Morphological evidence for the presence of the endogenous guinea-pig retravirus in the lymphoblasts of L2C leukemia[1]

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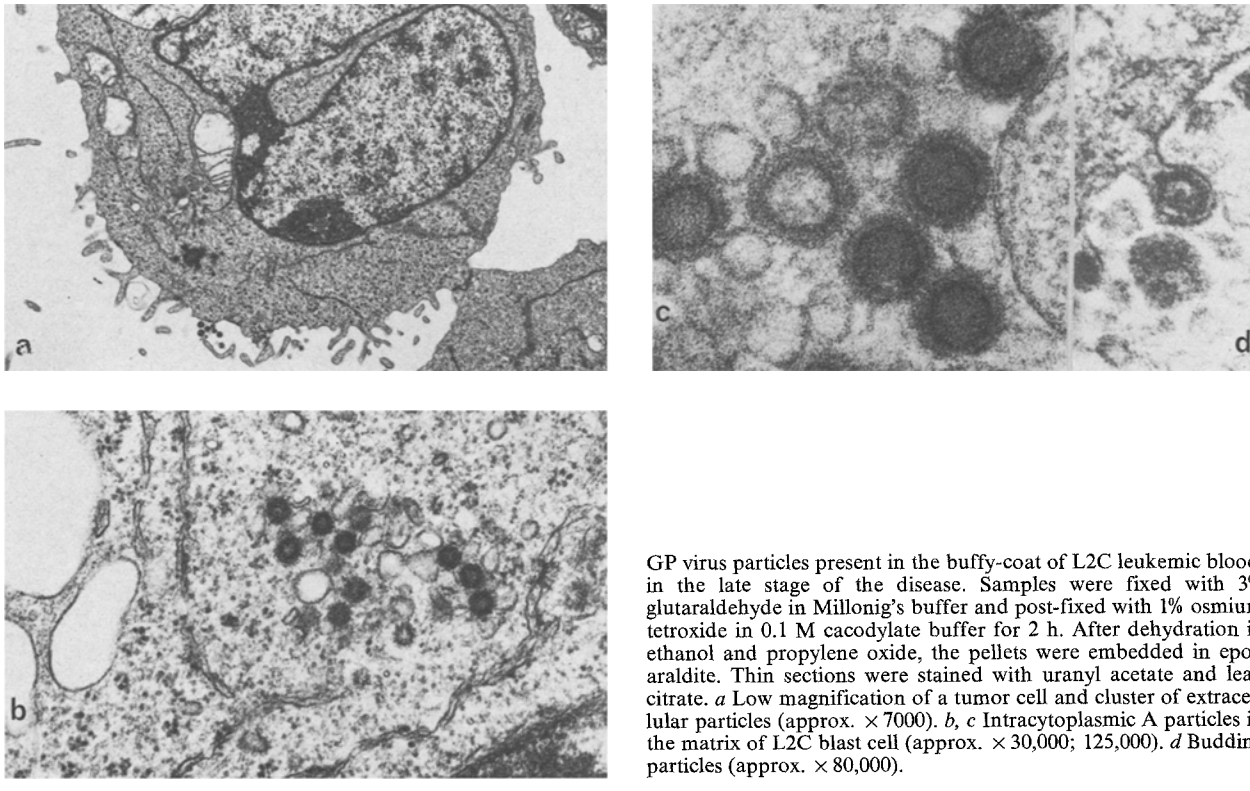
Summary. Peripheral blood samples of a transplantable guinea-pig leukemia revealed that, only during the terminal stages of the disease, leukemic cells produce virus particles indistinguishable from the RNA tumor viruses of the Retraviridae family genus *Oncorna* virus B.

L2C leukemia is an acute transplantable lymphoblastic leukemia in guinea-pigs. This leukemia is widely employed for a variety of virological, chemotherapeutic, pathological and immunotherapeutic studies and serves as a most useful model with clinical relevance to human acute leukemia[2–5].

Virus-like particles were found repeatedly in the cisternae (intracisternal A particles) of L2C tumor cells, in extracellular spaces and in plasma pellets[3,6–9]. Similar particles have been described in a chemically induced guinea-pig hepatoma[10], and in fetal tissues[11], spleen germinal centers[9], oocytes and oogonia of normal guinea-pigs[12]. Attempts to transmit leukemia or any other neoplastic disease by cell-free plasma or tumor extracts have proved negative[3]. Earlier reports claiming such cell-free transmission[13] have been disproved by the finding that all L2C tumors have retained since 1954 the original female karyotype regardless of the sex of the host animal[3,14].

Virus particles have been induced in tissue cultures of a wide variety of normal and neoplastic guinea-pig cells using 5-bromo-2′-deoxyuridine (BrdU)[15–19]. The guinea-pig virus (GPV) particles induced by BrdU are morphologically and biochemically similar to known RNA tumor viruses (retravirus)[3,15–19]. It was recently demonstrated that the BrdU-induced GPV is a true endogenous virus[20], and there is no direct relationship between GP virus and the intracisternal A particles seen in L2C leukemic cells[21]. Morphologically GPV is nearly indistinguishable from the mouse mammary tumor virus (MMTV) classified as B-type retravirus[19]. For example, intracytoplasmic A particles, one of the main features of cells producing MMTV, are present in BrdU-induced guinea-pig cells[19]. Intracytoplasmic A particles occur in the cytoplasmic matrix of the cell, and in some cases, become the core of B particles. Further, several biochemical and biophysical parameters of GPV are identical to those of MMTV[22] and are quite distinct from the comparable properties of the mammalian type C-retraviruses.

In the study reported here, we present morphological evidence for the appearance of the endogenous GP virus in the L2C leukemic cells. Examination of blood samples of the L2C leukemia for virus particles revealed that only during the terminal stages of the disease, leukemic cells show virus particles indistinguishable from the tissue-culture-induced GP virus. Although extracellular virus particles and intracytoplasmic A are observed frequently, budding particles are rare. The figure shows selected examples of thin sections of tumor cells obtained from the blood at terminal stages of the disease. Figure a presents a low magnification of a tumor cell and a cluster of extracellular particles. Figures b and c show clusters of typical intracytoplasmic A particles in lymphoblasts. Both electron dense shells are of approximately equal density. All the budding



GP virus particles present in the buffy-coat of L2C leukemic blood, in the late stage of the disease. Samples were fixed with 3% glutaraldehyde in Millonig's buffer and post-fixed with 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 h. After dehydration in ethanol and propylene oxide, the pellets were embedded in epon araldite. Thin sections were stained with uranyl acetate and lead citrate. *a* Low magnification of a tumor cell and cluster of extracellular particles (approx. × 7000). *b, c* Intracytoplasmic A particles in the matrix of L2C blast cell (approx. × 30,000; 125,000). *d* Budding particles (approx. × 80,000).

particles observed contained completely formed nucleoid (figure d). These morphological findings complement and extend the biochemical demonstration that only during the terminal stages of the disease are particulates with reverse transcriptase and 60–70S RNA indistinguishable from tissue-culture-induced GP-B type retravirus found[23]. This is also in line with the demonstration that BrdU-induced viral genes are transcribed in L2C leukemic cells[21].

The morphological evidence for the presence of the B-type retravirus in the late stages of leukemogenesis indicates that a positive correlation exists between the morphologically complete expression of endogenous viral genes in L2C leukemic cells and malignancy, particularly leukemia, in guinea-pigs.

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Effet de la neuraminidase sur l'activité phagocytaire d'*Amoeba proteus*
Effect of neuraminidase on phagocytosis activity in *Amoeba proteus*

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Summary. Phagocytosis activity of *Amoeba* is greatly reduced after treatment with neuraminidase, at concentrations that do not affect normal motile behavior.

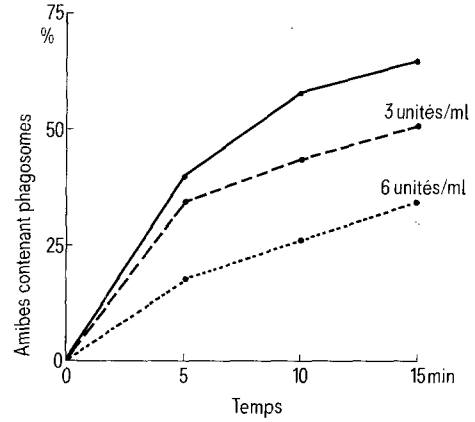
Presque toutes les cellules animales possèdent une couche de mucopolysaccharide (glycocalyx) sur la surface externe de leur membrane plasmique. Chez l'amibe d'eau douce, *Amoeba proteus*, ce revêtement est particulièrement important; son développement peut atteindre une épaisseur de 2000 Å[1]. Sa composition chimique est de nature polysaccharidique avec la présence, probable, d'acides sialiques[2,3].

Le rôle du glycocalyx de l'amibe dans certaines activités cellulaires telles que la pinocytose[4–7] et la chimiotaxie[8,9] semble maintenant bien établi. Cependant, son intervention dans les phénomènes phagocytaires – phénomène impliquant des mouvements protoplasmiques associés à la capture et à l'ingestion d'une proie – est moins bien connue. Les expériences de Lindberg[10] sont les seules, à notre connaissance, qui mettent en relief cette fonction du glycocalyx chez l'amibe. L'auteur observe une inhibition de la phagocytose chez des amibes immergées dans des solutions d'enzymes protéolytiques (pronase, trypsine, papaïne).

La présente note vise à préciser le rôle du glycocalyx dans la phagocytose chez *A. proteus*, en utilisant une enzyme plus spécifique; la neuraminidase.

Matériel et méthodes. Amoeba proteus a été cultivée suivant la méthode de Prescott et Carrier[11]. Les amibes subissent un jeûne de 48 h et sont lavées 5 fois dans leur milieu de culture (milieu de Chalkley[12]) avant chaque expérience. Les amibes traitées à la neuraminidase subissent en plus, une préincubation de 5 min dans l'enzyme (3 et 6 unités/ml de milieu Chalkley). Le pH du milieu est maintenu à 5.7. La neuraminidase de *Vibrio cholerae* provient de la maison General Biochemicals, Ohio.

Calcul de l'activité phagocytaire. Essentiellement, nous reprenons la méthode mise au point par Marsot et Couillard[13] pour la phagocytose de cellules dissociées de l'hydre, avec les modifications suivantes: a) Coloration vitale de *Tetrahymena pyriformis* (cilié). Les cellules préalablement lavées avec le milieu Chalkley (3 fois), sont resuspendues,



Effet de la neuraminidase sur la phagocytose de *Tetrahymena* par *Amoeba proteus*. Des populations identiques d'amibes (1500/ml) sont en présence d'un nombre toujours constant de ciliés (800/ml). La neuraminidase a été utilisée aux concentrations de 3 unités/ml (ligne brisée) et 6 unités/ml (ligne pointillée). La ligne unie représente l'expérience témoin (sans enzyme).