The MAL proteolipid is a component of the detergent-insoluble membrane subdomains of human T-lymphocytes

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The human *mal* gene, identified during a search for cDNAs selectively expressed during T-cell development, encodes a highly hydrophobic protein belonging to a group of proteins, termed proteolipids, characterized by their unusual property of being soluble in organic solvents used to extract cell lipids. To study the localization of the MAL protein we have prepared stable transfectants expressing the MAL protein tagged with a c-*myc* epitope (MAL/c-*myc*) using human epithelial A-498 cells. Immunofluorescence analysis suggested that MAL/c-*myc* is localized mainly to cholesterol-enriched structures with a post-Golgi location and, at low levels, in early endosomes. Moreover, extraction of A-498 cell membranes with Triton X-100 (TX100) and fractionation by centrifugation to equilibrium in sucrose gradients demostrated the presence of MAL/c-*myc* in the detergent-insoluble buoyant fraction, known to be enriched in glycolipids and cholesterol. To compare the behaviour of MAL in T-cells with that in epithelial A-498 cells, we prepared stably transfected cells expressing MAL/c-*myc* using human Jurkat T-cells. When TX100 extracts from Jurkat cells were subjected to centrifugation to equilibrium in sucrose gradients we found MAL exclusively in the floating fractions, together with molecules characteristic of the T-cell insoluble complexes, such as the tyrosine kinase p56*lck*, the glycosylphosphatidylinositol-anchored protein CD59 and the ganglioside GM1. These results, taken together, indicate that the MAL proteolipid is a component of the detergent-resistant membrane microdomains present in T-lymphocytes, and suggest that MAL might play a role in modulating the function of these microdomains during T-cell differentiation.

INTRODUCTION

Glycosylphosphatidylinositol (GPI)-anchored proteins exhibit tightly confined diffusion because they are sequestered in glycolipid-enriched regions [1]. The presence of a high content of both glycolipids and cholesterol makes these microdomains insoluble in non-ionic detergents such as Triton X-100 (TX100) [2]. This property has allowed the purification of large insoluble complexes using either centrifugation to equilibrium in sucrose gradients [3] or gel-filtration chomatography [4]. In epithelial Madin Darby canine kidney (MDCK) cells, insoluble complexes have been found at both the *trans*-Golgi network (TGN) and the plasma membrane. It has been proposed that the complexes at the TGN are involved in the transport of GPI-anchored proteins and glycolipids to the apical surface [5], which is highly enriched in these molecules. In T-cells, the existence of detergent-insoluble membrane microdomains at the cell surface is well documented [6]. As in epithelial cells, these complexes contain glycolipid and GPI-anchored proteins [4], and they are also highly enriched in the tyrosine kinase p56*lck* [4]. Myelin lipid composition is remarkably similar to that of detergent-insoluble complexes isolated from epithelial cells [3,7]. Schwann cells and oligodendrocytes polarize their surfaces into different membrane domains [8,9], and as a final result of this process myelin is formed. The similarities between the process of myelin formation and that of apical transport in MDCK cells suggest that the mechanisms operating in these processes are likely to be related.

The human MAL cDNA was cloned from a subtracted T-cell cDNA library, and encodes a highly hydrophobic protein with no overall homology with any protein sequence in the GenBank [10]. The human *mal* gene was mapped to chromosome 2 [11] and contains four exons, each encoding a hydrophobic, presumably membrane-associated, segment and its adjacent hydrophilic sequence [12]. The similarity among the hydrophobicity profiles of MAL and other proteins belonging or related to the proteolipid group of proteins [13] led to the demonstration that MAL clearly belongs to this class of proteins [14]. MAL expression also takes place in both Schwann cells and oligodendrocytes [15,16], and MAL has recently been found in detergent-resistant extracts from rat oligodendrocytes [15]. To investigate the localization and function of MAL, we have expressed the MAL protein tagged with a c-*myc* epitope (MAL/c-*myc*) in epithelial cells and in T-lymphocytes, and we have found MAL in membrane microdomains resistant to detergent solubilization. Finally, in a search for cell lines positive for MAL expression we have detected MAL mRNA expression in epithelial MDCK cells and found MAL in detergent resistant complexes (J. Millán, R. Puertollano, F. Li and M. A. Alonso, unpublished work), in agreement with its recent identification in TGN-derived transport vesicles [17]. Thus, MAL might have a general role in the functioning of the glycolipid-enriched microdomains present in myelin-forming cells, T-lymphocytes and epithelial MDCK cells.

EXPERIMENTAL

Materials

Mouse 25H8 monoclonal antibody (mAb) (IgM) [18], mouse MEM-43 (anti-CD59, IgG2a) mAb [4], and rabbit polyclonal

Abbreviations used: ATCC, American Type Culture Collection; BFA, brefeldin A; GPI, glycosylphosphatidylinositol; mAb, monoclonal antibody; MDCK, Madin Darby canine kidney; TGN, *trans*-Golgi network; TX100, Triton X-100.

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antibodies to p56*lck* [19] were generously given by Dr. I. Sandoval (Centro de Biología Molecular, Madrid, Spain), Dr. V. Horesji (Academy of Sciences, Prague, Czech Republic), and Dr. A. Veillette (McGill University, Montreal, Canada) respectively. Rat anti-tubulin mAb YL 1/2 was from Amersham Ibérica, S. A. (Madrid, Spain) and rabbit polyclonal antibodies to transferrin were from Dako A/S (Glostrup, Denmark). The mouse hybridoma, producing mAb 9E10 (IgG1) against the human c-*myc* epitope EQKLISEED [20], was purchased from the American Type Culture Collection (ATCC). Brefeldin A (BFA), filipin, digitonin, octyl-glucoside, TX100, iron-saturated human transferrin, mouse anti γ-adaptin mAb (IgG2b), and horseradish peroxidase-coupled cholera toxin B subunit were from Sigma (St. Louis, MO, U.S.A.). Peroxidase-conjugated antibodies were from Pierce (Rockford, IL, U.S.A.). Fluorescein- and Texas Red-conjugated antibodies were from Southern Biotech (Birmingham, AL, U.S.A.).

Cell culture conditions, DNA constructions and transfection

The cells used were epithelial A-498 cells (ATCC HB44) from human kidney and human lymphoblastoid Jurkat T-cells (ATCC TIB152). Cells were grown on Petri dishes or glass coverslips, in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) fetal bovine serum (Gibco-BRL, Gaithersburg, MD, U.S.A.), penicillin (50 units/ml) and streptomycin (50 μg/ml), at 37 °C in an atmosphere of air/CO_2 (19:1).

The insertion of the 9E10 c-*myc* epitope between the first and the second amino acids of MAL was carried out by amplification of the λMA5 insert [10] by PCR using the oligonucleotide primers N (5′-GGG CCC AGA TCT CAT ATG GAG CAG AAG CTG ATC TCC GAG GAA GAC CTG GCC CCC GCA GCG GCG ACG-3′) and C (5′-CCC GGG AGA TCT TTA TTA TGA AGA CTT CCA TCT-3′) (Isogen Bioscience, Amsterdam, The Netherlands). These primers contain sequences which anneal to the 5′- and 3′-ends of the MAL coding sequences respectively. In addition, primer N contains sequences encoding the 9E10 c-*myc* epitope, and both primers N and C contain one *Bgl*II site at their 5′-end. After amplification under standard conditions [21], the product was digested with *Bgl*II and cloned into the *Bam*HI site of the pSRα expression vector [22]. The sequence of the insert was verified to eliminate the possibility of amplification errors.

Transfections were carried out by standard protocols using either the DEAE-dextran method (A-498) or by electroporation (Jurkat cells) [21]. Selection of stable transfectants was carried out by treatment with 0.5 mg/ml G-418 sulphate (Gibco-BRL, Gaithersburg, MD, U.S.A.) for at least 4 weeks after transfection. Drug-resistant cells were selected, and positive clones were maintained in drug-free medium.

Immunofluorescence microscopy

Epithelial A-498 cells grown on coverslips, or Jurkat cells pelleted on coverslips, were washed twice with PBS, fixed in 3 % (w/v) paraformaldehyde in PBS for 15 min, rinsed in PBS and treated with 10 mM glycine in PBS for 10 min to quench the aldehyde groups. The cells were then permeabilized with 0.2 % (v/v) TX100 in PBS, rinsed, and incubated with 3 % (w/v) BSA in PBS for 20 min. Coverlips were then incubated with the primary antibody diluted in PBS/3 % BSA for 1 h, rinsed several times with PBS/3 % BSA, and incubated for a further 1 h with the appropriate fluorescent secondary antibody diluted in PBS/3 % BSA. After extensive washing, the coverslips were mounted on slides containing Mowiol (Aldrich, Milwaukee, WI, U.S.A.). The coverslips were photographed on Kodak TMAX 400 film with a Zeiss Axioskop photomicroscope using a 63 × objective.

Primary antibodies included mouse mAb 9E10 and 25H8 used as culture supernatants, and rat mAb YL 1/2 to tubulin used at 1:100. Rabbit polyclonal antibodies to transferrin were used at 1:20000. Secondary antibodies included Texas Red-conjugated goat anti-mouse IgG1 antibodies used at 1:1000, fluorescein isothiocyanate-conjugated goat anti-mouse IgG or IgM, or IgG2β at 1:200, and fluorescein isothiocyanate-conjugated goat anti-rabbit or anti-rat IgG antibodies (absorbed against mouse IgG) used at 1:100. Controls to assess the specificity and the lack of cross-labelling included incubations with irrelevant primary mAb or omission of either of the primary antibodies.

Detergent extraction procedures

TX100-insoluble complexes were prepared essentially as described by Brown and Rose [3]. Cells grown to confluency in 100 mm dishes were rinsed with PBS and lysed for 20 min in 1 ml of TNE/TX100 buffer [25 mM Tris/HCl (pH 7.5)/150 mM NaCl/5 mM EDTA/1 % TX100] at 4 °C. The lysate was scraped from the dishes with a rubber policeman, the dishes were rinsed with 1 ml of TNE/TX100 at 4 °C, and the lysate was homogenized by passing the sample through a 22 gauge needle. The lysate was finally brought to 40 % sucrose in a final volume of 4 ml and placed at the bottom of an 8 ml 5 %–30 % linear sucrose gradient. Gradients were centrifuged for 18 h at 100000 ***g*** at 4 °C in a Beckman SW41 rotor. Fractions of 1 ml were harvested from the bottom of the tube and aliquots were subjected to immunoblot analysis. Density was determined by measuring the refractive index of the fractions.

Immunoblot analysis, surface labelling and immunoprecipitation

Samples were subjected to SDS/PAGE in 15 % acrylamide gels under reducing conditions and transferred to Immobilon-P membranes (Millipore, Bedford, MA, U.S.A.). After blocking with 0.05 % (v/v) Tween 20 in PBS (PBS/Tween 20), membranes were incubated with mAb 9E10 culture supernatant at a ratio of 1:2, or anti-*lck* antibodies at 1:5000. After several washings, the membranes were incubated for 1 h with goat anti-mouse or anti-rabbit IgG antibodies coupled to horseradish peroxidase diluted at 1:5000 in PBS/Tween 20, washed extensively, and developed using an enhanced chemiluminescence Western blotting kit (ECL; Amersham).

For surface labelling, Jurkat cell cultures resuspended in ice-cold PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS-C/M), were incubated with 0.5 mg/ml sulfo-NHS-biotin (Pierce) in PBS-C/M for 30 min at 4 °C. The solution was removed and remaining unreacted biotin was quenched by incubation with ice-cold serum-free culture medium. After cell fractionation by centrifugation to equilibrium, the pooled fractions were immunoprecipitated with the indicated antibodies bound to protein G–Sepharose. Immunoprecipitates were washed, fractionated by SDS/PAGE under reducing conditions, and blotted. Blots were incubated with 1 μg/ml horseradish peroxidase-coupled streptavidin (Pierce) and developed using an ECL Western blotting kit.

RESULTS

To investigate both the localization and function of the MAL protein, a construct was made by adding DNA sequences encoding the c-*myc* epitope 9E10 between the first and the second codons of the MAL coding sequences, and this chimaeric MAL/c-*myc* cDNA was placed under the control of the eukary-

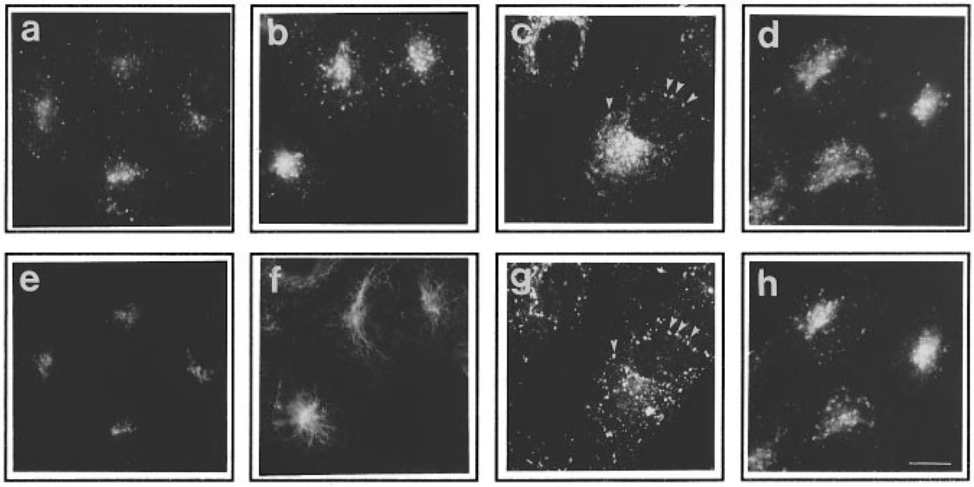

Figure 1 Localization of MAL/c-*myc* in epithelial A-498 transfected cells

Stably transfected A-498 cells were fixed with paraformaldehyde, permeabilized with 0.2% TX100 and subjected to indirect immunofluorescence analysis with anti-c-*myc* 9E10 mAb (**a**–**d**) and either 25H8 mAb (**e**), anti-tubulin mAb (**f**), anti-transferrin antibodies (**g**), or anti-γ-adaptin mAb (**h**) in double-labelling experiments. Arrowheads in (**c**) and (**g**) indicate some structures stained by both 9E10 and anti-transferrin antibodies. Scale bar, 3 μm.

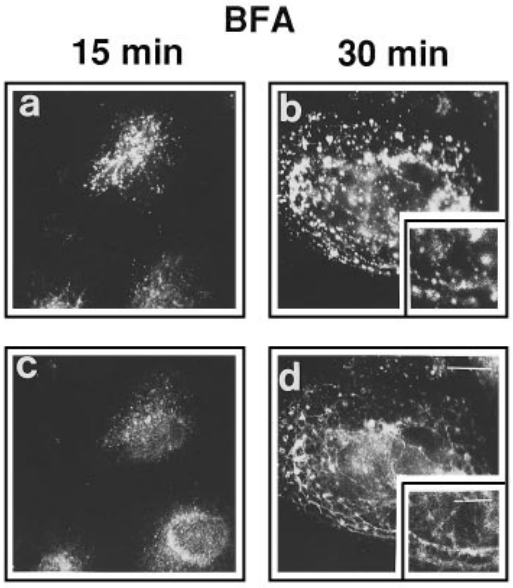

Figure 2 Effect of BFA on the distribution of MAL/c-*myc*

Stably transfected A-498 cells were treated with 5 μg/ml BFA for 15 min (**a**, **c**), or 30 min (**b**, **d**), fixed, permeabilized, and subjected to double-labelling immunofluorescence analysis using anti 9E10 mAb (**a**–**b**) and either 25H8 mAb (**c**) or anti-transferrin antibodies (**d**). Higher magnifications of regions from (**c**) and (**d**) are shown in their respective inserts. Controls of untreated cells are shown in Figure 1. Scale bar, 3 μm.

otic SRα promoter [22]. This construct was used to transfect human epithelial A-498 cells and the subcellular localization of the protein was followed by indirect immunofluorescence analysis using the 9E10 mAb specific for the c-*myc* epitope (Figure 1). To prevent massive overexpression of the gene and to get steady-state levels of the protein, stably transfected cells were selected by prolonged treatment with the aminoglycoside drug G-418. When the distribution of MAL/c-*myc* was analysed in stably transfected human epithelial A-498 cells (Figures 1a and 1d) we found a paranuclear staining and a punctate pattern corresponding to small vesicles. The paranuclear vesicular distribution of epitope-tagged MAL observed in A-498 cells overlapped partially with the pattern obtained with the 25H8 mAb (Figures 1a and 1e), which recognizes a Golgi-resident protein, and with the microtubule-organizing centre, as evidenced by staining with anti-tubulin antibodies (Figures 1b and 1f). The intracellular distribution of transferrin has been used routinely to identify early endosomes [23]. When cells loaded for 1 h with iron-saturated transferrin molecules were fixed and stained with anti-transferrin antibodies, the distribution of MAL/c-*myc* and transferrin were different, although a very limited colocalization of transferrin and MAL/c-*myc* was found (Figures 1c and 1g). Finally, a good colocalization of MAL/c-*myc* was found with γ-adaptin, a protein localized to the TGN [24]. To confirm these results all the samples were also analysed in a confocal immunofluorescence microscope (results not shown). To further clarify the assignment of MAL location, experiments with the fungal metabolite BFA were performed in an effort to distinguish between Golgi and post-Golgi compartments. It has been established that, shortly after addition, BFA causes the Golgi to redistribute to the endoplasmic reticulum [25] and the TGN to fuse with early endosomes, forming an extensive TGN/early endosomes network [26,27]. As shown in Figure 2, 15 min after BFA addition to transfected A-498 cells the 25H8 Golgi marker redistributed to the endoplasmic reticulum (Figure 2c) in a punctate pattern similar to that described for other resident Golgi proteins [26], whereas the MAL/c-*myc* protein started redistributing on rows of dots delineating tubules along the cytoplasm (Figure 2a). In cells treated with BFA for 30 min, transferrin molecules were found in continuous tubular processes (Figure 2d) characteristic of BFA-induced TGN/early endosome fusion [26]. Interestingly, MAL/c-*myc* molecules were found on discrete dots distributed along the tubular processes stained with anti-transferrin antibodies (Figures 2b and 2d). The low level of colocalization of MAL/c-*myc* and transferrin in control cells (Figures 1c and 1g) and the extensive colocalization between them found in BFA-

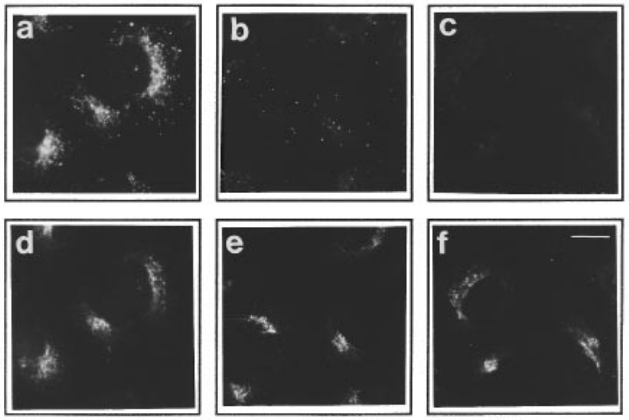

Figure 3 Effect of filipin and digitonin on MAL/c-*myc* staining

Stably transfected A-498 cells fixed with paraformaldehyde were untreated (**a**, **d**), or pre-treated with 5 μg/ml filipin (**b**, **e**) or 50 μg/ml digitonin (**c**, **f**) for 20 min before incubation with 0.2% TX100 for 10 min. Cells were finally processed for immunofluorescence analysis with 9E10 (**a**–**c**) or 25H8 mAb (**d**–**f**). Scale bar, 3 μm.

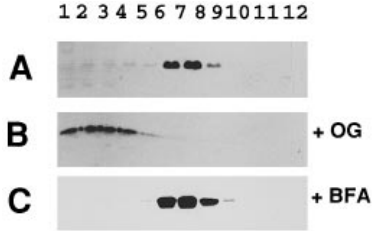

Figure 4 Presence of MAL/c-*myc* in TX100-insoluble complexes

A-498 cells stably expressing MAL/c-*myc* were untreated (**A**, **B**) or treated with with 5 μg/ml BFA for 30 min (**C**), extracted at 4 °C with either 1% TX100 (**A**, **C**) or 1% TX100 plus 60 mM octylglucoside (**B**) and homogenized. A 5%–30% sucrose linear gradient was formed over the lysates adjusted to 40% sucrose. After centrifugation to equilibrium, 1 ml fractions were harvested from the bottom of the tube. Fractions 1–4 correspond to the lysate fraction. Aliquots from each fraction were subjected to SDS/PAGE and analysed by immunoblotting with mAb 9E10.

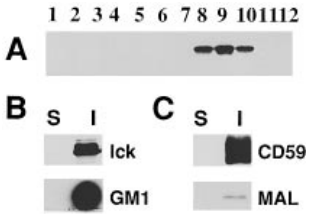

Figure 5 Presence of MA/c-*myc* in TX100-insoluble complexes in Jurkat T-cells

Jurkat cells stably expressing MAL/c-*myc* were surface-labelled with biotin, extracted at 4 °C with 1% TX100 and homogenized. The lysate was adjusted to 40% sucrose, and a 5%–30% linear gradient was formed over the lysates. After centrifugation to equilibrium, fractions were harvested from the bottom of the tube and analysed by immunoblotting with 9E10 mAb (**A**). Pools were made with fractions 1–4 and with fractions 8–10 and named as samples S (soluble) and I (insoluble) respectively. The same amount of total protein from samples S and I was subsequently analysed by immunoblotting with anti-*lck* antibodies, and by dot blotting with horseradish peroxidase-coupled cholera toxin B subunit to detect the ganglioside GM1 (**B**), or by immunoprecipitation with anti-CD59 or 9E10 mAb followed by biotin detection with streptavidin-peroxidase (**C**).

treated cells (Figures 2b and 2d), together with the colocalization of MAL/c-*myc* and γ-adaptin, suggest that MAL might be mainly localized to structures belonging to the TGN. Moreover, although MAL forms part of the tubular extensions in BFA-treated cells, MAL retains a local microenvironment that prevents its complete mixing with the TGN/early endosomes tubular network, as evidenced by the dotted pattern found for MAL in BFA-treated cells.

The localization of MAL to post-Golgi vesicles in epithelial cells, and the fact that MAL is a proteolipid, fit with the idea that MAL might be associated with glycolipids and cholesterol, which are known to be highly enriched in the TGN. To study this possible association we have used the fungal polyene antibiotic filipin and the cardiac glycoside digitonin, both known to interact with cellular cholesterol [28,29]. Cells were treated with these agents before incubation with TX100, and they were finally subjected to immunofluorescence analysis using anti c-*myc* mAb. Figure 3 shows that the treatment with TX100 of cells previously treated with filipin (Figure 3b) or digitonin (Figure 3c) produced a dramatic decrease in MAL/c-*myc* staining compared with that obtained with TX100 alone (Figure 3a). This suggests that cholesterol-binding agents affect MAL–lipid complexes rendering them TX100 soluble. The same treatments did not affect the staining obtained with the 25H8 mAb (Figure 3, d–f), indicating that the observed effects are specific to the local MAL microenvironment.

The existence of cholesterol- and glycolipid-enriched membrane subdomains that are insoluble in 1% TX100 at 4 °C [3] led us to investigate the presence of MAL/c-*myc* in TX100-resistant complexes. We used centrifugation to equilibrium on sucrose density gradients to separate lipid-rich resistant complexes from the TX100-soluble material [3]. Figure 4(A) shows that most of the MAL/c-*myc* protein was found at a low-density (1.09–1.10 g/ml) position, and only a very small amount was recovered from the 40% sucrose lysate (fractions 1–4) corresponding to the TX100-solubilized material. As the low-density fractions are known to be enriched in glycolipids and cholesterol, we carried out the same experiment in the presence of octylglucoside (Figure 4B), which is known to solubilize proteins associated with glycolipids [3]. In the presence of 60 mM octylglucoside, most of the MAL/c-*myc* protein was found in the 40% sucrose fractions, indicating that fully solubilized MAL/c-*myc* does not have a unexpectedly low density, and suggesting again that MAL is associated with glycolipids. Moreover, when a similar analysis was carried out in cells treated with BFA for 30 min, we found that MAL distribution was indistinguishable from that obtained in control cells. This suggests that MAL maintains its glycolipid- and cholesterol-rich microenvironment in the presence of BFA, which supports our former results showing a discontinuous distribution of MAL along the continous tubular processes corresponding to the fused TGN/early endosomes network (Figures 2b and 2d).

As the MAL cDNA was originally isolated from T-cells [10], we next addressed the question of whether MAL is also present in detergent-insoluble complexes in T-cells. When TX100-extracts from stably transfected Jurkat cells were subjected to centrifugation to equilibrium in sucrose gradients, we found that, similarly to the case in epithelial A-498 cells, MAL was present exclusively in the floating fractions (Figure 5A). Moreover, when equal amounts of protein from the pooled soluble fractions (fractions 1–4) and from the pooled insoluble complexes (frac-

Figure 6 Immunofluorescence analysis of MAL in transfected Jurkat cells

Jurkat cells stably expressing MAL/c-*myc* were fixed, permeabilized with TX100 and subjected to double-labelling immunofluorescence analysis with 25H8 (**a**) and 9E10 (**b**) mAbs. Cells were finally stained with Hoechst 33258 for 5 min to visualize the nuclei (**c**). Scale bar, 10 μm.

tions 8–10) were analysed, we observed, in agreement with previous reports [4], a high enrichment of the ganglioside GM1 in the insoluble fractions, as evidenced by dot blot analysis with horseradish peroxidase-coupled cholera toxin B subunit and in the tyrosine kinase p56*lck* (Figure 5B). As the 9E10 epitope was only accessible in permeabilized cells (results not shown), to investigate whether some MAL protein is also present in the plasma membrane, as it is the case in A-498 cells, we surface-labelled Jurkat cells with sulfo-NHS-biotin before extraction with TX100. After centrifugation to equilibrium, the soluble and insoluble pools were immunoprecipitated with anti-CD59 or 9E10 mAb, and the immunoprecipitates were finally analysed with streptavidin-coupled horseradish peroxidase. Figure 5(C) shows that surface-labelled CD59, a typical GPI-anchored protein, was highly enriched in the floating complexes. Moreover, some biotin labelling was detected in the MAL protein, suggesting that at least a fraction of MAL molecules was also present on the T-cell surface.

To examine the distribution of MAL in Jurkat cells, we carried out double-labelling immunofluorescence analysis. Figure 6 shows that, in agreement with the results in A-498 cells, MAL staining is mostly concentrated in the Golgi region (Figure 6b), as evidenced by its colocalization with the Golgi marker 25H8 (Figure 6a), and it is also present in a patched pattern at the cell periphery facing the concave side of the nucleus (Figure 6c).

DISCUSSION

Using a cDNA construct expressing the human MAL protein tagged with a c-*myc* epitope we have followed the localization of MAL protein in stably transfected epithelial cells. The association of MAL with glycolipids and cholesterol was demonstrated biochemically by the presence of MAL/c-*myc* in the buoyant detergent-resistant fraction obtained by centrifugation to equilibrium of cells extracted with TX100 [3]. The results of the immunofluorescence analyses in human epithelial A-498 cells were consistent with MAL/c-*myc* residing in post-Golgi vesicles and, at low levels, in early endosomes and in the plasma membrane. Supporting the view that MAL is embedded in a lipidic environment, we found that treatment with cholesterol-binding agents such as filipin and digitonin greatly perturbed the MAL/c-*myc* microenvironment, as evidenced by immunofluorescence analysis, suggesting that MAL is associated with cholesterol-enriched structures. In epithelial MDCK cells, insoluble membrane microdomains, possibly involved in protein and lipid sorting, have been found at the TGN [3]. Taken together, these results suggest that in epithelial cells MAL is mainly present in glycolipid- and cholesterol-enriched membrane microdomains belonging to the TGN, and at lower levels in early endosomes.

During transport to the surface in polarized cells, GPI-anchored proteins become resistant to solubilization by non-ionic detergents, due to their incorporation into detergent-insoluble intracellular membrane microdomains highly enriched in glycolipids and cholesterol [3]. It has been proposed that these microdomains or 'rafts' could form the scaffold onto which GPI-anchored proteins attach, either by direct interaction with the lipids or indirectly via interaction with the sorting machinery [5]. According to the proposal of a glycolipid-based sorting of apically destined proteins, the existence of specific proteins interacting with glycolipids and cholesterol, modulating the formation of microdomains and recruiting GPI-anchored proteins, is conceivable. Keeping in mind: (1) the unusual lipid-like behaviour of MAL; (2) its presence in buoyant detergent-insoluble complexes; (3) its localization to post-Golgi vesicles; and (4) its identification in transport vesicles in MDCK cells [17], it is plausible that MAL might play a role in MDCK cells as a component of the TGN-derived transport vesicle machinery, acting at the TGN, in the assembly of the 'rafts' or membrane microdomains by recruiting their lipid and/or protein components, and/or helping in the nucleation of the transport vesicles. Whether MAL is actively involved in the regulation of protein and/or glycolipid transport, or is just transported as a cargo protein in epithelial cells, remains to be elucidated.

As is the case in epithelial cells, GPI-anchored proteins in T-cells associate with lipids, forming membrane microdomains that can be recovered from detergent lysates in the form of insoluble complexes [4]. It is believed that the lipid composition of the T-cell-insoluble complexes is similar to that of the complexes isolated from epithelial cells [6]. There is evidence to suggest that lipid is essential for protein association to the complexes, as the non-ionic detergent, octyl-glucoside, an analogue of glycolipids, dissociates GPI-anchored proteins, whereas this detergent does not generally disrupt protein interactions resistant to TX100 [3]. In addition to GPI-anchored proteins, detergent-insoluble complexes from T-cells are also enriched in tyrosine kinases of the *src* family, such as p56*lck* or p59*fyn* [4,30], suggesting that they are involved in signal transduction. MAL expression is shared by T-lymphocytes and epithelial MDCK cells [17] and MAL is present in the detergent-resistant complexes in these two cell types. In MDCK cells a role for MAL as a component of the protein machinery of transport of GPI-anchored proteins and glycolipids is plausible; thus MAL might play a similar role in T-cells. Whether this role is related to the recruitment of certain proteins and/or lipids to the membrane microdomains during T-cell differentiation will be a matter for further investigation.

A recent report showing MAL expression in oligodendrocytes and Schwann cells [16], which are the cells responsible for myelin formation in the central and peripheral nervous system respectively, supports the view that MAL might have a more general role in glycolipid-enriched membrane microdomains. Moreover, MAL has been identified in oligodendrocytes as a component of the detergent-insoluble complexes [15]. Myelin is highly enriched in glycolipids, sphingomyelin and cholesterol [7], as are the TX100-insoluble complexes from epithelial cells [3]. This parallelism might reflect a similar mechanism in the process of recruitment of the myelin components and in the glycolipid 'raft' formation at the TGN in epithelial MDCK cells. The expression of MAL in both rat oligodendrocytes and Schwann cells opens up the possibility of a role for MAL in myelin formation.

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