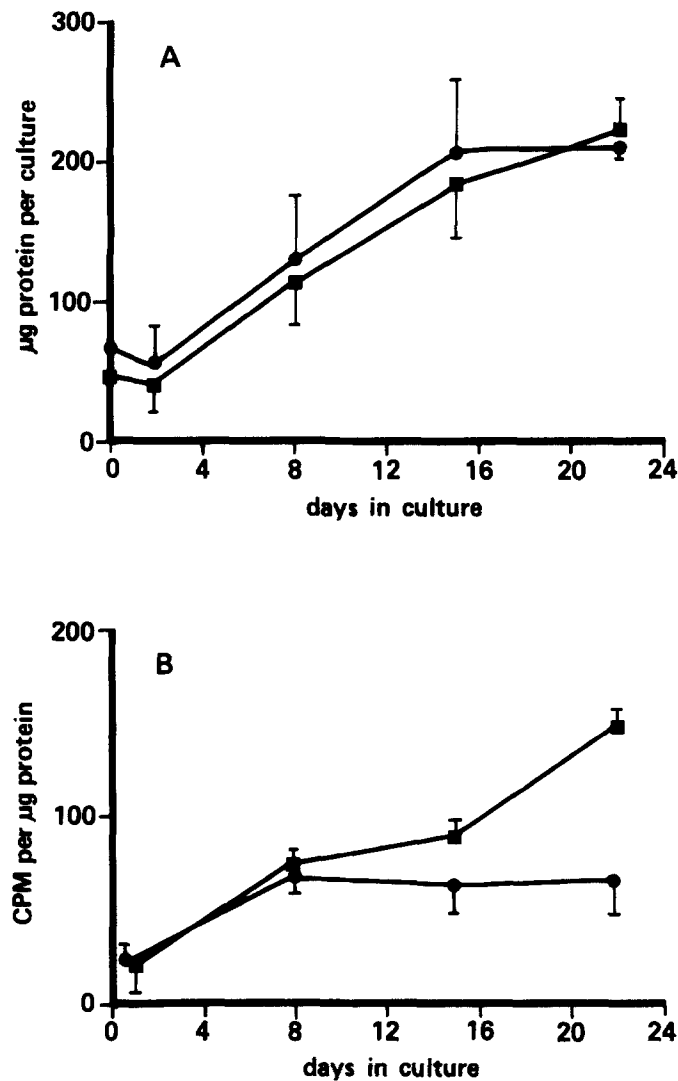


FIG. 1. Protein content (A) and $[^{14}\text{C}]$ glucosamine incorporation (B) into monocytes. Monocytes were cultured on glass (■) or on collagen (●). Results are means of 5 experiments \pm standard deviation.

Spontaneous Lysis of NHIK 3025 Cells. The serum concentration in control NHIK 3025 target cells influenced the extent of spontaneous release of radioactivity. The lower the serum concentration within a range of 2–10%, the higher the spontaneous release obtained (not shown). As a result some assays included control NHIK 3025 cells maintained in 2%, 5%, and 10% of serum and the range of spontaneous release was indicated (Figs. 3 and 4). In all other experiments 10% serum was used.

Time Course of Cytolysis. Fig. 3A and B demonstrate the time course of cytolysis of NHIK 3025 cells as monitored by the thymidine release assay. Monocytes cultured on glass, as well as monocytes cultured on microexudate-coated glass, induced slow cytolysis of target cells. Significant killing was obtained after 3–4 d of co-culture but

TABLE I
Cytotoxic Activity of Human Monocytes Against Tumor Cell Lines

Tumor	Monocytes in vitro*	Percent Release [†] (S.R.)		
		Glass	Collagen	Assay time
NHIK 3025	2 h	88 ± 4 (28 ± 2)	36 ± 2 (27 ± 3)	4 d
NHIK 3025	7 d	98 ± 6 (28 ± 2)	34 ± 3 (27 ± 4)	5 d
NHIK 3025	9 d	90 ± 9 (30 ± 3)	34 ± 3 (29 ± 3)	5 d
T24	2 h	60 ± 5 (28 ± 5)	32 ± 4 (26 ± 5)	4 d
T24	7 d	63 ± 6 (28 ± 5)	33 ± 4 (26 ± 5)	4 d
T24	9 d	77 ± 14 (32 ± 4)	36 ± 7 (32 ± 3)	4 d
NHIK 1485	2 h	98 ± 9 (45 ± 5)	42 ± 2 (43 ± 4)	3 d
NHIK 1485	7 d	80 ± 10 (45 ± 5)	42 ± 3 (43 ± 4)	3 d
HeLa	9 d	70 ± 2 (14 ± 2)	16 ± 2 (12 ± 2)	24 h
L929	9 d	67 ± 2 (19 ± 3)	12 ± 1 (12 ± 1)	24 h

2 × 10⁶ MNL seeded.

4 × 10⁴ target cells added to monocyte cultures.

* Time in culture before the addition of target cells.

† Mean release ± SD for 4-6 parallels. S.R., spontaneous release.

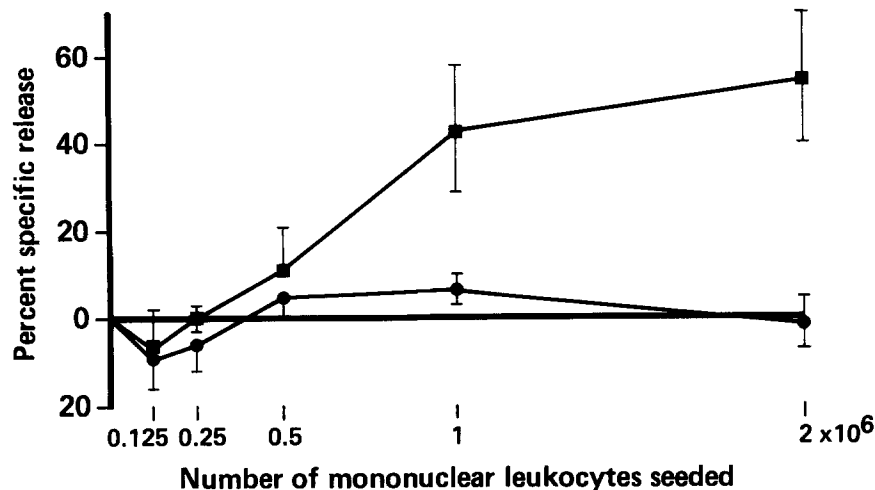


FIG. 2. Effect of monocyte culture density (number of MNL seeded) on lysis of 2 × 10⁴ NHIK 3025 cells (5-d assay). Monocytes were cultured on glass (■) or on collagen (●). Results are means of specific release of 4-6 parallels ± standard deviation. Spontaneous release was 30 ± 8%.

in most cases maximal killing was obtained by 5 d.

Monocytes cultured on collagen gels did not at any time induce release of radioactivity higher than the spontaneous release. Spontaneous release from NHIK 3025 cells seeded on collagen was the same as on glass (not shown). When the target cells were labeled with ⁵¹Cr the kinetics of killing were different. Significant specific release was obtained by 24 h of incubation of NHIK 3025 cells with glass-differentiated monocytes (Fig. 3C). Here too collagen-differentiated monocytes did not lyse the target cells. Spontaneous release from ⁵¹Cr NHIK 3025 on collagen was the same as on glass.

Effect of the Length of In Vitro Culture Time of Monocytes on Target Cell Cytolysis. Freshly explanted monocytes (2 h on glass) as well as monocytes cultured on glass from 1 d to

TABLE II
Cytolysis of NHIK 3025 Targets by Glass-differentiated Monocytes

Mononuclear cells (MNL) seeded	Target cells NHIK 3025	Percent ¹⁴ C released* (S.R.)		
		Day 1	Day 3	Day 5
1 × 10 ⁶	1 × 10 ⁴	12 ± 3 (10 ± 2)	55 ± 4 (33 ± 5)	67 ± 11 (70 ± 20)
1 × 10 ⁶	2 × 10 ⁴	13 ± 2 (8 ± 2)	42 ± 14 (24 ± 4)	80 ± 14 (35 ± 6)
1 × 10 ⁶	4 × 10 ⁴	11 ± 2 (12 ± 3)	66 ± 7 (19 ± 1)	98 ± 4 (25 ± 2)
2 × 10 ⁶	1 × 10 ⁴	23 ± 3 [‡]	41 ± 6	63 ± 11
2 × 10 ⁶	2 × 10 ⁴	15 ± 4	47 ± 8	89 ± 15
2 × 10 ⁶	4 × 10 ⁴	10 ± 7	56 ± 17	93 ± 12

S.R., spontaneous release.

* Mean ± SD of 4 parallels.

‡ Spontaneous release the same as above.

about 2 wk were capable of killing NHIK 3025 target cells. However, when monocytes maintained in vitro for longer periods of time were tested for target cell killing, no such activity was observed. By 15 or 16 d in vitro or longer (varying from donor to donor and in some cases from experiment to experiment), glass-cultured monocytes did not induce thymidine release higher than the spontaneous release from the target cell alone (Fig. 3).

The possibility that NK cells contaminating the freshly explanted monocytes were the cause of the cytotoxicity observed was excluded by the use of K-562 target cells. These cells, sensitive to human NK activity, were incubated with 2-h, 1-d, 2-d, and 7-d old monocytes for 6 h or longer. No significant killing of the [¹⁴C]thymidine or ⁵¹Cr-labeled K-562 target cells was observed (not shown).

Characterization of Monocyte-Target Cell Interaction by the Colony Formation Assay. The evaluation of cytotoxicity by means of the colony formation assay is presented in Table III. The incubation of glass-differentiated monocytes with NHIK 3025 cells for 24 h clearly reduced the number of viable NHIK 3025 cells. A reduction in the number of colonies formed is observed.

While collagen-differentiated monocytes did not appear to reduce the number of viable NHIK 3025 cells (Table III), the ability of these cells to grow in vitro seemed to be inhibited. An initial delay in cell growth gave rise to smaller colonies after 5 d in vitro. If these colonies were incubated in vitro for an extra 2-3 d the colonies reached the same size as control colonies at 5 d. Thus the inhibitory effect of collagen monocytes on target cells was cytostatic rather than cytotoxic and it appears to be reversible.

Morphological Evaluation of Monocyte-Target Cell Interaction. The addition of NHIK 3025 cells to monocyte cultures resulted in binding of the tumor cells to the culture surface (glass or collagen) or to the monocyte surface (Fig. 4A). By day 3 and 4 the NHIK 3025 cells had formed small colonies of 4-8 cells in control cultures (Fig. 4F). The cells were well spread on the glass and 98% were viable. When added to collagen-differentiated monocytes the NHIK 3025 cells gave smaller colonies and the cells were less spread (Fig. 4D). The collagen monocytes and NHIK 3025 cells were found next to each other, very often with some cell contact between them (Fig. 4E). Few dead cells were observed. If maintained in culture for 6 d or longer the NHIK 3025 cells overgrew the monocytes. NHIK 3025 cells incubated together with monocytes on

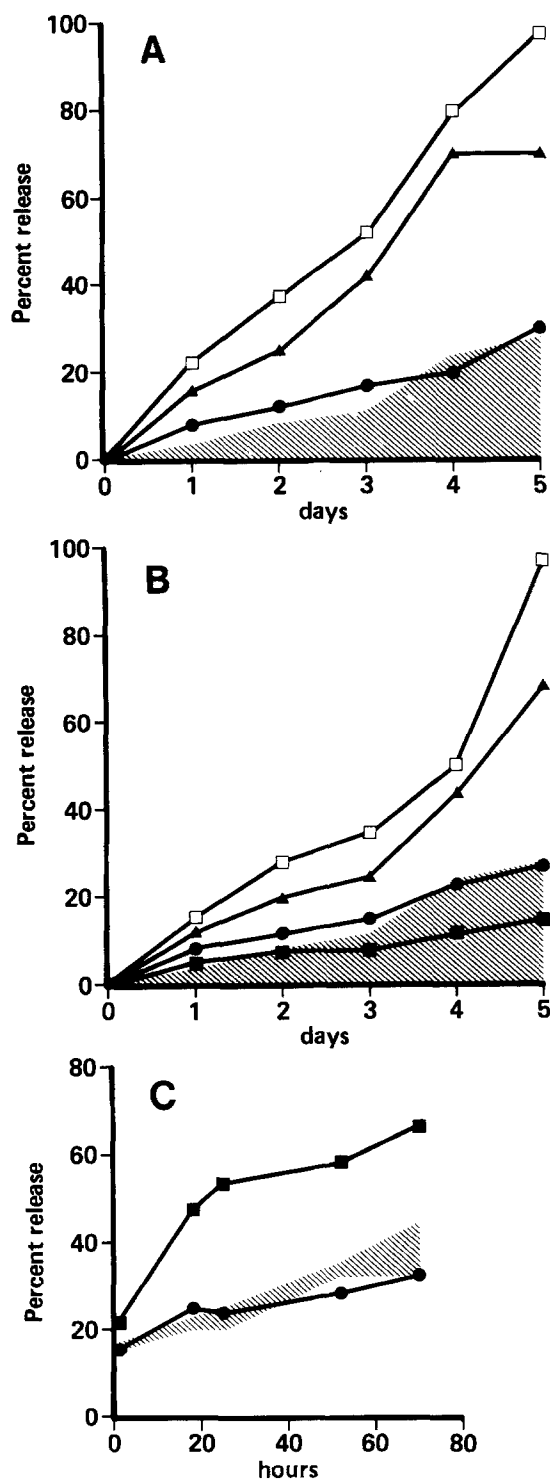


FIG. 3. The effect of the culture surface on cytotoxicity of monocytes against 4×10^4 [^{14}C]-thymidine-labeled (*A* and *B*) and ^{51}Cr -labeled (*C*) NHIK 3025 cells. (*A*) 7-d old monocytes cultured on glass (■), on microexudate-coated glass (▲), or on collagen (●). (*B*) 12-d old monocytes cultured on glass (■), microexudate-coated glass (▲), collagen (●), or 22-d old monocytes cultured on glass (□). The results are expressed as percent of incorporated ^{14}C released into the medium. The shaded areas indicate the range of spontaneous release. (*C*) 14-d old monocytes cultured on glass (■) or on collagen (●). The results are expressed as percent of incorporated ^{51}Cr released into the medium. The shaded area indicates the range of spontaneous release of ^{51}Cr .

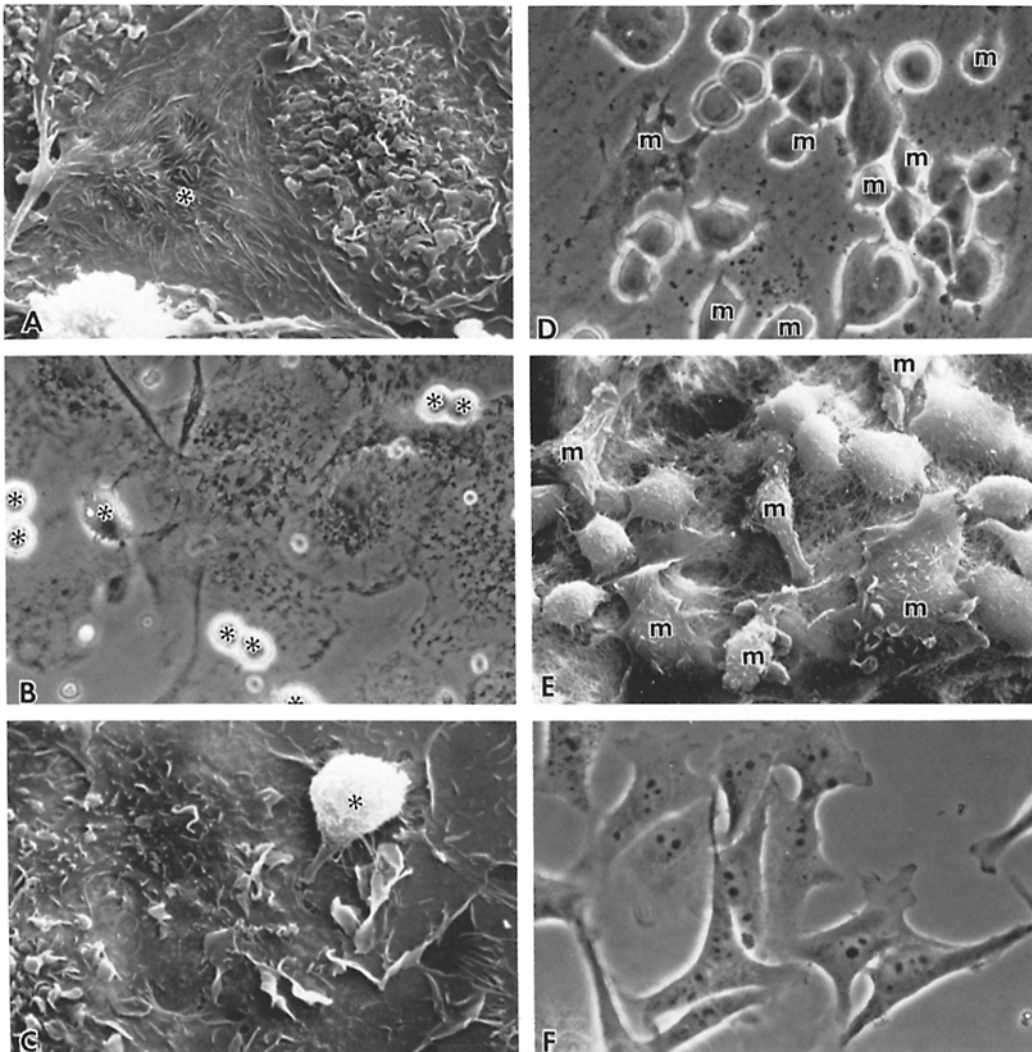


FIG. 4. Morphology of interaction between 10-d old monocytes and 2×10^4 NHIK 3025 target cells. (A) Scanning electron microscopy (SEM) of glass-differentiated monocytes incubated together with target cells for 6 h. Both cell types are well spread. The monocytes have many membrane folds and ruffling. The NHIK 3025 cells have long thin villi on their surface (*). $\times 1,100$. (B) Phase contrast microscopy of glass monocytes incubated with target cells for 2 d. The monocytes are well spread and ruffled. The target cells are rounded, often two cells together (*). $\times 440$. (C) SEM of glass monocytes incubated with target cells for 4 d. The monocytes are well spread. The target cells, bound to the monocyte surface are rounded (*). $\times 1,500$. (D) Phase contrast microscopy of collagen-differentiated monocytes (m) incubated with target cells for 4 d. Small colonies of NHIK 3025 cells are observed between the monocytes. $\times 440$. (E) SEM of collagen-differentiated monocytes (m) incubated with target cells for 2 d. Both cell types are spread on the collagen besides each other. $\times 600$. (F) Phase contrast microscopy of NHIK 3025 cells seeded on glass after 4 d. The cells are well spread. $\times 440$.

glass for 2 d seemed to be capable of at least one cell division (Fig. 4B). Some of the NHIK cells were found bound to the glass between the monocytes. After 1 d these cells were in colonies of 2-4 cells and had spread on the glass the way control NHIK

TABLE III
Cytotoxicity-colony Forming Units: 14-d old monocytes

Surface	Monocytes	NHIK	No. cells harvested, 24 h	Colonies, 5 d*
Plastic	None	1×10^4	2.5×10^4	1.7×10^4
Glass	None	1×10^4	2×10^4	1.4×10^4
Glass	2×10^6	1×10^4	0.5×10^4	0.3×10^4
Collagen	2×10^6	1×10^4	1.8×10^4	$1 \times 10^{4\ddagger}$ 0.2×10^4

* Medium diluted to 10^{-2} for seeding. Number of colonies presented corrected accordingly, total number given.

‡ Very small colonies.

3025 cells had (not shown). By 2 d most of the NHIK 3025 cells were found attached to the monocyte surface. These were rounded and most often single or in clumps of two cells (Fig. 4B). By 4 d of co-culture only few viable target cells were found. All the target cells found were rounded, mostly attached to the monocytes (Fig. 4C). If maintained in culture for 6 d or longer no viable NHIK 3025 cells were found in the monocyte cultures. No phagocytosis of viable target cells was observed. Killing appeared to be entirely extracellular.

When monocytes were seeded on very dilute (lose) collagen gels many fell through onto the glass below. The addition of target cells to these cultures gave rise to killing of all the target cells that fell through the collagen mesh. Only a few target cells remained on the collagen without any contact with monocytes on glass. These cells did not appear to be killed.

Discussion

The data presented here indicate that in vitro culture of human monocytes on glass surfaces induces the expression of extracellular cytotoxic activity of monocytes against tumor target cells. This activity was nonspecific, expressed against various cell lines. These observations are compatible with observations made by other investigators (1-5).

In contrast, when human monocytes were cultured on collagen gels, tumor cell cytotoxic activity was not significant. A possible limited cytostatic effect of these cells against the NHIK 3025 target cells was observed.

Morphological examination of the interaction of monocytes with the target cells supported the results obtained by the radioactivity release assays. While the target cells were clearly killed in the glass-differentiated monocyte cultures, they survived and multiplied in the collagen-differentiated monocyte cultures.

These differences between the two types of monocytes do not seem to stem from differences in culture density or general metabolic activity or viability of the cells. The protein content of the two types of cultures was similar (Fig. 1A). The numbers of monocyte in the cultures on glass were similar to those found in collagen-differentiated monocytes, and lysosomal enzyme activity was found to be high in both types of monocyte cultures (Kaplan, G., and T. Gauperaa, manuscript in preparation). On the other hand the rate of glucosamine incorporation was higher in monocytes differentiated on glass (Fig. 1B) pointing to a possible difference in the extent of activation of the two cell types. Morphological differences between the two types of

cultures (10) showed that the monocytes cultured on glass differentiated into epithelioidlike cells while monocytes on collagen differentiated into cells similar to resting tissue macrophages. This would suggest that human monocytes, although potentially capable of differentiating into cytotoxic cells, require a stimulus such as the contact with a foreign body (glass) to do so. In the absence of such a stimulus this activation would not occur.

The possibility that the collagen inhibited the cytotoxic activity of monocytes *in vitro*, was excluded by the observation that loose collagen gels that allowed most of the cells to fall through onto the glass below, did not interfere with target cell killing.

The fact that no phagocytosis of viable tumor cells was observed, and that target cells found with no contact with the glass monocytes (above them on the loose collagen gels) were not killed, suggested that the killing was extracellular and probably contact dependent.

These conclusions are compatible with numerous observations made in rodents showing that only activated macrophages, and not resident macrophages, are capable of killing target cells by an extracellular contact-dependent mechanism (24-30). The loss of cytotoxic activity by 2-wk old monocytes (on glass), suggests that during monocyte differentiation, a cytotoxic stage or type of cell is obtained and then lost. Comparison of this observation to morphological observations made on the same cells (10) suggests that the epithelioidlike cells could be the activated cytotoxic cells. Older epithelioid cells and multinucleated giant cells could be terminally differentiated noncytotoxic cells. Nakagawara et al. (22) have shown that human monocytes cultured *in vitro* on glass, secrete hydrogen peroxide (H_2O_2) and superoxide (O_2^-) into the culture medium. This activity is lost after ~10 d in culture. The H_2O_2 and O_2^- secreted into the culture medium could be the mediators of the cytolytic activity of the monocytes *in vitro*. It would be of interest to establish whether monocytes differentiated on collagen are capable of secreting H_2O_2 and O_2^- .

Whether monocytes differentiated on collagen can be stimulated to become cytotoxic is as yet unclear. Preliminary evidence suggests that these cells can be induced to differentiate morphologically to epithelioid cells and multinucleated giant cells by contact with small glass cover slips. Further investigations are under way in this laboratory.

The possibility that glass acts as a stimulant when in contact with monocytes is of major importance when monocyte differentiation and function are studied. Mechanisms of activation for tumoricidal and bactericidal activity should thus be studied in the absence of such a stimulant. The data presented here suggest that collagen gels could be used as the alternative culture surface for such studies.

Summary

Cultivation of human blood monocytes on glass gives rise to cells nonspecifically cytotoxic to tumor cells. If the monocytes are cultured on collagen gels with no contact with glass, no such cytotoxic activity is induced. Killing appeared to be extracellular and probably contact dependent.

The glass-induced cytotoxic activity was not related to protein content or cell viability. Rather, it appeared that the monocytes cultured on glass differentiated into cells resembling activated macrophages. On the other hand monocytes cultured on collagen differentiated into cells resembling resident tissue macrophages. These obser-

vations are compatible with numerous studies carried out in rodents, showing that activated macrophages, and not resident cells, are cytotoxic to tumor cells.

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