Antibody-Induced Modulation and Intracellular Transport of CD10 and CD19 Antigens in Human B-Cell Lines: An Immunofluorescence and Immunoelectron Microscopy Study

By Stanislaw Pulczynski, Anne Marie Boesen, and Olaf Myhre Jensen

Antibody-induced antigenic modulation (AIAM) of CD10 and CD19 was studied on NALM-6, RAJI, and JOK-1 cell lines using fluorescence microscopy (FM), flow cytometry (FCM), and immunoelectron microscopy (IEM). Cross-linking with monoclonal antibodies (MoAbs) induced rapid redistribution of CD10 and CD19 on the cell surface (FM) followed by internalization involving uptake through plasmalemmal pits, transfer through endosomal compartment (receptor-mediated endocytosis), and, finally, delivery to lysosomes for degradation or exocytosis and recycling (IEM). Significant quantitative differences regarding modulation and intracellular processing were shown by FCM and IEM. Thus, 35%, 30%, and 25% of CD10 compared with 80%, 60%, and 40% of CD19 were internalized in NALM-6, RAJI, and JOK-1 cells, respectively. Also, the rate of intracellular transfer as well as externalization and recycling was more pronounced in the case of CD10 than of CD19 and in the NALM-6 and RAJI cells compared with the JOK-1 cells. These differences may possibly reflect the functional significance of CD10 and CD19 as well as the stage of differentiation of the malignant B cells. Although both antigens can be useful in MoAb-targeted immunotherapy, our findings suggest that anti-CD19 MoAbs would be preferable for delivery of cytotoxic agents to malignant B cells.

MATERIALS AND METHODS

Cells. The human malignant cell lines of B lineage NALM-6, RAJI, and JOK-1 were used. The cells were kept in exponential growth phase at 37°C in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), glutamine, HEPES, and antibiotics in a humidified 5% CO2 atmosphere. Before use, the cells were removed from culture and washed twice in Hank’s Balanced Salt Solution (HBSS) containing 1% bovine serum albumin (BSA) and 2% heat-inactivated human AB serum (HBSS-BSA-AB).

Immunoreagents. Murine MoAbs J5 (anti-CD10/CALLA) and B4 (anti-CD19) were used for indirect IF and IEM. Reactivity of MoAbs with cells used in this study was determined by flow cytometry (FCM). The values of mean fluorescence intensity (arbitrary units) were: 152, 65, and 42 in the case of J5, and 92, 87, and 84 in the case of B4 in NALM-6, RAJI, and JOK-1 cells, respectively. The second Abs were fluorescein isothiocyanate (FITC)-conjugated rabbit antimouse IgG [RAM F(ab')2-FITC] and rabbit antimouse IgG coupled to protein A.

From the Department of Medicine and Hematology, University of Aarhus, Aarhus, Denmark.

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Address reprint requests to Stanislaw Pulczynski, MD, University Department of Pathology, Aarhus Amtssygehus, Aarhus, Denmark.

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1549
Experiments, the cells were labeled with J5 or B4 MoAb followed by incubation in culture medium at 37°C. At fixed time points varying from 30 minutes up to 24 hours, aliquots of 0.5 x 10⁶ cells were removed from the culture, washed, incubated with 50 µL RAM F(ab′)₂-FITC or with anti-CD10 and anti-CD19 MoAbs directly conjugated with FITC or phycoerythrin (PE) were used. J5 and B4 MoAbs were a gift from Dr J. Ritz and Dr L.M. Nadler (Dana-Farber Cancer Institute, Boston, MA), respectively. All other immunoreagents were purchased from Dakopatts A/S (Copenhagen, Denmark).

**IF and FC analysis.** Modulation of CD10 and CD19 was evaluated by measuring Ag-MoAb complexes remaining on the cells at given time intervals. The cells were chilled on ice and processed as described previously in detail. Two kinds of experiments were performed. (1) To measure the clearance of immunocomplexes from the cell surface, the cells were incubated with appropriately diluted J5 or B4 MoAbs at 4°C, washed twice in HBSS-BSA-AB, and incubated in culture medium at 37°C. At fixed time points varying from 30 minutes up to 24 hours, aliquots of 0.5 x 10⁶ cells were removed from the culture, washed, incubated with 50 µL RAM F(ab′)₂-FITC for 30 minutes at 4°C, washed again, fixed in buffered 1.5% paraformaldehyde solution, and stored at 4°C. (2) In parallel experiments, the cells were labeled with J5 or B4 MoAb followed by RAM F(ab′)₂-FITC or with anti-CD10 and anti-CD19 MoAb directly conjugated with FITC or PE, washed, cultured at 37°C, washed again, and fixed. Subsequent FC analysis, simultaneously measuring the amount of surface-bound and intracellularly located fluorescence at given time points, made it possible to determine the clearance of immunocomplexes from the cells due to externalization.

For each sample, 10,000 cells were analyzed by FCM on an EPICS Profile flow cytometer (Coulter Electronics, Luton, UK) to determine the percentage of positive cells and to calculate the mean fluorescence intensity (MFI) over 256 channels in a linear scale. Subtraction of background fluorescence was performed channel by channel using Cytologic software (Coulter Electronics). The degree of modulation (M) was calculated for each sample as the percentage of MFI in relation to unmodulated cells according to the equation:

\[ M(\%) = 100 - \left( \frac{\text{MFI (sample)} - \text{MFI (negative control)}}{\text{MFI (unmodulated cells)} - \text{MFI (negative control)}} \right) \]

For fluorescence microscopy (FM), the viable cells, labeled by direct or indirect method and cultured as described above, were removed from cultures at the above time points and viewed in a Leica Diaplan fluorescence microscope (Wetzlar, Germany).

**Immunogold labeling and processing for EM.** The chilled cells were washed, stained with saturating dilutions of MoAbs, washed, stained with RAM IgG G12, washed again, and incubated in culture medium for time intervals as mentioned above. After another wash, the cells were processed for EM as previously described. Briefly, they were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide, pretained with 2% uranyl acetate, and processed through warm agar. The resulting pellet was cut into 1-mm blocks, dehydrated in a graded series of ethanol, and embedded in epon. Ultrathin sections were cut on an LKB ultramicrotome III (LKB Biotechnologen AB, Bromma, Sweden) picked up on Formvar-coated copper grids, and stained in uranyl-acetate and lead citrate. The sections were viewed and photographed with a Philips EM 201 (Eindhoven, The Netherlands) operating at 60 kV.

**Quantitation of colloidal gold distribution.** Counting of gold particles was performed on micrographs at a final magnification of x20,160 using a x4 stand magnifier. In each sample, 30 to 50 equatorial cell sections were analyzed by counting gold particles bound to the cell surface and those located intracellularly. To evaluate the intracellular distribution of gold tracer in different intracellular compartments, separate quantitations were performed for cytoplasmic vesicles, multivesicular bodies, and lysosomes. The average number per cell section was calculated. Finally, the degree of internalization and intracellular distribution of tracer at given time intervals were expressed as a percentage of the total number of gold particles at the starting point of each experiment.

The general stereological requirements on establishing an estimate of the amount of gold particles on three-dimensional cells from counting the number on two-dimensional sections were fulfilled by means of the sampling procedure used in this study: homogeneous cell suspensions, uniform cell distribution, random orientation of the cells in agar, random selection of blocks obtained by cutting cell pellets, as well as random selection of ultrathin sections and cell profiles. The reproducibility and reliability of the quantitation method has been proven in a previous study.

**Controls.** Experiments using samples in which MoAbs were omitted or replaced by ascitic fluid from nonspecific secreting murine hybridoma 8A7 (kindly provided by Dr J.D. Griffin, Dana-Farber Cancer Institute, Boston, MA) were performed simultaneously with experiments to determine nonspecific binding. Neither IF or IEM showed any significant background staining. In experiments performed as positive controls, the modulation of both Ags was completely prevented by (1) fixation of cells in 1.5% solution of paraformaldehyde before immunolabeling; (2) addition of 0.2% sodium azide to the immunoreagents, washing buffers, and culture medium; and (3) incubation of labeled cells in culture medium at 0°C.

**Statistical analysis.** The means and standard deviations (SD) of results from several independent experiments were calculated. Data were compared using the correlation coefficient (r) and the Student's t-test.

**RESULTS.**

**FM.** Aliquots of viable cells stained by either direct or indirect IF were viewed by FM after incubation at 37°C. Surface redistribution, internalization, and exocytosis could be readily evaluated, showing differences depending on the cell line, but not on the IF technique used (Fig 1). This evaluation was qualitative rather than quantitative due to the rapid course of observed events and the fading of intracellular fluorescent grains; nevertheless, it yielded valuable information regarding the distribution of immunocomplexes. Thus, the cells initially showed a homogenous ring-like distribution of surface fluorescence, quickly changing into a patchy pattern (Fig 1a). Migration of fluorescence patches to one of the cell poles frequently resulted in cap formation, and uptake of some patches into the cells could be seen in all experiments (Fig 1b). Detachment of fluorescent grains directly from the cell surface as well as expulsion of intracellular fluorescent grains was frequently seen in NALM-6 and RAJI cells, but rarely in JOK-1 cells.

Two distinct patterns of surface redistribution and internalization were found, depending on cells involved, but independent of the MoAb used (Fig 1a and b). Thus, caps on NALM-6 cells were usually more widespread, occupying at least half of the cell surface and containing coarse patches of fluorescence. The same cells often showed rapid uptake of coarse fluorescent grains, which were quickly moving to the perinuclear area. In comparison, the caps observed on the RAJI and JOK-1 cells were smaller, showed a fine-grained structure, and internalized grains were mostly located within the cap area.
MODULATION OF CD10 AND CD19

Fig 1. Redistribution and internalization of CD10 and CD19 Ags after binding of specific MoAbs viewed by FM. (a) Uniform surface fluorescence at time 0. (b) Capping and intracellular localization of IF after 1 hour. Note the differences between cell lines. (c) Persistent capping in RAJ1 cells compared with patchy surface fluorescence in NALM-6 and JOK-1 cells after 24 hours of incubation at 37°C. Non-fluorescent cells were common, but intracellular IF could occasionally be seen. Fluorescence intensity in JOK-1/B4+RAM F(ab')2-FITC cells was not high enough to allow good photographic documentation.

At the later time points (6 to 24 hours), about 70% of RAJ1 cells were still fluorescent, showing capping and, occasionally, intracellular grains within the cap area, whereas the majority of NALM-6 cells had lost their fluorescence (Fig 1c). The few NALM-6 cells still positive showed no caps, but only 1 to 5 very coarse grains on the surface and occasionally single intracellular grains. The fluorescence pattern of JOK-1 cells at the end of the experiments was similar to that of NALM-6 cells.

The morphologic features of the above described events were very similar for both Ags, although CD19 seemed to be more avidly internalized and more frequently externalized than CD10.

FCM. Measurements of fluorescence intensity by FCM clearly showed that expression of CD10 and CD19 Ags was rapidly modulated in both types of experiments performed (Fig 2). First, cells were labeled with J5 or B4 MoAbs, incubated at 37°C, and subsequently stained with RAM F(ab')2-FITC to measure the amount of surface-bound Ag-MoAb complexes (reflecting noninternalized as well as recycled immunocomplexes). A rapid decrease in fluorescence intensity occurred during the first 2 to 3 hours of experiments (Fig 2, curves a). A longer incubation period caused only slight or no further reduction in fluorescence. Moreover, clearance of Ag-MoAb complexes was much more pronounced with CD10 than with CD19, especially in NALM-6 and RAJ1 cells (CD10, 85% and 71%; CD19, 60% and 47% after 24 hours, respectively). In contrast, only 33% to 40% of immunocomplexes were cleared from the surface in JOK-1 cells.

Second, experiments were performed in which cells were labeled with J5 or B4 and RAM F(ab')2-FITC before incubation at 37°C and analyzed by FCM at subsequent time intervals. This approach made it possible to determine both intracellular and surface-bound fluorescence at the same time, expressing the loss of immunocomplexes due to externalization (Fig 2, curves b). A 40% to 50% decrease in fluorescence intensity was observed in NALM-6 and RAJ1 cells with regard to both Ags, although CD19 was externalized faster than CD10. In contrast, little externalization was found in JOK-1 cells, not exceeding 8%. The possibility that only second Ab underwent externalization was ruled out, because the parallel experiments using direct technique with FITC- or PE-conjugated MoAbs yielded similar results.

Ultrastructural morphology. Cells fixed and processed for EM immediately after immunolabeling consequently showed a dispersed, circumferential pattern of labeling with single gold particles and small clusters attached to the plasma membrane as well as formation of plasmalemmal pits (Fig 3A and B). After 30 to 60 minutes of incubation at 37°C, pronounced clustering was observed, and gold particles were commonly found in plasmalemmal pits and within vesicles and tubular structures, localized in the peripheral cytoplasm (Fig 3C). Membrane-coating of pits and vesicles as well as uptake of intensively gold-labeled cell membranes by means of macropinocytosis, as seen in a previous study, were occasionally observed.

After 30 minutes to 3 hours of incubation at 37°C, gold-labeled uncoated vesicles were observed in the perinuclear region of the cytoplasm. They were often larger than peripheral vesicles, electron lucent, sometimes containing sparse, moderately dense material (Fig 3D), resembling the endosomes or receptosomes described previously. Accumulation of gold particles in endosomes was followed by the presence of tracer in multivesicular bodies after 1 to 3 hours (Fig 3E). Labeling of multivesicular bodies was most prominent in RAJ1/CD19 and NALM-6/CD19 experiments. At later time intervals, the number of gold particles located in vesicles and multivesicular bodies gradually decreased. Increasing accumulation of colloidal gold was observed in lysosomes after 2 hours of incubation and later on (Fig 3F). Transitional forms between multivesicular bodies and lysosomes were commonly seen after 2 to 3 hours of incubation.
Fig 2. Modulation of CD10 and CD19 Ags on B-cell lines analyzed by FCM. (a) The amount of Ag-MoAb complexes measured on the cell surface in cells labeled with J5 or B4 MoAbs, incubated in culture medium at 37°C. and then labeled with RAM F(ab')2-FITC. Note the rapid modulation in all experiments, pronounced clearance of CD10-J5 complexes as compared with CD19-B4 in respective cell lines, and differences between cell lines. The results reflect both unmodulated and recycled complexes at the given time points. (b) The amount of Ag-MoAb complexes representing simultaneous measurements of surface-bound and internalized Ag-MoAb complexes at the given time intervals in cells labeled with J5 or B4 MoAbs and RAM F(ab')2-FITC, or directly conjugated MoAbs, before incubation at 37°C. The decrease in fluorescence intensity, which reflects externalization, is more rapid in the case of CD19 than in the case of CD10 in NALM-6 and RAJI cells. Note the almost complete lack of externalization in JOK-1 cells. The time of incubation and MFI expressed as a percentage in relation to unmodulated cells are indicated on the x and y axes, respectively. Data shown represent mean values of at least five experiments. The SD at each time point did not exceed 10%.

Fusions between cytoplasmic vesicles and between vesicles and multivesicular bodies (but not with lysosomes) occurred in all cases. Gold particles were never found within the cisternes of Golgi apparatus. Fusions of labeled cytoplasmic vesicles and multivesicular bodies with the plasmalemma (resulting in externalization of immunocomplexes) were commonly seen in NALM-6 and RAJI cells modulating CD19, but less commonly during modulation of CD10. JOK-1 cells showed few signs of externalization. Although labeling of cell surface was decreased over time, colloidal gold could still be observed on the cell surface as late as 24 hours after the start of incubation, indicating an incomplete internalization of CD10 and CD19 Ags.

A close relation of the gold tracer to the cell surface and vesical membranes was usually observed (Fig 3A and D). In these locations, a distinct halo of moderately electron dense material was seen around each gold particle, reflecting the presence of a protein coat. In contrast, dense aggregates of gold particles were seen in lysosomes (Fig 3F), indicating that degradation of the protein coat and subsequent flocculation of the gold colloid had taken place. A minor degree of flocculation could occasionally be seen in endosomes and was a common finding in multivesicular bodies (Fig 3E).

Quantitative evaluation of gold marker distribution. Quantitative data were obtained by counting gold particles, thus enabling an evaluation of the extent and rate of internalization and of the subsequent intracellular transfer of immunocomplexes through cytoplasmic vesicles and multivesicular bodies into lysosomes. In all cases, a rapid decrease in cell surface labeling occurred during the first 1 to 3 hours of incubation, followed by a slight further reduction or a plateau (Fig 4, curves a). Generally, CD19 was cleared from the cell surface to a higher extent than CD10 (about 75% to 95% compared with 50% to 75% after 24 hours). Also, internal-
Fig 3. Internalization of CD19 Ag in NALM-6 cells. The cells were labeled with B4 MoAb + RAM IgG G12 before incubation in culture medium at 37°C. (A) Labeling of plasma membrane at time 0. Uptake of gold particles through plasmalemmal pits (B) at time 0 and (C) after 1 hour. (D) Gold-labeled endosome after 1 hour. Accumulation of gold particles within multivesicular bodies (E) after 3 hours and in lysosomes (F) after 6 hours. Note the coating of plasmalemmal pits (B and C), close relation of gold particles to endosomal membrane (D), dispersed labeling and some aggregation of tracer in multivesicular bodies (E), and pronounced flocculation of colloidal gold in lysosomes (F). (Original magnification ×73,000 [A], ×87,000 [B through F]).

Internalization of CD19 was more pronounced than of CD10 in NALM-6, RAJI, and JOK-1 cells (approximately 80%, 60%, and 40% and 35%, 30%, and 25%, respectively) (Fig 4, curves b). In addition, both Ags were internalized most rapidly in NALM-6 cells and slowest in JOK-1 cells. At later time points, a 50% to 70% decrease of intracellular labeling was observed in experiments involving NALM-6/CD19 and RAJI/CD19, indicating pronounced exocytosis.
A highly significant correlation between IEM data and the corresponding FCM data regarding clearance of immune complexes from the cell surface was found in experiments involving modulation of CD10 (r\text{NALM-6} = .96; r\text{RAJI} = .98; r\text{JOK-1} = .94), and less convincing concerning CD19 (r\text{NALM-6} = .76; r\text{RAJI} = .66; r\text{JOK-1} = .94).

The distribution of internalized gold tracer within intracellular compartments at different time points is shown in Fig. 5. Transient accumulation in cytoplasmic vesicles and multivesicular bodies was observed, followed by final accumulation in lysosomes. In RAJI and NALM-6 cells, transfer of CD19 complexes through endosomal compartment resulted in maximal labeling of lysosomes within 3 hours, whereas corresponding lysosomal accumulation of CD10 complexes required 6 hours. Likewise, lysosomal accumulation of gold tracer in JOK-1 cells took place significantly faster in the case of CD19 than of CD10, although these cells were generally characterized by a low rate of internalization, lack of exocytosis, and a slow intracellular traffic of internalized materials.

**DISCUSSION**

Although previous studies have dealt with modulation of CD10\textsuperscript{13,14} and CD19\textsuperscript{15,16} antigens, much remains to be known about this process in B cells at different stages of differentiation. In the present study we have shown: (1) that CD10 and CD19 Ags in malignant B-cell lines are invariably internalized through the pathway of receptor-mediated endocytosis, and (2) that significant differences exist regarding kinetics of internalization and intracellular processing between these Ags and between NALM-6, RAJI, and JOK-1 cell lines.

The various approaches applied to investigate the AM of CD10 and CD19 have complemented each other. Thus, the observation of viable cells by FM provided direct evidence of redistribution, internalization, and exocytosis of the Ags. Different redistribution patterns, which were shown to depend only on the cells studied, may possibly have practical implications in immunotherapy, because it is known that redistribution of immunocomplexes alone can diminish efficacy of complement-mediated lysis of Ab-coated B cells.\textsuperscript{6}

The IEM approach visualized the pathway of internalization at the ultrastructural level, enabling an evaluation of the intracellular fate of CD10 and CD19 Ags. The demonstration of gold tracer in close association with cell surface and vesicle membranes ascertained that this localization of tracer really reflected the binding of MoAbs to respective Ags. Flocculation of gold tracer, which seemed to be initiated in the endosomes, indicated that these structures determine the further fate of the internalized material, as previously suggested.\textsuperscript{14} Although the flocculation indirectly proves the ongoing destruction of secondary Abs, it by no way excludes the possibility that Ag-MoAb complexes are to some extent recycled to the cell sur-
MODULATION OF CD10 AND CD19

Fig 5. Distribution of gold particles within subcellular compartments during internalization of CD10 and CD19 Ags. Labeling of the cells and the axes is as in Fig 4. Data indicate transient accumulation of the gold tracer in cytoplasmic vesicles and multivesicular bodies followed by final accumulation in lysosomes. SD around the highest values did not exceed 15%. An increase to 50% around the lowest values could be seen. Note that transfer of gold tracer to lysosomes in respective cell lines is significantly faster in case of CD19 than of CD10.

Vesicles; (a) multivesicular bodies; (c) lysosomes.

The other ultrastructural features described are fully consistent with previous EM studies concerning internalization of CD10 Ags, as well as with the observations obtained in other systems of receptor-mediated endocytosis. Thus, there is no doubt that both CD10 and CD19 Ags use the same common pathway of receptor-mediated endocytosis.

The interpretation of the quantitative results appeared more complex. The data obtained by IEM indicated that all cell lines internalized CD19 to a higher degree than CD10, and that the extent of internalization of the respective Ags was as follows: NALM-6 > RAJI > JOK-1. Importantly, no correlation was found between the initial Ag density and the extent of internalization. On the other hand, the rate of internalization appeared to depend mainly on the cell line involved, because uptake of CD10 and CD19 Ags was much more rapid in NALM-6 and RAJI than in JOK-1 cells. These observations suggest that the kinetics of AIM in malignant B cells may be related to the stage of differentiation, as both extent and rate of internalization seem to decrease with increasing maturity of cells. However, this hypothesis requires further investigations with fresh cell material of B-cell origin.

The results obtained from the FCM investigations, in turn, showed discrepancies when compared with the respective IEM data. Thus, FCM measurements indicated a lesser surface clearance and a lower degree of internalization of CD19 complexes in NALM-6 and RAJI cells, whereas data concerning CD10 correlated well to each other. These discrepancies might be explained as follows. (1) The two approaches measured different parameters. The pre-embedding IEM enabled separate quantitation of Ags present on the cell surface and those located intracellularly at given time points, but not the intracellular events taking place after uncoupling of secondary Ab. On the other hand, FCM did not enable direct measurements of intracellular fluorescence. Moreover, measurements of surface fluorescence by FCM did not distinguish between the complexes that had not undergone internalization and those that had been recycled. In fact, the data shown (Fig 2, curves a) reflect the dynamic equilibrium between at least two biologic phenomena, ie, internalization and recycling. Add to this that possible shedding of Ags from the cell surface also might influence the results, although, to our knowledge, no report has been published concerning the shedding of CD10 and CD19 Ags in cells of B lineage. Therefore, a simple subtraction of the respective FCM data shown in Fig 2 (values on the curves b minus values shown on the curves a) cannot be used for the estimation of intracellular accumulation of internalized materials, unless one is sure that the Ag studied does not recycle at all. (2) Considerable differences exist between CD10 and CD19 with regard to intracellular processing of internalized immunocomplexes. Although uncoupling and recycling of CD10-J5 complexes...
in NALM-6 cells was suggested previously,\textsuperscript{15} the present results indicate that CD19 complexes underwent an even more rapid endosomal dissociation of secondary Ab (which subsequently could easily be traced in lysosomes, where it was destructed) followed by a more pronounced recycling of CD19-B4 complexes to the cell surface. Such a hypothesis is further supported by the fact that the transfer through intracellular compartments (Fig 5) as well as externalization (Fig 2, curves b) of CD19 were more rapid than of CD10.

Previous studies on AIM of B-cell Ags have also shown discrepancies between comparable data obtained by different methods. Press et al.,\textsuperscript{16} using a two-step immunoperoxidase staining for IEM combined with \textsuperscript{131}I-labeled MoAbs to study AIM of seven Ags on Daudi cells, generally found a more rapid clearance of Ags from the cell surface when assessed by measurements of surface-bound radioactivity as compared with IEM (eg, approximately 50% and 95% of CD19 was detected on the cell surface after 0.5 to 1 hour, respectively). On the other hand, the extent of internalization was much higher when assessed by IEM than by measurements of intracellular radioactivity. Thus, in the case of CD19, IEM demonstrated intracellular labeling increasing during the time (approximately 45% after 0.5 hours and 95% after 18 hours), indicating that no externalization occurred, whereas measurement of intracellular radioactivity showed only a minor degree of internalization with a maximum of approximately 15% after 1 hour, followed by decrease to 0% after 24 hours.

Differences in the kinetics of antibody-induced antigenic modulation between CD10 and CD19 might possibly reflect the different functions of these molecules, although this issue could not be addressed in the present study. CD10, which has been found identical with endopeptidase-24,11,12 is still a very useful diagnostic tool and an attractive target for immunotherapy, despite its wide expression on a variety of normal and neoplastic cells. CD19 has been shown to be a member of the Ig supergene family,\textsuperscript{23} which is involved in transmembrane signalling. Although its natural ligand is not known and the intracellular events involved are only partly understood,

\textsuperscript{24,25} CD19 is a promising target for MoAb therapy because (1) specific cytotoxicity of anti-CD19 immunotoxins to malignant B cells has been reported,\textsuperscript{11,16} (2) CD19 is B-lineage restricted and widely expressed in B-cell malignancies,\textsuperscript{4} and (3) anti-CD19 immunotoxins are not lethal to normal hematopoietic progenitor cells.\textsuperscript{11} Successful in vitro purging of bone marrow in a tumor cell line model and in patients with B-cell lymphoma was recently reported by Gribben et al.,\textsuperscript{27} who used J5 alone or in combination with other anti-B-cell MoAbs in the presence of complement. Promising results were also obtained in patients with CNS-ALL after treatment with a cocktail of radioimmunoconjugates targeting CD10 and CD19 Ags.\textsuperscript{28} Although both CD10 and CD19 can thus be used in immunotherapy, our results indicate that targeting of CD19 with immunotoxins and radioimmunoconjugates might prove to be more effective in comparison with CD10 because of a more pronounced internalization and a more rapid intracellular processing. On the other hand, immunotoxins directed against myeloid tumor-associated Ags were recently shown to lack cytotoxicity despite a high extent of internalization in target cells, presumably due to rapid intracellular degradation.\textsuperscript{12} These observations indicate that a high Ag expression and a high extent of internalization are not the only factors responsible for susceptibility of target cells to MoAb therapy.\textsuperscript{11,12}

In conclusion, the immunogold technique used for IEM was extremely useful for the delineation of the intracellular transport and distribution of CD10 and CD19 in B-cell lines. Additional information about the fate of the internalized immunocomplexes after destruction of the protein coat on the gold particles with regard to uncoupling, recycling, and externalization was provided by combining IEM and FCM data. Further studies using separate visualization of Ags and MoAbs on cell sections are needed to elucidate the mentioned steps in the intracellular processing of CD10 and CD19 Ags.

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S Pulczynski, AM Boesen and OM Jensen