The Association of Nuclear Blebs with Aneuploidy in Human Acute Leukemia

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SUMMARY

In our laboratory, bone marrow studies that monitored acute leukemia patients in the active phases of the disease and in the remission state demonstrated an apparent association between aneuploidy and a specific ultrastructural cellular abnormality (nuclear blebs). In order to determine the extent of this correlation, bone marrow specimens from patients with acute leukemia were investigated by simultaneous cytogenetic and ultrastructural analyses. As an additional parameter of this study, a quantitative ultrastructural analysis was carried out in bone marrow samples from 20 of the acute leukemia patients, as well as from 6 normal controls. Results from these correlated studies show that, during the active phase of the disease, there is a definite correlation between aneuploidy and a high frequency of nuclear blebs. Successful response to therapy is accompanied by a marked reduction or total disappearance of the chromosomally and ultrastructurally abnormal cells. Conversely, impending relapse in these individuals is indicated by the reappearance of both nuclear abnormalities. These cytological alterations may provide a useful tool for evaluating patient response to therapy.

INTRODUCTION

For the past several years, at our Institution, a multidisciplined ultrastructural and cytogenetic study has been conducted on sequential bone marrow specimens from acute leukemia patients before, during, and after chemotherapy. During this study, the presence of an ultrastructural alteration consisting of blebs, pockets, or projections was observed on the nuclear surface of the immature myeloid cells in many of these leukemic samples. Ultrastructural examination of these processes revealed that areas of cytoplasmic material were enclosed within the bulging projection, and these areas extended beyond the normal contour of the nucleus. In this respect, these blebs were unlike the nucleolus-associated invaginations reported by Burns et al. (4). The nuclear changes found in the cells of these patients closely resembled those described by Achong and Epstein (1, 7) and Epstein et al. (8) as a consistent inherent feature of the Burkitt lymphoma. Dorfman (5, 6) has also reported the appearance of such nuclear blebs in cells from 2 patients with malignant lymphoma. Anderson (3) and McDuffie (12) independently observed similar structures in the bone marrow of untreated leukemic patients. In addition to these reports of inherent alterations due to a disease process, several investigators have shown that a group of DNA-inhibiting chemotherapeutic agents can also induce the formation of nuclear blebs. Stalzer et al. (17), for instance, described the appearance of these alterations in bone marrow cells following 5-fluorouracil treatment in patients with neoplastic disease. Nuclear bleb formation in human bone marrow cells following administration of exceptionally high doses of cytosine arabinoside was initially reported by our laboratory in 1967 (2). We also demonstrated that the addition of this drug to short-term in vitro cultures of bone marrow cells leads to the appearance of similar nuclear changes.

Further investigation of this phenomenon in the bone marrow of leukemic patients revealed that the blebs appeared more frequently in those specimens exhibiting chromosomal abnormalities. The incidence of these chromosomal alterations in the hematopoietic tissue from acute leukemic patients seen at this Institution was found to be approximately 41% (19). The present study was designed to investigate further the apparent association between aneuploidy and nuclear blebs in the bone marrow cells obtained from these leukemic patients.

MATERIALS AND METHODS

Bone marrow specimens for combined cytogenetic and ultrastructural examination were obtained from patients with acute leukemia at the time of diagnosis and during the remission and relapse phases of the disease prior to initiation of the next course of drug therapy. The cytogenetic techniques used for chromosome analyses have been previously described (19). Bone marrow samples for electron microscopic observation were concentrated, using Wintrobe tubes. Fixation was carried out in 2.5% glutaraldehyde in Sörensen's phosphate buffer, 300 mOsmoles, for 1 hr at 25°. The specimens were washed in phosphate buffer and postfixed in 1% osmium tetroxide at 4°. The cell pellets were dehydrated in graded acetones and embedded in Epon 812. Sections were cut with a diamond knife mounted on a Porter-Blum ultramicrotome and, subsequently, were stained with uranyl acetate for 1 hr at room temperature and then with Reynolds lead citrate for 2 min. The specimen was examined with a Siemens-Elmiskop 1A at 80 kV.

A quantitative study was carried out in bone marrow

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samples obtained from 26 individuals. In each sample 1000 cell sections exhibiting full nuclear surfaces unobstructed by grid bars were counted. In order to differentiate between true nuclear blebs and various other nuclear invaginations, only those structures observed as membrane-bound pockets containing cytoplasm that extended beyond the normal contour of the nucleus were considered.

RESULTS

At the termination of this study, a total of 152 bone marrow samples from adult individuals had been examined by cytogenetic and electron microscopic techniques. Six of this number represented normal marrows, 4 from control individuals and 2 from patients with nonhematological malignant disease (Table 1). Eighty-nine of the remaining 146 bone marrow samples (133 acute granulocytic leukemia; 13 acute lymphocytic leukemia) displayed an aneuploid karyotype, while 57 had a diploid karyotype. Initial investigation of these specimens disclosed numerous nuclear blebs associated with each of the aneuploid bone marrows, whereas the diploid marrows exhibited only a sporadic occurrence of the structural abnormality. These results indicated a distinct correlation between the existence of chromosomal abnormalities and a high frequency of nuclear blebs in acute leukemia.

To determine the extent of this association between nuclear blebs and aneuploidy, a quantitative ultrastructural study was done on bone marrow samples from 26 individuals, grouped as follows: 10 acute leukemia patients with diploid karyotypes, 10 acute leukemia patients with aneuploid karyotypes, and 6 normal controls. The results of these quantitative studies in the 20 leukemic bone marrows indicated that in each instance the mean number of nuclear blebs observed in the aneuploid leukemias during the active phase of the disease was significantly higher (59.4/1000) than that found in similar staged diploid leukemias (2.5/1000) (Table 1).

These nuclear blebs were found to be 0.9 to 1.5 \( \mu \text{m} \) in diameter and consisted of a dense membrane circling out into the cytoplasm, enclosing amorphous material (Fig. 1). The membrane itself consisted of a dense core measuring about 400Å, bounded on each side by a clear membrane continuous with the nuclear membrane. The blebs often contained cytoplasmic granules or organelles (Figs. 2 and 3).

In acute leukemia patients bearing chromosomal alterations, sequential studies indicated that, in most of these individuals, a good remission was marked by the early disappearance of the abnormal clones, while impending relapse was preceded by the
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reappearance of the same aneuploid or pseudodiploid clone (19). Similar sequential ultrastructural investigations performed in our laboratory on the same bone marrow samples that were studied cytogenetically demonstrated that the increased frequency of nuclear blebs in the hematopoietic tissue of acute leukemias was closely associated with the presence of several different categories of cytogenetic abnormalities. Repeated examination of bone marrow from both normal individuals and those with nonhematological malignant disease failed to reveal the presence of the nuclear bleb, whereas those with acute leukemia and a diploid karyotype demonstrated only low frequencies of the structural alterations (Table 1). In both of these latter categories, the patients were on therapeutic regimens similar to that of the aneuploid leukemia patients and were also examined in the acute and remission phases of their disease.

Figs. 4 and 5 show a typical blebbed cell and a representative idiogram of an abnormal cytogenetic clone (46,XX,—C,+D,+E,—G) found in the initial bone marrow specimen obtained from Patient C. C. prior to therapy. This bone marrow sample contained a high percentage of leukemic blasts (40%) and exhibited a high frequency of nuclear blebbed cells, as well as the pseudodiploid clone. The next 2 illustrations, prepared from bone marrow cells obtained at the time of remission, show a total disappearance of the nuclear blebbed elements and of the cytogenetically abnormal clone (Figs. 6 and 7). This patient was maintained in remission for a period of 14 months and, during that time, was free of both nuclear abnormalities. At the time of her relapse, the nuclear blebbed cells and the identical cytogenetic clone returned (Figs. 8 and 9). It was impossible to achieve remission again, and the patient maintained both abnormalities until she expired. This case was of particular interest, since reappearance of the nuclear blebs was noted several weeks prior to relapse, as judged by light microscopy.

Bleb formation appears to begin as a veil-like projection extending forth from the nucleus into the cytoplasm (Fig. 10). This veil-like projection bears both nuclear membranes intact and maintains a central core of nucleoplasm continuous with that in the main body of the nucleus. The still-intact membrane then approaches the surface of the nucleus (Fig. 11), and the nuclear membranes appear to undergo dissolution and reformation at the contact site (Fig. 12). The outer membrane of the bleb is at this time continuous with that of the nucleus. The next micrograph (Fig. 13) shows the process completed. The bleb is enclosed within the surface of the nucleus but is isolated from it by both layers of the nuclear membrane. Excessive whorls of membrane in the area of loop formation (Fig. 14) are found to be a consistent feature of the bleb genesis. The sequential process of bleb formation as shown in these micrographs is reminiscent of that illustrated by Fawcett (9) in his electron microscopic study of pinocytosis by the plasma membrane. Occasionally, 2 veil-like projections extending from the nucleus are found to overlap and reattach, resulting in blebs isolated by a double set of nuclear-cored membrane (Fig. 15). These structures are similar to those demonstrated by Toro and Olah (18) in cells of guinea pig thymus.

DISCUSSION

The quantitative data presented herein suggest that there is a positive correlation between the presence of a high frequency of ultrastructural nuclear abnormalities and the existence of aneuploidy in bone marrow cells of acute leukemia patients during the active phase of their disease. It is interesting to note that Weber et al. (20) have also reported the occurrence of nuclear pockets in lymphocytes of normal and leukemic adult cattle. Although no cytogenetic studies were done, these authors found that the leukemic animals had 6 times as many lymphocytes with nuclear pockets as did the animals with normal hemograms. A subsequent study by the same investigators (21) showed that those animals with an increased incidence of nuclear pockets produced C-type virus particles.

In addition to megaloblastosis and cytogenetic abnormalities, Stalzer et al. (17) included the nuclear bleb as a morphological effect of 5-fluorouracil treatment. It is known that 5-fluorouracil blocks the conversion of 5-fluorodeoxyuridine-5-phosphate to thymidine, thus inhibiting DNA synthesis. The blebs reported in megaloblastic anemia probably reflect the importance of Vitamin B12 in the synthesis of thymine and, subsequently, DNA. Our earlier work (2) demonstrated the presence of these bleb structures in patients undergoing i.v.-push therapy with high levels of cytosine arabinoside, a drug that inhibits the conversion of cytidine diphosphate to deoxycytidine diphosphate and thus specifically inhibits the de novo biosynthetic pathway to DNA. Therefore, it is possible to theorize that these drug-induced nuclear abnormalities, reflecting a disturbance of nucleoprotein synthesis, are identical with the nuclear blebs recently demonstrated to be present in certain lymphomas (1, 5, 6–8, 13) and which we have shown in this study to exist in certain acute leukemias.

The appearance of nuclear alterations in these neoplastic disorders probably reflects a similar disturbance of nucleoprotein synthesis due to unbalanced cell growth related to the disease state. Since the blebs are always associated with excessive whorls of nuclear membrane in the area of genesis, we can speculate that the bleb probably represents continued nuclear membrane production at a period when nucleoplasm volume is stable. Haynes et al. (10) concluded that nuclear asymmetry is a requisite but is not the only factor in determining the formation of envelope-limited monolayers in the form of blebs. Although nuclear blebs have infrequently been reported in cells from normal individuals, the structures are found in the peripheral blood rather than in the bone marrow, and might well reflect an occasional altered cell at the nonproliferative level (11, 14, 15).

As shown in these preliminary findings, during the active phase of the disease there is a definite correlation between a high frequency of nuclear blebs and aneuploidy in bone marrow cells from acute leukemia patients. Conversely, successful therapy and/or remission is accompanied by a reduced incidence or disappearance of the nuclear alteration, while reappearance of the blebbed cells is an indication of relapse. The results of this investigation, then, suggest that correlated electron microscopic and cytogenetic techniques may be useful in identifying the leukemic cells during both the mitotic and interphase stages. Accurate identification of the leukemic blebs is essential for the evaluation of the patient’s response to therapy. In addition, the fact that these nuclear blebs observed in the neoplastic cells are morphologically similar to those induced by DNA-inhibiting drugs is suggestive
of a deranged DNA metabolism in these leukemic blasts. Confirmation of the existence of such metabolic defects would be highly important in the planning of a more rational and a specific form of therapy for the acute leukemia patient carrying these cytological markers.

REFERENCES


Fig. 1. Immature myeloid cell from an acute leukemia patient, demonstrating the appearance of a nuclear bleb (single arrow) and a nuclear body (double arrow). x 19,250.

Fig. 2. Nuclear bleb containing a varied assortment of cytoplasmic granules. x 18,000.

Fig. 3. Concentric cisternae enclosed within a nuclear bleb. x 30,190.

Fig. 4. Myeloblast from an acute granulocytic leukemia patient in the initial disease state exhibiting 2 blebs on the nuclear surface. x 18,050.

Fig. 5. Idiogram prepared concomitantly from the bone marrow sample (Fig. 4), demonstrating a 46,XX,—C,+D,+E,—G clone present in active disease.

Fig. 6. Typical promyelocyte illustrating the absence of nuclear blebs in the remission state of Patient C. C. x 10,500.

Fig. 7. Normal diploid (46,XX) idiogram prepared from the sample (Fig. 6) following remission induction.

Fig. 8. Myeloblast with nuclear blebs from a bone marrow sample of Patient C. C. at the time of relapse. x 20,000.

Fig. 9. Idiogram demonstrating the return of the 46,XX,—D,+D,+E,—G clone in the relapse sample from Patient C. C.

Fig. 10. Veil-like extension of the nucleus protruding into the cytoplasm. x 12,800.

Fig. 11. Nuclear extension isolating a small cytoplasmic area; arrow, membrane whorl typically observed at the site of membrane contact. x 11,000.

Fig. 12. Membrane reformation at the nuclear extension-nuclear membrane contact site. x 15,000.

Fig. 13. Cytoplasmic area enclosed within the nucleus but isolated from the nucleoplasm by both nuclear membranes. Arrow, residual membrane at the bleb contact site. x 11,000.

Fig. 14. Enlarged detail in the area of bleb formation illustrating the association of the membrane whorl (arrow) with the outer nuclear membrane. x 48,600.

Fig. 15. Double membrane blebs resulting from 2 overlapping nuclear veils. x 64,000.
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Images 10, 11, 12, and 13 show electron micrographs of leukemic cells with nuclear blebs.
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