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Enforced Adhesion of Hematopoietic Cells to Culture Dish Induces Endomitosis and Polyploidy

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Abstract

Cells of epithelial or endothelial lineage when forced to grow in suspension undergo the detachment-induced death termed “anoikis”. In the present study we explored the reverse situation namely the effect of enforcement of hematopoietic lineage cells that are normally maintained in suspension to grow attached. Towards this end murine L1210 or human HL-60 and Jurkat leukemia cells were cultured in slide chambers coated with poly-L- or poly-D- lysine, or with compound 48/80, the polycations attracting them electrostatically. Within minutes after the transfer L1210 cells strongly adhered to bottom surface of the dish and shortly thereafter binuclear-, and later on, polynuclear-cells become apparent. The frequency of nuclei per cell was increasing with time and polykaryons with up to 16 nuclei and high DNA ploidy (DI = 16.0) were apparent after 48 h. After 4 days the size (volume) of some polykaryons exceeded by over 340-fold the volume of mononuclear cells. The presence of mitotic figures and abnormal mitotic spindles in adhering polykaryons provided evidence of the impeded cytokinesis that led to endomitosis. Most polykaryons excluded trypan blue, had balanced growth (unchanged protein/DNA ratio compared to monokaryons), and showed no evidence of apoptosis. Individual nuclei within each polykaryon replicated DNA in synchrony. The strong cell attachment and aborted cytokinesis were cell line specific since no significant endomitosis was observed in Jurkat- or HL-60- cells which did not strongly attach to polycation-coated surfaces. Defective cytokinesis and induction of polyploidy by this mechanism, if occurs in vivo (e.g., mediated by integrins), may lead to aneuploidy and therefore have tumorigenic consequences. The phenomenon offers novel experimental model for induction of polyploidy and provides alternative to cytocholasin B to prevent cytokinesis in the mutagenicity cytokinesis-blocked micronucleus (CBMN) assay.

Keywords

Polykaryon; cytokinesis; endomitosis; endoreplication; polylysine; compound 48/80; polycations; laser scanning cytometry; aneuploidy; micronucleus

INTRODUCTION

Cells of epithelial or endothelial lineage that grow in cultures adhering to the flasks when enforced to grow in suspension undergo the detachment-induced death defined as “anoikis”, which shows characteristic features of apoptosis.[1–3] Their attachment, thus, is essential for survival and apoptosis appears to be triggered by interruption of phosphoinositide-3 kinase mediated signaling from cell surface.[4–7] Much less is known about reverse situation, namely

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how growth of hematopoietic cells that are normally cultured in suspension is affected by enforcing their attachment to solid surfaces. The aim of the present study, therefore, was to characterize growth of cells of leukemic lines under conditions of the enforced cell attachment to the solid surface. The attachment was imposed by using the polycation-coated culture dishes to which during culturing cells adhered by electrostatic forces.[8,9] Of the three leukemic cell lines that were investigated only murine lymphocytic L1210 cells attached strongly to the coated surfaces; the attachment of human T-lymphocytic Jurkat or promyelocytic HL-60 cells was less pronounced. There was no evidence of significant apoptosis or "anoikis"- like cell death that could be attributed to cell attachment. However, cytokinesis of the attached L1210 cells was impaired leading to endomitosis and formation of polykaryons. This is a novel mechanism of polyploidy induction, and it may play a role in some physiological and pathological processes of hematopoietic tissue. It may also be used as an experimental model for induction of polyploidy, complementing the alternative approaches that rely on cell fusion or use of chemical agents targeting cytokinesis.

MATERIALS AND METHODS

Cells and cell treatments

The murine leukemic cell line L1210 was originally obtained from Dr. Bayard D. Clarkson of the Memorial Sloan-Kettering Cancer Center; their doubling time in culture is approximately 12 h.[10] Human lymphoma Jurkat cells were kindly provided by Dr. Douglas R. Green of La Jolla Institute for Allergy and Immunology, San Diego, CA. Human promyelocytic leukemic HL-60 cells were obtained from American Type Culture Collection (ATCC; Manassas, VA). The cells were grown in suspension in 25 ml FALCON flasks (Becton Dickinson Co., Franklin Lakes, NJ) in RPMI 1640 supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (all from GIBCO/BRL Life Technologies, Inc., Grand Island, NY) at 37.5°C in an atmosphere of 5 cells per ml in culture and the 5% CO_2 in air; there were fewer than 5×10^5 cells were in exponential and asynchronous phase of growth. At the onset of experiments cells from suspension cultures were transferred into poly-L-lysine or poly-D-lysine Sigma Chemical Co., St. Louis, MO) coated 2-well culture slides (Biocoat Cellware from Becton Dickinson, Bedford, MA) or 25 ml FALCON flasks, for various time as shown in figure legends. In some cultures, 10 μg/ml compound 48/80 (Sigma) was administrated into the culture.

Laser scanning cytometry (LSC)

L1210 or Jurkat cells were fixed in 80% ethanol and kept in −20°C for 2–24 hours before they were stained either with 1 μg/ml 4,6,-diamidino-2-phenylindole (DAPI; Molecular Probes, Inc., Eugene, OR) alone or with DAPI and 1 μg/ml fluorescein isothiocyanate (FITC; Molecular Probes). Cells grown in suspension in flasks were cyto-centrifuged onto slides. Cellular green (FITC) and blue (DAPI) fluorescence emission was measured using LSC (CompuCyte, Cambridge, MA) utilizing standard filter settings; green fluorescence of FITC and blue of DAPI was excited with 488-nm argon ion and violet diode lasers, respectively.[11] The intensities of maximal pixel and integrated fluorescence were measured and recorded for each cell. Because of large variation in DNA content between cells growing in normal flasks and polylysine-coated slides, logarithmic scale was used in some measurement to display and determine the number of DNA ploidy levels on the DNA content frequency histograms. At least 3,000 cells were measured per sample.

Fluorescence microscopy

Cells were fixed in 80% ethanol for at least 2 hours at −20°C and then were rinsed twice in PBS. The DNA content and cellular protein expressions were determined by immunofluorescence analysis by using either DAPI or 1 μg/ml 7-aminoactinomycin D (7-

AAD; Molecular Probes, Eugene, OR) and FITC. To determine the pattern of localization of microtubules L1210 cells were grown on polylysine-coated slides for 24 hours and then fixed in 100% methanol at −20°C for 10 minutes; some cultures were treated with 50 nM vinblastine (Sigma) for the final 4 h before fixation. Bromo-2′-deoxyuridine (BrdU; Sigma) at 30 μM concentration was added to some cultures 60 min before their fixation in 100% methanol at −20°C. The slides were subsequently rinsed twice in PBS and treated with 0.1% Triton X-100 in PBS for 5 min on ice. After blocking with 1% BSA in PBS for 30 min, the cells were incubated in 1% BSA containing FITC-conjugated α-tubulin mAb (clone DM1A, Sigma) for 45 minutes at room temperature in the dark. The cells incubated with BrdU were treated with 2M HCl for 20 min to partially denature DNA before incubation with 1:20 anti-BrdU monoclonal PRB1-U antibody (Phoenix Flow Systems, San Diego, CA) for 30 minutes. Then the cells were incubated with 1:200 Rhodamine Red-tagged goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, INC., West Grove, PA) for 45 minutes. The cells were then counterstained with 1 μg/ml DAPI. Cellular morphology and fluorescence were examined under a Nikon Microphot FXA microscope, utilizing a 40X objective.

Assay for cell growth and viability of polykaryons detached from polylysine-coated flasks

L1210 cells were initially seeded onto poly-L-lysine-coated flasks and maintained in culture for 48 h. The attached cells were subsequently detached by trypsinization using standard 0.05% trypsin—0.53 mM EDTA solution in HBSS without sodium bicarbonate, Ca^{+2} and Mg^{+2} (Mediatech Inc., Herndon, VA). However, to successfully detach cells from the polylysine-coated flasks the incubation with trypsin had to be extended to 30 min followed by vigorous shake-off. The detached cells were washed with medium to remove any residual trypsin and resuspended in RPMI 1640 containing 10% fetal calf serum in a FALCON 15 ml polystyrene conical tube. The large polykaryons that sedimented to the bottom of the tube at 1x*g* within 60 min were collected and cultured for the following 3 days; their morphology and survival were monitored by interference microscopy.

All experiments were repeated at least three times.

RESULTS

When L1210 cells suspended in tissue culture medium were plated onto microscope slides (“CultureSlides”) coated with poly-L- or poly-D- lysine, or compound 48/80 and transferred for culturing within minutes the cells started to adhere to the slides and after 1–2 h most cells become firmly attached. The attached cells excluded trypan blue and propidium iodide (PI), and when examined by interference microscopy showed strong light refractive properties, typical of live cells. No evidence of apoptosis such as undulations of plasma membrane characteristic of shedding apoptotic bodies, chromatin condensation or cell shrinkage could be seen among the attached cells. The apoptotic index, estimated by characteristic changes in cell morphology[12] was similar among the cells growing attached to polylysine-coated slides as compared to cells growing in suspension (<7%). Many cells were undergoing mitosis, and mitotic index in the population of adhering cells was also similar to that of the cells cultured in suspension. However, unlike the cells that normally grow attached, such as fibroblasts or cells of endothelial lineage,[13] L1210 cells growing on polycation-coated slides did not show “rounding-up” and detachment during mitosis. Figure 1 illustrates characteristic patterns of mitosis of the attached cells during the initial several hours after the attachment. The interesting feature of mitosis of these cells was the apparent lack of cytokinesis. Thus, while cells in anaphase and telophase were distinctly present, no evidence of the contracting ring and the cleavage furrow in the mid-section of most mitotic cells that would indicate on progression through cytokinesis, was apparent. In fact, in many mitotic cells the mid-section was wide and flat, distinctly physically attached to the surface of the culture chamber. Numerous post-mitotic

cells were binuclear, some having highly condensed chromatin in both nuclei while other, apparently being later after mitosis, less condensed chromatin, similar to that of the most mononuclear interphase cells on the slide. Examination of L1210 cells by microscopy during the initial 2–12 hours of culturing, thus, provided strong evidence that their attachment to the slides coated with poly-D-lysine impaired cytokinesis and led to endomitosis (Fig. 1). Cytokinesis of L1210 cells was also prevented during their growth when attached to poly-L-lysine coated slides or attached in the presence of compound 48/80 (not shown).

It should be noted that endomitosis was observed in the case of cells in which the metaphase plate was perpendicular to the surface of adherence. Mitotic cells that were attached at the site of the spindle pole and thus their metaphase (equatorial) plate was oriented horizontally vis-à-vis the adherence surface appeared to undergo normal cytokinesis (Fig. 1E).

Figure 2, illustrates formation of L1210 polykaryons in cultures maintained on poly-D-lysine coated slides (A,C) or in the presence of compound 48/80 (B) for 48 h. High variability in cell size and number of nuclei per cell was apparent in these cultures, which contained small mononuclear cells as well as polykaryons, some having up to 32 nuclei. In large polykaryons the nuclei were distributed on cell periphery and were distinctly absent in the center. Most nuclei in polykaryons appeared to be larger than the nuclei in mononuclear cells. There was no evidence of significant apoptosis among the attached cells regardless whether the attachment was induced by polylysine or compound 48/80, or regardless on number of nuclei per cell.

The variability in cell size was still larger in the cultures maintained for longer periods of time. Figure 3, shows size differences between mononuclear cells and polykaryons after 96 h of culturing when unfixed cells in suspension (i.e., spherical in shape) are examined by interference contrast microscopy. The cells shown in Figure 3, were initially maintained as attached for 48 h, they were then detached by trypsinization and subsequently grown in suspension for additional 48 h. The polykaryons remain viable and cell cycle progression and endomitosis continue, as cells grow in size following their transfer to suspension culture. Diameter of the polykaryon shown in this figure, whose size is representative of many other polykaryons seen after 96 h of culturing, is at least 7-fold- and its volume 344-fold- greater than diameter and volume of the average mononuclear cell, respectively.

Additional evidence for endomitosis of the adhering cells was provided by their DNA content measurement using LSC (Fig. 4). Namely, it was quite evident from the frequency histograms representing cellular DNA content that there was a progressive increase in proportion of tetraploid cells with DNA index (DI) = 2.0 among the attached cells during the culturing. In contrast, there was a predominance of DI = 1.0 cells and distinct decrease in proportion of S and DI = 2.0 cells among the cells that were detaching in these cultures, compared to control.

DNA content frequency histograms of L1210 cells growing attached for 48 h show the presence of polyploid cells with distinct DI = 1.0, 2.0, 4.0 and even 16.0 peaks (Fig. 5). Concurrently with DNA content which was estimated based on the intensity of DAPI fluorescence (integrated over mono- or poly-karyons) we have also measured protein content (intensity of FITC fluorescence, integral) of these cells. The protein/DNA ratio of polykaryons was similar to that of mononuclear cells. Thus, when protein/DNA ratio of mononuclear cells with DNA content DI = 1.0 was measured by LSC in four experiments and defined in each measurement as 1.00, this ratio for the polykaryons with DI = 2.0 and 4.0 was 0.97 ± 0.04 and 0.99 ± 0.06, respectively.

Figure 6, illustrates intracellular distribution of microtubules in interphase- and mitotic-polykaryons. In nearly all interphase polykaryons the centrosomes were located as a single aggregate in the center of the cell, in the space devoid of nuclei as the latter had peripheral location. The arrays of individual microtubules were rather uniformly distributed over whole

polykaryons, in the pattern resembling their normal distribution in mononuclear cells. Several distinct centrosomes were apparent in mitotic polykaryons. From each centrosome the polar spindle microtubules protruded into the center of polykaryon. Treatment of cells with vinblastine led to disassembly of centrosomes and microtubules, both in mono- and poly-karyons (not shown), DNA replication in individual nuclei in polykaryons was synchronous (Fig. 7). Thus, following 60 min pulse-incubation with BrdU either all nuclei in the polykaryon were labeled, or all were unlabeled. Also the degree of labeling of individual nuclei, most likely reflecting the overlap between time-window of DNA replication and BrdU accessibility, was strikingly similar for all nuclei per polykaryon. This would indicate that the synchrony of DNA replication was nearly perfect.

It should be noted that about 60% polykaryons were labeled after 60 min exposure to BrdU. Essentially the same proportion of mononuclear L1210 cells was also labeled, which is consistent with approximately 12 h generation time of L1210 cells[10] and with the rate of accumulation of cells with high DI (Fig. 5). This would indicate that despite of the impaired cytokinesis the cell cycle kinetics in polykaryons remains unchanged relative to mononuclear cells.

No distinct evidence of polykaryons or DNA-polyploid cells that could be detected either by microscopy or DNA content measurement by LSC, respectively, was observed in Jurkat cultures maintained up to 48 h on the polycation-coated slides (not shown). However, compared to L1210, Jurkat cells appeared to be less strongly attached since significant number of cells were floating in the medium, and vigorous shaking of the cultures dislodged many of the attached cells. HL-60 cells did not attach at all and nearly all were mononuclear.

DISCUSSION

The present data demonstrate that attachment of hematopoietic cells imposed electrostatically during culturing neither induced their apoptosis nor significantly affected the cell cycle progression. In the case of L1210 cells, however, while the attached cells were progressing through the cycle some were unable to undergo cytokinesis. This led to endomitosis and formation of polykaryons. It appears that chemical composition of the polycation used to enforce cell adhesion was not critical because induction of endomitosis was observed in cultures set on poly-L-, and poly-D- lysine as well as compound 48/80-coated slides. Compound 48/80 is a mixture of synthetic polymers derived from N-methyl-methoxy-phenylethylamine, and because of its ability to interact with plasma membrane and induce cell degranulation is widely used in studies of mast cells and eosinophils.[13,15] In fact, we have already observed before[16] that compound 48/80 can impair cytokinesis of murine leukemic cells, but at that time no mechanistic explanation for this phenomenon was provided. It should be noted that compound 48/80, which at the concentration presently used showed no detectable toxicity to the studied cells, induced cell adherence not only when applied for slide precoating but also when added directly into the cultures.

The present observations suggest that endomitosis of the attached cells occurred when mitotic spindle was in horizontal rather than vertical orientation vis-à-vis surface of the attachment, i.e., the equatorial metaphase plate was perpendicular to the surface. During late telophase of the so attached cells there was no evidence of the contracting ring or cleavage furrow, the features indicating the initiation of cytokinesis. On the contrary, midsections of the cells were wide and distinctly attached to the surface of the slide. On the other hand mitotic cells that were attached at the site of the spindle pole and thus their mitotic spindle was in perpendicular orientation to surface of adherence, appeared to progress successfully through cytokinesis (Fig. 1). The predominance of $G_{0/1}$ cells among the detached cells that were collected during culturing (Fig. 4) suggests that one of the progeny of cells undergoing cytokinesis was

transiently floating in the medium while the other could be still attached. Such phenomenon has been recently described and it was proposed that collection of the detaching cells provides populations of postmitotic cells synchronized in the cell cycle.[17,18]

Polykaryons as large as containing 16 nuclei (DI = 16.0) were formed already after 48 h of growth on polylysine-coated slides (Fig. 5). Because doubling (generation) time of our L1210 cells grown in suspension is approximately 12 h,[10] the presence of polykaryons with 16 nuclei after 48 h indicates that compared to mononuclear cells in suspension, the rate of cell cycle progression of the polykaryons was unchanged, even as cytokinesis was abolished. After detachment by trypsinization and transfer to new cultures these large polykaryons remained viable and continued to grow in size for the next 3 days. It should be noted, however, that L1210 cells attached to poly-D-lysine-coated slides were more resistant to trypsinization compared to most cell types that normally grow attached in cultures.

Among the tested leukemic lines only murine L1210 cells strongly adhered to polycation-coated surfaces and were undergoing endomitosis. Human promyelocytic HL-60 cells did not adhere to these surfaces and their growth was unaffected. Human T-cell leukemic Jurkat cells, while showing some degree of attachment, did not undergo endomitosis, and their growth also was not affected. The strength of electrostatic attachment, thus, varies between cell types, most likely depending on the electronegativity of their surface, known to reflect degree of sialylation of surface glycoproteins.[9] It should be noted, however, that sialic acid present on cell surface plays a minor role in maintenance of the transmembrane potential and ionic permeabilities.[19]

Formation of polykarons is quite common in vivo and it occurs either by mechanism of cell fusion, endoreplication/endomitosis or abortive cell cycle.[20] Cell fusion is a programmed step that leads to production of terminally differentiated cells in some cell types such as skeletal muscle or osteoclasts.[21,22] Cell fusion also occurs during viral infections,[23] formation of granulomas,[24] and was recently proposed as a mechanism explaining differentiation of the transplanted stem cells.[25] The classic example of polykaryon formation by endomitosis is seen during megakaryocytopoiesis, when after DNA replication the cells progress through the cycle up to anaphase A but anaphase B, telophase and cytokinesis do not occur.[26–28] The mechanism of endoreplication which occurs in pathological situations rather than is programmed (as in the case of megakaryocytes) was recently classified as “abortive cell cycle”.[20]

Can the mechanism of polykaryon formation by the enforcement of attachment of the cell plasma membrane, as presently observed in the case of L1210 cells, be of relevance in vivo? Some observations suggest that this mechanism may indeed play a role in the induction of polyploidy. Thus, for example, intercellular matrix and strong cell adhesion to fibronectin via integrins is critical in megakaryocytopoiesis.[26,29] Also, in the phenomenon defined as “foreign body tumorigenesis” strong cell adherence to glass or plastic surfaces implanted to rodents was reported to contribute towards development of sarcomas on these surfaces.[30,31] It is possible that abortive cytokinesis of the cells adhering to these surfaces led to formation of polyploid cells, which in turn led to aneuploidy,[31] with all the consequences of genome instability and cancer development.[20,32]

Cytochalasin B is commonly used to impair cytokinesis, particularly to generate binucleated cells in the “cytokinesis-blocked micronucleus” (CBMN) assays of mutagenicity.[33–35] The present data suggest that impairment of cytokinesis by enforcement of cell attachment may provide an alternative approach to generate binucleated cells that may be used in the CBMN assay. An advantage of this approach is the absence of the chemical agent such as cytochalasin B which prevents cytokinesis but may interfere with the tested mutagens affecting their effectiveness in inducing micronuclei. It should be kept in mind, however, that as in the case

of Jurkat or HL-60, cells of some hemopoietic lines may not attach strongly enough to undergo endomitosis.

Acknowledgements

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References

1. Frish SM, Screaton RA. Anoikis mechanisms. Curr Opin Cell Biol 2001;13:555–62. [PubMed: 11544023]
2. Grossman J. Molecular mechanisms of "detachment-induced apoptosis - anoikis". Apoptosis 2002;7:247–60. [PubMed: 11997669]
3. Valentijn AJ, Zouq N, Gilmore AP. Anoikis. Biochem Soc Trans 2004;32:421–5. [PubMed: 15157151]
4. Khwaja A, Rodriguez-Viciana P, Wennstrom S, Warne PH, Downward J. Matrix adhesion and Ras transformation both activate a phosphoinositide 3-OH kinase and protein kinase B/Akt cellular survival pathway. EMBO J 1997;16:2878–93.
5. Applin AE, Howe AK, Juliano RL. Cell adhesion molecules, signal transduction and cell growth. Curr Opin Cell Biol 1999;11:737–44. [PubMed: 10600702]
6. Giancotti F, Ruoslanti E. Integrin signaling. Science 1999;285:1028–35. [PubMed: 10446041]
7. Zhan M, Zhao H, Han ZC. Signalling mechanisms of anoikis. Histol Histopathol 2004;19:973–83. [PubMed: 15168359]
8. Smetana K Jr, Vytasek R, Stol M. Electrostatic interaction influences cell adhesion. Int J Hematol 1992;56:219–23. [PubMed: 1477334]
9. Marquez M, Nilsson S, Lennartsson L, Liu Z, Tammela T, Raitanen M, Holmberg AR. Charge-dependent targeting: Results in six tumor cell lines. Anticancer Res 2004;24:1347–51. [PubMed: 15274294]
10. Del Bino G, Skierski SJ, Darzynkiewicz Z. Diverse effects of camptothecin, an inhibitor of topoisomerase I, on the cell cycle of lymphocytic (L1210, MOLT-4) and myelogenous (HL-60, KG1) leukemic cells. Cancer Res 1990;50:5746–50. [PubMed: 2168279]
11. Darzynkiewicz Z, Bedner E, Gorczyca W, Melamed MR. Laser scanning cytometry. A new instrumentation with many applications. Exp Cell Res 2000;249:1–12. [PubMed: 10328948]
12. Darzynkiewicz Z, Bruno S, Del Bino G, Gorczyca W, Hotz MA, Lassota P, Traganos F. Features of apoptotic cells measured by flow cytometry. Cytometry 1992;13:795–808. [PubMed: 1333943]
13. Gaffney EV. The accumulation and selective detachment of mitotic cells. Meth Cell Biol 1975;9:71–84.
14. Takayama I, Fujii Y, Ohno S, Fujino MA. Morphological study of rat mast cells stimulated with compound 48/80 at different temperatures. Histochemistry 1994;102:83–87. [PubMed: 7822217]
15. Chahdi A, Frandforfer PF, Beaven MA. Compound 48/80 activates mast cell phospholipase D via heterotrimeric GTP-binding proteins. J Pharmacol Exp Ther 2000;292:122–30. [PubMed: 10604938]
16. Darzynkiewicz Z, Carter S. Compound 48/80 impairs cytokinesis in murine leukemic cells. J Cell Physiol 1984;119:1–6. [PubMed: 6546236]
17. Thornton M, Eward KL, Helmstetter CE. Production of minimally disturbed synchronous cultures of hematopoietic cells. Biotechniques 2002;32:1098–1105. [PubMed: 12019783]
18. Helmstetter CE, Thornton M, Romero A, Eward KL. Synchrony in human, mouse and bacterial cell cultures. A comparison Cell Cycle 2003;2:42–45.
19. Pape L, Kristensen BI, Bengtson O. Sialic acid, electrophoretic mobility and transmembrane potential of the Amphiuma red cell. Biochim Biophys Acta 1975;406:516–25. [PubMed: 241417]
20. Storchova Z, Pellman D. From polyploidy to aneuploidy, genome instability and cancer. Nature Reviews: Molec Cell Biol 2004;5:45–54.
21. Taylor MV. Muscle differentiation: How two cells become one. Curr Biol 2002;12:R224–8. [PubMed: 11909553]
22. Vignery A. Osteoclasts and giant cells: Macrophage-macrophage fusion mechanism. Int J Exp Pathol 2000;81:291–304. [PubMed: 11168677]

23. Hernandez LD, Hoffman LD, Wolfsberg TG, White J. Virus-cell and cell-cell fusion. Annu Rev Cell Dev Biol 1996;12:627–61. [PubMed: 8970739]
24. Most J, Spotl L, Mayr G, Gasser A, Sarti A, Dierich MP. Formation of multinucleated giant cells in vitro is dependent on the stage of monocyte to macrophage maturation. Blood 98:662–71.
25. Vassilopoulos, Wang RR, Russel DW. Transplanted bone marrow regenerates liver by cell fusion. Nature 2003;422:901–4. [PubMed: 12665833]
26. Gewirtz AM. Megakaryocytopoiesis: The state of the art. Thromb Haemostasis 1995;74:204–9. [PubMed: 8578458]
27. Wang Q, Tongyi Liu T, Fang Y, Xie S, Huang X, Mahmood R, Ramasiwamy M, Sakamoto KM, Darzynkiewicz Z, Xu M, Dai W. BUBR-1 deficiency results in abnormal megakaryopoiesis. Blood 2004;103:1278–85. [PubMed: 14576056]
28. Huang X, Ruan Q, Fang Y, Traganos F, Darzynkiewicz Z, Dai W. Physical and functional interactions between mitotic kinases during polyploidization and megakaryocytic differentiation. Cell Cycle 2004;3(7):946–951. [PubMed: 15190214]
29. Berthier R, Jaquier-Sarlin M, Schweitzer A, Block MR, Molla A. Adhesion of mature polyploidy megakaryocytes to fibronectin is mediated by β1 integrins and leads to cell damage. Exp Cell Res 1998;242:315–27. [PubMed: 9665829]
30. Ferguson DJ. Cellular attachment to implanted foreign bodies in relation to tumorigenesis. Cancer Res 1977;37:4367–71. [PubMed: 922728]
31. Rachko D, Brand KG. Chromosomal aberrations in foreign body tumorigenesis of mice. Proc Soc Exp Biol Med 1983;172:382–8. [PubMed: 6844345]
32. Duesberg P, Li R. Multistep carcinogenesis: A chain reaction of aneuploidizations. Cell Cycle 2003;2:202–10. [PubMed: 12734426]
33. Rosefort C, Fauth E, Zankl H. Micronuclei induced by aneugens and clastogens in mononuclear and binucleate cells using the cytokinesis block assay. Mutagenesis 2004;19:277–84. [PubMed: 15215326]
34. Fenech M. The in vitro micronucleus technique. Mutat Res 200;455:81–95. [PubMed: 11113469]
35. Smolewski P, Ruan Q, Vellon L, Darzynkiewicz Z. The micronuclei assay by laser scanning cytometry. Cytometry 2001;45:19–26. [PubMed: 11598943]

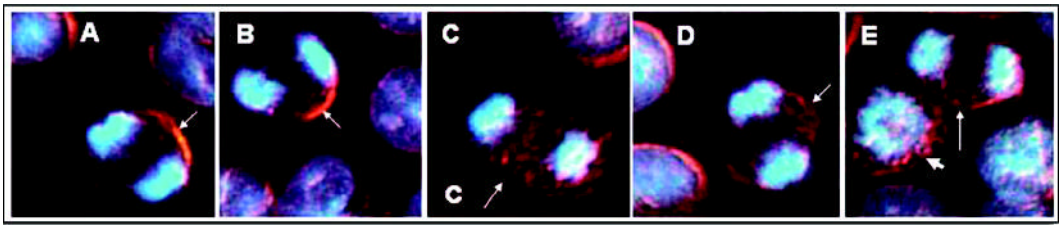

Figure 1.

Failure to undergo cytokinesis during the first mitosis of L1210 cells growing on polycation-coated slides. The cells from suspension cultures were seeded onto poly-D-lysine coated slides and cultured for 2 (A and B), 4 (C and D) or 6 h (E) and then fixed. The attached cells were stained with DAPI and examined under fluorescence microscope using incident UV light illumination combined with differential interference (Nomarski) contrast. The mitotic cells shown in individual panels are in telophase; the thin arrows point out to the midsection of the cell which shows no evidence of narrowing. The thick arrow in (E) points out to the mitotic cell with metaphase plate in horizontal position vis-à-vis surface of attachment.

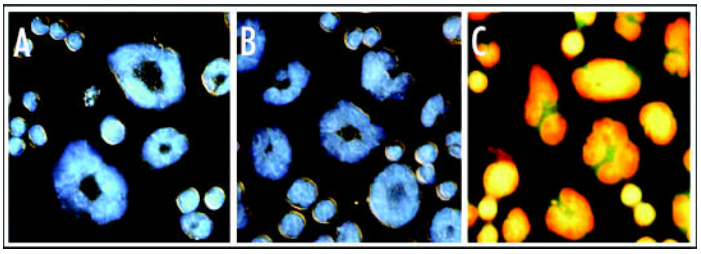

Figure 2.
Formation of polykaryons during growth of L1210 cells attached to polylysine-coated slides. The cells were seeded either on poly-D-lysine-coated slides (A and C) or compound 48/80 was included into the culture (B) and the attached cells were examined 48 h after the seeding. The cells in (A and B) were examined as described in legend to Figure 1. The cells in panel C were stained with 7-AAD and FITC and their green (protein) and red (DNA) fluorescence was excited with blue light incident illumination.

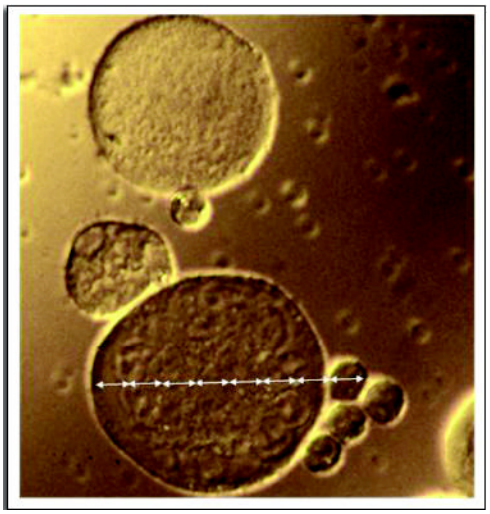

Figure 3.
Differential interference contrast photographs of L1210 cells grown attached for 48 h and subsequently maintained in suspension for additional 48 h. Note the presence of three large polykaryons and five small monokaryons.

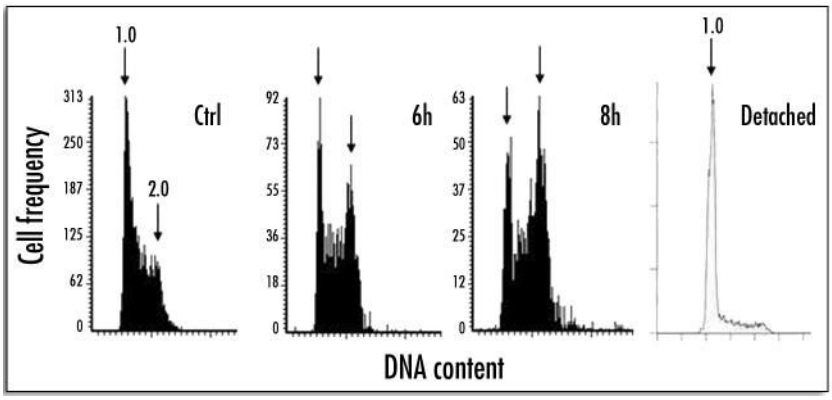

Figure 4.

Cellular DNA content frequency distributions of L1210 cells, untreated (Ctrl), cultured for 6 and 8 h on poly-D-lysine-coated slides, and cells that detached from the coated slides during culturing and were floating in the medium. Positions of the peaks of the cells with DNA index (DI) = 1.0 and DI = 2.0 are marked by the arrows. Note progressive increase in frequency of cells with DI = 2.0 as a function of time of cell growth upon their attachment. The attached and detached cells were measured by LSC and flow cytometry, respectively.

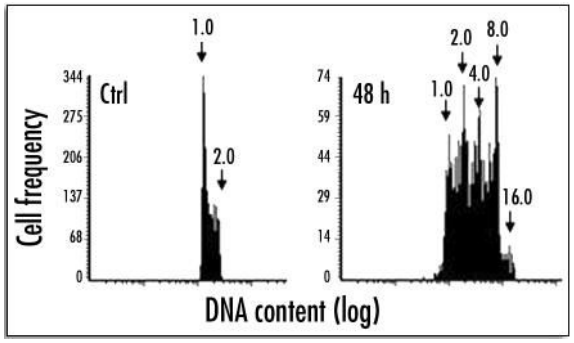

Figure 5.
Cellular DNA content of L1210 cells, untreated (Ctrl) or grown for 48 h on poly-D-lysine-coated slides. The presence of polykaryons with varying DNA content, up to DI = 16.0, is apparent among the attached cells. This wide DNA content distribution necessitated the use of exponential (log) DNA content-coordinate.

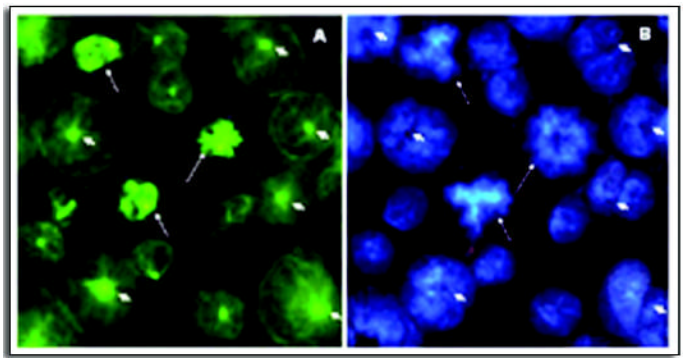

Figure 6.

Localization of microtubules in interphase- and mitotic- polykaryons. L1210 cells grown attached to poly-D-lysine-coated slides for 24 h were fixed and immunostained for α-tubulin (A); their DNA was counterstained with DAPI (B). Note the presence of a single microtubules organizing center (MTOC) in the centers of the interphase polykaryons (thick arrows). Up to four centrosomes are present in mitotic polykaryons (thin arrows). In the two (left) mitotic polykaryons mitotic spindles are parallel to the surface of the attachment, in the right polykaryon they are perpendicular.

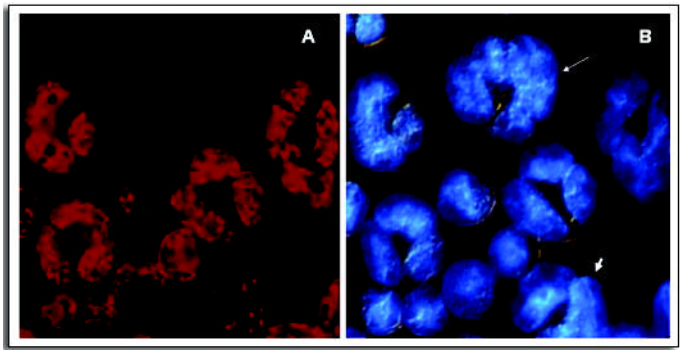

Figure 7.
Synchrony in DNA replication in individual nuclei in polykaryons. L1210 cells grown attached to poly-D-lysine coated slides for 24 h were incubated for 60 min in the presence of BrdU and then fixed. The incorporated BrdU was detected immunocytochemically (A), DNA was counterstained with DAPI (B). The unlabeled polykaryon is marked with thin arrow; the thick arrow marks the weakly labeled polykaryon, in which DNA replication apparently was overlapping for short time interval with the pulse of BrdU.