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Malaria-Suppressible Expression of the Anti-Apoptotic Triple GTPase mGIMAP8

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Abstract The IMAP/IAN family of AIG1-like GTPases is conserved among vertebrates and angiosperm plants and has been postulated to regulate apoptosis, particularly in context with diseases such as cancer, diabetes, and infections. The human genes were recently renamed as *gimap* for GTPase of the immunity associated protein (GIMAP) family. Here we extend this new nomenclature to the murine *gimap* gene family. All *gimap* genes of the mouse are clustered on chromosome 6B with eight functional members and one pseudogene. The mGIMAP proteins contain one GTP-binding site and display molecular masses between 33 and 38 kDa except for the very unusual 77 kDa mGIMAP8 protein, which is the first characterized protein containing three GTP-binding domains. Northern blot analysis revealed expression of m*gimap8* predominantly in the thymus. The low expression level observed in the spleen was further suppressed by *Plasmodium chabaudi* malaria. Confocal laser scanning microscopy demonstrated localization of mGIMAP8 at ER, Golgi, and mitochondria. Overexpression of mGIMAP8 could significantly impair anisomycin-induced activation of caspase 3. Our data support the view that mGIMAP8 exerts an anti-apoptotic effect in the immune system and is involved in responses to infections. J. Cell. Biochem. 96: 339–348, 2005.

Key words: *gimap* genes; *ian* genes; genomic organization; malaria response; apoptosis

GTPases are ubiquitously distributed and control a bundle of various cellular functions by regulating diverse processes, as for example, translation, polymerization of actin cytoskeleton, vesicle transport and fusion, nuclear import and export, etc. In addition, GTPases are inherent constituents of many signal transduction pathways, which integrate input from different extracellular signals by activating enzymes producing major second messengers like cyclic AMP, inositol-1,4,5-triposphate (IP_3) or diacylglycerol or by activating MAP kinase pathways. GTPases belonging to a novel family have been recently identified in angiosperm plants and vertebrates. The prototype has been first described as AIG1 in *Arabidopsis thaliana* [Reuber and Ausubel, 1996].

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The mammalian AIG1-like GTPases were described under various designations as IAP, IMAP, IAN, or IROD. In addition, one IAN designation has been used for different human genes, and designations for IAN genes of different species do not always represent orthologs. In order to avoid the increasing confusion and to introduce gene names conforming to the guidelines for human gene nomenclature [Wain et al., 2002], the name GTPase of the immunity associated protein family (GIMAP) has been recently introduced [Krücken et al., 2005]. These approved gene names were here adopted for the murine orthologs and will be used throughout this study.

Though most of these AIG1-like GTPases have not yet been fully characterized, the hitherto available information indicates their association with diverse diseases. For instance, AIG1 has been implicated in the development of a protective hypersensitivity reaction after infection of *Arabidopsis thaliana* with the bacterial pathogen *Pseudomonas syringae* [Reuber and Ausubel, 1996]. Expression of *gimap1* was found to be induced in the spleen of mice infected with *Plasmodium chabaudi*

malaria [Krücken et al., 1997, 1999]. A mutation in *gimap3* was shown to be responsible for T-cell lymphopenia leading to autoimmune diabetes in the BB rat [Hornum et al., 2002; MacMurray et al., 2002]. Overexpression of *gimap4* and *gimap5* genes has been described in several leukemias and lymphomas [Cambot et al., 2002; Zenz et al., 2004]. This study comparatively describes all mouse *gimap* genes and provides first evidence for an anti-apoptotic function of the very exceptional m*gimap8* gene, which encodes the only known GTPase with three GTP-binding domains.

MATERIALS AND METHODS

Database Screening and Sequence Analysis

EST and cDNA entries in GenBank® with homology to known *gimap* genes were identified using BLASTN [Altschul et al., 1990] and TBLASTN [Altschul et al., 1997]. Deduced protein sequences were analyzed with the programs PSORTII [Nakai and Horton, 1999], Coils [Lupas et al., 1991], Mitoprot [Claros and Vincens, 1996], InterProScan [Mulder et al., 2003], and GOR [Garnier et al., 1996].

Mice and Infections

Inbred C57BL/10 mice were bred under specific pathogen-free conditions in our animal facility. *Plasmodium chabaudi* parasites were maintained by weekly passages in NMRI mice by intraperitoneal injection of 1×10^6 parasitized erythrocytes [Wunderlich et al., 1991].

Cell Culture

Chinese hamster ovary CHO-K1 (DSMZ no. ACC 110) and NIH 3T3 (ATCC no. CRL-1658) murine embryonic fibroblast cell lines were grown at 37°C in a humid atmosphere containing 5% CO_2 in Ham's F12 or DMEM medium (Invitrogen), respectively, supplemented with 10% fetal calf serum (PAA Laboratories), 50 U/ml penicillin, and 50 μg/ml streptomycin.

Isolation of RNA

Spleens were removed from mice, immediately homogenized in Trizol (Invitrogen) and homogenates were stored at −80°C. RNA was isolated according to the manufacturer's instructions. PolyA$^+$ RNA was isolated using the OligoTex kit (Qiagen, Hilden).

Reverse Transcription PCR

First strand cDNA was generated from 1 μg RNA by incubation with 25 U AMV reverse transcriptase (Roche) in 25 μl 1 × AMV buffer containing 0.4 mM dNTPs, 5 mM DTT, and 2.6 μM random hexamer primer. For PCR, 5 μl of the RT reaction were used as template in 50 μl reactions containing 0.4 mM dNTPs and 0.3 μM of each gene-specific primer in 1 × expand high fidelity buffer with Mg^{2+} (Roche). As gene specific primers we used either 5′-AATATGGATTGAAAGATGGATTGGCTTTACAGAA-3′ and 5′-GCAGCAAAGTCTCTCAAGGGCTTAGAAGTAAGTC-3′ for m*gimap6*, 5′-TGCATTCAGATCTTTAGAGCAGCTTGCCTTG-3′ and 5′-TGACCACGAATGTTCTGTCAGGATTGTTTATG-3′ for m*gimap7*, 5′-ATGGCGACTTCATCCCACCAAGGAGCAGCT-3′ and 5′-TTTAAATGCCATAGTAATTTGGCCTAGTGTTGATT-3′ for m*gimap8*. After denaturation for 2 min at 94°C, 2.6 U expand high fidelity enzyme mix were added followed by 35 cycles of 15 s 94°C, 90 s 65°C, and 4 min 68°C. For m*gimap6*, a nested PCR had to be performed using the same upstream primer and the downstream primer 5′-TAATGGTTGCCTCCTCAGGGCCTTGCTATT-3′ and 1 μl of the first PCR as template. PCR products were cloned in pCR2.1 TOPO or pcDNA3.1 V5His TOPO vectors (Invitrogen) and sequenced as described previously [Krücken et al., 1999].

Northern Hybridizations

A multiple tissue Northern blot containing polyA$^+$ RNA from BALB/c mice (2 μg per lane) was purchased from Origene Technologies (Rockville). Northern blotting with splenic RNA from *P. chabaudi*-infected C57BL/10 mice was performed using 7.5 μg polyA$^+$ RNA as described recently [Krücken et al., 2005].

Confocal Laser Scanning Microscopy

CHO-K1 cells were transfected with the vector pGIMAP8-V5His expressing mGIMAP8 in frame with a COOH-terminal V5-6 × His-tag encoded by the pcDNA3.1 V5His TOPO vector (Invitrogen). After 24 h, cells were fixed for 10 min with 1% formaldehyde in PBS at room temperature. Cells were permeabilized in PBS containing 0.05% Tween® 80, incubated for 1 h at room temperature with a monoclonal mouse anti-V5 antibody (Invitrogen) (50 μg/ml in PBS, 0.05% Tween® 80), washed three times with

PBS, and stained with a goat anti-mouse secondary antibody coupled to either Alexa Fluor 488 (20 μg/ml) or Alexa Fluor 568 (2 μg/ml) (Molecular Probes) for 1 h. Specimens were mounted with 10% Mowiol® 4–88 (Polyscience), 25% glycerol, and 2% 1,2-diazobicyclo-(2,2,2) octane (DABCO) (Merk) in 0.1M TrisHCl (pH 8). Subcellular compartments were counterstained with propidium iodide for nuclei, MitoTracker (Molecular Probes) for mitochondria, rabbit anti-calnexin (Stressgene) for endoplasmic reticulum, rabbit anti-giantin (CRP, Cumberland) for Golgi, or ConcanavalinA-Rhodamin for plasma membrane as described previously [Krücken et al., 1999; Saeger et al., 2001; Stamm et al., 2002]. Localization of calnexin or giantin was visualized with goat anti-rabbit IgG coupled to Alexa Fluor 488 (20 μg/ml) (Molecular Probes). Samples were analyzed with the Confocal Laser Scanning microscope Leica TCS version 1.5.451 (Leica Lasertechnik).

Apoptose Assay

NIH 3T3 or CHO-K1 cells were transfected with equal amounts of pCaspaseSensor vector (Clontech) and either pGIMAP8V5His or pcDNA3.1 V5His TOPO without insert. Cells were grown for 24 h before anisomycin (Calbiochem) was added to a final concentration of 33 μg/ml for 3 h (NIH 3T3 cells) or 66 μg/ml for 5 h (CHO-K1 cells) to induce apoptosis in about 20% of the cells. Anisomycin concentrations were optimized for both cell lines in preliminary experiments. Controls received only the vehicle DMSO (0.1% final concentration). Finally, cells were counterstained with DAPI, fixed with methanol, and analyzed using a Leica DM LB fluorescence microscope.

RESULTS

Mouse Gimap Gene Family

All GTPases of the immunity associated protein family are characterized by the central AIG1-domain (InterPro acc. no. IPR006703), which contains the five motifs G1–G5 involved in contact to GTP/GDP in all GTP-binding proteins [Sprang, 1997] (Fig. 1). In addition, there is a conserved box (CB) with a hydrophobic core, which is predicted to adopt an extended beta sheet structure [Garnier et al., 1996]. The CB consensus sequence for the murine GIMAP proteins is $L_{50}L_{70}xxP_{80}G_{80}P_{80}H_{80}A_{60}L_{50}L_{70}L_{100}$-$V_{100}T_{50}Q_{70}L_{80}G_{80}R_{80}\Theta_{90}T_{90}xE_{80}\Psi_{100}$, where Θ indicates an aromatic, Ψ an acidic amino acid, x a non-conserved position, and numbers reveal the percentage of occurrence among the AIG1-domains of the mouse GIMAP proteins. The corresponding region between G3 and G4 of the GTPase H-ras forms indeed a beta sheet structure and is not exposed to the surface of the molecule, but transverses through the central core of the GTPase domain as detected by X-ray diffraction [Krengel et al., 1990].

The mouse *gimap* genes are clustered on chromosome 6B as presented in Figure 2. These data have been compiled from GenBank/EBI database entries and from our own PCR-derived clones. The TBLASTN program identifies 11 sequence regions with similarity to AIG1 in the mouse gene cluster. However, there are in fact only eight functional m*gimap* genes and one pseudogene (m*gimap10-ps*), which corresponds to the AIG1-like region 8 in Figure 2. At the proximal end of the *gimap* gene cluster, three AIG1-like sequences are part of a single unusual gene. This m*gimap8* gene encodes a protein with three GTP-binding domains (Fig. 2). The comparison of the mouse *gimap* cluster with the human *gimap* gene cluster on the syntenic locus 7q36.1 reveals that this triple GTPase encoding gene is conserved between both species. In addition, there are orthologs to all mouse *gimap* genes in the human genome with the exceptions of the pseudogene m*gimap10-ps* and the m*gimap9* gene. The latter is closely related to m*gimap6* and h*gimap6*. The m*gimap3* gene is functional, whereas the ortholog h*gimap3* is a pseudogene [Krücken et al., 2004]. On the other hand, the human locus contains one gene, that is, h*gimap2*, which is absent from the mouse genome. The order and the orientation of all conserved *gimap* genes are maintained between mouse and man. However, the intergenic regions differ remarkably in size with the consequence that the human *gimap* cluster is about twice as large as the mouse *gimap* cluster (Fig. 2).

The proteins encoded by mouse *gimap* genes are presented schematically in Figure 3. All mGIMAP proteins are expected to contain at least one small coiled-coil region within the COOH-terminal part of the AIG1-domain. However, mGIMAP7, mGIMAP9, mGIMAP3, and mGIMAP5 have a second putative coiled-coil region immediately downstream of the AIG1-domain, whereas mGIMAP4 has a long continuous coiled-coil region. The proteins mGIMAP1

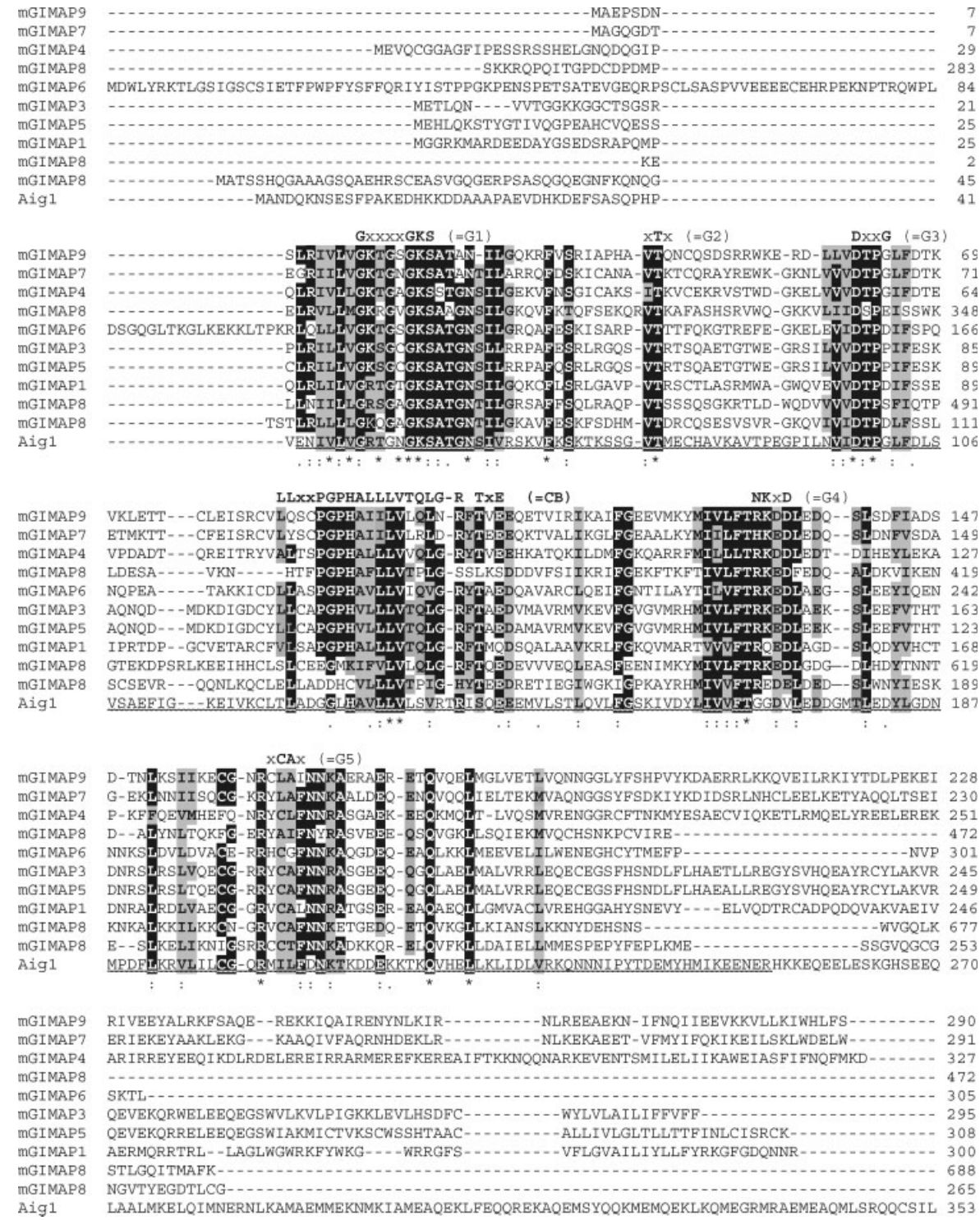

Fig. 1. The sequences of mGIMAP proteins and AIG1 from *A. thaliana* were aligned using ClustalW. A consensus sequence of the conserved box (CB) characteristic for all AIG1-like proteins and the five motifs G1–G5 involved in nucleotide binding with the corresponding motifs of the human H-ras GTPase are indicated above the sequences. In the consensus sequence, Θ indicates an aromatic and Ψ an acidic amino acid, while x marks a non-conserved position. The AIG1-domain as detected by InterProScan is underlined. The sequence of mGIMAP8 was divided in three parts to allow alignment of its three AIG1-domains.

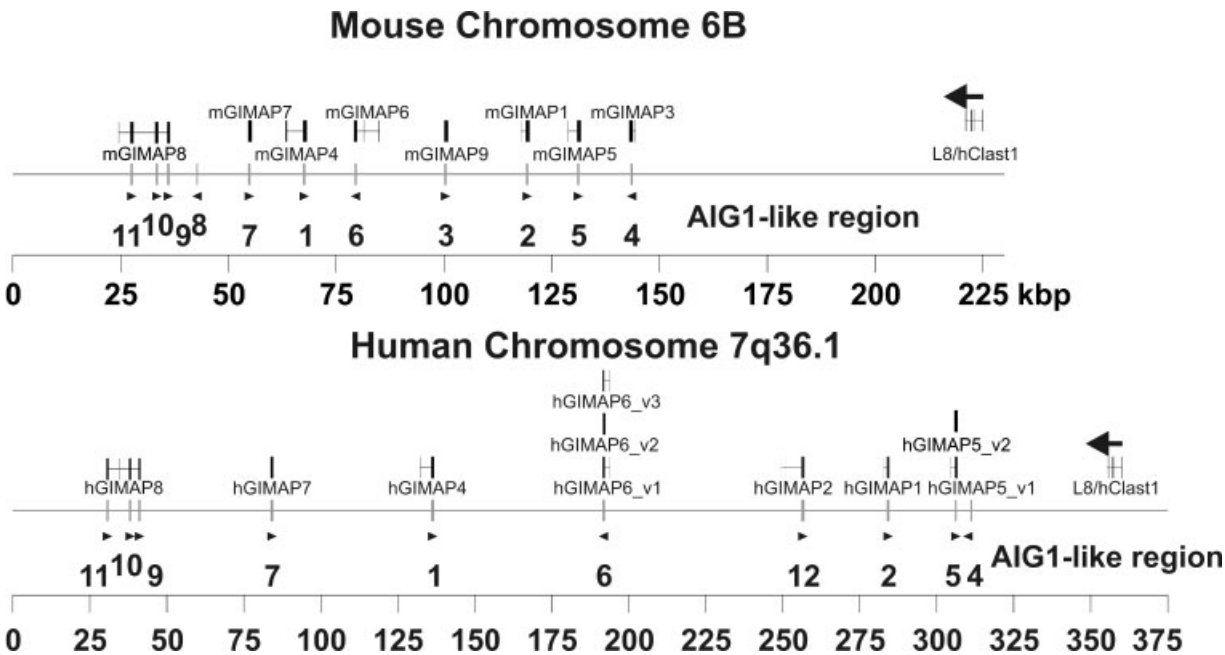

Fig. 2. Genomic organization of the mouse *gimap* gene cluster. The **upper panel** shows the exon/intron structure of the coding regions in the murine *gimap* gene cluster on chromosome 6B. The distribution and designation of regions with similarity to AIG1 are shown and numbered according to MacMurray et al. [6]. For comparison, the **lower panel** shows the human *gimap* gene cluster on chromosome 7q36.1 and the exon/intron structure of the published h*gimap* genes.

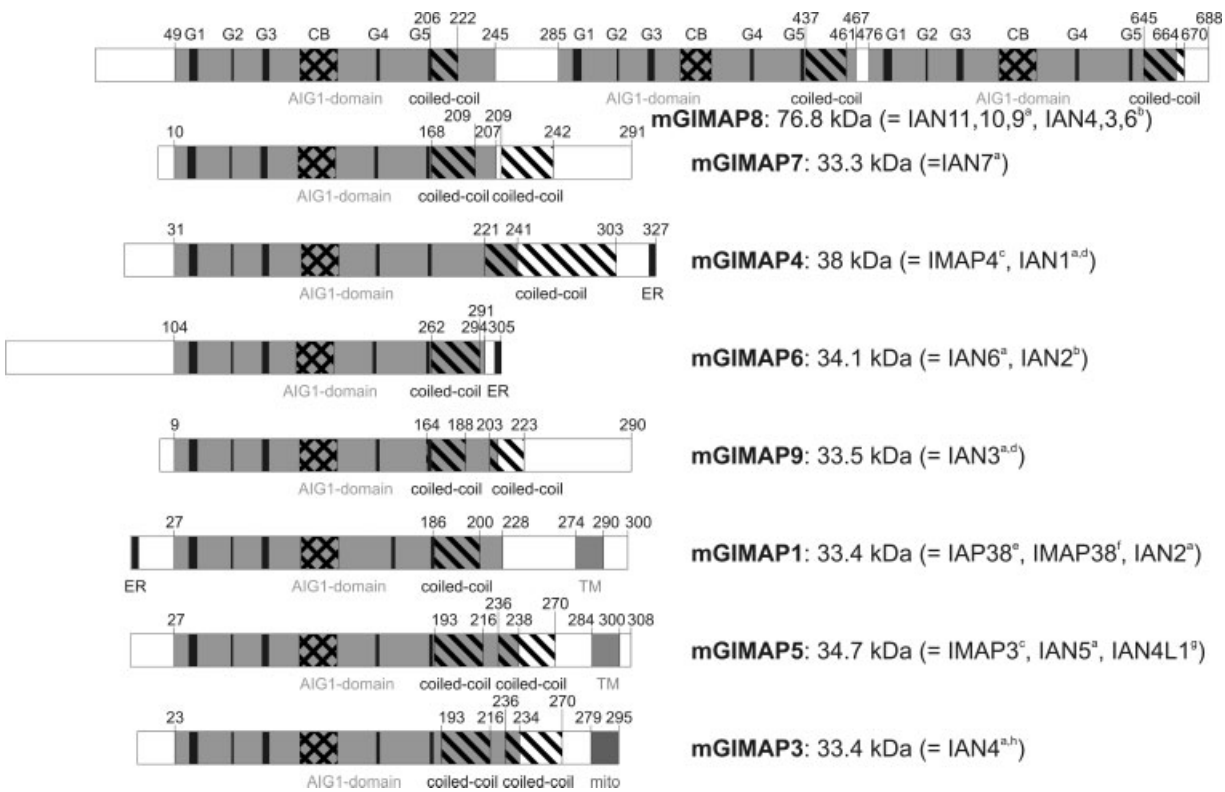

Fig. 3. Schematic illustration of the mouse GIMAP proteins. The AIG1-domain contains the five motifs G1–G5 characteristic for all GTPases and the conserved box (CB) characteristic for the AIG1-domain GTPases. Regions with similarity to known ER localization signals (ER), putative coiled-coil or transmembrane (TM) regions are also marked. Synonyms previously used for these proteins are given in brackets. [e,f]Krücken et al., 1997, 1999; [d]Poirier et al., 1999; [h]Dahéron et al., 2001; [b]Cambot et al., 2002; [a]MacMurray et al., 2002; [c]Stamm et al., 2002; [g]Pandarpurkar et al., 2003.

and mGIMAP5 are predicted to contain a transmembrane helix in their COOH-terminus, while the hydrophobic stretch at the immediate COOH-terminus of mGIMAP3 was shown to be responsible for transport to the outer mitochondrial membrane [Dahéron et al., 2001]. The program PSORTII [Nakai and Horton, 1999] detects motifs with weak similarity to ER localization signals of transmembrane proteins at the termini of mGIMAP1, mGIMAP4, and mGIMAP6 (Fig. 3).

Expression of m*gimap8*

Genes encoding three GTP-binding domains as m*gimap8* have not been described to date. The complete ORF of m*gimap8* was amplified by RT-PCR from splenic RNA (Fig. 4A) confirming the presence of three AIG1-like sequences in a single transcript. Using a multiple tissue Northern blot containing polyA$^+$ RNA, an abundant 4.2 kb and a minor 2.9 kb m*gimap8* transcript were detected in the thymus using the complete m*gimap8* cDNA as probe (Fig. 4B). The larger mRNA was weakly detectable in heart, skin, and lung, but undetectable in all other organs tested including the spleen.

In order to detect a possible responsiveness of m*gimap8* expression to infection, C57BL/10 mice were infected with *P. chabaudi* malaria for 8 days, when parasitemia is maximal. The spleen is the major defense organ against malaria, and m*gimap8* mRNA levels in the spleens of infected mice were about 80% lower than in non-infected mice (Fig. 4C). Expression of m*gimap8* was detectable only after prolonged exposure of the blot containing 7.5 μg polyA$^+$ RNA.

To analyze subcellular localization of mGIMAP8 protein using confocal laser scanning microscopy, CHO-K1 cells were transiently transfected with an expression vector encoding an mGIMAP8 protein with a COOH-terminal V5-6 × His tag. This mGIMAP8 protein was not found in nuclei or at the plasma membrane but rather at ER, Golgi, and mitochondria as detected by strong colocalization with calnexin, giantin, and Mito-Tracker, respectively (Fig. 5).

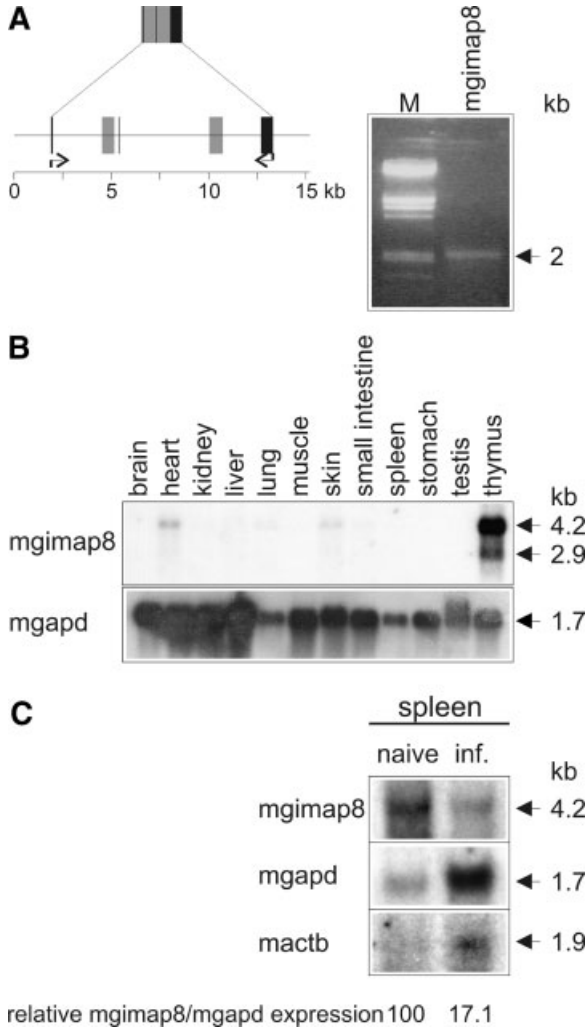

Fig. 4. Expression analysis of m*gimap8*. **A**: The complete ORF of m*gimap8* was amplified by RT-PCR from splenic RNA. Localization of primers and expected product sizes for cDNA and genomic PCR products are indicated in the scheme. **B**: A multiple tissue Northern blot containing 2 μg polyA$^+$ RNA per lane was hybridized with a m*gimap8* probe corresponding to the complete ORF and, after stripping, with a glyceraldehyde-3-phosphate dehydrogenase (mgapd) and probe. **C**: A Northern blot containing 7.5 μg of polyA$^+$ RNA from the spleen of C57BL/10 mice on days 0 and 8 after infection with 10^6 *P. chabaudi*-parasitized erythrocytes. The filter was hybridized with the gimap8, and mgapd and probes. To ensure that differences in mgapd signal intensity were due to unequal amounts of RNA and not regulated expression of mgapd, the blot was also hybridized to a β actin probe (mactb).

Anti-Apoptotic Effect of mGIMAP8

In a first attempt to identify potential effects of mGIMAP8 on apoptosis we analyzed caspase 3 activation after treatment of cells with the pro-apoptotic agent anisomycin. The pCaspase-3-Sensor vector expressing an EYFP fusion protein with localization directed to cytoplasm and nucleoli was used to identify apoptotic cells. Upon apoptosis, caspase 3 is activated, removes a nuclear export signal and a remaining nuclear localization signal of EYFP directs its import into the nucleus. Figure 6A shows one apoptotic NIH 3T3 cell (arrow) with EYFP in the nucleus

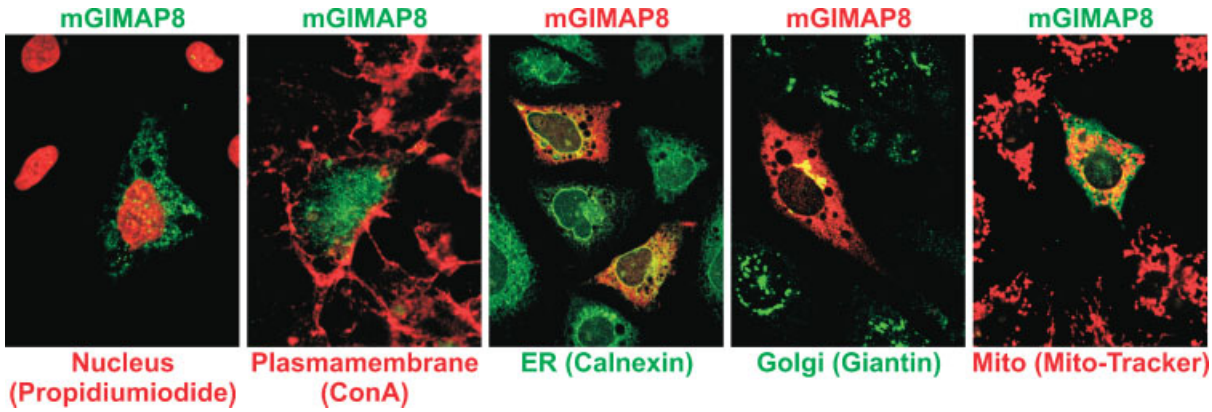

Fig. 5. Subcellular localization of mGIMAP8 protein. CHO-K1 cells were transfected with expression plasmids encoding mGIMAP8 protein with a COOH-terminal V5-6 × His tag. Subcellular compartments and mGIMAP8 are shown in the colors indicated.

surrounded by several non-apoptotic cells. CHO-K1 and NIH 3T3 cells were cotransfected with the mGIMAP8 expression vector and the pCaspase3 sensor vector and treated with anisomycin as an inducer of apoptosis. In order to detect any possible effects of mGIMAP8 on apoptosis, such experimental concentrations of anisomycin were chosen, which result in about 20% apoptotic cells. Overexpression of mGIMAP8 had no effect on the low rate of spontaneous apoptosis. However, after anisomycin treatment, the number of apoptotic cells was reduced in NIH 3T3 fibroblasts by nearly 30% ($P < 0.01$) when mGIMAP8 was overexpressed (Fig. 6B). Even in CHO-K1 cells, which are more resistant to the pro-apoptotic action of anisomycin, a significant reduction of apoptotic cell number by about 13% was observed ($P < 0.05$).

DISCUSSION

The members of the GIMAP protein family contain a GTP-binding domain and exhibit a similar overall structure—except for GIMAP8, which possesses three GTP-binding sites. To our knowledge, this is the first protein described with three GTP-binding domains. However, an ortholog to m*gimap8* exists in the human and rat genome and we have confirmed expression of an mRNA encoding these three GTP-binding domains here in the mouse spleen. Though there are a small number of proteins with two GTP-binding domains in protein databases, only very limited information concerning their function is available. For the highly conserved bacterial engA protein with two consecutive homologous GTPase domains a role in assembly or stability of the 70S ribosome has been suggested [Tan et al., 2002]. Moreover, it has been shown that a mutation in one of the GTPase domains modulates hydrolytic activity of the other [Robinson et al., 2002]. This suggests that an allosteric cross-talk takes place between both domains [Robinson et al., 2002]. In mammals, a novel subfamily of atypical Rho GTPases located in the mitochondrion has been recently characterized [Fransson et al., 2003]. These Miro (mitochondrial Rho) proteins possess a Rho-like and an unrelated GTPase domain flanking two EF hand Ca^{2+} binding motifs. Transfection of COS-7 cells with a gain of function Miro-1 mutant leads to breakdown of the mitochondrial network, accumulation of mitochondria in a perinuclear position, and increased apoptosis [Fransson et al., 2003].

Though the functions of mGIMAP8 may be diverse, we could demonstrate for the first time an anti-apoptotic effect of mGIMAP8 at least on anisomycin-induced programmed cell death using overexpression in two different cell lines. If mGIMAP8 also modifies the response to other pro-apoptotic stimuli will be evaluated in a stably transfected cell line we are currently creating. The anti-apoptotic effect of mGIMAP8 is consistent with findings showing a role for other GIMAP GTPases in apoptotic processes. First, a pro-apoptotic role of mGIMAP1 is suggested by its upregulation during p53-induced apoptosis in the mouse myeloid leukemia cell line LTR6 [Kannan et al., 2001] and extremely low expression in several cancer cell lines [Stamm et al., 2002]. Remarkably, expression of m*gimap1* is strongly induced in the spleen during malaria infections in mice [Krücken

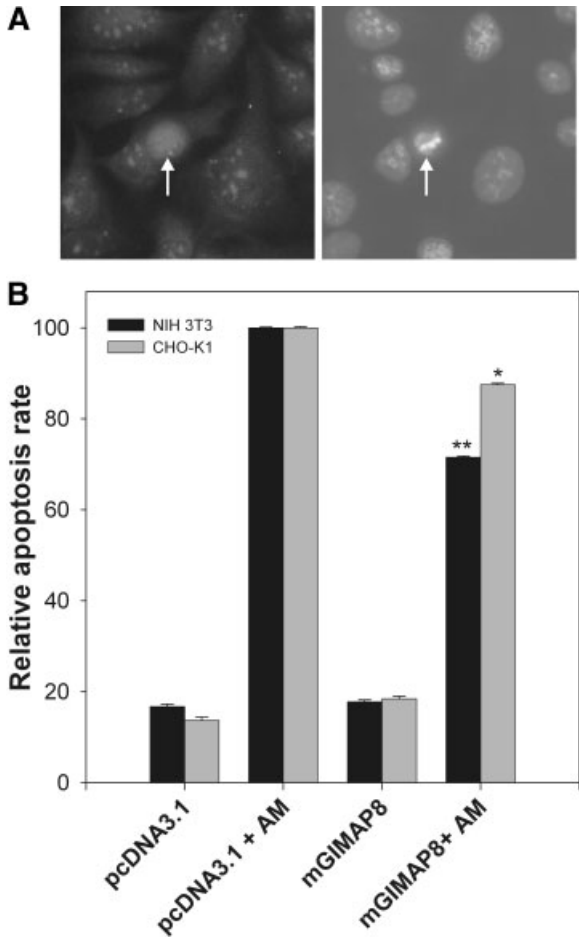

Fig. 6. Modulation of apoptosis rate by mGIMAP8. **A**: Apoptotic cells were detected by nuclear localization of an EYFP fusion protein expressed by the pCaspase3-Sensor vector. Cells were fixed with methanol and nuclei were counterstained with DAPI. A typical result for NIH 3T3 cells is shown. Bars represent 50 μm. **B**: NIH 3T3 and CHO-K1 cells were transfected with equal amounts of pCaspase-Sensor and either an mGIMAP8 expression vector (mGIMAP8) or the empty expression vector without insert (pcDNA3.1). After 24 h, cells were treated with anisomycin (AM) or vehicle, fixed and stained with DAPI before apoptotic cells were counted. Four independent experiments with a total number of about 2,500–4,000 transfected cells in each group were combined. The frequency of apoptosis in anisomycin-treated cells transfected with empty vector was set to 100% for each cell line. Error bars represent standard error between independent proportions.

et al., 1997, 1999] when a significant number of spleen cells become apoptotic [Helmby et al., 2000; Wunderlich et al., 2005]. Second, a mutation in rat *gimap3* causes T-cell lymphopenia due to apoptosis of recent thymic emigrants [Hornum et al., 2002; MacMurray et al., 2002; Michalkiewicz et al., 2004] confirming an anti-apoptotic function of GIMAP3. Indeed, rGIMAP3 is necessary for mitochondrial integrity and survival of T-cells [Pandarpurkar et al., 2003]. Third, the closely related human GIMAP5 exerts a strong inhibitory effect on apoptosis induced by okadaic acid and γ-radiation but not on that induced by several other agents [Sandal et al., 2003].

The mGIMAP8 protein is situated at ER, Golgi, and mitochondria, presumably at the cytoplasmic site of the membrane. While GIMAP3 was unequivocally found at mitochondria [Dahéron et al., 2001; Pandarpurkar et al., 2003], Sandal et al. [2003] localized the closely related hGIMAP5 at the ER and Golgi but not at mitochondria whereas mitochondrial localization of the same protein was reported by Zenz et al. [2004]. ER and mitochondria are the most important intracellular stores of Ca^{2+}, and hGIMAP5 selectively inhibits apoptotic pathways that share a calcium calmodulin kinase II-dependent step [Sandal et al., 2003].

In addition to their role in Ca^{2+} signaling, ER and mitochondria harbor several pro- and anti-apoptotic regulatory proteins [Breckenridge et al., 2003] and Ca^{2+} release from ER contributes to activation of caspases [Rizzuto et al., 2003]. Indeed, an intricate interplay between ER and mitochondria has been described to initiate apoptosis in response to ER stress, ceramide, ionizing radiation, and overexpression of BAX [Breckenridge et al., 2003]. Proteins like BCL-2, BCL-x_L, BAX, BAK [Breckenridge et al., 2003], GIMAP8, and also GIMAP5, which are found in both compartments are obvious candidates for regulation of such processes. For instance, ER-localized BCL-2 is selectively able to inhibit those apoptotic processes that involve mitochondrial depolarization prior to cytochrom c release [Breckenridge et al., 2003] and T-cell apoptosis due to loss of rGIMAP3 is also associated with mitochondrial depolarization [Pandarpurkar et al., 2003], implicating GIMAP proteins in similar regulatory circuits as BCL-2 family members.

Expression of m*gimap8* is predominantly found in the thymus. This implicates a function of m*gimap8* in selection of thymocytes as previously shown for m*gimap4* [Poirier et al., 1999]. In contrast to m*gimap4*, however, only a very low expression of m*gimap8* was found in the spleen indicating that m*gimap8* might play only a minor role for the physiology of peripheral T-cells. Nevertheless, a function of GIMAP8 in peripheral T-cells cannot be excluded since mRNA levels of h*gimap8* in the spleen are much higher than in the thymus

[Krücken et al., 2004]. In the spleen, there is a strong upregulation of m*gimap1* mRNA during *P. chabaudi* malaria [Krücken et al., 1997, 1999] and increased levels of m*gimap1* mRNA described in context with p53-induced apoptosis [Kannan et al., 2001] suggest a pro-apoptotic function of mGIMAP1. By contrast, m*gimap8* is downregulated in the spleen during *P. chabaudi* malaria and has clearly anti-apoptotic effects. These opposing properties of m*gimap1* and m*gimap8* suggest that several GIMAP GTPases exert complex control mechanism on cellular survival during malaria and probably also during other infections.

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