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Cellular Localizations and Processing of the Two Molecular Forms of the Hodgkin-associated Ki-1 (CD30) Antigen

The Protein Kinase Ki-1/57 Occurs in the Nucleus

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The Ki-1 antibody not only detects a Hodgkin-associated membrane molecule of 120 kd (Ki-1/120 = CD30), but also reacts with an independently synthesized molecule of 57 kd (Ki-1/57) that only occurs intracellularly. Hodgkin's disease–derived cell lines L428 and L540 contain both Ki-1–reactive antigens, whereas others, e.g., U266/B1 myeloma cells, only express the intracellular Ki-1/57. The present immunoelectronmicroscopic analysis detected the Ki-1/57 antigen of U266/B1 cells not only in the cytoplasm, but also in association with the nuclear envelope, chromatin structures, and nucleoli. This Ki-1/57–specific type of labeling also was observed in L428 and L540 cells that, in contrast to U266/B1 cells, showed an additional staining of cell membranes and cytoplasmic vesicles. These results were confirmed by two independent methods: 1) cytocentrifuge preparations of isolated nuclei of L540 cells showed a spotted Ki-1–specific labeling, 2) immunoprecipitations demonstrated that the Ki-1/57, but not the Ki-1/120 antigen, was transferred into the nuclei of L540 and U266/B1 cells, whereas the Ki-1/120 antigen with its 90-kd precursor remained in the non-nuclei fraction of L540 cells. (Am J Pathol 1992, 140:473–482)

The KI-1 antigen (CD30) was originally discovered as a marker of Hodgkin's (H) and Reed–Sternberg (RS) cells in all types of Hodgkin's disease[1,2] and on a few non-Hodgkin's lymphomas.[3] *In vitro*, however, the CD30 antigen is expressed on virus-transformed B and T lymphocytes,[4–7] on a variety of established cell lines,[4,5,8] on activated normal lymphocytes,[2,4,6] and on monocyte-derived macrophages.[9]

The Ki-1 antibody reacts with two molecules: a 120-kd membrane-associated glycosylated phosphoprotein (Ki-1/120) and a 57-kd nonglycosylated phosphoprotein (Ki-1/57), which only occurs intracellularly. Both molecules are phosphorylated at serine residues.[8] The Ki-1/120 and Ki-1/57 are synthesized independently of each other and they are not necessarily coexpressed, eg, the cell lines Raji and Daudi (Burkitt lymphomas), U266/B1 (myeloma), or HL-60 (promyelocytic leukemia) synthesize the intracellular Ki-1/57, but not the membrane-bound Ki-1/120.[8] The Ki-1/57 antigen is a protein kinase that showed autophosphorylation and could also phosphorylate histones, whereas such an enzymatic activity could not be demonstrated for the Ki-1/120 membrane antigen.[10] This molecule develops from an intracellular precursor of 90 kd by *N*- and *O*-glycosidic glycosylation.[7,8,11] Later on, it is shed from the cell surface as a soluble Ki-1 antigen of approximately 90 kd,[8,12,13] which is of diagnostic value.[12–14]

The goal of the present study was to analyze the cellular distribution of the two Ki-1–reactive molecules by immunoelectronmicroscopic means and cell fractionation techniques in combination with light microscopy and immunoprecipitation.

Materials and Methods

Antibodies

The reactivity of the Ki-1 antibody ($\gamma 3$, κ) has been described elsewhere.[1–3,7,8] Two other monoclonal antibod-

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ies of the γ3 isotype were used for specificity controls: 1) H141-31 (γ3, κ), which reacts with the private specificity of the H-2D^b antigen[15] and 2) 1/2 (γ3, κ), which is an anti-sheep red blood cell (SRBC) antibody.[16]

Cell Lines

The Ki-1 antigen was isolated and analyzed from two Hodgkin's disease–derived cell lines L428 and L540[5] and the myeloma cell line U266/Bl.[17] They were cultivated under standard conditions using RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), which had been tested for its growth-promoting ability.

Preparation of Ultrathin Cryosections for Electron Microscopy

The cells were harvested by centrifugation, washed once in phosphate-buffered saline (PBS), and fixed with 0.5% glutardialdehyde-containing PBS for 30 minutes at 4°C. After two further washes in PBS and suspension in prewarmed PBS (37°C) containing 5% gelatin (Merck, Darmstadt, Germany), the cells were centrifuged immediately and the cell pellet was solidified by incubation for 20 minutes on ice. The pellet was cut into blocks of 2 mm which were incubated in increasing concentrations of 10%, 30%, 50%, and 70% sucrose solution in water, each for 90 minutes at 4°C. The blocks were fixed to a specimen holder and frozen in liquid nitrogen. Ultrathin cryosections were prepared with an Ultracut E, FC-4D (Reichert-Jung, Germany) and collected on formvar-coated nickel grids.[18]

Immunolabeling of Cryosections

The cryosections were incubated for 30 minutes at room temperature with culture supernatant fluid or ascites fluid of the primary antibody Ki-1 that had been diluted to an appropriate concentration in PBS supplemented with 0.1% gelatin and 0.5% bovine serum albumin (PBG). The grids then were washed six times (each for 5 minutes) in PBG and incubated for 1 hour at room temperature with Staphylococcal protein A (SpA) coupled to 15-nm gold particles (diluted 1:30 in PBG; Janssen Pharmaceutica, Beerse, Belgium). The cryosections were washed again in PBG and fixed once more with 2.5% glutardialdehyde-containing PBS for 3 minutes. Finally the sections were contrasted with 4% uranyl acetate and examined in a transmission electron microscope (Siemens Elmiskop 101).

Immunolabeling of Cytospins

The Ki-1 antigen was detected in isolated nuclei with the aid of a three-step immunoperoxidase technique (Vector Laboratories, Burlingame, CA). Acetone-fixed cytocentrifuge preparations were incubated with the primary monoclonal antibody Ki-1 diluted to an optimal concentration in PBS containing 1% bovine serum albumin for 30 minutes. After careful washing in NaCl/TRIS buffer (pH 7.4), the cytospins were incubated for 30 minutes with peroxidase-conjugated rabbit anti-mouse IgG (diluted 1:15 in serum-supplemented PBS). The cytospin preparations were washed twice with NaCl/TRIS buffer and incubated for 30 minutes with peroxidase-conjugated goat anti-rabbit IgG (diluted 1:15 in serum-supplemented PBS). The Graham-Karnovsky peroxidase reaction was performed with 3,3'-diaminobenzidine (0.6 mg/ml; Sigma Chemie, München, Germany) and 0.01% hydrogen peroxide at room temperature for 10 minutes. The cytospins were counterstained with hemalaun and finally coverslipped using glycergel (Dakopatts; Hamburg, Germany).

Labeling of Cells with ^{35}S-methionine

The cells (5×10^6/ml) were preincubated for 30 minutes in methionine-free minimal essential medium (MEM) (Flow, Meckenheim, Germany) supplemented with 2 mmol/l (millimolar) glutamine and 10% dialyzed FCS. After washing, the cells were suspended in 1 ml of methionine-free MEM and 500 μCi ^{35}S-methionine (specific activity > 800 Ci/mmol; Amersham Buchler, Braunschweig, Germany) were added. The cells were cultured at 37°C for a short pulse of 10 minutes and an additional chase time of 60 minutes in normal culture medium.[8]

Preparation of Nuclei

The nuclei of the cells were prepared as described by Marzluff and Huang[19] with slight modifications. The cells were harvested by centrifugation and suspended in buffer 1 (0.32 mol/l [molar] sucrose, 3.0 mmol/l $CaCl_2$, 2.0 mmol/l magnesium acetate, 0.1 mmol/l ethylenediaminetetra-acetic acid [EDTA], 0.1% Triton X-100, and 10.0 mmol/l TRIS-HCl, pH 8.0; in contrast to the method of Marzluff and Huang,[19] dithiothreitol [DTT] was omitted) at a concentration of 1×10^7 cells/ml. Half of the cells had been labeled with ^{35}S-methionine, and the rest was nonradioactively labeled. The cells were homogenized with 10 to 15 strokes in a Dounce homogenizer (loose-fitting pestle). The homogenate was diluted with 1 vol buffer 2 (2.0 mol/l sucrose, 5.0 mmol/l magnesium acetate, 0.1

mmol/l EDTA, 10.0 mmol/l TRIS-HCl, pH 8.0; again, DTT was omitted), layered on 4 ml of buffer 2, and centrifuged at 30,000*g* for 45 minutes at 4°C. The top layer plus the interface were collected and the same volume of lysis buffer (PBS containing 2% Triton X-100, 2 mmol/l EDTA, pH 8.0) and 10 μl of phenylmethylsulfonyl fluoride (saturated solution in 2-propanol) were added. The sucrose cushion of buffer 2 was removed. The sedimented nuclei were suspended in 0.2 ml PBS, and 1 ml lysis buffer was added. Both fractions were incubated for 20 minutes on ice, and insoluble material was removed by centrifugation (15,000*g*, 30 minutes). The Ki-1 antigen was isolated from both lysates as described below.

Immunoprecipitation of the Ki-1 Antigen

The Ki-1 antigen was immunopreciptitated as described.[8,10] Briefly, the Ki-1 antibody was added to the lysates of the nuclei and the non-nuclei fraction. The immune complexes were isolated by affinity chromatography on staphylococcal protein A-Sepharose CL-4B (SpA-S) (Pharmacia, Freiburg, Germany) and the antigenic molecules were analyzed by autoradiography after separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), according to the method described by Laemmli[20] with modifications previously described.[7,8,10]

Results

Cellular Localization of the Ki-1 Antigen in Hodgkin-analogous L428 and L540 Cells

First we analyzed the intracellular localization of the Ki-1 (CD30) antigen in the two Hodgkin-analogous cell lines L428 and L540. The Ki-1 antigen could be detected on the cell membranes of L428 (Figure 1a, b) as well as of L540 cells (Figure 2b, c) and on intracellular vesicles of different sizes, eg, in L428 cells (Figure 1a, b). Moreover an additional Ki-1–specific staining was found as small clusters of densely packed gold particles in the cytoplasm, e.g., in L540 cells (Figure 2a, b), but also in the nuclei of the two cell lines. In Figure 2d, such a cluster is located in the condensed chromatin of L540 cells. Other localizations of nuclear Ki-1–specific stainings are depicted in Figure 4 (see below).

Cellular Localization of the Ki-1 Antigen in U266/B1 Myeloma Cells

In U266/B1 myeloma cells that exclusively express the intracellular 57-kd antigen,[8,10] neither a Ki-1–specific labeling of cell membranes nor labeling of intracellular vesicles like those in L428 (Figure 1a, b) or L540 cells (data not shown) could be observed. In U266/B1 cells, Ki-1–specific clusters of gold particles were found in the cytoplasm and in the nucleus (Figure 3). These clusters had a rather identical appearance and intracellular distribution like those in the Hodgkin-analogous cell lines, as shown for L540 cells (Figure 2b, d).

Nuclear Localization of the Ki-1/57 Antigen

The Ki-1–specific accumulations of densely packed gold particles in the various nuclear localizations seemed to be identical in all three cell lines tested. These Ki-1–specific nuclear labelings are exemplified in Figure 4. They have been observed at the nuclear envelope (Figure 4a; in U266/B1 cells), in heterochromatin (Figure 4b; in U266/B1 cells), in euchromatin structures (Figure 4c; in L428 cells) and in the nucleolus (Figure 4d; in L540 cells).

Specificity Control of the Cellular Labeling of the Ki-1 Antigen

The labeling of the Ki-1 antigen in the immunoelectronmicroscopic analysis of L428, L540, and U266/B1 cells, depicted in Figures 1 through 4, was specific for the following reasons: 1) The observed structures were never seen in the absence of the primary Ki-1 antibody; ie, with the gold-coupled SpA alone, we never observed the characteristic accumulations of gold grains shown in Figures 1 through 4. 2) The control preparation with the anti-H2 antibody H141-31[15] or the anti-SRBC antibody 1/2[16] were also entirely negative.

Detection of the Ki-1 Antigen in Cytocentrifuge Preparations of Isolated Nuclei of L540 Cells

For light microscopic investigation, cytocentrifuge preparations of isolated nuclei of L540 cells were prepared and stained for the Ki-1 antigen. About 50% of the isolated nuclei showed a Ki-1–specific labeling (Figure 5). The nuclear Ki-1/57 antigen could be detected as spots that occurred singly or rather concentrated. When different layers of the cells were successively focused during the microscopic examination, it appeared as though the Ki-1–reactive spots formed an interconnected structure. These spots were not evenly distributed, but appeared in a certain sector of the nucleus only.

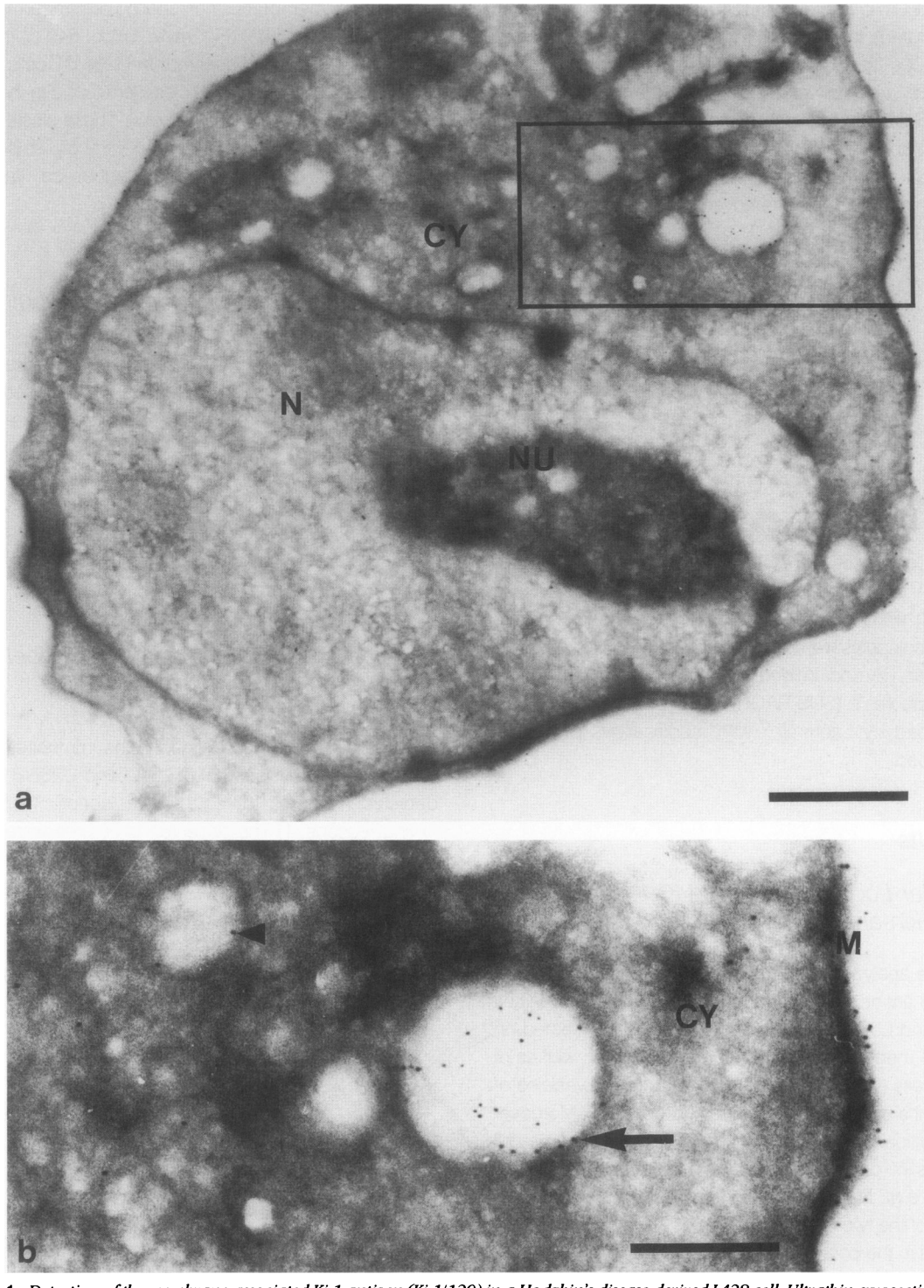

Figure 1. *Detection of the membrane-associated Ki-1 antigen (Ki-1/120) in a Hodgkin's disease-derived L428 cell. Ultrathin cryosections were labeled with the Ki-1 antibody and protein A-coupled gold particles (15 nm) as secondary reagent.* **a**: *Overview (bar = 1 μm).* **b**: *Part of the cell shown in* (**a**). *Gold particles can be observed on the cell surface and on membranes of intracellular vesicles (arrow, arrow-head) (bar = 0.5 μm). (M = plasma membrane, CY = cytoplasm, N = nucleus, NU = nucleolus).*

Isolation of the Ki-1 Antigen from the Nuclei and Non-nuclei Fractions

L540 and U266/B1 cells were pulse-labeled with ^{35}S-methionine. After the addition of complete medium and a further incubation of 1 hour, the cells were harvested and a fourfold amount of nonlabeled cells was added. The nuclei were prepared and separated from the non-nuclei fraction containing the cytoplasm and the cell membranes. From both fractions, the Ki-1–reactive antigens

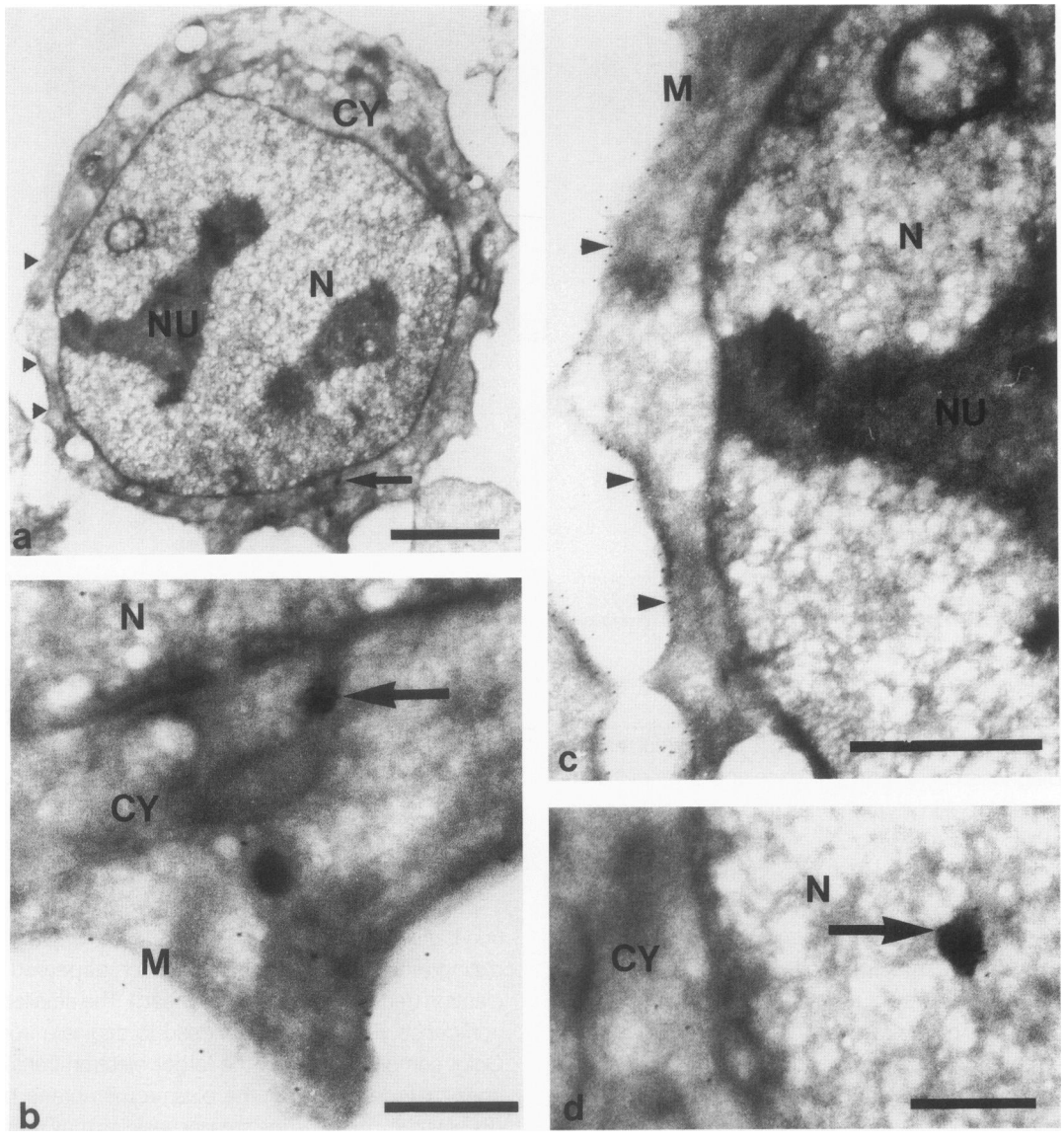

Figure 2. *Detection of the membrane-associated Ki-1/120 and the intracellular Ki-1/57 antigens in Hodgkin-analogous L540 cells. Ultrathin cryosections were labeled with the Ki-1 antibody and protein A-coupled gold particles (15 nm) as secondary reagent.* **a**: *Overview (bar = 1 μm).* **b**: *Part of the cell shown in* (**a**) *with immunogold labeling for the Ki-1/57 in the cytoplasm (arrow) and staining for the Ki-1/120 on the cell surface (bar = 1 μm).* **c**: *Part of the cell shown in* (**a**) *with immunogold labeling of the Ki-1/120 on the cell membrane (bar = 1 μm).* **d**: *Part of a L540 cell with immunogold labeling of the Ki-1/57 in the nucleus, here in condensed chromatin (arrow) (bar = 0.5 μm). (M = plasma membrane, CY = cytoplasm, N = nucleus, NU = nucleolus).*

were immunoprecipitated, separated by SDS-PAGE, and analyzed by autoradiography. The result of such an experiment is shown in Figure 6. From the non-nuclei fraction of L540 cells (lane 2), the membrane-associated Ki-1/120 antigen could be isolated together with its 90-kd precursor and the 105-kd molecule that is a degradation product of the 120-kd molecule.[7,8] The intracellular Ki-1/57 antigen was not found, however. In contrast, only the Ki-1/57 antigen (and possibly a barely visible 120-kd band) could be isolated from the nuclei fraction of L540 cells (lane 1). In U266/B1 cells, no Ki-1–reactive molecule could be isolated from the non-nuclei fraction (lane 4), and only the Ki-1/57 antigen was immunoprecipitated from the separated nuclei (lane 3).

In addition, we wanted direct proof whether or not the newly synthesized cytoplasmic 57-kd protein was redirected into the nucleus. L540 cells were pulse labeled for 10 minutes with ^{35}S-methionine, and the Ki-1–reactive antigens were immunoprecipitated from the cytoplasmic and the nuclear fraction either immediately or after chase periods of 1 and 3 hours, respectively. The isolated molecules were separated by SDS-PAGE and analyzed by autoradiography (Figure 7). After the pulse period, the radiolabeled Ki-1/57 molecule was present in the cytoplasm (lane 1), but not in the nucleus (lane 4). After a chase period of 1 or 3 hours, however, the Ki-1/57 could be isolated from the nuclear (lanes 5 and 6), but not the cytoplasmic, non-nuclear fraction (lanes 2 and 3).

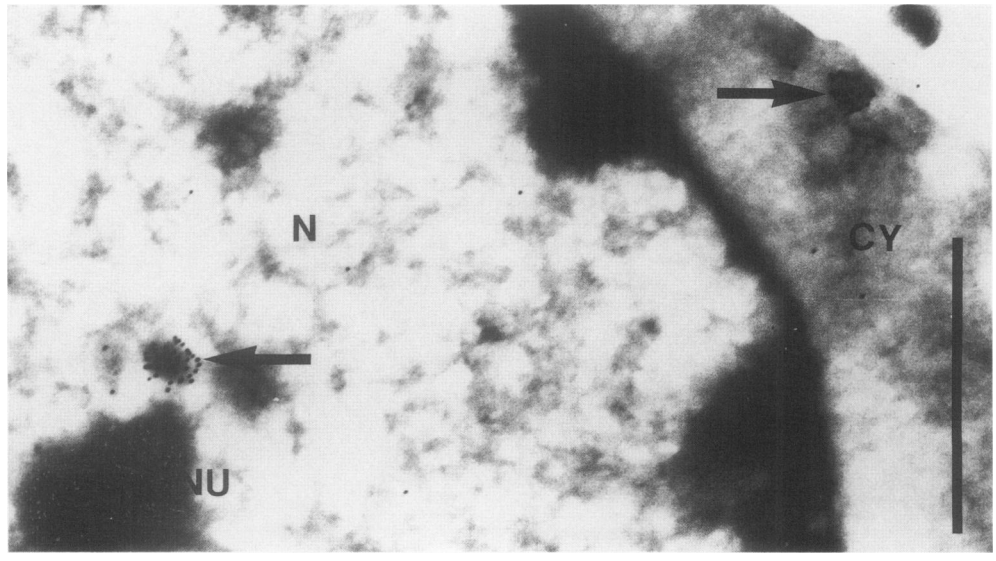

Figure 3. *Labeling of the Ki-1/57 antigen in a U266/B1 myeloma cell. Ultrathin cryosections were stained with the Ki-1 antibody and protein A-coupled gold particles (15 nm) as secondary reagent. The Ki-1/57 appears in the cytoplasm as well as in the nucleus, here in the nucleolus-associated chromatin (bar = 1 μm). (CY = cytoplasm, N = nucleus, NU = nucleolus).*

Discussion

The Ki-1 (CD30) antigen has proven to be a useful marker in the diagnosis of Hodgkin's disease and large-cell anaplastic non-Hodgkin's lymphomas of B- or T-cell type.[3] *In vitro* studies have given arguments for the Ki-1 antigen as an activation marker. The Ki-1 antigen could be found not only on Hodgkin's disease-derived cells lines,[1,2,5,21,22] but also on activated normal lymphocytes,[3,4,6] *in vitro* monocyte-derived macrophages,[9] virally transformed cells,[4,5–7] and a variety of permanent cell lines.[4,5,8] Most of these reports have not taken into account the fact that the Ki-1 antibody reacts with two independently synthesized molecules, ie, the membrane-associated Ki-1/120 and the intracellular Ki-1/57.[8] Because both Ki-1–reactive molecules are not necessarily coexpressed in a cell,[8,10] it has to be clarified which of the two Ki-1–reactive antigens is expressed *on* or *in* the various Ki-1–positive cell types.

The observation that the Ki-1/57 antigen may occur in an organelle-like staining in U266/B1 cells required study of the cellular localization of the Ki-1 antigen at the ultrastructural level: 1) in Hodgkin's disease-derived cell lines L428 and L540, which contain both Ki-1–reactive antigens, and 2) in U266/B1 myeloma cells, which only synthesize the intracellular molecule Ki-1/57.[8] For detection of the Ki-1–reactive antigens by immunoelectronmicroscopic means, we tested different modifications of preembedding and postembedding methods, varying the fixation procedures[23–25] or embedding of the cells with Araldit[25–27] (Serva, Heidelberg, Germany) or Lowicryl[25,28,29] (Chemische Werke Lowi, Waldkraiburg, Germany). None of these methods allowed the detection of the intracellular Ki-1/57 antigen. We therefore had to be content with the rather poor structural distinctiveness of the immunoelectronmicroscopic labeling of the ultrathin cryosections presented.

These cryosections of L428 and L540 cells showed a Ki-1–specific labeling of cell membranes (Figures 1, 2), whereas U266/B1 cells remained negative in this respect. L428 and L540 cells contained three different Ki-1–positive cytoplasmic compartments. Rather large electron-translucent organelles (Figure 1b, arrow) could be distinguished from smaller ones, which appeared more electron dense (Figure 1b, arrowhead). The smaller electron-dense vesicles are supposed to originate from the Golgi complex, whereas the larger electron-translucent ones may develop from the plasma membrane by endocytosis.[30,31] Hence the smaller vesicles may represent transport forms of Ki-1/120 antigen on the way to the cell membrane,[7,8] and the larger electron-translucent vesicles might contain endocytosed Ki-1/120 antigen from the cell membrane. These two cytoplasmic forms of the Ki-1 antigen could not be detected in U266/B1 cells.

A third form of the Ki-1 antigen in the cytoplasm appeared as clusters of densely packed gold particles in L428 and L540 cells (Figure 2b). Because this type of a Ki-1–specific labeling also was observed in U266/B1 cells (Figure 3), we regard it as specific for the Ki-1/57 antigen. These observations confirmed previous results that had demonstrated that the Ki-1/120 molecule is present on the cell membrane, whereas the Ki-1/57 molecule remains intracellular.[8,10]

Because the cytoplasmic clusters of gold particles in U266/B1 cells often seemed to be located just under the cell membrane (Figure 3), we surmised that the Ki-1/57 might be secreted into the cell supernatant, comparable

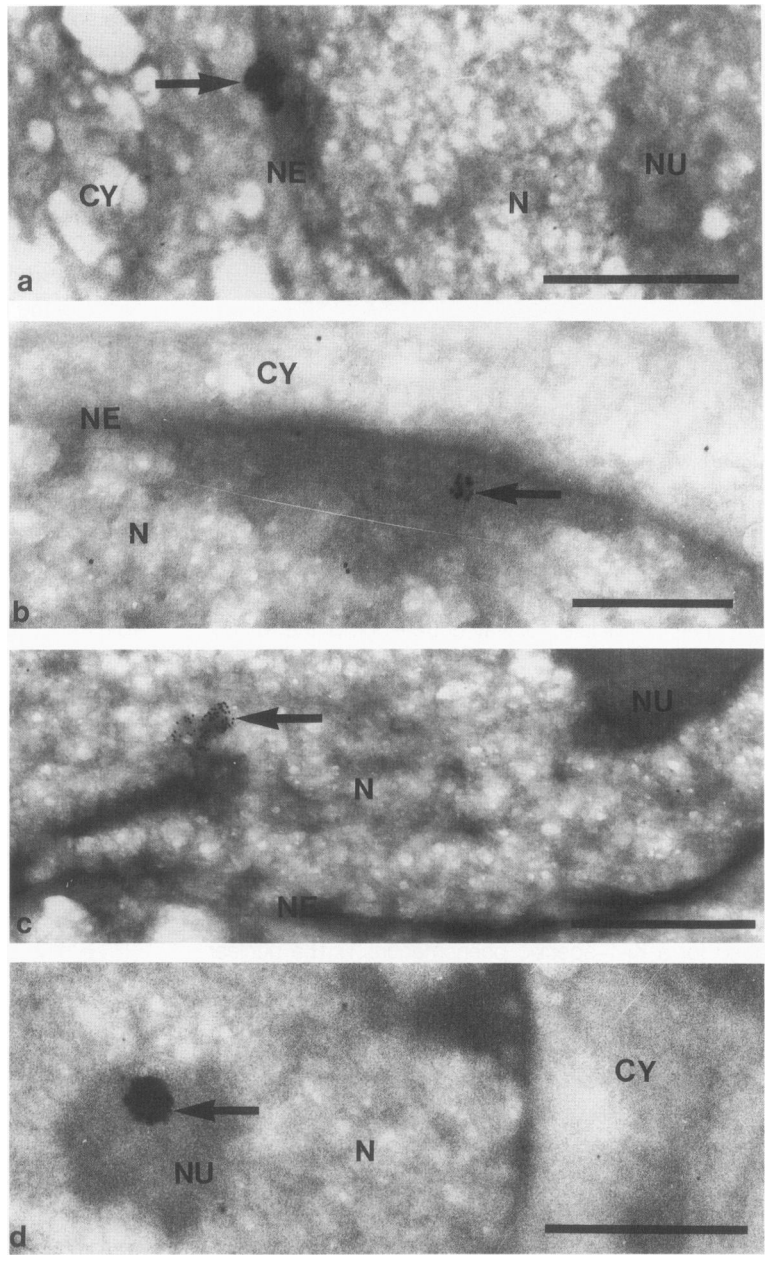

Figure 4. *Detection of the Ki-1/57 antigen in nuclei of the three different cell lines tested. Ultrathin cryosections were labeled with the Ki-1 antibody and protein A-coupled gold particles (15 nm) as secondary reagent. The Ki-1/57 antigen is depicted* (a) *on the nuclear envelope of U266/B1 cells;* (b) *in the heterochromatin of U266/B1 cells;* (c) *in the euchromatin of L428 cells; and* (d) *within the nucleolus of L540 cells (bar = 1 μm). (CY = cytoplasm, NE = nuclear envolope, N = nucleus, NU = nucleolus).*

to the occurrence of a soluble form of the membrane-associated Ki-1 antigen.[8,12–14] All efforts to isolate a Ki-1–reactive molecule from the supernatant fluid of U266/B1 cells were unsuccessful, however. Therefore we assume that these clusters of densely packed Ki-1–specific gold particles in L428, L540, and U266/B1 cells possibly represent polyribosomes on which the Ki-1/57 molecule is synthesized.

An unexpected result of this investigation was the discovery of a Ki-1–specific labeling in the nuclei of all three cell lines tested, ie, the Hodgkin's disease-derived cell lines L428 and L540 and in the myeloma cell line U266/B1 (Figures 2 through 5). This Ki-1–specific labeling appeared in various locations in the nuclei. These clusters were seen at the nuclear envelope (Figure 4a), in the heterochromatin (Figure 4b), in euchromatin structures (Figure 4c), and in the nucleolus (Figure 4d).

Very rarely, we observed bandlike elongated Ki-1–specific labelings in the nuclei which could begin in the vicinity of the nuclear envelope and could reach out to the nucleolus (data not shown). Such structures were rare events and are difficult to interpret. We hypothesize that they might represent longitudinal sections through elongated structures that were otherwise seen in cross sections as round dense clusters of Ki-1–specific gold particles. Conceivably these elongated Ki-1–specific label-

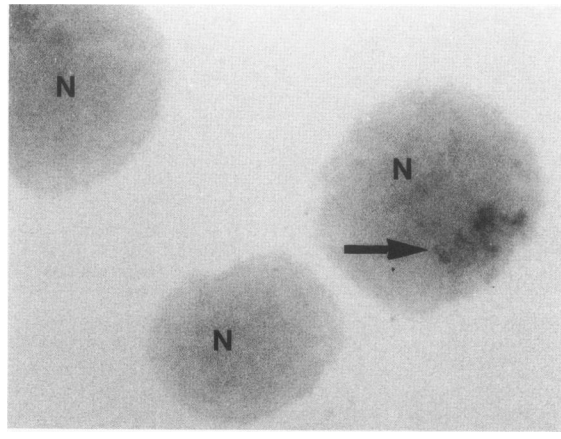

Figure 5. *Detection of the Ki-1/57 antigen within isolated nuclei of Hodgkin's disease-derived L540 cells. Cytospin preparations of the nuclei were reacted with the Ki-1 antibody followed by the reaction with peroxidase-conjugated rabbit anti-mouse immunoglobulin. The enzyme reaction showed the Ki-1/57 antigen (arrow) to be restricted to a certain section of the nuclei (×1000).*

ings may 1) indicate the way of transport of the Ki-1/57 antigen into the nucleolus or 2) demonstrate an indirect functional association with the nucleolus. In both respects, the function of the Ki-1/57 antigen might be connected to the nuclear matrix.

The nuclear localization of the Ki-1/57 antigen was an unexpected and hence a questionable result. In addition to the fact that this result could be confirmed by light microscopy and immunoprecipitations (Figures 5 through 7; see below), however, the following points argued for a Ki-1–specific phenomenon: 1) The nuclear labeling depended entirely on the primary antibody Ki-1, whereas the secondary gold-coupled SpA alone never produced such or even similar pictures. 2) The isotype-matched control antibodies were also entirely negative. 3) The expression of the Ki-1 antigen was also studied in mitogen-stimulated peripheral lymphocytes.[25] The Ki-1/57–specific labelings in the different positions of the nucleus and the nucleolus also were observed in phytohemagglutinin-*stimulated* lymphocytes. In contrast, freshly prepared *unstimulated* lymphocytes were negative for all kinds of Ki-1–specific labeling *despite* the application of the Ki-1 antibody (Hansen et al, in preparation). The latter fact we regard as an additional persuasive argument *for* the specificity of the nuclear accumulations of gold particles for the Ki-1/57 antigen and *against* the suspicion of an artifact.

Moreover the results of the electronmicroscopic studies were confirmed in two other ways: 1) When isolated nuclei were stained for the Ki-1 antigen, about half of them showed a spotted positive reaction (Figure 5). These Ki-1–reactive spots were not evenly distributed, occurring only in a certain sector of the nuclei. Sometimes the Ki-1–reactive spots were found in high concentrations. During the microscopic examination, it appeared as though the Ki-1–reactive spots formed an interconnected structure similar to the elongated Ki-1–specific labelings mentioned above. 2) When L540 or U266/B1 cells were separated into nuclei and non-nuclei fractions, the Ki-1/57 antigen could almost exclusively be isolated from the nuclei together with a questionable 120-kd band (Figure 6). The non-nuclei fraction contained the 90-, 105-, and 120-kd Ki-1 molecules, whereas a 57-kd band could not be detected. This almost complete absence of the Ki-1/57 molecule in the non-nuclei fraction after a 1-hour chase period could be confirmed by an additional pulse-chase experiment (Figure 7). After a 10-minute pulse with ^{35}S-methionine, the 57-kd antigen could be isolated from the cytoplasmic (lane 1), but not from the nuclear fraction of L540 cells (lane 4). After chase periods of 1 or 3 hours, however, the radioactively labeled Ki-1/57 molecules had been transferred to the nucleus. Hence 57-kd bands could be immunoprecipitated from the nuclear (Figure 7, lanes 5 and 6), but not from the cytoplasmic fraction (lanes 2 and 3).

When the Ki-1–reactive molecules were immunopre-

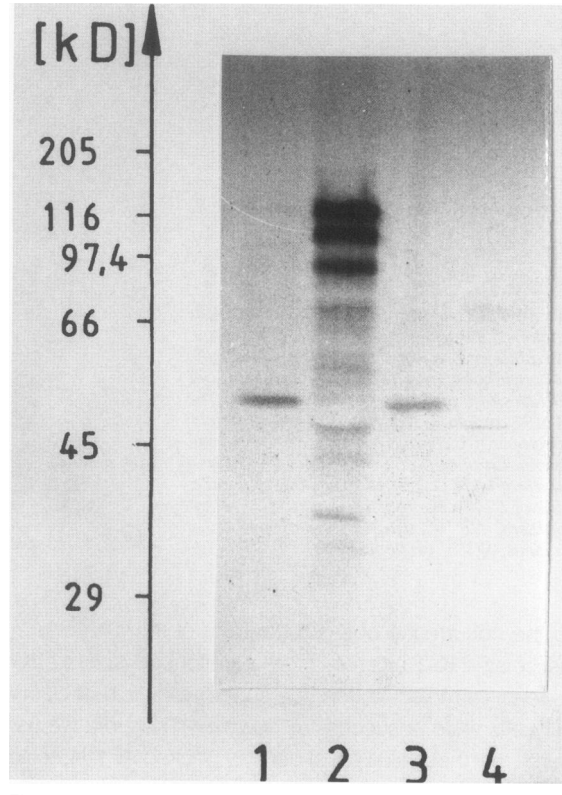

Figure 6. *Isolation of the Ki-1 antigen from nuclear (lanes 1 and 3) and non-nuclear fractions (lanes 2 and 4) of Hodgkin-analogous L540 (lanes 1 and 2) and U266/B1 myeloma cells (lanes 3 and 4). The cells were labeled with ^{35}S-methionine for a short pulse. After an additional chase period of 1 h, the Ki-1 antigen was isolated from the nuclear and the non-nuclear fractions, separated by SDS-PAGE (7.5–15% gradient gels) under reducing conditions and analyzed by autoradiography. The molecular mass markers were: β-galactosidase, 116 kDa; phosphorylase b, 97.4 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa and carbonic anhydrase, 29 kDa.*

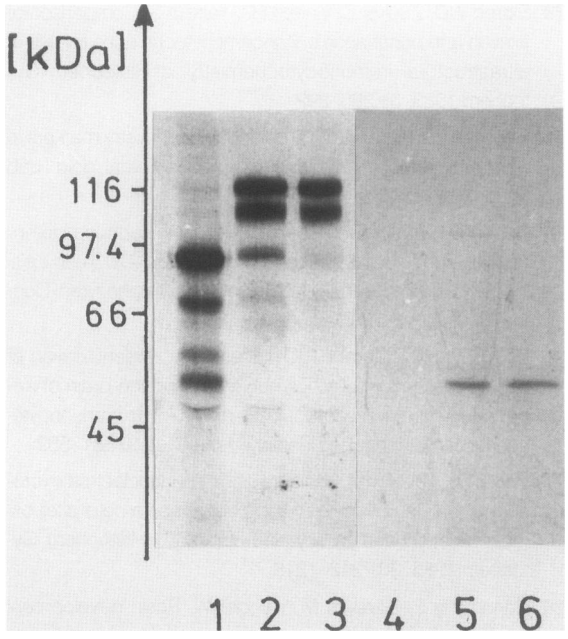

Figure 7. *Isolation of Ki-1-reactive antigens from nuclear (lanes 4–6) and non-nuclear fractions (lanes 1–3) of Hodgkin-analogous L540 cells during a pulse-chase experiment. L540 cells were labeled with ^{35}S-methionine for a short pulse of 10 min and/or cultured for different periods of chase time. The Ki-1-reactive molecules were immunoprecipitated immediately after the pulse (lanes 1 and 4) or after chase periods of 1 hour (lanes 2 and 5) and 3 hours (lanes 3 and 6). After separation by SDS–PAGE (7.5–15% gradient gels) under reducing conditions, the radioactively labeled molecules were visualized by autoradiography. The molecular mass markers were as in Figure 6.*

cipitated after separation of L540 cells into nuclear and non-nuclear fractions, two additional molecules were isolated with relative molecular masses between the Ki-1/57 and the 90-kd precursor of the CD30. Those additional two bands were especially prominent after the pulse (Figure 7, lane 1) and disappeared during the chase period. Because these molecules could only be isolated after separation of the cells into nuclear and cytoplasmic fractions, we regard them as artifacts due to those special experimental conditions. They may represent Ki-1–reactive degradation products of the 84-kd apoprotein precursor of the 120-kd CD30 antigen.[7,8]

Taken together, these data demonstrate that the Ki-1/57 antigen is transported to various compartments of the nucleus and the nucleolus. We therefore assume that the function of the Ki-1/57 is directly or indirectly associated with nucleolar functions.

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