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Role of Monocyte Cytotoxic Factor in Cytolysis of Actinomycin D-treated WEHI 164 Cells Mediated by Freshly Isolated Human Adherent Mononuclear Blood Cells[1]

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ABSTRACT

The role of the monocyte cytotoxic factor (CF) in cytolysis of untreated and actinomycin D (Act D)-treated WEHI 164 cells by freshly isolated human adherent mononuclear cells has been investigated in this study. Murine WEHI 164 cells were used as target cells because of their sensitivity to lysis mediated by monocytes and their resistance to natural killer cells. Monocytes as well as monocyte supernatants mediated cytolysis of WEHI 164 cells. Cytolysis was enhanced by Act D treatment of target cells. The addition of lipopolysaccharide to monocytes accelerated the progression of cytolysis of Act D-treated WEHI 164 cells mediated by monocytes. A polyclonal rabbit antiserum against CF inhibited the cytolytic activity of monocytes and monocyte supernatants against untreated as well as Act D-treated WEHI 164 cells. At low effector:target ratios, the cytolysis was totally abrogated by CF antiserum. Depletion of natural killer cells from adherent cells by the monoclonal antibody Leu 11b and rabbit complement did not reduce cytolysis of Act D-treated WEHI 164 cells. Immunofluorescence microscopy revealed that CF antiserum stained the plasma membrane of freshly isolated monocytes, suggesting that CF is a membrane-associated molecule. Our data indicate that CF is an important effector molecule in cytolysis mediated by freshly isolated monocytes against untreated and Act D-treated WEHI 164 cells.

INTRODUCTION

The mechanism of monocyte-mediated cytolysis is not completely understood. Monocyte cytotoxic protein factors have been implicated as effector molecules (1–5). We have earlier reported that lymphokine-activated human macrophage-like cells, induced by prolonged *in vitro* culture of monocytes, release a M_r 40,000 CF,[3] which has been purified by ion exchange chromatography, chromatofocusing, and gel filtration (4). A polyclonal antiserum to purified CF (5) inhibits the cytolysis of K562 cells induced by γ-interferon-activated macrophage-like monocytes (6).

However, adherent blood cells undergo changes in cytotoxic capacity associated with the maturation from monocytes to macrophage-like cells during *in vitro* culturing (7–11). Freshly isolated human adherent mononuclear cells exhibit strong spontaneous cytolytic activity against a variety of tumor target cells (10, 12–18), which decreases abruptly after the first 24 h of culture (10, 19). The cytotoxicity of *in vitro* cultured, macrophage-like monocytes is augmented by endotoxin and lymphokines (3, 5, 6, 10, 20). The cytolysis induced by lymphokine-activated, macrophage-like cultured monocytes might differ in mechanism from the spontaneous killing exerted by freshly isolated monocytes. Thus, in the present study, we have investigated the contribution of CF to cytolysis mediated by freshly isolated monocytes using antiserum to purified CF (5).

The murine fibrosarcoma WEHI 164 cells were used as target cells in this study as they are sensitive to rapid spontaneous cytolysis mediated by freshly isolated monocytes (17, 18) and resistant to cytotoxicity mediated by NK cells (17, 18) which may contaminate the adherent cell population (21). Preincubation with Act D renders WEHI 164 cells even more susceptible to killing by freshly isolated human monocytes (17, 18), a phenomenon which has been termed DDCC. Furthermore, previous work has demonstrated that cytolysis mediated by CF is greatly enhanced by Act D treatment of WEHI 164 cells (22). These characteristics make WEHI 164 cells and the DDCC assay uniquely suited for the study of the role of monocyte-derived CF in the spontaneous lysis mediated by freshly isolated adherent cells.

MATERIALS AND METHODS

Human sera were from the Blood Bank, Trondheim, Norway; phenol water extracted *Escherichia coli* LPS 026:B6, α-naphthyl acetate, and actinomycin D from Sigma Chemical Co., St. Louis, MO. FCS, RPMI 1640, trypsin, and HBSS were obtained from Gibco Biocult, Glasgow, Scotland; $Na_2{}^{51}CrO_4$ and Texas red-linked anti-rabbit IgG were from Amersham, Buckinghamshire, United Kingdom; poly(2-hydroxyethylmetacrylate), type NCC, Lot 110, was from Hydron Lab., Inc., New Brunswick, NJ. MoAb anti-Leu 11b and fluorescein isothiocyanate-conjugated goat anti-mouse IgG were purchased from Becton Dickinson Monoclonal Center, Inc., Mountain View, CA, and MoAb Mo5 was from Coulter Electronics Limited, Luton, United Kingdom. Peroxidase-F(ab)$_2$ of goat anti-rabbit IgG (heavy and light) was from ZYMED, San Francisco, CA. The nylon wool, LP-1-Leukopak leukocyte filter was from Fenwal Lab., Mortron Grove, IL; rabbit complement was from Behringwerke AG, Marburg, Germany. Diafine for developing photographs was obtained from Acufine, Inc., Chicago, IL. Culture wells and dishes were purchased from Costar, Cambridge, MA; Petri dishes were from Nunc, Roskilde, Denmark; Teflon centrifuge tubes, type 3114, Oak Ridge, were from Nalge Co., Rochester, NY; and glass syringes, No. 90391, were from Elios Vantini, Padova, Italy. Culture medium was RPMI 1640 supplemented with 0.1 mM L-glutamine, gentamicin (40 μg/ml), and the given percentage of pooled human AB Rh-positive serum or 10% heat-inactivated (56°C, 30 min) FCS (FCS-M).

Human Monocytes. Mononuclear cells were separated from defibrinated venous blood of *Bacillus Calmette-Guérin*-vaccinated[4] healthy volunteers by Ficoll-Isopaque centrifugation (23). The mononuclear cells were washed twice in HBSS and suspended to a concentration of 4×10^6 cells/ml in RPMI 1640 with 25% human serum. Monocytes and nonadherent cells were separated by plastic adherence in 35-mm tissue culture dishes (Costar No. 3506), 1.5-ml cell suspension being added to each well. Nonadherent mononuclear cells were removed after 90-min incubation. Adherent cells were washed 3 times with HBSS and detached by 5-min exposure to 0.02% EDTA in Ca^{2+}/Mg^{2+}-free HBSS.

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[3] The abbreviations used are: CF, cytotoxic factor; NK, natural killer; Act D, actinomycin D; DDCC, drug-dependent cellular cytotoxicity; LPS, lipopolysaccharide; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; MoAb, monoclonal antibody; FCS-M, medium with 10% heat-inactivated FCS; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; E:T, effector:target; TNF, tumor necrosis factor.

[4] Almost all Norwegian citizens are vaccinated with *B. Calmette-Guérin* at age 13. The donors used in our study are between 25 and 35 yr old; thus the interval between vaccination and blood testing is more than 10 yr. We do not know the importance of vaccination to our experimental results.

Collected cells were finally washed twice in HBSS. This treatment did not appreciably affect the viability of adherent cells, which was >98% as determined by trypan blue exclusion. Adherent cells were >93% monocytes as assessed by staining for α-naphthyl acetate esterase (11, 24). Adherent cells were suspended in RPMI 1640 with 10% FCS (FCS-M), 1×10^6 cells/ml for cytotoxicity studies.

Human Lymphocytes. Nonadherent cells were further depleted of monocytes by passage through nylon wool columns (25, 26). Only nylon wool-passaged nonadherent cells were used in cytolysis assays. After nylon wool passage less than 2% of cells were monocytes, assessed by α-naphthyl acetate esterase staining.

Complement-mediated Lysis of NK Cells. In order to deplete NK cells from the adherent cell population, complement-mediated lysis of NK cells was performed. Adherent cells were incubated with MoAb Leu 11b (5 μl of stock solution added to 1×10^6 cells in 50 μl of PBS) for 1 h at 4°C. The cells were washed twice in HBSS and incubated with rabbit complement in RPMI 1640 (500 μl/1×10^6 cells, 1/4 dilution) for 1 h at 4°C with occasional shaking. Finally, the cells were washed twice in HBSS and readjusted to 1×10^6 cells/ml in FCS-M. Controls were cells treated with complement or untreated cells. Viability of monocytes was >98% after this treatment as determined by trypan blue exclusion.

Target Cells. WEHI 164 is a murine fibrosarcoma cell line chemically induced in BALB/c mice (27). The cells were kindly provided by H. W. Löms Ziegler-Heitbrock (Institute of Immunology, University of Munich, Germany). Cells were passaged in RPMI 1640 with 10% FCS and grown in suspension in polystyrene Petri dishes (Nunc 240142) for 3 days before cytolysis assay. K562 human erythroleukemia cells (28) were grown in suspension cultures in RPMI 1640 with 10% human serum.

^{51}Cr Labeling. WEHI 164 cells in RPMI 1640 (1×10^6 cells/500 μl) were labeled with 100 μCi of ^{51}Cr for 90 min in 16-mm wells (Costar No. 3524) coated with poly(2-hydroxyethylmetacrylate) to prevent adherence, as described (29). K562 cells (2×10^6 cells/200 μl of RPMI 1640) were labeled with 200 μCi of ^{51}Cr for 90 min. Finally, the cells were washed twice in HBSS and suspended in FCS-M (5×10^4 cells/ml).

Preincubation with Act D. WEHI 164 cells were incubated with Act D (1 μg/ml) in FCS-M for 3 h as described (17, 18). Incubation was done in Teflon centrifuge tubes to prevent adhesion.

Preparation of Monocyte Supernatants. Adherent cells detached by EDTA were suspended in FCS-M (1×10^6 cells/ml). Cell suspension (100 μl) was added to 6-mm culture wells (Costar No. 3799) and incubated with 100 μl of FCS-M or FCS-M with LPS (0.2 μg/ml). Supernatants were aspirated after 2–3 min or 1, 2, 3, or 6 h; centrifuged ($400 \times g$, 10 min); and stored at −20°C. The cytolytic activity of monocyte supernatants was determined in a 6-h ^{51}Cr release assay.

Cytolysis Assay. Effector cells (100 μl) in appropriate dilution in FCS-M or monocyte supernatant (100 μl) was added to round-bottomed microculture wells (Costar No. 3799) together with 100 μl of ^{51}Cr-labeled target cells. The assays were terminated after 3, 6, or 9 h. The microculture plate was centrifuged ($400 \times g$, 10 min), 100 μl of the supernatant were removed, and γ-emission was counted in a LKB 1270 gamma counter. Experimental release (E) was determined in the supernatant of target cells incubated with effector cells or monocyte supernatants, and spontaneous release (S), in supernatants from target cells in control medium. Total release (T) was determined in the supernatants of target cells receiving 50 μl of 2.5% sodium dodecyl sulfate 0.5 h before termination of the assay. In some experiments the effect of CF antiserum or normal rabbit serum was tested. HBSS, in appropriate amounts, was added to all wells 0.5 h before termination of the assay to obtain a final volume of 250 μl in each well. Specific lysis (SL) was calculated as

$$\% \text{ of } SL = \frac{E - S}{T - S} \times 100$$

CF Antiserum. Purification of CF was performed as described (4). A rabbit was given multiple intracutaneous injections of purified CF (5). The antiserum used in our study was obtained after the fifth injection, 8 mo after the immunization schedule started (5). The antiserum blocks the cytostatic (5) and cytolytic (22) activity of CF-containing monocyte supernatants. The antiserum does not inhibit the cytolytic effects of lymphotoxin against WEHI 164 cells produced by lymphocytes exposed to phytohemagglutinin.[5] Furthermore, monocyte-mediated lysis of antibody-coated human RBC, which probably is partly mediated by hydrogen peroxide, superoxide, and hydroxyl radicals (30, 31), is not inhibited by CF antiserum (results not shown). CF antiserum does not stain OKM 1-negative lymphocytes, U937 cells, undifferentiated HL-60 cells, NHIK 3025 epithelial-like cells, and K562 cells (6).

Immunofluorescence Microscopy. Detached adherent cells were washed in PBS and incubated with MoAb Leu 11b or Mo5 for 20 min at room temperature (5 μl of stock solution per 1×10^6 cells in 50 μl of PBS). The cells were then washed once in PBS and incubated with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (1 μl of stock solution per 1×10^6 cells in 50 μl of PBS) for 20 min at room temperature. After a wash in PBS, the cells were transferred to 16-mm culture wells (Costar No. 3524) with poly-L-lysine-coated glass coverslips and centrifuged ($100 \times g$, 5 min). Subsequently, cells were fixed in 2% paraformaldehyde in PBS for 20 min at 4°C and mounted on glass slides. The specimens were examined in a Leitz Orthoplan epifluorescence microscope with ×63/1.40 objective, and the percentage of fluorescent cells was determined.

Adherent cells detached by EDTA were plated in 16-mm wells with glass coverslips and incubated at 37°C for 2.5 h. The coverslips were then washed in PBS and fixed in 2% paraformaldehyde in PBS for 20 min at room temperature. After fixation the coverslips were washed in PBS, incubated for 30 min at 37°C with CF antiserum or normal rabbit serum diluted 1:30 in PBS, and then washed extensively in PBS before incubation with Texas red-linked anti-rabbit IgG diluted 1:10 in PBS for 30 min at 37°C. Finally, the coverslips were extensively washed in PBS, mounted on glass slides, and examined in the microscope. Photographs were recorded on Kodak Tri-X pan film and developed in Diafine.

ELISA of Binding of CF Antiserum to Adherent Cells. Adherent cells detached by EDTA were plated in poly-L-lysine-coated microculture wells (10^5/well). The cells were incubated at 37°C for 2.5 h and thereafter fixed in 2% paraformaldehyde in PBS for 20 min at room temperature. After fixation the wells were washed in PBS, incubated for 30 min at 37°C with CF antiserum or normal rabbit serum diluted 1:50 in PBS, and then washed 3 times in PBS before incubation with peroxidase-F(ab)$_2$ of goat anti-rabbit IgG (heavy and light) diluted 1:1000 in PBS for 30 min at 37°C. The microculture wells were then washed 4 times with PBS and received 100 μl of *o*-phenylenediamine substrate solution (8 mg of *o*-phenylenediamine in 20 ml of 0.1 M citrate phosphate buffer, pH 5.0, with 5 μl of H_2O_2). The reaction was terminated with 100 μl of concentrated H_2SO_4, and absorbance was measured at 450 nm (A_{450}) in a Dynatech MR600 microplate reader.

Statistics. Results are given as the mean ± SD of triplicate determinations in single experiments. Results of n separate experiments are given as the mean ± SE.

RESULTS

Cell Lysis Induced by Monocytes and Nonadherent Cells. Monocytes detached by EDTA induced a moderate lysis of WEHI 164 cells within 6 h (Fig. 1). When WEHI 164 cells were treated with Act D, cytolysis was greatly augmented (Fig. 1). Monocytes mediated cytolysis of Act D-treated WEHI 164 cells at very low E:T ratios, well below 1:1 (Fig. 2). Increasing the E:T ratio above 20:1 did not improve cytolysis (results not shown). Cytolysis of untreated WEHI 164 cells induced by the nonadherent cell population was negligible after 6 h (2.1 ± 0.5%, $n = 4$) and was not improved by Act D treatment of target cells (Fig. 2). Cytolysis of K562 cells mediated by nonadherent cells was 45.2 ± 0.5% ($n = 5$) in a 6-h ^{51}Cr release assay.

Cell Lysis Induced by Monocyte Supernatants. Monocyte-

[5] Unpublished results.

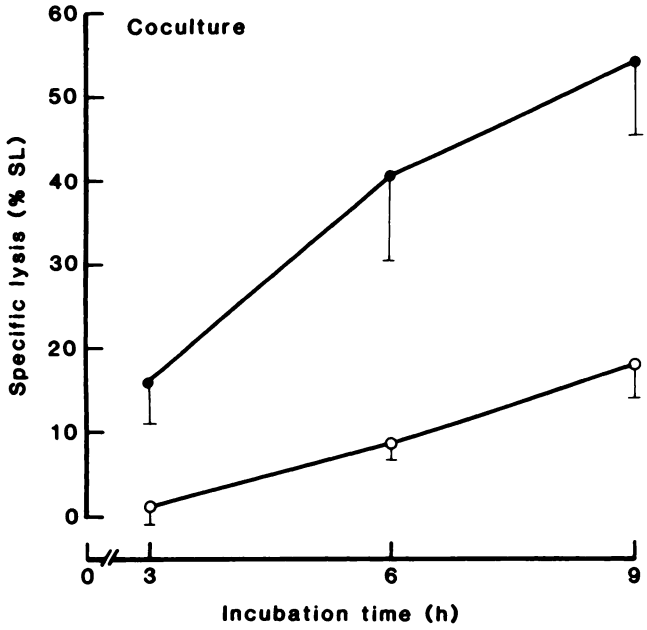

Fig. 1. Monocyte-mediated lysis of untreated WEHI 164 cells (○) and Act D-treated WEHI 164 cells (●). Cells were incubated with monocytes for 3, 6, and 9 h before measuring ^{51}Cr release. E:T ratio was 20:1. Spontaneous release of Act D-treated WEHI 164 cells after 6 h was 27.3 ± 3.1% and after 9 h was 35.2 ± 3.2%. *Points*, mean; *bars*, SE; $n = 4$.

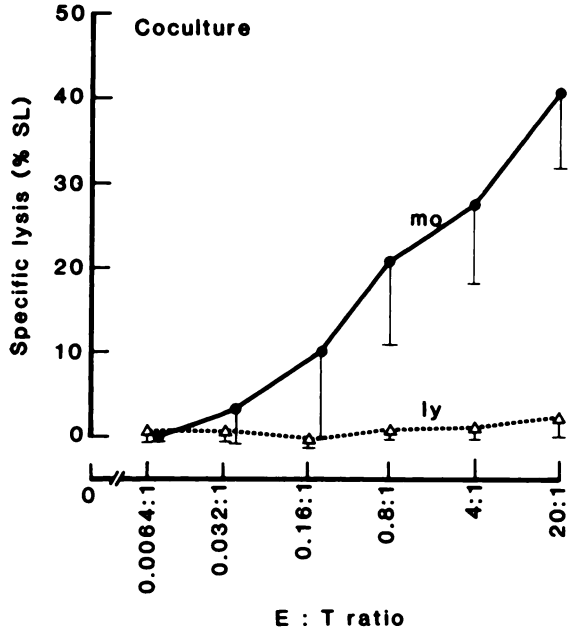

Fig. 2. Cytolysis of Act D-treated WEHI 164 cells mediated by monocytes (*mo*, ●) and lymphocytes (*ly*, △) at different E:T ratios in a 6-h ^{51}Cr release assay. *Points*, mean; *bars*, SE; $n = 4$.

mediated cytolysis did not depend upon direct effector:target cell contact as supernatants from monocytes incubated in FCS-M for 6 h induced cytolysis of Act D-treated WEHI 164 cells in a 6-h ^{51}Cr release assay (Table 1). The level of cytotoxic activity in monocyte supernatants was comparable to cytolysis observed in the parallel cocultures with direct effector:target cell contact (Table 1).

Influence of LPS on the Cytolytic Activity of Freshly Isolated Monocytes. Release of CF from *in vitro* cultured, differentiated monocytes is enhanced by adding LPS to the cultures (5). In order to investigate whether LPS induced a similar release phenomenon accompanied by increased cytotoxicity in freshly

Table 1 *Percentage of specific cytolysis of Act D-treated WEHI 164 cells mediated by monocytes and monocyte supernatants*

LPS in the absence of monocyte supernatant did not mediate cytolysis of Act D-treated WEHI 164 cells. No significant cytolysis was induced by supernatants from monocytes exposed to LPS for 2–3 min.

Duration of ^{51}Cr release assay (h)	% of specific cytolysis			
	Monocytes[a]	Monocytes exposed to LPS	Monocyte supernatants[b]	Supernatants from monocytes exposed to LPS
1	−1.7 ± 0.5[c]	0.3 ± 0.4	1.8 ± 0.4	0.8 ± 0.6
2	−0.1 ± 1.1	4.1 ± 2.0	1.2 ± 0.2	1.9 ± 0.4
3	8.0 ± 3.6	22.4 ± 4.8	8.6 ± 3.8	12.2 ± 2.1
6	45.3 ± 10.9	58.0 ± 4.2	51.5 ± 7.0	60.5 ± 5.4

[a] Monocytes were added to Act D-treated WEHI 164 cells (E:T = 20:1) in FCS-M without or together with LPS (0.1 μg/ml).
[b] Supernatants from monocytes cultured in FCS-M without or with LPS (0.1 μg/ml) were collected after 6-h incubation and transferred to Act D-treated WEHI 164 cells.
[c] Mean ± SE, $n = 4$.

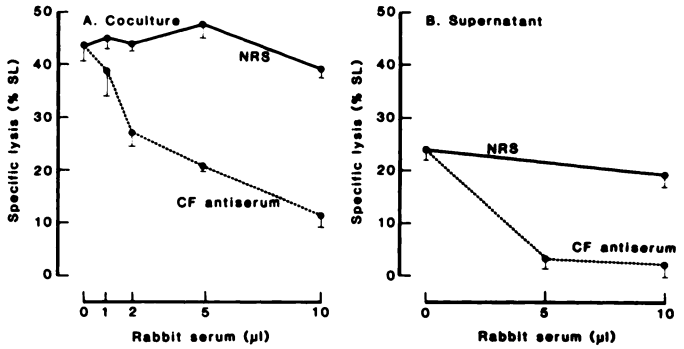

Fig. 3. *A*, effect of CF antiserum (●---●) and normal rabbit serum (*NRS*) (●——●) on monocyte-mediated cytolysis of Act D-treated WEHI 164 cells in a 6-h ^{51}Cr release assay. E:T ratio was 20:1. *Points*, mean; *bars*, SE; $n = 4$. *B*, effect of CF antiserum (●---●) and normal rabbit serum (*NRS*) (●——●) on cytolysis of Act D-treated WEHI 164 cells in a 6-h ^{51}Cr release assay induced by supernatants from monocytes exposed to LPS (0.1 μg/ml) for 2 h. *Points*, mean; *bars*, SE; $n = 4$.

isolated monocytes, LPS was added to adherent cells. LPS addition to the cocultures accelerated the progression of monocyte-mediated cytolysis of Act D-treated WEHI 164 cells (Table 1). Experiments with supernatants from monocytes exposed to LPS for various times demonstrated that this acceleration was due to a more rapid release of cytotoxic activity from monocytes in the presence of LPS (data not shown). Untreated, freshly isolated monocytes eventually released nearly the same amount of cumulative cytotoxic activity as LPS-treated monocytes (Table 1, 6 h).

Effects of CF Antiserum on Cytolysis Induced by Monocytes and Monocyte Supernatants. Antiserum against CF inhibited the cytolytic activity of monocytes. The specific lysis of untreated WEHI 164 cells after 6-h incubation was reduced from 14.7 ± 2.3% to 5.1 ± 2.7% by adding 5 μl of CF antiserum (SE; $n = 4$). The antiserum profoundly inhibited the cytolytic activity of monocytes against Act D-treated WEHI 164 cells (Fig. 3*A*) as well as that of supernatants (Fig. 3*B*). The cytolytic activity of monocytes was abrogated by adding 10 μl of CF antiserum to cocultures at low E:T ratios (Fig. 4). Ten μl of rabbit serum did not exert toxic effects upon monocytes, as assessed by morphology and trypan blue exclusion (viability > 95% at the end of incubation).

Immunofluorescence Localization. Immunofluorescence microscopy using CF antiserum and anti-rabbit IgG revealed that CF was accumulated on the plasma membrane of freshly isolated monocytes (Fig. 5*A*). Monocytes exposed to LPS did not show an increase in membrane-bound CF compared to freshly isolated, untreated monocytes (not shown). Normal rabbit serum did not bind to the plasma membrane of monocytes (Fig.

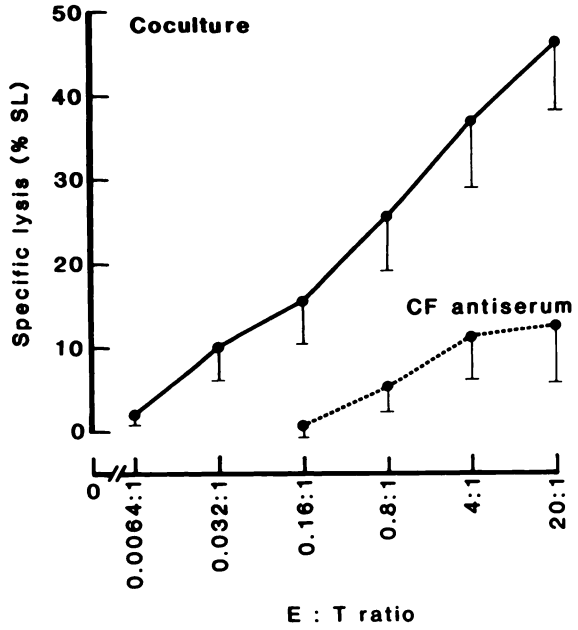

Fig. 4. Monocyte-mediated cytolysis of Act D-treated WEHI 164 cells measured in the presence of (●---●) and absence of (●——●) CF antiserum. Cytolysis was measured in a 6-h ^{51}Cr release assay at different E:T ratios. *Points*, mean; *bars*, SE; $n = 4$.

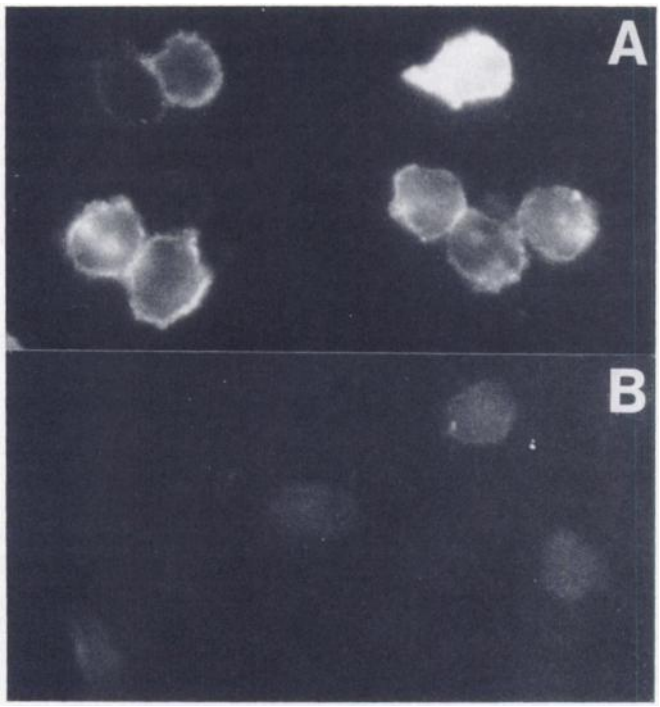

Fig. 5. Indirect immunofluorescence microscopy of adherent cells incubated with CF antiserum (*A*) and normal rabbit serum (*B*). The film was exposed for 20 s in both *A* and *B* and subsequently printed with equal exposure times making the immunofluorescence from *A* and *B* directly comparable. × 1000.

5*B*). Binding of CF antiserum to monocytes was confirmed and quantified by ELISA (A_{450} = 0.679 ± 0.036/A_{450} = 0.102 ± 0.020, CF antiserum/normal rabbit serum, respectively; SE, n = 6). The immunofluorescence microscopy was performed on washed, fixed, and nonpermeabilized monocytes, indicating that CF is located on the outer plasma membrane.

Identification of Effector Cells. To elucidate the nature of effector cells, the adherent cell population was characterized by MoAb Leu 11b (NK cells) and Mo5 (monocytes). In the adherent cell population 94.3 ± 1.7% of the cells expressed the Mo5 antigen, and 3.3 ± 0.03% of the cells expressed the Leu 11b antigen (SE, n = 3). In order to examine the contribution of NK cells to cytolysis mediated by adherent cells, we performed experiments with lysis of NK cells by Leu 11b and rabbit complement (Table 2). Lysis of the NK-sensitive K562 cells was almost abrogated by NK cell depletion, while cytolysis of Act D-treated WEHI 164 cells was not reduced (Table 2).

DISCUSSION

Previous work with macrophage-like human monocytes cultured for some days *in vitro* before activation with lymphokines or γ-interferon has shown that CF is an important mediator of the slowly progressing cytolytic activity against human tumor cells (6, 10, 20). The present study of freshly isolated monocytes, not treated by any "activating" procedure besides isolation by adherence, suggests that the rapid spontaneous cytolysis of WEHI 164 cells is due to a similar mechanism. We found that antiserum raised against purified CF inhibited lysis of both untreated and Act D-treated WEHI 164 cells in cocultures with monocytes, suggesting that CF participates in cytolysis. As cytolysis of untreated (data not shown) as well as Act D-treated WEHI 164 cells was profoundly inhibited by CF antiserum, the participation of CF is not dependent on Act D treatment of target cells. Moreover, the cytolytic activity mediated by a low number of monocytes could be totally abolished by CF antiserum, suggesting that CF may be responsible for all cytolytic activity in this experimental setting. In agreement with these observations, most of the cytolytic potential of an adherent cell culture could be accounted for by the cytolytic activity found in the corresponding supernatant. The soluble cytotoxic activity was inhibited by antiserum to CF, demonstrating that the cytolytic activity of the supernatant is at least partly due to CF.

Adding LPS to cultured, macrophage-like monocytes has been shown to induce release of CF from monocytes (5). Similar observations were made in this study with the DDCC assay and freshly isolated monocytes as effector cells. It appeared that LPS accelerated the secretion of CF. Untreated, freshly isolated human monocytes released nearly the same amount of cytotoxic activity as LPS-treated monocytes when secretion was allowed for more than 6 h. We speculate that the isolation procedure may be a strong and nearly maximal stimulus for CF synthesis and secretion. An alternative hypothesis may be that monocytes circulate *in vivo* in an "activated" state. The low levels of cytotoxicity and CF secretion found in nonactivated, cultured macrophage-like monocytes (11) indicate that adaption to *in vitro* culture induces a down regulation of rather high levels of cytotoxicity found in the present study.

Previous work has shown that the membrane-associated CF on macrophage-like, cultured monocytes increases considerably when the cells are activated to a cytotoxic state by γ-interferon (20), suggesting a correlation between membrane expression of CF and cytotoxic activity. In accordance with this, immunoflu-

Table 2 *Effect of NK cell depletion on specific cytolysis mediated by adherent cells*

Act D-treated WEHI 164 cells and NK-sensitive K562 cells were used as target cells in a 6-h ^{51}Cr release assay. E:T ratio = 20:1.

	Adherent cell treatment	
Target cell	Complement	Leu 11b + complement
WEHI 164	19.2 ± 1.5[a]	24.7 ± 1.3
K562	68.1 ± 2.4	6.6 ± 3.3

[a] Mean ± SD, n = 1. One of two similar experiments is presented.

orescence microscopy and ELISA in this study revealed that freshly isolated monocytes with high cytotoxic potential had more CF present on their plasma membrane than nonactivated, *in vitro* cultured monocytes with low cytotoxic potential.

Freshly isolated adherent mononuclear cells are contaminated with 1–8% NK cells (21), which may contribute to the spontaneous cytolysis of NK-sensitive target cells (10, 19). This was confirmed by our observation that cytolysis of K562 cells was inhibited by NK cell depletion. The lack of effect of this maneuver on the cytolysis of WEHI 164 cells confirms earlier observations that WEHI 164 cells are resistant to NK cell cytotoxicity (17, 18). The results obtained with the WEHI 164 assay thus seem to reflect solely the cytotoxic activity of monocytes.

At least some of the CF activity may be due to human TNF, the gene of which has recently been cloned (32–35). TNF (33, 34) as CF (5) is produced by monocytes exposed to LPS. HL-60 cells differentiated with 4β-phorbol-12β-myristate-13α-acetate produce TNF (32). HL-60 cells differentiated with 4β-phorbol-12β-myristate-13α-acetate release a cytotoxic activity into supernatants which is inhibited by CF antiserum.[6] Molecular weight of CF and TNF as determined by gel filtration is 40,000–45,000 (4, 32, 35), and their isoelectric points are between 5.0 and 6.0 (4, 32). The relationship between CF and TNF remains a subject for further investigation. Studying mechanisms of cytolysis by CF in DDCC may offer information useful for therapeutic use of recombinant TNF in combination with chemotherapeutic drugs.

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