Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 481–486
BBRC
www.elsevier.com/locate/ybbrc

GIMAP5 regulates mitochondrial integrity from a distinct subcellular compartment

Mamadou Keita [a], Chantal Leblanc [a], David Andrews [b], Sheela Ramanathan [a,*]

[a] *Immunology Division, Department of Pediatrics, FMSS, University of Sherbrooke, 3001-12th Avenue North, Sherbrooke, Que., Canada J1H5N4*
[b] *Departments of Biochemistry and Biomedical Sciences and Medicine, McMaster University, Hamilton, Ont., Canada L8N3Z5*

Received 10 July 2007
Available online 23 July 2007

Abstract

Spontaneous apoptosis of T lymphocytes results in marked lymphopenia in the Biobreeding diabetes-prone (BB-DP) rat leading to the development of autoimmune type 1 diabetes. The lymphopenia phenotype in these rats is linked to the *lyp* locus. The *lyp* allele harbors a frameshift mutation within the gene encoding 'GTPase of immunity-associated nucleotide binding protein 5' (GIMAP5). Mechanisms underlying the pro-survival function of GIMAP5 in T lymphocytes are unclear. Overexpression studies have shown that GIMAP5 localizes within mitochondria and the endoplasmic reticulum (ER). We have used an antiserum raised against GIMAP5 to define its localization in rat primary T lymphocytes. We present evidence that endogenous GIMAP5 is associated with a sedimentable subcellular fraction that is distinct from mitochondria and the ER. These data are further supported by confocal microscopy using a GIMAP5 construct with an intact C-terminal membrane anchor. Nonetheless, T cells isolated from *GIMAP5*$^{lyp/lyp}$ rats display rapid loss of mitochondrial membrane potential. Our findings suggest that GIMAP5 regulates T lymphocyte survival by mechanisms that operate upstream of mitochondria.

Keywords: GIMAP5; Lymphopenia; BB-DP rats; Type 1 diabetes; Mitochondria; T cells

Biobreeding diabetes-prone (BB-DP) strain of rats develops insulin dependent autoimmune type 1 diabetes that is associated with RT1u haplotype of the MHC class II locus and a second locus designated as *lyp* on chromosome 4 [1–3]. Homozygosity for the *lyp* mutation (*lyp/lyp*) in BB-DP rats causes a 5-to10-fold reduction in the number of T cells in secondary lymphoid organs [4,5]. The *lyp* mutation affects all T cell lineages [6–8]. Lymphopenia caused by the *lyp* mutation is essential for diabetes induction in BB-DP rats [1]. We and others have shown that T lymphocytes in BB-DP rats display a short life span and die by apoptosis unless they are activated by antigen [9–12].

The *lyp* allele arises from a frameshift mutation within the gene encoding 'GTPase of immunity-associated nucleotide binding protein 5' (GIMAP5) [13,14]. The mutation introduces a premature stop codon resulting in a truncated protein (Fig. 1A) Homologues of rat GIMAP5 (rGIMAP5) are expressed in mouse and human T cells [13,14]. The GIMAP family of proteins is characterized by an AIG1 domain that contains an unconventional GTP-binding motif and a coiled-coil domain [15,16]. In addition, some GIMAP proteins (GIMAP1, 3, and 5) possess a C-terminal hydrophobic domain that could serve to target them to intracellular membranes [17–19]. In fact, overexpression studies have shown that GIMAP5 localizes in mitochondria, endoplasmic reticulum (ER), the Golgi apparatus, and centrosomes [20–22].

Abbreviations: BB-DP, Biobreeding diabetes-prone; ER, endoplasmic reticulum; GIMAP5, GTPase of immunity-associated nucleotide binding protein 5; rGIMAP5, rat GIMAP5; IAN5, immune associated nucleotide-binding protein 5; TMRE, tetramethylrhodamine ethyl ester.

* Corresponding author. Fax: +1 819 564 5215.
E-mail address: Sheela.Ramanathan@Usherbrooke.ca (S. Ramanathan).

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.07.048

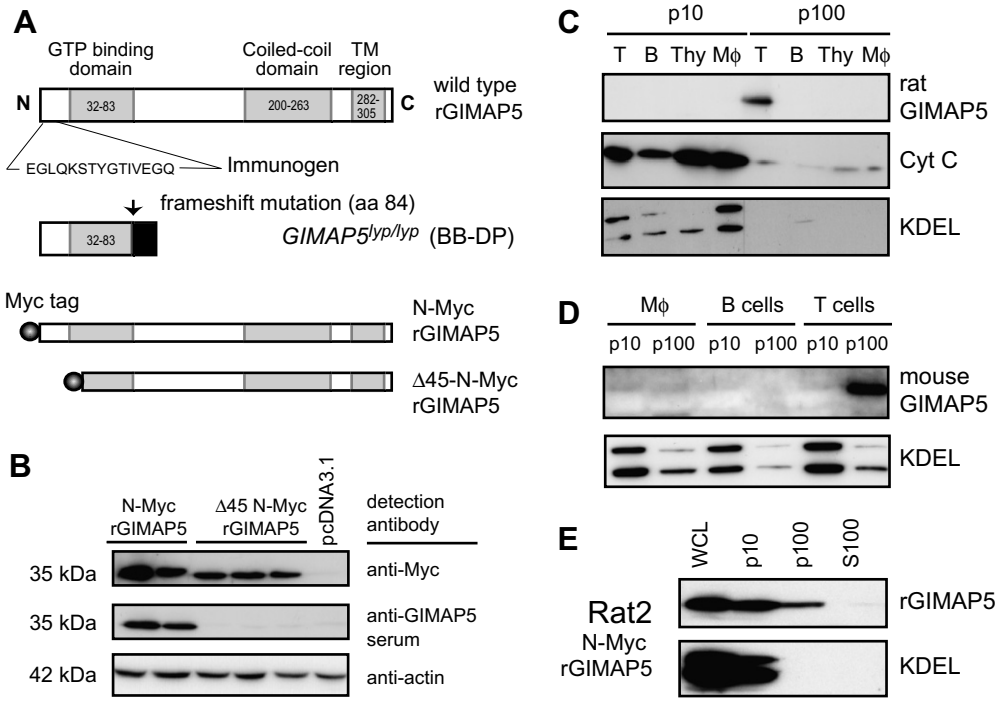

Fig. 1. Expression of GIMAP5 in rodent T cells. (A) rGIMAP5 shares with other GIMAP members an unconventional GTP binding domain, a coiled-coil region, and a putative transmembrane region. In BB-DP rats, a frameshift mutation (lyp) at codon 84 truncates the protein. A 14-amino acid peptide of rGIMAP5 (GenBank Accession No. AY055776) coupled to KLH was used to immunize rabbits. Constructs of full-length and truncated rGIMAP5 (N-Myc-rGIMAP5 and Δ45-N-Myc-rGIMAP5), both carrying a Myc epitope tag at the N-terminus, were used to transfect Rat2 cells. (B) Rat2 fibroblasts were transfected with N-Myc-rGIMAP5, Δ45-N-Myc-rGIMAP5 or the empty vector and stable cell lines were established. Cell lysates were analyzed by Western blot using anti-Myc or rGIMAP5 antiserum as shown. (C,D) P10 and P100 fractions obtained from T and B lymphocytes, thymocytes (Thy) or peritoneal macrophages (Mφ) of normal (ACI.1u *gimap5*$^{+/+}$) rats (C) and C57Bl/6 mice (D) were evaluated for the expression of GIMAP5 protein, mitochondrial cytochrome c or GRP94 and GRP96 proteins containing the ER-retaining KDEL sequence. (E) P10 and P100 fractions of Rat2 fibroblasts overexpressing N-Myc-rGIMAP5 were analyzed for the distribution of GIMAP5.

Consistent with the pro-survival function of GIMAP5 in rat T lymphocytes, overexpressed hGIMAP5 confers protection against apoptosis induced by okadaic acid and gamma-radiation in HEK293T cells [21]. However, T lymphocytes from BB-DP rats do not show increased sensitivity to gamma radiation [23], whereas siRNA-mediated knockdown of mIAN5 increases apoptosis of 23-1-8 T cells caused by IL-2 withdrawal [18]. Surprisingly, overexpression of wild type or the mutant BB-DP allele of rGIMAP5 also induces spontaneous apoptosis of T cells [20].

The mechanisms by which GIMAP5 regulates the survival of T lymphocytes remain unclear. Whereas overexpression studies have significantly contributed to molecular characterization of GIMAP5, investigations on the expression and functions of endogenous GIMAP5 protein are scanty [24]. Furthermore, most overexpression studies have used GIMAP5 constructs that carried an epitope tag or EGFP fused to the C-terminus [18,20–22], a modification that can potentially interfere with the putative membrane targeting sequence. As a first step to elucidate the mechanisms underlying the pro-survival function of endogenous GIMAP5 we have generated an antiserum against rGIMAP5 raised in the rabbit. In parallel, we have cloned a rGIMAP5 construct with a Myc epitope tag at the N-terminus to minimize possible interference with its subcellular localization. Our data indicate that rGIMAP5 is present outside the ER and mitochondria in a distinct sedimentable subcellular fraction, yet it regulates mitochondrial membrane integrity.

Materials and methods

Animals. C57BL/6 mice were purchased from Charles River Canada. ACI.1u *gimap5*$^{lyp/lyp}$ rats and ACI.1u *gimap5*$^{+/+}$ control rats were kindly provided by Dr. P. Poussier (University of Toronto, Canada). All experiments involving animals were approved by the Institutional Ethics Committee.

Antibodies and reagents. Antiserum against GIMAP5 protein was generated by immunizing rabbits with a 14-amino acid long peptide at the N-terminus of rGIMAP5 (Fig. 1A). Anti-Myc Ab was from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Anti-Rat CD3-FITC and organelle specific antibodies were purchased from BD Biosciences (Mississauga, ON, Canada). Fluorescent secondary antibodies, mitotracker, and tetramethylrhodamine ethyl ester (TMRE) were obtained from Molecular Probes (Invitrogen, Carlsbad, CA).

Cloning and stable expression. rGIMAP5 was cloned from cDNA prepared from rat T lymphocytes and subcloned into the pCDNA3.1 vector either as wild type protein or lacking first 45 amino acids. Both clones carried a Myc epitope tag at the N-terminus (Fig. 1A). Rat2 fibroblasts were transfected with either one of these constructs or with the empty vector. Stable lines expressing the wild type and the truncated proteins were established by G418 drug selection (Fig. 1B).

Cell isolation, subcellular fractionation, and Western blot. Total thymocytes, rat and mouse T and B lymphocytes purified by magnetic cell sorting (Militenyi Biotech, USA), and peritoneal macrophages were used for subcellular fractionation. All procedures, illustrated in Fig. 2A, were carried out at 4 °C. The cells were resuspended at 3×10^7/mL density in a lysis buffer (250 mM sucrose, 20 mM Hepes, pH 7.3, 2 mM $MgCl_2$, 1 mM EGTA, and 1 mM DTT) containing protease inhibitors [25]. The cell suspensions were lysed by the nitrogen cavitation method (Parr Instrument Co., Moline, IL) at 300 psi for 20 min. Lysates generated by slow release of pressure were spun at 1000*g* to remove nuclei and unbroken cells. The postnuclear supernatants were centrifuged at 10,000*g* for 15 min to obtain supernatant (S10) and pellet (P10) fractions. The S10 fraction was centrifuged at 100,000*g* for 1 h in a Beckman Optima analytical ultracentrifuge using a MLS50 rotor to generate pellet (P100) and supernatant (S100) fractions. Alternatively, the S10 fraction (0.5 mL) was placed over a discontinuous gradient of 38%, 30%, and 20% sucrose and centrifuged at 100,000*g* for 1 h [26]. One milliliter fractions were collected from the top and precipitated with equal volume of 20% trichloroacetic acid. For Western blot analysis, subcellular fractions representing equivalent number of cells were separated by denaturing SDS–PAGE, transferred to Immobilon membrane (Millipore, USA), and probed with relevant antibodies.

Cell culture and confocal microscopy. Rat2 fibroblasts were maintained in DMEM supplemented with 10% serum at 37 °C in 5% CO_2 incubator. Rat2 fibroblasts expressing N-Myc-rGIMAP5 were grown on glass coverslips overnight and fixed with 4% paraformaldehyde in PBS. The cells were permeabilized by treatment with 0.1% Triton X-100 (PBS–TX) in PBS, blocked with 5% evaporated skimmed milk in PBS–TX, and incubated serially with primary and secondary antibodies diluted in the blocking buffer. Confocal microscopy was carried out using an Olympus 1X81 FV1000 microscope. Images were processed using the Olympus Flouoview software.

Measurement of mitochondrial membrane potential. LN cells from ACI.1u *gimap5*$^{+/+}$ and ACI.1u *gimap5*$^{-/-}$ rats, freshly isolated or incubated at 37 °C for 8 h, were loaded with TMRE for 15 min in dark. The dye-loaded cells were stained by anti-CD3-FITC and fluorescence signal was collected in FL1 (for CD3) and FL2 (for TMRE) channels using a FACScan flow cytometer. TMRE signal in gated T cells (CD3$^+$) was evaluated to determine the integrity of mitochondrial membrane.

Results and discussion

Expression of endogenous GIMAP5 in primary rat cells

Overexpressed human, rat, and mouse GIMAP5 have been shown to localize in mitochondria, ER, the Golgi apparatus, and centrosome [18,20–22]. Whether endogenous GIMAP5 protein displays similar cellular distribution remains unclear. Antibody against endogenous GIMAP5 is not available. We therefore immunized rabbits with a peptide (EGLQKSTYGTIVEGQ) corresponding to position 2–15 of rGIMAP5 (GenBank Accession No. AY055776) that is unique to GIMAP5 (Fig. 1A). The reactivity of anti-GIMAP5 antiserum was tested as follows. Rat2 fibroblasts were stably transfected with expression constructs of full-length rGIMAP5 (N-Myc-rGIMAP5) or Δ45-rGIMAP5 (Δ45-N-Myc-rGIMAP5) lacking the first 45 amino acids (Fig. 1A). Western blot analysis of the transfected cells revealed that the rGIMAP5 antiserum recognized a protein of 35 kDa corresponding to the full-length rGIMAP5 (Fig. 1B). As expected, the antiserum failed to recognize the truncated protein (Fig. 1B).

Transcripts of rGIMAP5 have been detected in CD4$^+$CD8$^+$ double positive (DP) immature thymocytes, CD4$^+$ and CD8$^+$ mature single positive (SP) thymocytes, and peripheral T lymphocytes, but not in B cells [23]. To test the expression of endogenous rGIMAP5, lysates of freshly isolated T cells from *gimap5*$^{+/+}$ rats were analyzed by Western blot using the anti-rGIMAP5 antiserum. rGIMAP5 protein was undetectable in whole cell lysates even after loading 5×10^6 cell equivalent of proteins (data not shown). To investigate whether the failure to detect endogenous rGIMAP5 resulted from a very low level of

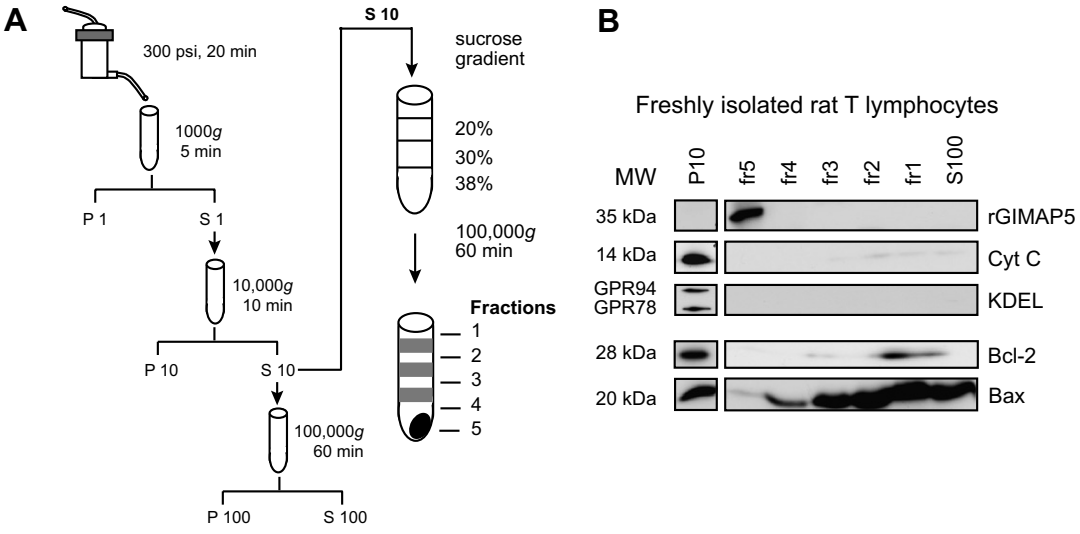

Fig. 2. Enrichment of GIMAP5 in a sedimentable subcellular compartment distinct from mitochondria and ER in primary rat T lymphocytes. (A) Freshly isolated rat T lymphocytes were lysed by the nitrogen cavitation method. The post-nuclear supernatant (S1) was centrifuged at 10,000*g* to obtain the P10 fraction. The S10 supernatant was centrifuged at 100,000*g* to obtain P100 and S100 fractions. Alternatively, the S10 fraction was subjected to discontinuous sucrose density gradient centrifugation to separate distinct subcellular fractions. (B) The various fractions were evaluated by Western blot for the distribution of GIMAP5, cytochrome c (Cyt c), KDEL sequence-containing proteins, Bcl-2 and Bax.

expression, we carried out subcellular fractionation to enrich organelles that may harbor rGIMAP5, as detailed in the Materials and methods section and in Fig. 2A. GIMAP5 was detected exclusively in the P100 fraction of T lymphocytes but not in thymocytes, macrophages or B cells, indicating that rGIMAP5 protein is constitutively expressed in rat peripheral T lymphocytes, which is consistent with its pro-survival function in these cells. Since DP thymocytes that express GIMAP5 mRNA [23] represent 90% of the total thymocyte population, the failure to detect GIMAP5 protein in thymocyte lysates (Fig. 1C) suggests that expression of rGIMAP5 protein may be subject to post-transcriptional regulation during maturation of T lymphocytes within the thymus.

Contrary to the published reports using overexpressed GIMAP5 [18,20–22], our results showed that endogenous GIMAP5 protein was not enriched in the endoplasmic reticulum (ER) or mitochondria. Protein markers of these organelles, GRP78 and GPR84, which contain the ER retention sequence KDEL, and cytochrome c were found predominantly in the P10 fraction, whereas GIMAP5 was found exclusively in the P100 fraction (Fig. 1C). Due to the very low cell yield from ACI.1u *gimap5*$^{lyp/lyp}$ rats, the presence or the absence of the truncated GIMAP5 protein could not be verified. The anti-rGIMAP5 antiserum also recognized the mouse and human GIMAP5 proteins in the P100 fraction of mouse T cells and Jurkat cells (Fig. 1D and data not shown). Our findings on the subcellular distribution of the endogenous GIMAP5 in rat T lymphocytes suggest that rGIMAP5 mediates its anti-apoptotic functions from outside of mitochondria and the ER.

Rat GIMAP5 is associated with a distinct cellular compartment in primary T cells

To characterize the subcellular location of rGIMAP5, the S10 fraction of rat T lymphocyte lysate was subjected to discontinuous sucrose density gradient centrifugation as depicted in Fig. 2A. The various fractions were analyzed by Western blot for the distribution of rGIMAP5 and organelle markers. As shown in Fig. 2B, cytochrome c and KDEL-containing protein markers were not found in any of the fractions. It has been shown previously that GIMAP5 physically interacted with Bcl-2 in co-immunoprecipitation assays [18]. A major proportion of Bcl-2 was found in the P10 fraction (Fig. 2B), consistent with its mitochondrial association [27]. A small amount of Bcl-2 was also found in fractions 1 and 2 of the sucrose gradient whereas rGIMAP5 was found exclusively in the pellet fraction (Fig. 2B). Bax was recovered throughout the gradient in soluble (fraction 1) and membrane-associated (fractions 2–4) fractions, although a barely detectable amount was associated with the pellet fraction containing rGIMAP5. These findings strongly indicate that endogenous rGIMAP5 is neither associated with mitochondria or ER, nor is a soluble protein. Our data are consistent with the interpretation that rGIMAP5 may be associated with a large molecular complex or with a low-density vesicular organelle.

rGIMAP5 is localized outside of the mitochondria and the ER

To examine whether the discrepancy in the subcellular distribution of endogenous and overexpressed GIMAP5 proteins was due to overexpression [18,20–22], Rat2 fibroblasts overexpressing N-Myc-rGIMAP5 were subjected to lysis using the nitrogen cavitation method. In the N-Myc-rGIMAP5 construct, the Myc tag was placed at the N-terminus to preserve the putative membrane anchor function of the C-terminus. Although a major fraction of the overexpressed rGIMAP5 was found in the P10 fraction containing the KDEL protein markers, a significant amount was also found in the P100 fraction that did not contain the KDEL proteins (Fig. 1E). These observations suggest that the recombinant rGIMAP5 with an intact C-terminal membrane anchor targets to its right cellular compartment, which is outside of mitochondria and the ER. Therefore, we suggest that the enrichment of overexpressed GIMAP5 in mitochondria, the ER, and other organelles (Fig. 1E) [18,20–22] may arise not only from perturbation of the membrane anchor by an epitope tag fused at the C-terminus but may also result from protein overexpression.

To further rule out the possibility that the cell fractionation procedure that we have employed in this study may disrupt the association of N-Myc-rGIMAP5 with mitochondria and the ER resulting in its relocation to the P100 fraction, we analyzed the expression of N-Myc-rGIMAP5 in Rat2 fibroblasts by confocal microscopy. Although N-Myc-rGIMAP5 showed enrichment in the subcellular fraction containing the ER proteins (Fig. 1E), the results of confocal microscopy were inconsistent with co-localization in this cellular organelle (Fig. 3). N-Myc-rGIMAP5 did not localize in the ER, mitochondria or the Golgi complex whereas a clear localization of Bcl-2 in mitochondria was readily observed (Fig. 3). In addition, N-Myc-GIMAP5 did not show significant co-localization with Bcl-2 or Bax (Fig. 3 and data not shown). These findings further support our contention that overexpressed rGIMAP5 protein with an unmodified C-terminus behaved similarly to the endogenous rGIMAP5 and therefore did not localize in the ER or mitochondria.

Loss of mitochondrial membrane potential in the absence of GIMAP5

T lymphocytes generated in BB-DP rats lacking functional GIMAP5 die rapidly *in vivo* following their maturation in the thymus in a caspase independent manner [4]. To investigate whether the lack of GIMAP5 impaired mitochondrial functions, we evaluated mitochondrial membrane potential in T lymphocytes isolated from *gimap5*$^{lyp/lyp}$ rats in which a frameshift mutation of the

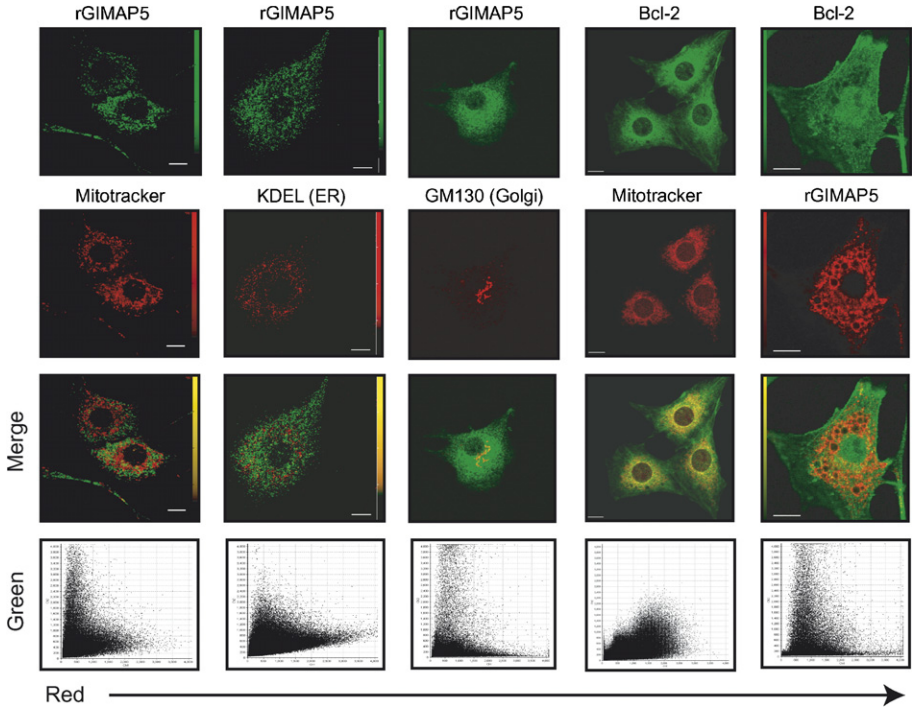

Fig. 3. rGIMAP5 does not co-localize with mitochondria, ER or Bcl-2. A stable Rat2 fibroblast line expressing N-Myc-rGIMAP5, grown on glass coverslips, was fixed and stained for rGIMAP5 using anti-Myc Ab, along with specific markers for the indicated organelles (A) or Bcl-2 (B). The green fluorescence and the red fluorescence images were superimposed to obtain the merged image. Overlap between mitotracker and Bcl-2 served as a positive control. A quantitative measure of signal overlap is used to assess the distribution of GIMAP5 in the organelles. (For interpretation of color in this figure legend, the reader is referred to the web version of this article.)

GIMAP5 reading frame introduces a premature stop codon [13,14] (Fig. 1A). Whereas T lymphocytes from control *gimap5*$^{+/+}$ rats showed intact mitochondrial membrane potential as revealed by the loss of the TMRE dye only in less than 5% of cells that did not augment even after 8-h incubation *in vitro*, freshly isolated T cells from *gimap5*$^{lyp/lyp}$ rats already contained more than 16% of the cell population that failed to retain the TMRE. The percentage of TMRE-negative cells increased to 26% after 8 h of incubation (Fig. 4). These observations indicated that even though rGIMAP5 neither localizes in mitochondria nor associates with Bcl-2, it is required to maintain the mitochondrial membrane integrity in rat T lymphocytes.

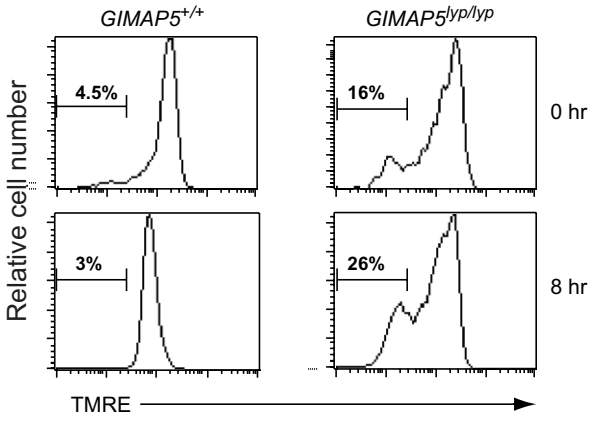

Fig. 4. Absence of rGIMAP5 results in the loss of mitochondrial membrane potential in primary rat T lymphocytes. Lymph node cells isolated from ACI.1u *gimap5*$^{+/+}$ and ACI.1u *gimap5*$^{lyp/lyp}$ rats, freshly isolated or after incubation at 37 °C for 8 h, were loaded with TMRE for 15 min in the dark. The dye-loaded cells were stained by anti-CD3-FITC and analyzed by flow cytometry. TMRE signal in gated T cells (CD3^{+}) was evaluated to determine the integrity of mitochondrial membrane. The values represent the percentage of cells that have lost the mitochondrial potential and thus fail to retain TMRE.

Conclusions

Based on the lymphopenic phenotype of the BB-DP rat, a pro-survival function has been attributed to rGIMAP5 [16]. Overexpression studies have shown that GIMAP5 confers resistance to apoptosis induced by okadaic acid, and is localized in mitochondria and the ER. Another study has shown that overexpressed GIMAP5 promotes apoptosis, raising the possibility that differential subcellular localization may alter the normal function of a pro-survival protein as has been shown for the ER-targeted Bcl-2 [25]. Our results strongly suggest that the molecular basis of the pro-survival function of rGIMAP5 in T lymphocytes is more complex than the mechanisms proposed based on protein overexpression studies. It also remains unclear how mitogenic activation overcomes the requirement for GIMAP5 and delays apoptosis of *gimap5*$^{lyp/lyp}$ (BB-DP) rat T cells [9,20]. Our findings that rGIMAP5 regulates mitochondrial membrane potential despite residing in a

subcellular compartment distinct from mitochondria suggest that GIMAP5 regulates T cell survival upstream of mitochondria.

Acknowledgments

This work was supported by the Natural Sciences and Engineering Research Council of Canada Discovery Grant to S.R (312777-05). We thank Dr. Philippe Poussier for the generous supply of rats and Dr. Subburaj Ilangumaran and Dr. Gilles Dupuis for helpful discussions.

References

[1] R. Jackson, N. Rassi, T. Crump, B. Haynes, G.S. Eisenbarth, The BB diabetic rat. Profound T-cell lymphocytopenia, Diabetes 30 (1981) 887–889.

[2] H.J. Jacob, A. Pettersson, D. Wilson, Y. Mao, A. Lernmark, E.S. Lander, Genetic dissection of autoimmune type I diabetes in the BB rat, Nature Genetics 2 (1992) 56–60.

[3] E. Colle, R.D. Guttmann, T. Seemayer, Spontaneous diabetes mellitus syndrome in the rat. I. Association with the major histocompatibility complex, The Journal of Experimental Medicine 154 (1981) 1237–1242.

[4] S. Ramanathan, P. Poussier, BB rat lyp mutation and Type 1 diabetes, Immunological Reviews 184 (2001) 161–171.

[5] P. Poussier, A.F. Nakhooda, J.A. Falk, C. Lee, E.B. Marliss, Lymphopenia and abnormal lymphocyte subsets in the "BB" rat: relationship to the diabetic syndrome, Endocrinology 110 (1982) 1825–1827.

[6] P. Poussier, T. Ning, T. Murphy, D. Dabrowski, S. Ramanathan, Impaired post-thymic development of regulatory CD4+25+T cells contributes to diabetes pathogenesis in BB rats, Journal of Immunology 174 (2005) 4081–4089.

[7] S. Ramanathan, L. Marandi, P. Poussier, Evidence for the extrathymic origin of intestinal TCRgammadelta(+) T cells in normal rats and for an impairment of this differentiation pathway in BB rats, Journal of Immunology 168 (2002) 2182–2187.

[8] N.N. Iwakoshi, I. Goldschneider, F. Tausche, J.P. Mordes, A.A. Rossini, D.L. Greiner, High frequency apoptosis of recent thymic emigrants in the liver of lymphopenic diabetes-prone BioBreeding rats, Journal of Immunology 160 (1998) 5838–5850.

[9] S. Ramanathan, K. Norwich, P. Poussier, Antigen activation rescues recent thymic emigrants from programmed cell death in the BB rat, Journal of Immunology 160 (1998) 5757–5764.

[10] G. Hernandez-Hoyos, S. Joseph, N.G. Miller, G.W. Butcher, The lymphopenia mutation of the BB rat causes inappropriate apoptosis of mature thymocytes, European Journal of Immunology 29 (1999) 1832–1841.

[11] H. Groen, F.A. Klatter, N.H. Brons, A.S. Wubbena, P. Nieuwenhuis, J. Kampinga, High-frequency, but reduced absolute numbers of recent thymic migrants among peripheral blood T lymphocytes in diabetes-prone BB rats, Cellular Immunology 163 (1995) 113–119.

[12] H.H. Zadeh, D.L. Greiner, D.Y. Wu, F. Tausche, I. Goldschneider, Abnormalities in the export and fate of recent thymic emigrants in diabetes-prone BB/W rats, Autoimmunity 24 (1996) 35–46.

[13] L. Hornum, J. Romer, H. Markholst, The diabetes-prone BB rat carries a frameshift mutation in Ian4, a positional candidate of Iddm1, Diabetes 51 (2002) 1972–1979.

[14] A.J. MacMurray, D.H. Moralejo, A.E. Kwitek, E.A. Rutledge, B. Van Yserloo, P. Gohlke, S.J. Speros, B. Snyder, J. Schaefer, S. Bieg, J. Jiang, R.A. Ettinger, J. Fuller, T.L. Daniels, A. Pettersson, K. Orlebeke, B. Birren, H.J. Jacob, E.S. Lander, A. Lernmark, Lymphopenia in the BB rat model of type 1 diabetes is due to a mutation in a novel immune-associated nucleotide (Ian)-related gene, Genome Research 12 (2002) 1029–1039.

[15] J. Krucken, R.M. Schroetel, I.U. Muller, N. Saidani, P. Marinovski, W.P. Benten, O. Stamm, F. Wunderlich, Comparative analysis of the human gimap gene cluster encoding a novel GTPase family, Gene 341 (2004) 291–304.

[16] T. Nitta, Y. Takahama, The lymphocyte guard-IANs: regulation of lymphocyte survival by IAN/GIMAP family proteins, Trends in Immunology 28 (2007) 58–65.

[17] L. Daheron, T. Zenz, L.D. Siracusa, C. Brenner, B. Calabretta, Molecular cloning of Ian4: a BCR/ABL-induced gene that encodes an outer membrane mitochondrial protein with GTP-binding activity, Nucleic Acids Research 29 (2001) 1308–1316.

[18] T. Nitta, M. Nasreen, T. Seike, A. Goji, I. Ohigashi, T. Miyazaki, T. Ohta, M. Kanno, Y. Takahama, IAN family critically regulates survival and development of T lymphocytes, PLoS Biology 4 (2006) e103.

[19] O. Stamm, J. Krucken, H.P. Schmitt-Wrede, W.P. Benten, F. Wunderlich, Human ortholog to mouse gene imap38 encoding an ER-localizable G-protein belongs to a gene family clustered on chromosome 7q32-36, Gene 282 (2002) 159–167.

[20] U. Dalberg, H. Markholst, L. Hornum, Both Gimap5 and the diabetogenic BBDP allele of Gimap5 induce apoptosis in T cells, International Immunology 19 (2007) 447–453.

[21] T. Sandal, L. Aumo, L. Hedin, B.T. Gjertsen, S.O. Doskeland, Irod/Ian5: an inhibitor of gamma-radiation- and okadaic acid-induced apoptosis, Molecular Biology of the Cell 14 (2003) 3292–3304.

[22] T. Zenz, A. Roessner, A. Thomas, S. Frohling, H. Dohner, B. Calabretta, L. Daheron, hIan5: the human ortholog to the rat Ian4/Iddm1/lyp is a new member of the Ian family that is overexpressed in B-cell lymphoid malignancies, Genes and Immunity 5 (2004) 109–116.

[23] C. Dion, C. Carter, L. Hepburn, W.J. Coadwell, G. Morgan, M. Graham, N. Pugh, G. Anderson, G.W. Butcher, J.R. Miller, Expression of the Ian family of putative GTPases during T cell development and description of an Ian with three sets of GTP/GDP-binding motifs, International Immunology 17 (2005) 1257–1268.

[24] M. Pandarpurkar, L. Wilson-Fritch, S. Corvera, H. Markholst, L. Hornum, D.L. Greiner, J.P. Mordes, A.A. Rossini, R. Bortell, Ian4 is required for mitochondrial integrity and T cell survival, Proceedings of the National Academy of Sciences of the United States of America 100 (2003) 10382–10387.

[25] M.G. Annis, N. Zamzami, W. Zhu, L.Z. Penn, G. Kroemer, B. Leber, D.W. Andrews, Endoplasmic reticulum localized Bcl-2 prevents apoptosis when redistribution of cytochrome c is a late event, Oncogene 20 (2001) 1939–1952.

[26] J. Vidugiriene, A.K. Menon, Early lipid intermediates in glycosylphosphatidylinositol anchor assembly are synthesized in the ER and located in the cytoplasmic leaflet of the ER membrane bilayer, The Journal of Cell Biology 121 (1993) 987–996.

[27] S. Krajewski, S. Tanaka, S. Takayama, M.J. Schibler, W. Fenton, J.C. Reed, Investigation of the subcellular distribution of the bcl-2 oncoprotein: residence in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes, Cancer Research 53 (1993) 4701–4714.