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Structural Changes in the Megakaryocytes of Patients Infected with the Human Immune Deficiency Virus (HIV-1)

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Although immune mechanisms are known to be partially responsible for the thrombocytopenia of patients infected with HIV-1, an understanding of the mechanism underlying this disorder is incomplete. A casual observation that bone marrow biopsies of HIV-infected individuals seem to exhibit an unusually large number of denuded megakaryocyte nuclei (DN-MK) prompted a study comparing MK of 20 HIV-seropositive individuals with those of 10 patients with HIV-negative idiopathic thrombocytopenic purpura and 10 hematologically normal subjects. In normal marrows the number of DN-MK averaged 2.1 ± 0.5 SE per 10 low power field. In patients with ITP the average number was 6.5 ± 1.4 SEM, whereas HIV-ITP marrows had an average of 42.5 ± 3.7 SEM. Electron microscopy of AIDS megakaryocytes exhibited ballooning of the peripheral zone to an extent not seen by us in any other myelodysplastic syndromes. These observations support the concept that the pathophysiology affecting MK/platelets in HIV-infection should not be equated with the destructive process underlying other immune thrombocytopenias. (Am J Pathol 1989, 134:1295–1303)

The bone marrow of patients afflicted with acquired immune deficiency syndrome (AIDS) shows numerous abnormalities. To a large extent these are nonspecific, although some observers have reported that the reticulin pattern and the distribution of fat cells may distinguish AIDS bone marrows from those obtained from patients with other disease entities.[1] The present study concerned the question whether the platelet antibodies and immune complexes found in many patients with human immune deficiency virus (HIV) associated thrombocytopenia[2] cause the same type of structural damage to megakaryocytes/platelets as is sometimes seen in chronic idiopathic thrombocytopenic purpura or other immune thrombocytopenias. This question was raised because of the casual observation that routine AIDS bone marrow biopsies seemed to exhibit an unusually large number of denuded megakaryocyte nuclei (DN-MK) even in the absence of peripheral blood thrombocytopenia.[3] Moreover, in the course of electron microscopic studies of AIDS bone marrows, we were impressed by the aberrant ultrastructure of the megakaryocytes. This appeared to differ from the structure seen in the MK of patients with idiopathic thrombocytopenic purpura. To substantiate these observations a more systematic study was undertaken. The results of this analysis suggest that the pathophysiology affecting megakaryocytes/platelets in HIV-infected individuals or patients with overt AIDS cannot be equated with the destructive process responsible for other immune thrombocytopenias.

Materials and Methods

Bone marrow biopsies of 20 AIDS patients, 10 patients with ITP, and 10 hematologically normal individuals whose marrows were obtained in the course of a work-up for unrelated diseases were retrieved from the slide files of the Department of Pathology at NYU Medical Center. The specimens were hematoxylin and eosin (H & E) stained biopsy sections selected at random. They were read by an observer uninformed of the diagnosis. The absolute number of megakaryocytes and denuded megakaryocyte nuclei (DN) per 10 low-power fields (LP) were counted with the help of a reticle placed in the ×10 ocular of the microscope, which divided the field into 100 squares. Whenever identification of a denuded mega-

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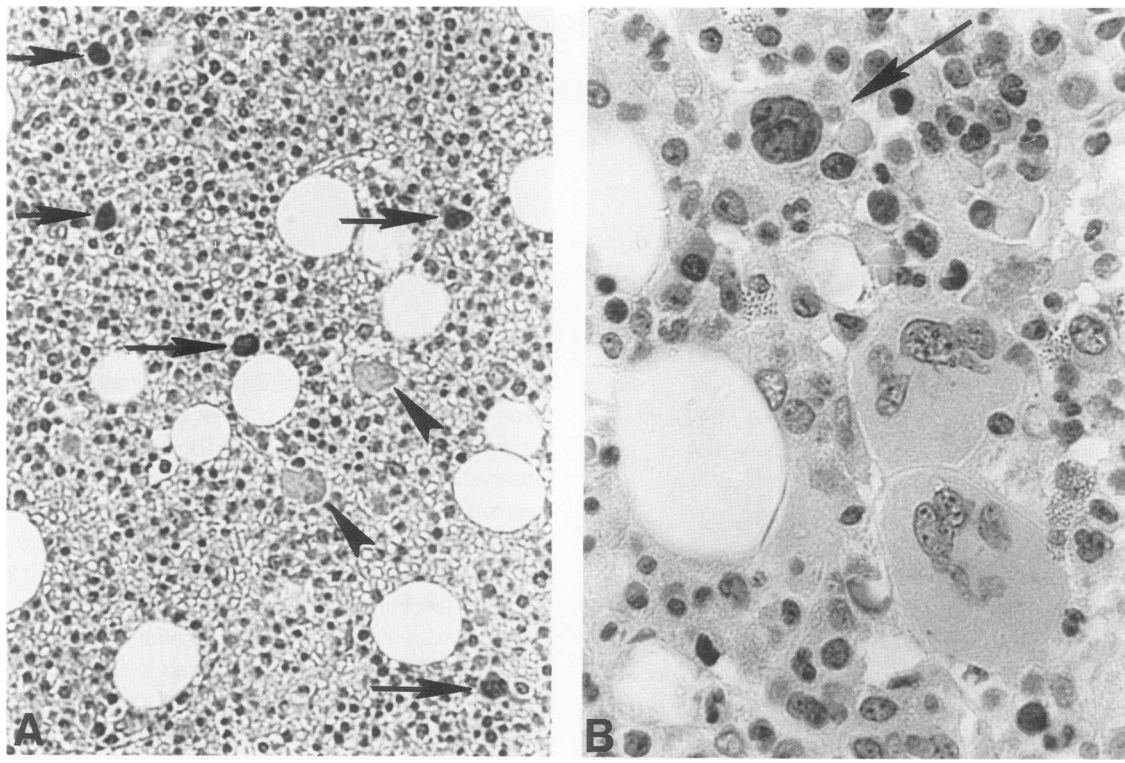

Figure 1. *Photomicrographs of a bone marrow biopsy specimen obtained from a patient with AIDS embedded in paraffin and stained with H & E.* **A:** *Low-power field shows 5 large pyknotic megakaryocyte nuclei (arrows) and 2 intact megakaryocyte (arrowheads) (×300).* **B:** *High-power field of the same specimen shows the contracted megakaryocyte nucleus (arrow) to better advantage. The intact megakaryocytes appear to have relatively smooth circumferences (×830).*

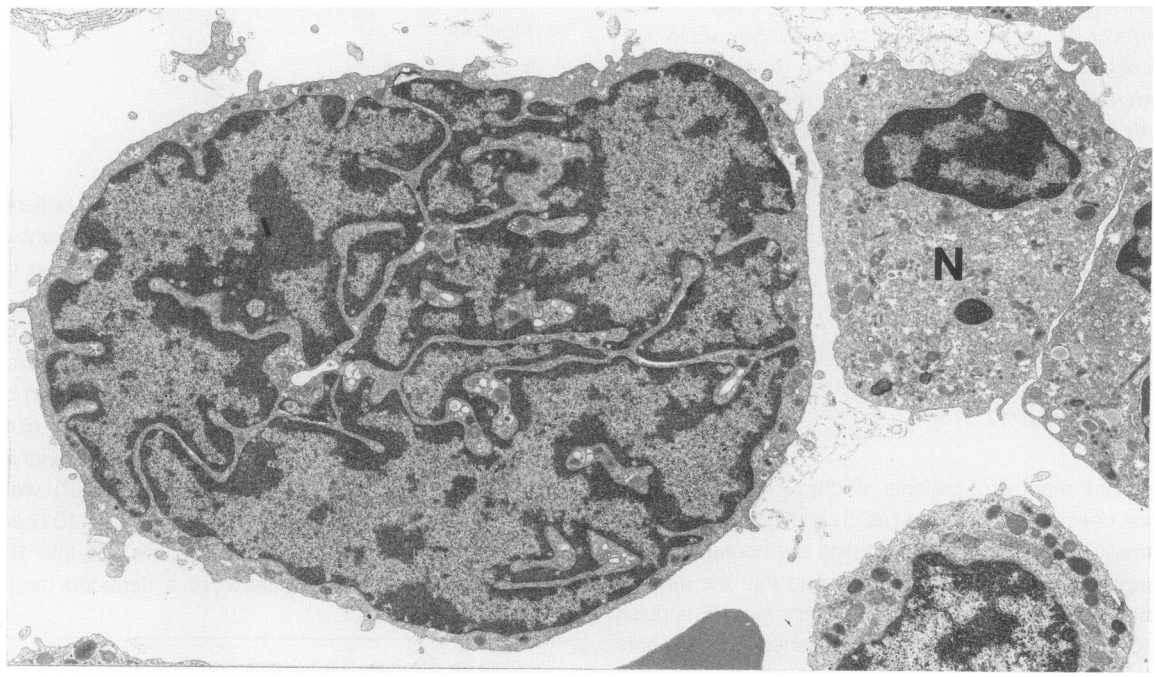

Figure 2. *Electron micrograph of a denuded megakaryocyte nucleus illustrates segmentation typical of this polyploid cell. Moreover, the distribution and quantity of heterochromatin indicates maturity. Neutrophil (N) provides standard for comparison of size (×6500).*

Table 1. *Absolute Number of Megakaryocytes and Denuded Nuclei in Marrow Biopsies*

Normal			HIV-positive			ITP, HIV-negative	
Pt. #	MK	DN	MK	Plt × 10	DN	MK	DN
1	140	4	153	172	12	169	1
2	141	0	155	483	26	237	3
3	154	4	181	–	47	242	2
4	156	1	185	67	62	270	3
5	159	2	187	–	21	283	4
6	165	1	197	119*	28	288	9
7	170	0	200	192*	49	288	9
8	191	3	210	190	38	289	10
9	203	2	211	145	56	317	8
10	269	4	235	241	35	515	16
11	Avg, 178.8	Avg, 2.1	258	197	55	Avg, 289.8	Avg, 6.5
12	±11.6	±0.5	261	195*	32	±26.8	±1.4
13			262	272	62		
14			264	34	84		
15			266	262	45		
16			278	136*	52		
17			285	–	33		
18			299	–	26		
19			345	78	39		
20			346	140	47		
				Avg, 238.9 ±12.3	Avg, 42.5 ±3.7		

Number of intact megakaryocytes (MK) and denuded nuclei ± SEM per 10 LPF in bone marrow biopsies of 10 normal, 20 AIDS, and 10 ITP (HIV-negative) individuals. The platelet counts (Plt) recorded were obtained on the same day as the marrow specimens.

* Patients whose platelet counts varied by 100,000 within less than 2 weeks. Platelet counts of HIV-negative ITP patients were all <100,000. (*P* value of DN in normals *vs.* AIDS was <0.001, *P* value of DN in ITP *vs.* AIDS was <0.001, *P* value of DN in ITP *vs.* normal was <0.4 >0.3.

karyocyte (MK) nucleus was uncertain at low power, a ×100 oil immersion lens was used in a parfocal plane.

Statistical Analysis

Except where specifically indicated, the student's *t*-test was used to determine statistical significance of the difference between sets of data obtained.

Electron Microscopy

Aliquots of fresh bone marrow specimens collected for diagnostic reasons were placed into 3% glutaraldehyde. Dehydration and embedding for thin sectioning was done by methods used routinely in this laboratory.[4] Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop I electron microscope at instrument magnifications ranging from 4000 to 30,000.

Results

A low-power field of an AIDS bone marrow biopsy is shown in Figure 1A. The large pyknotic nuclei are readily apparent. At high power the identity of the multilobed nucleus characteristic of mature megakaryocytes is easily recognized (Figure 1B). It may be noted that the denuded

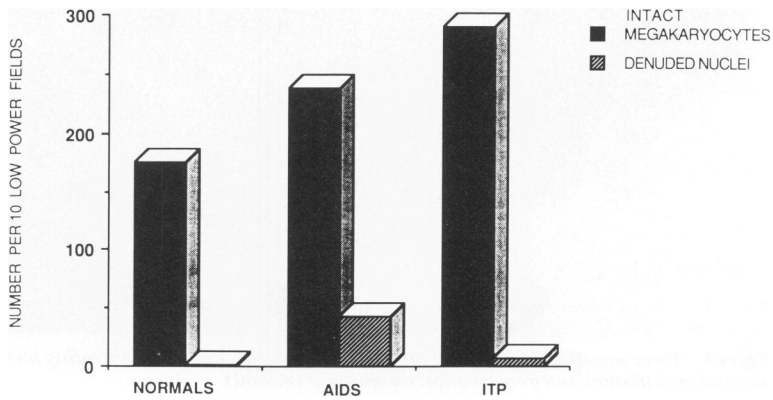

Figure 3. *Histogram showing the average numbers of intact megakaryocytes and denuded nuclei for 10 normal, 20 HIV-positive, and 10 ITP HIV-negative samples. The standard errors of these observations are found in Table 1. The difference between the number of denuded MK nuclei in AIDS marrows and marrows from patients with HIV-negative ITP was highly significant (P < 0.001). The same P value applied to DN in normal marrows when compared with AIDS. The difference between the number of DN in normals and ITP marrows was not significant (P < 0.4 > 0.3).*

Table 2. *Results on Repeated Marrow Biopsies*

Patient	MK	MK-DN	Plt/mm3	% DN
GM	153	12	172,000	7.3
	346	47	140,000	12.0
RO	192	28	119,000	12.4
	200	49	192,000	19.7
JT	258	55	107,000	17.6
	185	62	67,000	25.1
GK	211	56	145,000	21.0
	181	47	ND	20.6

Data showing three AIDS patients who had bone marrow biopsies 2 months apart (GM, RO, JT) and one patient (GK) whose biopsy was repeated after 18 months. The platelet counts recorded were done on the same day as the marrow biopsy. However, RO had a platelet count of 18,000 2 weeks later without any other changes in clinical or laboratory parameters. Only GK received AZT at the time of the biopsy.
ND, not determined.

nuclei were always more contracted or "pyknotic" than the nuclei of intact megakaryocytes. This also held true for normal bone marrow samples, in which only few DN were encountered. The identification of such contracted DN was, however, never in doubt at higher resolution (Figure 1B) or on electron microscopy (Figure 2). Moreover, on electron microscopy it became apparent that the DN were remnants of MK, which based on their size and morphology, had attained a ploidy commensurate with the capacity for thrombocytopoiesis.[5]

The results of the MK and DN-MK counts are recorded in Table 1. In normal marrow biopsies an average of 178.8 ± 11.6 SEM megakaryocytes were seen per 10 LP and the number of denuded nuclei (DN-MK) ranged from 0 to 4. The average number of denuded nuclei in normal marrow biopsies was 2.1 ± 0.5 SEM. In patients with chronic ITP, the number of MK per 10 LP was usually increased, averaging 289.8 ± 26.8 SEM per 10 LP, and the absolute number of DN was higher than in normal marrows, ranging from 1 to 16 with an average of 6.5 ± 1.4 SEM. The difference in the number of DN in ITP marrows compared

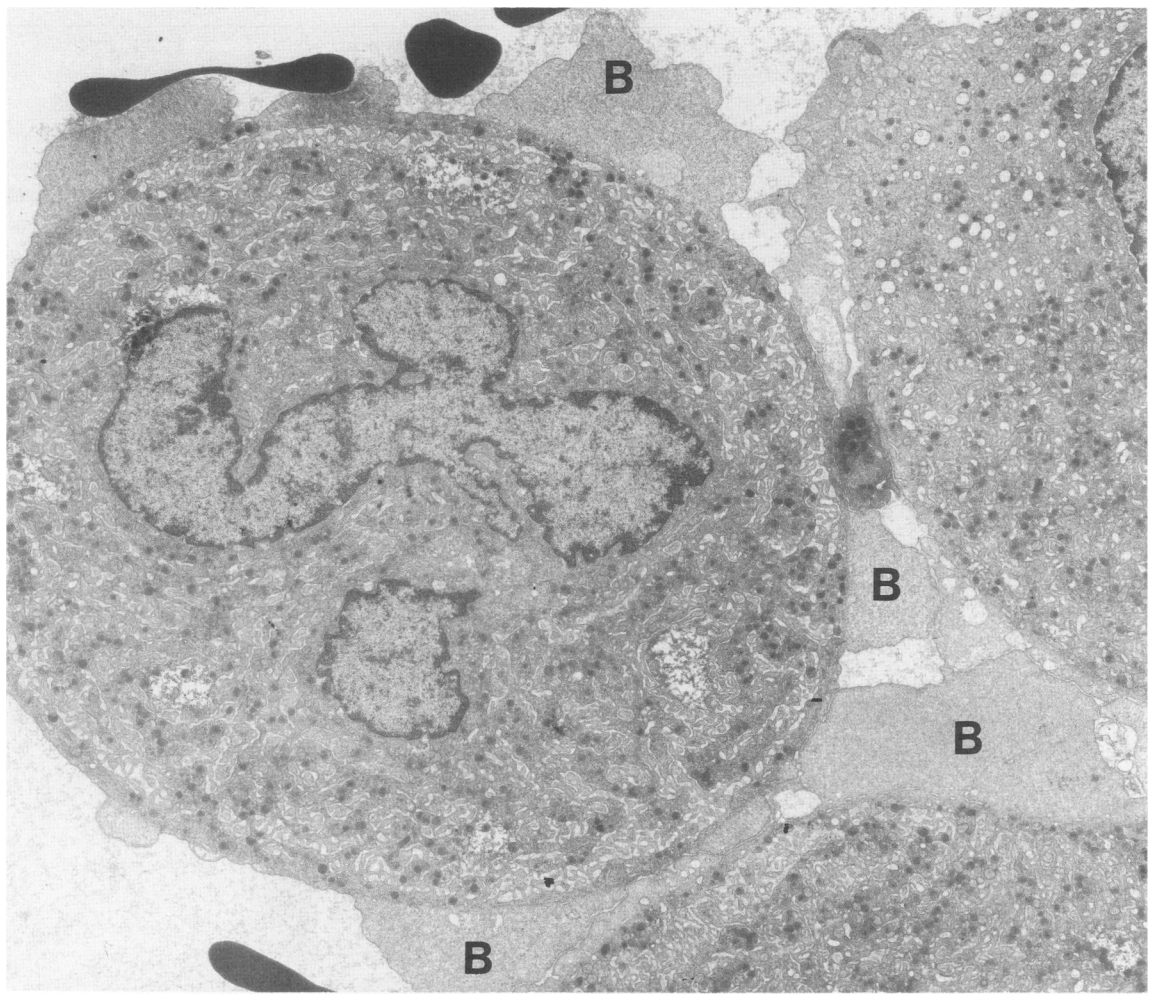

Figure 4. *Three megakaryocytes from marrow of a patient with AIDS illustrating blebbing of the peripheral zone (B). The cells show normal granulation and demarcation membranes (×4800).*

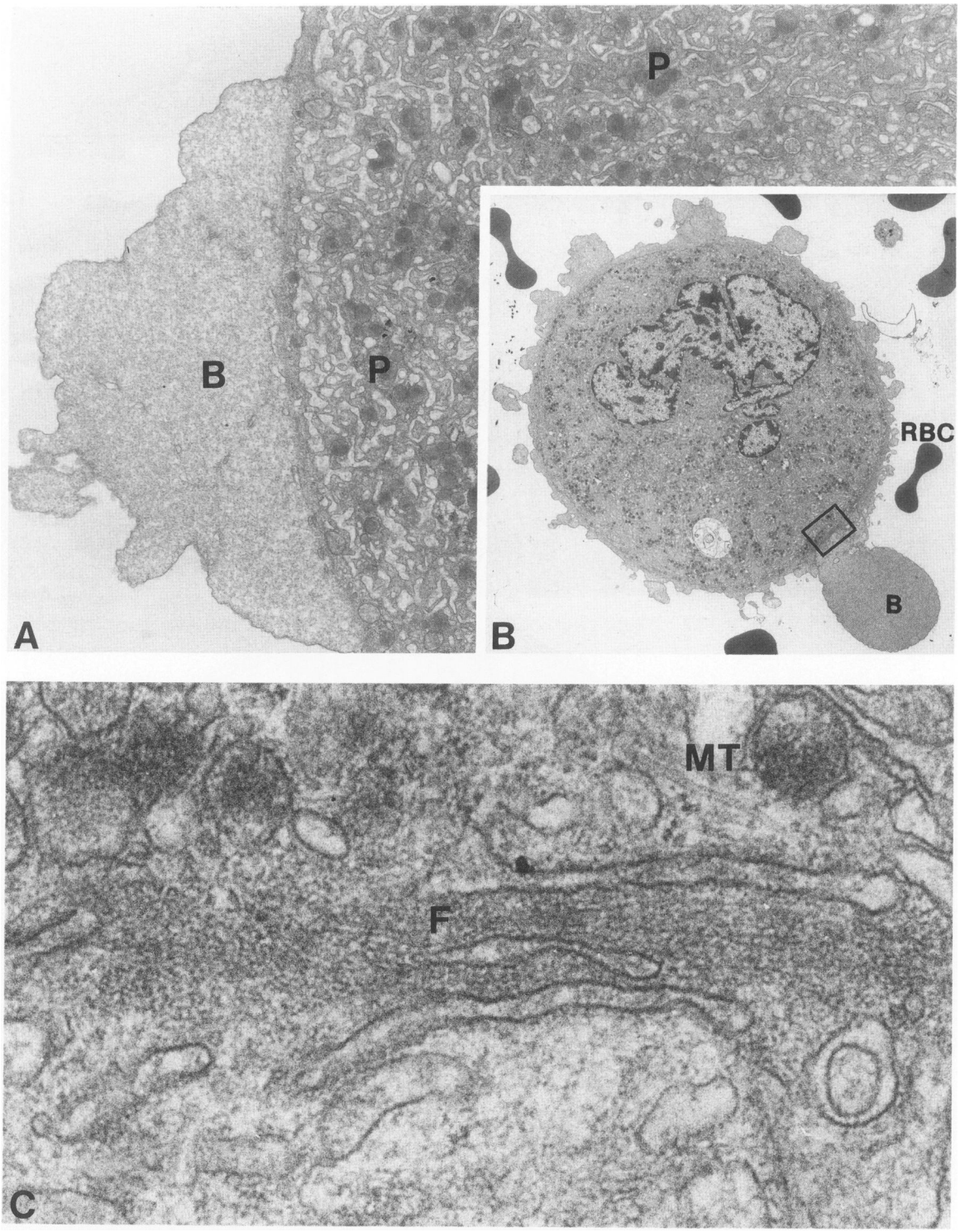

Figure 5. *Details of megakaryocytes from AIDS marrow illustrating large blebs and band of filaments (F) that appear to separate the blebs from the intermediate zone.* **A:** *The demarcated platelet fields (P) show excellent preservation while the "bleb" (B) is devoid of organelles (×10,000).* **B:** *A well-preserved megakaryocyte with a "bleb" that extends by more than 10mm from the main body of the cell (compare with erythrocyte, RBC) (×2000).* **C:** *The bundle of filaments within the inset that separates the "bleb" from the intermediate zone at higher resolution (×75,000). MT, microtubule.*

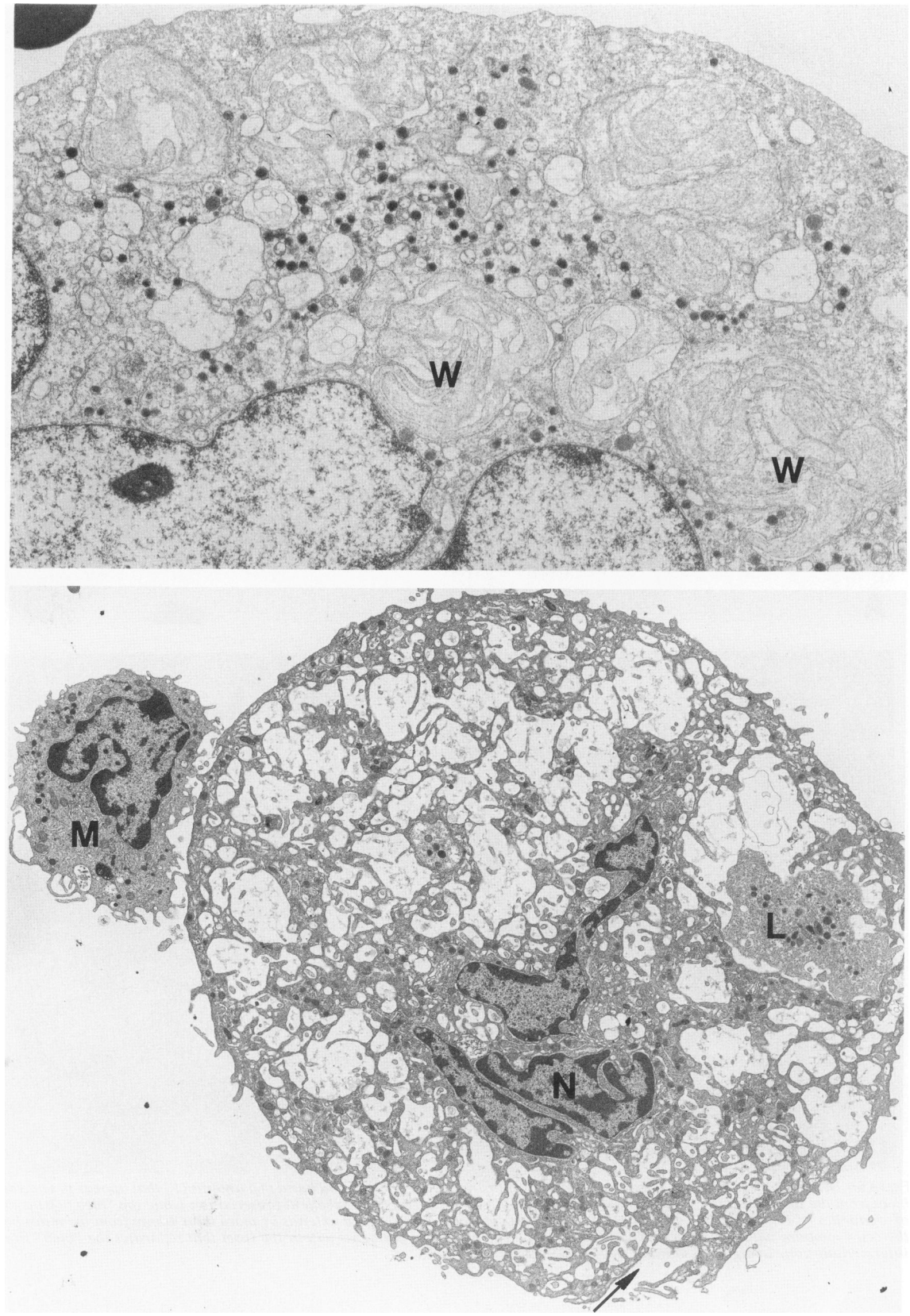
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Figure 6. *Detail of an AIDS megakaryocyte obtained from one of three patients whose megakaryocytes presented cytoplasmic "whorls" (W). Although continuity with the plasma membrane has not been proven, the "whorls" are hypothesized to consist of invaginated surface processes. These contain thin filaments but no other organelles and thus also constitute portions of the peripheral zone (×76,000).* **Figure 7.** *Megakaryocyte obtained from a patient with HIV-seronegative idiopathic thrombocytopenic purpura. This cell shows the "lacy" appearance characteristic of damaged megakaryocytes in this condition. Note that the peripheral zone is thin. The cell also illustrates emperipolesis. A detail of a leukocyte (L) is seen within the dilated demarcation membrane system. A monocyte (M) is seen in close opposition to the MK. Monocytes are frequently seen to phagocytose injured MK in ITP marrows (×4400).*[5]

with the number seen in normals was not significant ($P < 0.4 > 0.3$). On the other hand, marrows of HIV-infected individuals, while having normal or only slightly increased absolute numbers of MK (average, 238.9 ± 12.3 SE per 10 LP; range, 153 to 346) proved to have a much higher number DN (Table 1, Figure 3). The difference between the number of denuded MK in AIDS specimens *vs.* ITP marrows was highly significant ($P < 0.001$).

Sequential biopsy specimens were available for four patients in this study. In each instance there was an increase in the number of denuded nuclei over time (Table 2).

The most striking ultrastructural feature of AIDS megakaryocytes was ballooning or blebbing of the peripheral zone (Figures 4, 5, and 6). The peripheral zone of normal megakaryocytes presents a thin rim of cytoplasm that rarely exceeds 1 to 2 μm in width. As a rule, this zone is devoid of organelles or demarcation membranes but may show some thin filaments.[4,5] In many AIDS megakaryocytes the marginal zone appeared markedly swollen, in some areas extending 6 to 8 μm beyond the intermediate zone, which contains the organelles (Figures 4 and 5). While thin filaments are randomly distributed throughout the marginal zone of normal megakaryocytes, in AIDS megakaryocytes a band of filaments is sometimes seen interior to this zone, encircling the region of the cytoplasm that contains the organelles (Figure 5).

In addition to the blebbing of the peripheral zone and the denuded nuclei, some AIDS specimens presented cytoplasmic abnormalities that, to the best of our knowledge, have not been described in megakaryocytes before. One of these (Figure 6) was seen in a few MK in each of three patients. It consisted of whorls of cytoplasm containing filaments but no other organelles. The whorls most likely represented intravacuolar processes folded upon themselves or surface projections that had been interiorized.

The ultrastructure of megakaryocytes from patients with ITP has been described by others[7,8] as well as by ourselves[6] and will not be reviewed in detail here. The megakaryocytes of the majority of patients with ITP appear normal. Those that are damaged tend to have a "lacy" appearance and a very thin peripheral zone that is often interrupted, thereby establishing large openings in continuity with the dilated demarcation membrane system. Emperiopolesis of leukocytes into these channels is often seen (Figure 7).

Discussion

Not infrequently, thrombocytopenia is the first hematologic disorder manifested in patients seropositive for antibody to HIV. The underlying mechanism of this phenomenon is not completely understood.[9,10] Antibodies adsorbed to platelets as well as circulating immune complexes have been reported by several investigators and considerable effort has been made to distinguish HIV-related thrombocytopenia from the idiopathic (ITP) or lupus erythematosus-associated type.[2] The obvious question is whether megakaryocyte/platelets themselves are directly or indirectly damaged by HIV infection or whether the pathologic process is solely attributable to immune mechanisms. The observations reported here leave no doubt that the number of denuded megakaryocyte nuclei is significantly higher in HIV-infected individuals than in patients with ITP. This phenomenon becomes even more apparent with time when marrow sampling is repeated on the same individual (Table 2). Blebbing of the peripheral zone affects MK with well demarcated platelet fields (Figure 4), an observation that leads to the assumption that cell damage is inflicted relatively late in maturation. What is the fate of DN-MK in healthy humans? Presumably, the nuclei of effete cells are eliminated by cells belonging to the macrophage phagocytic system (MPS) located in the marrow or the periphery, eg, in the lung.[11] Could an HIV-impaired MPS account for the unusually large number of DN-MK seen in AIDS marrows? This would explain why there is a much larger number of denuded magakaryocyte nuclei in the marrows of HIV-infected individuals than in the specimens of patients with ITP. Moreover, we have reported elsewhere[6] that some antibodies may react with epitopes found on the surface membrane of MK, but not on platelets. In some patients this could account for injury to MK without a reduction in circulating platelets. On the other hand, some observations mitigate against the assumption that megakaryocyte damage in AIDS is due exclusively to immune processes. First, in contrast to patients with ITP, the platelet lifespan in patients with AIDS is not necessarily shortened. As mentioned, the increase

in the number of denuded nuclei also was seen in HIV-infected patients with normal platelet counts. In fact, there was no correlation between the number of DN and the number of circulating platelets. Second, thrombocytopenic patients receiving azidothymidine (AZT) may have a rise in their platelet counts within 1 to 2 weeks after initiation of therapy.[13–15] It is not likely that AZT treatment affects the level of platelet antibodies known to have a half-life in excess of 2 weeks. Therefore, the possibility that AZT could affect viral replication within megakaryocytes must be entertained. Indeed, a preliminary report suggests that AZT has a more salubrious effect on platelet production than platelet survival.[12] Yet in the absence of any evidence for viral infection of megakaryocytes, a direct effect of HIV on this cell lineage is only speculative. As has been true for lymphocytes freshly isolated from the peripheral blood of patients with AIDS, no virus particles have been seen by us on electron microscopy of megakaryocytes obtained from 65 patients with the syndrome. Therefore, it is unlikely that the virus replicates in megakaryocytes although it may be integrated in their genome. This proposition would still require an explanation of how megakaryocytes become infected. Preliminary immunofluorescence studies in this laboratory have revealed few if any CD_4 receptors on MK plasma membranes. However, megakaryocytes are known to take up microorganisms as well as autologous cells by Fc receptor-mediated endocytosis and by emperipolesis.[5,6] Therefore, HIV could gain entry to this cell in a variety of ways. Because DNA synthesis and nuclear endoreduplication takes place almost during the entire life span of the cell, HIV could integrate in the MK genome at various ploidy stages. This could determine the level of differentiation at which viral epitopes become expressed on intracellular and/or surface membranes. We have reported that the surface membrane of megakaryocytes differs structurally and antigenically from the plasma membrane of circulating platelets.[4,6,17] Other investigators also have shown that platelet antibodies used to induce thrombocytopenia in mice did not damage the megakaryocytes of such animals even ultrastructurally.[18] Therefore, HIV-negative ITP megakaryocytes with an intact peripheral zone may not be injured by antibodies directed against antigens associated with platelets. This seems to be the case. As mentioned before, most MK in HIV-negative ITP appear normal. The damaged cells in HIV-negative ITP have usually reached an advanced stage of ploidy and are releasing platelets. As illustrated elsewhere,[4,6,17] only at this stage of maturation can plasma proteins, including antibodies, gain access to the interior of the cell when demarcation membranes or "platelet fields" may be affected. Presumably HIV infection of megakaryocytes could occur at any stage of ploidy, and viral epitopes could be expressed at the surface of the cell as well as on internally located demarcation membranes. The association of HIV epitopes with the megakaryocyte surface membrane, even when the cells have not yet reached the stage of thrombocytopoiesis, could be responsible for the "blebbing" of the peripheral zone in the absence of injury to platelet fields. Therapy directed against virus replication may intervene with the expression of such epitopes on the MK surface, making the existence of circulating antibodies irrelevant to platelet production. This kind of mechanism would account for the response to antiviral agents without simultaneous elimination of antibody. In any event, the studies presented here have shown that the structural damage to megakaryocytes in HIV-infected individuals differs from that seen in patients with ITP. Whether the explanation for this difference is correct awaits *in situ* hybridization with HIV probes and the ultrastructural localization of HIV epitopes in megakaryocytes of HIV-seropositive thrombocytopenic patients.

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