Virus-Like Particles in Acute Lymphoblastic Leukemia

Virus particles have been revealed in lymphoreticular sarcoma and multiple myeloma[1,2]. There is also a strong clue that human leukemia can be caused by virus[3]. However, intracellular virus-like particles have not been previously reported from leukemic bone marrow.

A 5-year-old girl had splenic and hepatic enlargement with temperature elevations. The white blood cell count was low. Study of the bone marrow aspirate and peripheral blood smears confirmed the diagnosis of acute lymphoblastic leukemia.

Bone marrow aspirates were obtained for electron microscopy during the patients preleukemic phase, and at intervals of 1, 4 and 12 weeks after the onset of the leukemic phase. Cytoplasmic inclusions were observed within the cytoplasm of many mononuclear cells (Figures 1, 3, 4). In the cytoplasmic inclusions, dense virus-like particles were present. They were enclosed by a

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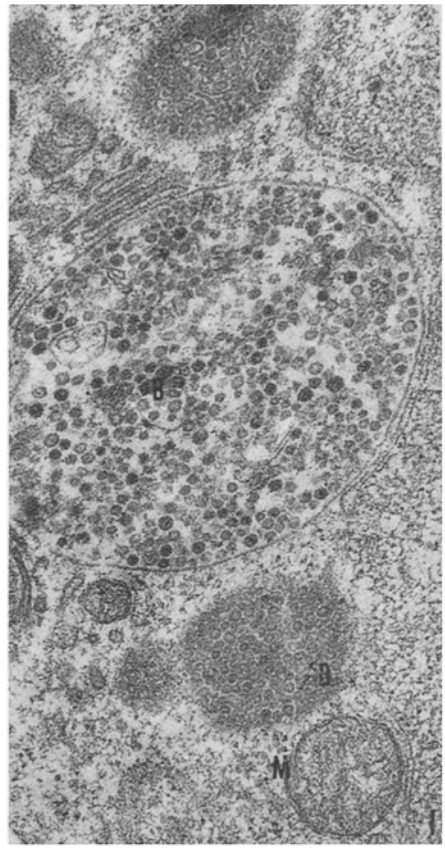

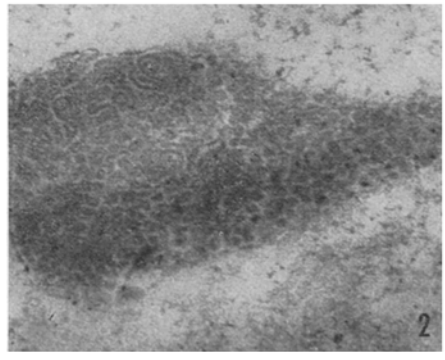

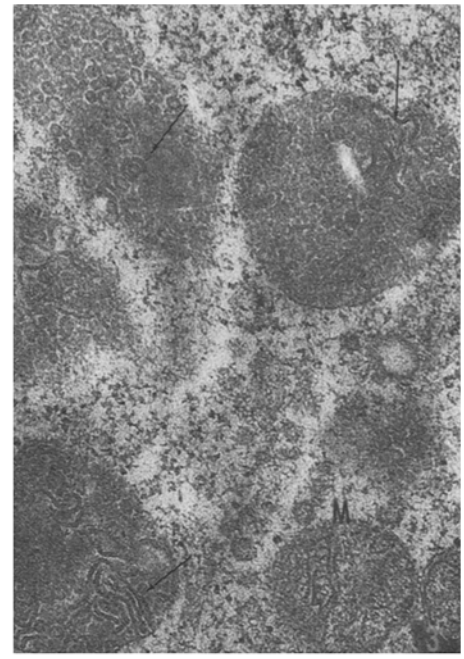

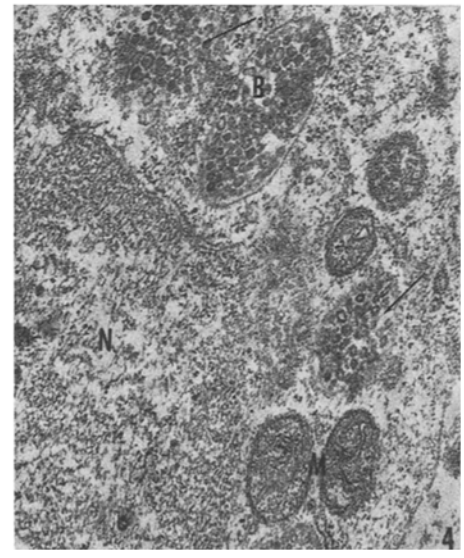

Fig. 1. Mononuclear cells from bone marrow showing virus like particles within cytoplasmic inclusions (*B*). *M*, mitochondrion; *N*, nucleus. × 65,000.

Fig. 2. Whorled and tortuous membranes within the inclusions. Budding of virus like particles from the membranes was suggested. × 50,000.

Fig. 3. Some inclusions showing a whorled membrane (arrow) may represent transitional forms between mitochondria and virus containing inclusions. *M*, mitochondrion. × 65,000.

Fig. 4. The limiting membranes of some inclusions are absent (arrow). The virus like particles can be seen in the cytoplasm. *B*, virus containing inclusion; *M*, mitochondrion; *N*, nucleus. × 32,000.

single limiting membrane. The particles were rather uniform in size approximately 400 Å in diameter and were composed of a thick electron-dense outer wall and an electron-lucent center. Less than 1% of the particles contained small nucleoids which resembled the A-type particles as described by BERNHARD[4].

In some cytoplasmic inclusions, whorled membranes were formed (Figure 3). Particles lying between them were occassionally seen. Some inclusions consisted entirely of whorled membranes (Figure 2). In a few instances, budding of virus-like particles from the membranes was suggested.

The origin of the membrane-bounded virus containing inclusions may possibly be from mitochondria. Some inclusions have the size as mitochondria, some inclusions have only a few whorled membranes with a few particles (Figure 3). These may be suggested as transitional forms between the mitochondria and virus containing inclusions[5].

Zusammenfassung. In Knochenmark-Monocyten eines fünfjährigen Mädchens konnten im Cytoplasma Einschlüsse beobachtet werden, die mit gewundenen Membranen und virusähnlichen Partikeln gefüllt waren.

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2 May 1972.

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[5] Technical assistance of J. MEADOR is greatly appreciated.

Antigen Binding Rosette Forming Cells in a Friend Virus-Induced Leukemia

The suppression of immunological functions induced by infection of experimental animals with RNA tumorigenic viruses is a poorly understood phenomenon[1]. Infection with a virus before administration of antigen has a greater suppressive effect of the subsequent antibody response than does infection after immunization[2,3]. Furthermore, spleen cells from a virus-infected donor show decreased immunological potential when transferred adoptively to an immunoincompetent recipient[4]. These findings imply that the leukemogenic virus causes a defect at the level of immunologically competent antigen reactive cells (ARC). Does the virus eliminate the ARCs by destroying them, or do the ARCs remain preserved in a state of functional paralysis prevented from differentiating into antibody production upon antigenic stimulation? The following experiments were undertaken in an attempt to distinguish between the two aforementioned alternative mechanisms of viral inhibition of the antibody response. The effect of Friend leukemia virus (FLV) on rosette forming cells, which are capable of binding antigen (heterologous erythrocytes) in vitro, was chosen as a model system.

Six- to twelve-week-old male Balb/c mice (Flow Laboratories, Rockville, Md.) were used. Friend leukemia virus was obtained from Dr. W. CEGLOWSKI, The Pennsylvania State University[3,4].

Rosette forming cells (RFC) in mouse spleen were enumerated by incubation of a spleen cell suspension with sheep red blood cells (sheep RBC) according to the technique of BIOZZI et al.[5], with two modifications: a) buffered Hanks' balanced salt solution was used for cell suspension instead of EDTA buffer and b) cells were

Table I. Effect of previous FLV infection[a] on the response of specific plaque forming cells (PFC) and rosette forming cells (RFC) to immunization with 10^8 sheep RBC

Interval[b]	PFC		RFC/10^6 cells
(days)	per spleen	per 10^6 cells[c]	
1	66700 (± 10950)[d]	420 (± 91)	38000 (± 26400)
Control	66600 (± 26200)	426 (± 132)	24000 (± 7600)
5[e]	7700 (± 2100)	39 (± 10)	8400 (± 3300)
Control[e]	68600 (± 27370)	430 (± 40)	26400 (± 1000)

Immune responses were assayed in the spleen 4 days after immunization. [a] Friend leukemia virus (FLV)[3] was prepared as a 10% (w/v) homogenate of spleens from infected Balb/c mice, in Puck's saline (Gibco, N.Y.), pH 7.4. The homogenate was subjected to 2 centrifugations (1,500 *g*, 15 min, 4 °C and at 7,000 *g*, 10 min, 4 °C, respectively). The final supernatant fraction was filtered through a Millipore filter (0.8 μm) and frozen at −80 °C. Activity of the virus was determined by the spleen colony assay[14]. Each mouse received i.v. 5,000–10,000 focus forming units of FLV. [b] Interval in days between infection with FLV and immunization with SRBC. Controls were uninfected mice of the same age. [c] Calculated on the basis of approximately 10^6 cells in 0.1 ml of 1% (v/v) spleen cells suspension. [d] Arithmetic mean from 5–10 individual mice (± standard deviation). [e] Difference between infected and control animals in both PFC and RFC responses, respectively, is significant ($p < 0.01$) as calculated by a rank test[15].

Table II. Number of background plaque forming cells (PFC) and rosette forming cells (RFC) in spleens of non-immunized, FLV-infected mice

Interval after infection (days)[a]	PFC per spleen	per 10^6 cells[b]	RFC/10^6 cells
3			820 (± 53)[c]
Control			1040 (± 258)
5	277 (± 260)[d]	2 (± 1.3)	1700 (± 245)
Control	28 (± 28)[d]	<1[e]	1000 (± 447)

[a] For preparation and infection with FLV see Table 1; controls were uninfected mice of the same age. [b] Calculated on the basis of approximately 10^6 cells in 0.1 ml of 1% (v/v) spleen cell suspension. [c] Arithmetic mean from 6 individual mice (± s.d.). [d] Statistically significant difference between infected and control group ($p < 0.01$) by the rank test. [e] 1 to 5 PFC detected in 0.1 ml of 10% suspension (10^7 cells).

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