Effect of Human Tumor Cells on Platelet Aggregation: Potential Relevance to Pattern of Metastasis

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ABSTRACT

Tumor metastasis may be facilitated by interaction of tumor cells with platelets. It is not known, however, whether solid tumors which have predisposition to pulmonary metastasis affect platelets differently than lymphoid tumors, which rarely spread to lungs. We therefore examined the effects of cultured osteogenic sarcoma (MG-63, U2-OS), as well as leukemia (NALM-16, LAZ-221, K-562) and lymphoma (RAJI, MOLT 4) cells, on human platelet aggregation. Human osteogenic sarcoma (MG-63) cells alone induced platelet aggregation, whereas U2-OS cells induced platelet aggregation only after preincubation of platelets with subthreshold concentrations of epinephrine. In contrast, neither leukemia nor lymphoma cells affected platelet aggregation. These observations suggest that the platelet proaggregatory potential of tumor cells is variable and that the platelet stimulatory effects of osteogenic sarcoma cells may relate to their high risk of pulmonary metastasis.

INTRODUCTION

Tumor metastasis represents a complex process by which tumor cells escape from the primary site, invade surrounding tissue, extravasate, and traverse blood vessels or lymphatic channels to proliferate in distant sites (1). Although many tumor cells circulate in peripheral blood, only some tumor cells successfully implant and metastasize in lungs. There is evidence that hematogenous spread of certain tumors may be facilitated by the interaction of tumor cells with platelets (2). Whether other tumor cells which metastasize via the blood stream or tumor cells which circulate in blood but do not metastasize predominantly via a hematogenous route also interact with platelets is not known. We therefore compared platelet-tumor cell interactions of human osteogenic sarcoma cells which metastasize to lungs with other tumors which circulate in blood but which rarely result in pulmonary metastasis.

MATERIALS AND METHODS

Tumor Cell Lines. Human osteogenic sarcoma cells, MG-63 and U2-OS, were obtained from the American Type Tissue Collection (Rockville, MD). The cells were grown in Dulbecco's modified Eagle's medium and were supplemented with 15% fetal bovine serum, penicillin, and streptomycin, 100 μg/ml. Adherent cell populations were brought to a single cell suspension by a 5-min incubation in phosphate buffered saline and counted in a hematocytometer. There was more than 95% cell viability in every experiment. Cells were maintained at 4°C until use in platelet aggregation experiments.

Human leukemia cells, NALM-16 and LAZ-221, were obtained from Roswell Park Memorial Institute, Buffalo, NY. NALM-16 is a null cell leukemia and LAZ-221 is a pre-B-cell leukemia. The cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM l-glutamine, and 1% antibiotic-antimycotic mixture (GIBCO, Grand Island, NY). Cultures were split at a 1:5 ratio at approximately weekly intervals. An in vitro chronic myelogenous leukemia cell line, K-562, was obtained from the American Type Tissue Collection, and was grown in RPMI 1640 and 10% fetal bovine serum.

Burkitt's lymphoma (RAJI) and T-cell lymphoma cells (MOLT 4) were obtained from the American Type Tissue Collection, and were grown in suspension in RPMI 1640 and 10% fetal bovine serum.

Preparation of Tumor Cells for Platelet Aggregation Studies. Suspensions of tumor cells were adjusted to a final cell concentration of 0.5 to 4 x 10^6 cells/ml. Tumor cell viability was determined prior to each experiment at 0.5-h intervals. Viability was measured by the trypan blue dye exclusion method and was over 95% throughout each experiment. Cells were maintained at 4°C until use in platelet aggregation studies.

Control Experiments. In control experiments, latex particles were used to examine the effect of inert particles on platelet aggregation. Latex particles, 0.8 μm, (Difco Laboratories, Detroit, MI) were suspended in normal saline to a final concentration of 1-2 x 10^9 particles/ml. As another control, normal human lymphocytes were also used to determine their effects on platelet aggregation. In these experiments, blood was obtained from laboratory personnel who had not ingested any medications within the previous 10-day period. Lymphocytes were isolated by layering 10 ml of whole blood collected in heparin (10 units/ml) over 10 ml of monocyte polymorphonuclear resolving medium (Flow Laboratories, Oak Grove, IL). Blood was then centrifuged at 300 x g for 10 min and the lymphocyte fraction was removed. The identity of these cells as lymphocytes was confirmed by light microscopy.

Platelet Aggregation. Platelet aggregation was measured by light transmission as described previously (3). Blood was obtained from normal human donors and collected in heparin (10 units/ml). Blood was centrifuged at 150 x g for 8 min to obtain PRP. The remaining blood was centrifuged at 1500 x g for 30 min to obtain platelet poor plasma. Platelet poor plasma was used to calibrate light transmission at 100%. The platelet count in PRP was adjusted between 2 and 3 x 10^11/ml for each experiment. Aggregation was measured in a Sienco Aggregometer (Sienco Co., Denver, CO). PRP was incubated at 37°C with normal saline or tumor cells (0.5 to 4 x 10^6 cells/ml). In other experiments, light transmission was measured before and 5 min after addition of aggregating agents ADP (0.02 to 2.0 μM) or epinephrine (0.055 to 5.5 μM) to PRP-tumor cell suspensions. The aggregating agents were used in subthreshold concentrations, defined as the maximal amount of aggregant which failed to induce platelet aggregation of more than 20%. The final concentration of tumor cells, lymphocytes, or latex particles in each cuvet was 0.5-4 x 10^6 cells/ml.

RESULTS

Effects of Latex Particles or Normal Lymphocytes on Platelet Aggregation. Latex particles alone did not induce platelet aggregation. These particles also did not affect platelet aggregation in response to ADP or epinephrine. Similarly, human lymphocytes from normal donors did not cause spontaneous platelet aggregation and had no effect on ADP or epinephrine induced platelet aggregation.

Differential Effects of Tumor Cells on Platelet Aggregation.

3 The abbreviation used is: PRP, platelet rich plasma.

3115
Human osteogenic sarcoma (MG-63) cells caused platelet aggregation in a concentration dependent fashion. Increasing the number of tumor cells (0.5 to 2 x 10^6 cells/ml) caused progressive increase in platelet aggregation with a maximum effect at 2 x 10^6 cells/ml (Fig. 1). Platelet aggregation occurred in response to MG-63 cells in 5 of 6 donors tested. The magnitude of platelet aggregation caused by 2 x 10^6 cells/ml was similar to that caused by threshold concentrations of epinephrine. In these subjects, tumor cell induced aggregation was complete and irreversible (Fig. 2). Preincubation of PRP with subthreshold concentrations of epinephrine or ADP did not increase platelet aggregation caused by tumor cells (2 x 10^6 cells/ml) suggesting that maximal platelet aggregation had already been achieved. In PRP from one donor, the same concentration of tumor cells added directly to the PRP did not induce platelet aggregation, but it did so when PRP was preincubated with subthreshold concentrations of epinephrine. Similarly, using subthreshold concentrations of tumor cells (i.e., 0.5 x 10^6 cells/ml) and of epinephrine, no potentiation of aggregation was observed. In contrast to MG-63 cells, U2-OS cells incubated with PRP alone did not induce aggregation in any PRP sample. However, in all cases U2-OS cells caused complete aggregation when PRP was preincubated with subthreshold concentrations of epinephrine. Although preincubation of PRP with subthreshold concentrations of epinephrine increased responsiveness of platelets to U2-OS cells, subthreshold concentrations of ADP did not (Table 1).

There was no aggregation of platelets in response to the leukemia or lymphoma lines studied. Preincubation of platelets with subthreshold concentration of epinephrine or ADP failed to increase platelet responsiveness to tumor cells, even if the tumor cells were increased to 1 x 10^7 cells/ml (Table 1).

DISCUSSION

In this study, two human osteogenic sarcoma cell lines exhibited platelet aggregation stimulatory activity, whereas human leukemia and lymphoma cells did not. The MG-63 human osteogenic sarcoma cells caused immediate and complete platelet aggregation in 5 of 6 PRP samples. In the remaining PRP sample MG-63 cells caused complete aggregation when PRP was preincubated with subthreshold concentrations of epinephrine. The U2-OS human osteogenic sarcoma cells potentiated human platelet aggregation in all cases when PRP was preincubated with subthreshold concentrations of epinephrine. In vivo, platelets are continually exposed to epinephrine. Thus, the preincubation of platelets with low concentrations of epinephrine in vitro may parallel an in vivo physiological state as shown in our previous studies (3). Although preincubation of platelets with subthreshold epinephrine sensitized platelets to effects of tumor cells, preincubation with subthreshold ADP did not, suggesting that the epinephrine pathway of platelet activation may be important in platelet-tumor cell interactions.

In contrast to the stimulatory effects of osteogenic sarcoma cells on platelets, none of the lymphoid lines studied demonstrated the ability to stimulate platelet aggregation. We evaluated several variants of lymphoid cells to determine if certain lymphoid tumors were more stimulatory than others. In these experiments, neither null cell, pre-B, lymphoid, or myelogenous leukemia or B- or T-cell lymphoma demonstrated any stimulatory activity on platelets.
that the heterogeneity of responsiveness of platelets to tumor cells could be genetically determined. On the basis of the results of our studies, we suggest that the variability in responsiveness of platelets to some tumor cell lines can be reduced by pretreatment of platelets with very low concentrations of epinephrine. We also speculate that the stimulation of platelets by osteogenic sarcoma cells could relate to their tendency to metastasize to lungs. The evidence that platelets may facilitate tumor metastasis was originally suggested by Gasic et al. (5) who reported a reduction in ascites tumor cell metastasis following thrombocytopenia in the host. Since that time, other investigators have suggested a similar potential role of platelets in contributing to tumor metastasis (4, 6, 7).

Platelets may enhance tumor metastasis by one of several mechanisms. Platelet aggregates surrounding tumor cells may shield tumor cells from immunosurveillance (8). At the same time, platelets can enhance adhesion of tumor cells to endothelium or to deendothelialized surfaces via platelet bridges (9, 10). In addition, platelets can facilitate tumor cell growth by release of mitogenic factors (11). Whether these processes occur in human tumors which circulate primarily in blood vessels is not known, nor is it known if the ability of tumor cells to interact with platelets affects the risk of spread and ultimate site of metastasis.

The role of platelets in metastasis of specific tumors has not been clearly demonstrated. Leukemia and lymphoma cells may circulate in blood but rarely cause pulmonary metastasis. The stimulatory effect of osteogenic sarcoma cells on platelets, and the lack of such effect by leukemia or lymphoma cells, may relate to the pattern of metastasis of these tumors. Osteogenic sarcoma cells preferentially metastasize to lung whereas in contrast leukemia and lymphoma cells rarely do so. This difference in site of metastasis may relate to the inability of leukemia and lymphoma cells to stimulate platelets. The lack of interaction of these tumor cells with platelets may render these cells less likely to attach to vascular endothelium, a critical step in pulmonary tumor metastasis (1).

It is important to emphasize that the platelet aggregating activity of osteogenic sarcoma cells may represent only one mechanism by which tumor cell metastasis is facilitated. Metastasis of tumor cells is a complex cascade of events. Tumor cells must first proliferate at the initial site of disease, aided by growth factors, angiogenesis, and blood vessel permeability factors (14). Subsequently, cells detach from the primary tumor, a process which depends on size, type and histology of tumor, and presence of enzymes such as collagenase, plasmin, and cathepsin D-like cysteine proteases. Invasion of cells into the lymphatic or blood vessel compartments also relates to type and histology of tumor as well as similar enzymes. Once cells circulate, they must arrest at a particular vascular or lymphatic bed prior to metastasis. The coagulation processes (i.e., fibrin formation and platelet aggregation) are important at this step in organizing cells into cell aggregates and in stabilizing these aggregates to the vessel wall. Throughout these steps in metastasis, host factors remain critical, since the immune status of the host, presence of other diseases or other areas of metastasis, genetics, and other factors will affect actual determination of metastasis in a given individual.

REFERENCES

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