Monocytes are thought to play a role in host resistance to tumor cell growth in animals and humans. In addition, platelets are known to be involved in tumor metastases. To investigate the interaction of these two cell types and their effect on tumor cells, human monocytes and platelets were examined using an in vitro monocyte–tumor cell cytotoxicity assay. Monocytes alone resulted in 32% ± 1.5 (mean ± SEM) tumor cell kill. When platelets were added to monocytes in a 1:1 ratio, an increase in cytotoxicity to 61% ± 3.2 was observed. The cytotoxicity noted when platelets were added to a fixed number of monocytes and tumor cells was dependent on the number of platelets added. A decrease in cytotoxicity from 32% ± 1.5 to 12% ± 2.3 was observed when contaminating platelets were removed from monocyte preparations. Platelets added to tumor cells in the absence of any monocytes were also toxic, resulting in a maximum kill of 95% at a 4:1 platelet/tumor cell ratio. Secreted products of freshly isolated platelets may be responsible for much of the observed cytotoxicity, since supernatants from the platelets were toxic for tumor cells. Platelets pretreated with a cyclooxygenase inhibitor (ASA) or a lipoxygenase inhibitor had decreased cytotoxicity compared with untreated platelets. Our results indicate that products of platelet arachidonate metabolism are toxic for tumor cell lines. They also suggest that the role of the platelet must be considered when studying monocyte–tumor cell cytotoxicity.

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MATERIALS AND METHODS

Preparation of Human Monocytes

Fresh heparinized blood was obtained from normal human volunteer donors. Peripheral blood mononuclear cells were isolated by Ficoll-Hypaque gradient separation and allowed to adhere to culture flasks in RPMI 1640 with 10% newborn calf serum (NCS) in 5% CO2 at 37 °C for one hour. Monocytes were removed from flasks by adding phosphate-buffered saline, 0.1 mol/L EDTA (PBS EDTA), pH 7.2, and then resuspended in culture media, and plated in microtiter wells. In other experiments, the monocytes were washed twice with 100% autologous serum to remove contaminating platelets before being resuspended in culture medium, counted, and plated. This method is similar to that described by Pawlowski et al, except that the use of EDTA and the ten-minute incubations have been omitted. Mononuclear cells obtained by adherence were 95% nonspecific esterase-positive and 88% to 90% ingested latex. These criteria define a relatively pure population of monocytes, as previously demonstrated. Nevertheless, the adherent cell population washed by autologous serum had less than a 1:1 platelet/monocyte ratio, while in nonwashed monocyte preparations the mean platelet/monocyte ratio was 3:1, as determined by evaluation of Wright-Giemsa stained cytopsin slides.
PLATELET CYTOTOXICITY OF TUMOR CELLS

Tumor Cells

Two adherent tumor cell lines, Malme, a human melanoma cell line, and 786, a human renal cell carcinoma line, were used as targets. Tumor cells were cultured in flasks overnight with \(^{(3)}\H\) thymidine, (specific activity 20 Ci/mmol/L, New England Nuclear, Boston), removed with PBS EDTA, and resuspended in culture media. \(^{(3)}\H\) thymidine-labeled tumor cell targets were plated in microtiter wells at a final concentration of 2.5 x 10⁶ cells per well.

Platelets

Platelet-rich plasma (PRP) was prepared from citrated whole blood by centrifugation and further concentrated by additional centrifugation at 4°C after the addition of 0.1 mL (0.1 mol/L EDTA) per mL of PRP. Fewer than ten lymphocytes were present per milliliter of PRP. Platelets were resuspended in culture media at a concentration of 5 x 10⁵/mL. Gel-filtered platelets were prepared by filtration on Sepharose 2B columns and eluted with a modified Lindon’s buffer. Platelet supernatants were obtained by allowing 1 x 10⁶ platelets in 200 µL of media to adhere in microtiter wells for two to four hours in 5% CO₂ at 37°C. The supernatants were aspirated, centrifuged to remove any remaining platelets, and used immediately.

Platelet Inhibition Studies

To test the effect of acetyl salicylic acid (ASA) on platelet-mediated cytotoxicity, we exposed platelets to 5 x 10⁻⁴ mol/L ASA (Sigma Chemical Co, St Louis) for 15 minutes, washed and then assayed for tumor cell cytotoxicity. To test the effect of platelet lipoxygenase inhibitors on platelet-mediated tumor cell cytotoxicity, we utilized nordihydroguaiaretic acid (NDGA) and 4, 7, 10, 13 Eicosatetraynoic acid (ETYA) (both kindly provided by Dr Gundu Rao) in final concentrations of 1.5 x 10⁻⁶ mol/L and 10 x 10⁻⁶ mol/L, respectively. Platelets (5 x 10⁶/mL) were incubated with either inhibitor or both for 20 minutes at 37°C prior to performing the tumor cell cytotoxicity assay. Lipoxygenase inhibitors remained in the system during the assay, as it is known that the effect is easily and almost immediately reversible by washing.

In Vitro Tumor Cell Cytotoxicity Assay

To examine the effects of monocytes plus platelets, platelets alone, or their secreted products on human tumor cell lines, we utilized a previously described tumor cell cytotoxicity assay.\(^{14}\) In brief, human monocytes were allowed to adhere to microtiter wells (5 x 10⁶ cells per well) and labeled tumor cells (Malme or 786) were added in a 20:1 effector to target (E/T) ratio. We find this ratio to be optimal for reproducible monocyte–tumor cell cytotoxicity. The cell mixtures were incubated for 48 hours at 37°C and 5% CO₂, wells were washed with fresh media, and residual radioactivity counted. Percent cytocidal activity was calculated using the formula:

\[
\frac{1 - \text{cpm effector} + \text{target}}{\text{cpm target}} \times 100.
\]

In some experiments, various numbers of platelets were added to the monocyte–tumor cell mixture; in others, platelets alone or platelet supernatants alone were added directly to the labeled tumor cells, and subsequent tumor cell cytotoxicity was determined.

RESULTS

Effects of Monocytes, Monocytes Plus Platelets, and Platelets Alone on Tumor Cells

Monocytes alone resulted in a mean tumor cell cytotoxicity of 32% ± 1.5 (n = 10). When additional platelets (5 x 10⁶ per well) were added to these monocytes, cytotoxicity increased to 61% ± 3.2 (n = 6) (P < .01, Fig 1). Dose–response curves were obtained by adding increasing numbers of platelets to constant numbers of monocytes (5 x 10⁶ per well) and tumor cells (2.5 x 10⁶ per well). Cytotoxicity increased with the addition of increasing numbers of platelets and reached a maximum of 78% at a 1:1 platelet/monocyte ratio. A representative experiment is shown in Fig 2. Data in the above experiments were obtained using nonwashed monocyte preparations.

After washing freshly isolated monocytes with 100% autologous serum, the platelet/monocyte ratio decreased from 3:1 to 0.5:1 and cytotoxicity decreased from 32% ± 1.5 to 12% ± 2.3 (n = 5, P < .01). The addition of either PRP or gel-filtered platelets to the washed monocytes to provide a 1:1 platelet/monocyte ratio restored cytotoxicity to the level noted in the unwashed monocyte preparation (Fig 1).

Addition of increasing numbers of platelets to tumor cells (n = 8) in the absence of monocytes resulted in increasing tumor cell cytotoxicity with maximum cytotoxicity of 95% ± 1.08 observed at a 8:1 platelet/tumor cell ratio (Fig 3). Gel-filtered platelets and PRP were equally toxic for tumor cells, indicating that plasma factors were not responsible for this phenomenon (data not shown). Platelet supernatants used in final concentrations ranging from 5% to 50% of the culture media resulted in a dose-dependent increase in tumor cell cytotoxicity. This cytotoxicity reached 58.3% ± 7.5% at 50% of the culture media (N = 6). A representative experiment is shown in Fig 4.

Effect of Cyclooxygenase and Lipoxygenase Inhibitors on Platelet-Mediated Cytotoxicity

Inhibitors of the cyclooxygenase and lipoxygenase pathways of arachidonate metabolism reduced platelet-mediated cytotoxicity. The mean cytotoxicity of platelets preincubated with ASA (5 x 10⁻⁴ mol/L) and added to tumor cells in a 20:1 ratio, was 42 ± 8.3% (n = 7) compared with a control mean of 60 ± 4.9% (n = 19) (P < .10). A similar degree of inhibition was

![Fig 1. Percentage of cytotoxicity of monocytes, monocytes plus platelets, monocytes washed with autologous serum, and washed monocytes plus platelets (n = 6). Data are expressed as mean ± SEM.](image-url)
observed with NDGA. Preincubation of platelets with both ASA ($5 \times 10^{-4}$ mol/L) and NDGA ($1.5 \times 10^{-6}$ mol/L) resulted in a significant decrease in cytotoxicity to $20\% \pm 6.0\%$ ($n = 7; P < .01$). Variable inhibition was observed with ETYA, a problem noted by other investigators when using this inhibitor.\(^{13}\)

**DISCUSSION**

These observations indicate that platelets and/or their secreted products are toxic for human tumor cell lines. Monocyte preparations free of platelet contamination result in less tumor cell kill than monocyte preparations from which platelets have not been removed. Platelets alone, as PRP or gel-filtered platelets, and supernatants obtained from either of these platelet preparations were also effective mediators of tumor cell lysis. The use of a cyclooxygenase inhibitor (ASA) or both cyclooxygenase and lipoxygenase inhibitors (ASA and NDGA) result in decreased platelet-induced cytotoxicity. These results suggest that arachidonate metabolites may be involved in this toxic event.

Although considered important primarily as mediators of hemostasis, there is evidence to suggest that blood platelets and/or platelet products are capable of mediating a variety of cellular effects not directly related to hemostasis. In addition to the well-known cellular growth stimulation induced by platelet-derived growth factor,\(^{13}\) cellular growth inhibition, killing, and lysis have been attributed to platelets and platelet products. Inhibition of tumor growth in vitro has been observed following the addition of prostaglandins $D_2$, $E_1$, and $E_2$ \(^{16,18}\) products of the cyclooxygenase pathway of platelet prostaglandin metabolism. Lipoxygenase pathway products have also been implicated in cellular growth inhibition and lysis. The lipoxygenase pathway product, 12 hydroxyeicosatetraenoic acid (12 HETE), has been shown to be inhibitory for human neuroblastoma cells.\(^{19}\) Cell lysis by natural killer (NK) cells may also involve lipoxygenase pathway metabolites. Inhibition of lipoxygenase by BW775C and NDGA resulted in inhibition of NK-mediated lysis of K562 and Molt 4 cells, without influencing target cell binding.\(^{13}\) No inhibition was noted at levels sufficient to inhibit cyclooxygenase but not lipoxygenase.\(^{13}\)

More direct evidence in support of our observations of platelet-mediated cytotoxicity is the fact that platelets have been found to be capable of lysing complement-sensitized human erythrocytes.\(^{20}\) Also of interest is the observation that platelets from patients infested with schistosoma mansoni, but not from normal controls, kill schistosomes in vitro.\(^{21}\)

This study extends previous observations that indicate platelets are capable of exerting significant effects on cell growth and viability. Although the studies completed to date do not precisely define the mechanism by which platelets mediate tumor cell cytotoxicity, they strongly suggest that secretory products, perhaps arachidonate metabolites, are responsible. The apparent additive effect of cyclooxygenase and lipoxygenase inhibitors is of interest in view of the fact that lipoxygenase inhibitors have been shown to inhibit platelet adhesion and primary wave aggregation in ASA-incubated platelets.\(^{22}\) In addition, our study
highlights the possible contribution of platelets in in vitro monocyte–tumor cell lysis assays. Platelets generally have not been removed from monocyte preparations used in tumor cell cytotoxicity assays, presumably because they have not previously been known to possess effector functions in such situations. Our results indicate that it is important to consider the contribution of the platelet when analyzing monocyte–tumor cell cytotoxicity studies. In addition, they suggest the possibility that platelets play a role in host defense against neoplasia.

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Human platelets exert cytotoxic effects on tumor cells

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