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Jakmip1 Is Expressed upon T Cell Differentiation and Has an Inhibitory Function in Cytotoxic T Lymphocytes

Valentina Libri,*‡ Dörte Schulte,* Amber van Stijn,§ Josiane Ragimbeau,* Lars Rogge,† and Sandra Pellegrini2*†

Jakmip1 belongs to a family of three related genes encoding proteins rich in coiled-coils. Jakmip1 is expressed predominantly in neuronal and lymphoid cells and colocalizes with microtubules. We have studied the expression of Jakmip1 mRNA and protein in distinct subsets of human primary lymphocytes. Jakmip1 is absent in naive CD8+ and CD4+ T lymphocytes from peripheral blood but is highly expressed in Ag-experienced T cells. In cord blood T lymphocytes, induction of Jakmip1 occurs upon TCR/CD28 stimulation and parallels induction of effector proteins, such as granzyme B and perforin. Further analysis of CD8+ and CD4+ T cell subsets showed a higher expression of Jakmip1 in the effector CCR7+ and CD27-T cell subpopulations. In a gene expression follow-up of the development of CMV-specific CD8+ response, Jakmip1 emerged as one of the most highly up-regulated genes from primary infection to latent stage. To investigate the relationship between Jakmip1 and effector function, we monitored cytotoxicity of primary CD8+ T cells silenced for Jakmip1 or transduced with the full-length protein or the N-terminal region. Our findings point to Jakmip1 being a novel effector memory gene restraining T cell-mediated cytotoxicity. 

Immunological memory is an essential trait of the adaptive immune system as it enables a rapid and robust response to previously encountered pathogens. Indeed, memory T lymphocytes have a lower threshold of activation than naive cells, less stringent requirements, and higher effector potential (1, 2). Following Ag encounter in the lymph node, naive T cells undergo clonal expansion and progressively leave the lymph node to reach peripheral inflamed tissues (3, 4), sequentially losing the lymph node homing receptors CCR7 and CD62L and the costimulatory requirements, and higher effector potential (1, 2). Fol-

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receptor. We identified Jakmip1 as a partner of Tyk2, a member of the Janus tyrosine kinase family (19). Jakmip1 belongs to a family of three related genes conserved in vertebrates. It encodes a 73 kDa coiled-coil protein with a highly basic N-terminal region that targets the protein on microtubules and a C-terminal regulatory region that can be posttranslationally modified by serine/threonine phosphorylation. Imaging studies of endogenous Jakmip1 in the Jurkat T cell line revealed a prominent localization along microtubules and at the microtubule organizing center (19).

In this study, we have investigated the expression of Jakmip1 in distinct subsets of human primary T lymphocytes. We provide evidence that Jakmip1 is maximally expressed in the terminally differentiated human CD8$^+$ TEMRA subset described as possessing the highest effector potential. Through functional studies, performed in primary CD8$^+$ T cells and based on lentiviral-mediated silencing of Jakmip1 or ectopic expression of its N-terminal region, we demonstrate that Jakmip inhibits target cell killing.

Materials and Methods

Purification and stimulation of T cells

Blood samples from healthy donors were obtained from Etablissement Français du Sang, Paris, France in accordance with a convention signed with the Institut Pasteur. Studies with material of human origin have been reviewed and approved by appropriate institutional review committees. Human peripheral blood and cord blood mononuclear cells were isolated by Ficoll density gradient centrifugation. CD4$^+$ and CD8$^+$ T cells were purified by positive selection with anti-CD8 and anti-CD4 paramagnetic clone CD28.2). For lentiviral transduction, CD8 T cells were replaced with fresh medium supplemented with IL-2, which was re-added 24 h before the experiment. Transduction efficiency was measured by FACS.

Generation of CD8$^+$ T cell lines

Freshly purified CD8$^+$ T lymphocytes were activated with Dynabeads and transduced with FEIGW empty, FEIGW-Jakmip1 full length or FEIGW-N-terminal. The percentage of EGFP+ cells varied from 50% for empty vector, <7% for full length, and 15% for N-terminal transduced cells. EGFP+ cells from the three transductions were sorted and amplified by three cycles of stimulation (11–13 days each) with anti-CD3 (OKT3 0.5 μg/ml) and PBMC irradiated with 40 Gy γ-radiation. The medium was supplemented with IL-2 (5 ng/ml), IL-7 (10 ng/ml), and IL-15 (5 ng/ml) whose concentration was tapered before re-stimulation. The three cytokines were from R&D Systems.

Flow cytometric analyses and sorting

The following mAbs (BD Biosciences) were used for flow cytometry: anti-CD3-PE, CD8-Alexa487, CD4-FITC, CCR7-PE, CD27-PE, CD45RA- FITC, CD45RO-PE, and CD28-PE. Stained cells were analyzed by using FACSscan or FACSSorter (BD Biosciences) and FCS express software (Tree Star). Memory subsets were sorted using FACSaria (BD Biosciences). Immediate re-analysis of the isolated populations revealed on average >96% purity. FACS analysis and sorting were performed on gated CD8$^+$ bright cells, allowing the exclusion of residual contaminating NK cells in the sorted populations.

Microarray analysis

Tetramer complexes. Allophycocyanin-conjugated HLA-A2 tetramer loaded with the CMV pp65-derived NLVPTGGMATV peptide and allophycocyanin-conjugated HLA-B7 tetramer loaded with the CMV pp65-derived TPRVTGGGAM peptide were obtained from Sanquin.

Cells. Isolated PBMC were cryopreserved in liquid nitrogen until the day of analysis. To isolate naive T cells, CD8$^+$ were isolated by positive selection with microbeads and stored overnight at 4°C in 10% NBCS serum containing medium. CD8$^+$ T cells were labeled with CD27- FITC (7C9, home-made), CD45RA-RD1 (Beckman Coulter) and CD8-allophycocyanin (BD Pharmingen) and FACS sorted in naive CD8$^+$ T cells (CD8$^+$ CD45RA$^+$CD27$^+$). To isolate CMV-specific CD8$^+$ effector cells at the peak of the CMV response, PBMC were stained with HLA-DR-FITC, CD38-PE (BD Biosciences), and CD8-allophycocyanin (BD Pharmingen). HLA-A2-DQ9 (DQ8)CD8$^+$ cells were sorted using FACSaria. To obtain CMV-specific CD8$^+$ cells in the latency phase, PBMC obtained 40–60 wk after transplantation were stained with allophycocyanin-conjugated tetramers and subsequently allophycocyanin microbeads (Miltenyi Biotec) were used to isolate the cells. Similarly, tetramer-binding cells from healthy blood donors were included to represent the long-term latency CMV-specific cells. Upon re-analysis the purified populations contained between 95 and 97% tetramer-binding cells.

RNA processing for microarray analysis. mRNA was amplified using the MessageAmp II kit (Ambion). Labeling, hybridization, and data extraction were performed at ServiceXS. Hybridization was performed on two-color Human Whole Genome 44K Oligo Microarrays from Agilent Technologies.

Microarray imaging and data analysis. The microarray slides were scanned using the Agilent dual laser DNA microarray scanner. Default settings of Agilent Feature Extraction preprocessing protocols were used to obtain normalized expression values from the raw scans. Exact protocol and scanner settings are described in the Agilent Feature Extraction Software User Manual 7.5 (http://chem.agilent.com/scripts/LiteraturePDF.asp?iWHD=37629). The default Agilent normalization procedure, called Linear & Lowess, was applied. Rosetta Resolver (Rosetta Biosoftware) was used for analysis of the data. Complete datasets in Ref. 30. Microarray data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) and are accessible through GEO Series accession GSE12589.

Quantification of mRNA levels. Total RNA was purified with RNeasy columns (Qiagen). Reverse transcription were primed with random primers and performed using the MLV reverse transcriptase (Invitrogen). The mRNA levels of Jakmip1, granzyme B, perforin, IFN-γ, and Tyk2 were determined by real-time quantitative PCR (RT-qPCR) with TaqMan probes using a protocol provided by the manufacturer (Applied Biosystems). Each PCR was run in triplicate and normalized to 18S RNA amplification levels in each sample and calculated relative to expression of the target gene in unstimulated (naive) T cells.

Protein analysis. For Western blot, ∼1 × 10$^6$ of cells were lysed in 50 μl of RIPA buffer and protease inhibitors. Blots were incubated first with
Results

Jakmip1 is absent in naive T cells and is induced upon TCR stimulation

Jakmip1 protein expression was measured in naive and Ag-experienced T lymphocytes. For this, CD45RA and CD45RO surface markers were used to identify and sort the CD45RA+/CD45RO− subset and the Ag-primed CD45RA+/CD45RO+ subset from CD8+ and CD4+ T cells freshly purified from human peripheral blood (Fig. 1A). A stringent gating strategy was used to eliminate cells with intermediate phenotypes. The analysis postsorting showed a high purity of the sorted populations (Fig. 1A). Total protein lysates were analyzed ex vivo by Western blot using Jakmip1 and Tyk2 Abs. Although the level of Tyk2 was comparable in the two subsets, Jakmip1 was strongly up-regulated in memory cells (Fig. 1B). This differential expression was observed for both CD8+ and CD4+ T cells.

Since CD45RA down-regulation has been shown to be reversible, in particular in CD8+ T cells, the CD45RA+/CD45RO− subset is not exclusively composed of pure naive T cells but also Jakmip1 detected in this subset (Fig. 1B) could be due to primed cells that re-express CD45RA. Therefore, we analyzed Jakmip1 level in truly naive T cells from human cord blood, before and after in vitro TCR stimulation. CD8+ lymphocytes were purified from human cord blood and stimulated with CD3 and CD28 Abs, in the absence of supplemented cytokines. Jakmip1 protein levels were analyzed in cells harvested 1, 3, and 6 days after activation (Fig. 2A). Although undetectable in naive cells, Jakmip1 was detected at day 3 and had increased at day 6. Tyk2 was expressed in naive T cells and its level remained constant following TCR stimulation. Comparable results were obtained with CD4+ T cells (data not shown). RNA samples from the same donor were used for RT-qPCR measurements of Jakmip1, granzyme B, perforin, and IFN-γ transcripts. All these transcripts were at the limit of detection in unstimulated naive CD8+ T cells. At day 1 post-stimulation, Jakmip1 mRNA level increased with respect to naive cells (Fig. 2B). At day 3, the fold increase varied from 4 to 13 depending on the donor (n = 3). Transcripts of effector molecules were all induced after CD3/CD28 stimulation with differing kinetics and extent of induction (Fig. 2B). Granzyme B mRNA was strongly induced at day 1 and augmented at day 3. Perforin mRNA also increased at each time point, though to a lesser extent. IFN-γ mRNA was rapidly induced but then quickly decreased. In conclusion, Jakmip1, granzyme B, and perforin transcripts were all induced in naive CD8+ T cells from cord blood following in vitro CD3/CD28 stimulation.

In the same donor, naive/memory phenotypic changes occurring after in vitro stimulation were also analyzed. As shown in Fig. 2C, the CD45RA/CD45RO phenotype evolved gradually from a naive profile (day 0) to an intermediate profile at day 3 and finally to a memory profile at day 6. Interestingly, at the protein level, Jakmip1 became detectable when the switch between naive and memory began (day 3 in Fig. 2, A and C) and increased at day 6 when the switch was complete. Altogether, these data demonstrate that Jakmip1 expression is induced by T cell activation and parallels differentiation toward a memory phenotype.

Jakmip1 expression peaks in effector memory and effector T cell subsets

To further address the correlation between Jakmip1 and T cell differentiation, we analyzed Jakmip1 expression in vivo-generated memory cells at different stages of differentiation. CD8+ T cells from peripheral blood were doubly stained for CD45RA and

CD8+ T cells were freshly purified from human peripheral blood and stained with CD45RA and CD45RO Abs. Naive and memory T cells were enriched by sorting, respectively, CD45 RA+RO− and CD45 RA+RO+ subpopulations with the indicated gating strategy. Percentages obtained for each population are indicated. Purity of the sorted cells is shown on the right. B, Jakmip1 protein levels were measured in total lysates (15 μg) of ex vivo sorted subsets from CD8+ and CD4+ T cells. Tyk2 was used as control. Similar results were obtained with three different healthy donors.
CCR7 (Fig. 3A, upper panel). Percentages of TN (CD45RA+/H11001+/CCR7+/H11001), TCM (CD45RA+/H11002+/CCR7+/H11001), and TEMRA (CD45RA+/H11001+/CCR7+/H11002) subpopulations showed donor to donor variations. Highly pure populations obtained by sorting were used for RT-qPCR analyses of Jakmip1, granzyme B, perforin, and IFN-γ transcripts (Fig. 3A, lower panel). Consistently, in all analyzed donors (n = 3), Jakmip1 mRNA increased from the TN to the TCM subset and reached the highest level in the CCR7+/H11002 effector memory and effector subsets (TEM and TEMRA). These latter also expressed the highest level of granzyme B, perforin, and IFN-γ effector molecules. Thus, Jakmip1 expression is maximal in CD8+/H11001 T lymphocytes with the greatest effector potential.

Our results pointed to CCR7 as an appropriate marker for separating functional subpopulations of CD8+ T cells. Nevertheless, some studies have shown that expression of CCR7 does not correlate with effector functions of memory CD8+ T cells (7, 22, 23). Therefore, to validate the results obtained with CCR7, we sorted CD8+/H11001 T cell subsets using CD45RA in combination with CD27, another commonly used marker (Fig. 3B, lower panel). As observed with CCR7, following CD45RA/CD27 staining, four CD8+/H11001 T lymphocyte subpopulations could be distinguished: TN (CD45RA+/CD27+), TCM (CD45RA+/CD27+), TEM (CD45RA+/CD27+), and TEMRA (CD45RA+/CD27+). The analysis of the sorted populations by RT-qPCR confirmed the very low abundance of granzyme B, perforin, and IFN-γ mRNA in CD8+ naive cells and their substantial increase in TCM (Fig. 3B, lower panel). Moreover, the CD27− subpopulations (TEM and TEMRA) showed the highest level of effector transcripts, confirming the effector profile of these cells. In these subsets Jakmip1 mRNA reached its peak of expression.

Analogous separation was performed with CD4+ T lymphocytes from peripheral blood. As already described for healthy donors (8), in CD4+ cells the TEMRA subset was poorly represented and hence only three populations could be obtained (Fig. 4A, upper panel). The granzyme B, perforin, and IFN-γ transcripts distribution among the sorted populations identified well the functionally distinct CD4+ T cell subsets. Jakmip1 mRNA expression was maximal in TEM (Fig. 4A, lower panel). Results obtained from analyses of CD4+ T cells stained with CD45RA/CD27 correlated well with those obtained with CCR7, in terms of number of subpopulations observed and distribution of Jakmip1, granzyme B, perforin, and IFN-γ transcripts (Fig. 4B).
Altogether, these results showed that Jakmip1 expression is regulated depending on the stage of T cell differentiation. Moreover, Jakmip1 can be classed among genes with an “effector memory signature,” reaching its maximal expression in the terminally differentiated TEMRA subset.

Jakmip1 is highly expressed in CMV-specific CD8\(^+\) T cells

Detailed studies of virus-specific CD8\(^+\) T cells from primary infection to establishment of viral latency in human chronic infections have demonstrated substantial enrichment of a particular phenotype according to the viral specificity (EBV, CMV, HIV-1, and hepatitis C virus) (5, 24–26). It has been described that CMV seropositive individuals are characterized by a high frequency of cells of the TEMRA subset (CD45RA\(^-\)CCR7\(^-\)CD27\(^-\)) (27–29). Since the maximal Jakmip1 transcript level was detected in this subset in healthy donors, we monitored its level during the course of CMV infection.

FIGURE 3. Differential expression of Jakmip1 in CD8\(^+\) memory subsets. A, Upper panel, CD8\(^+\) T cells purified from human peripheral blood and stained with CD45RA and CCR7 Abs. The labeling profile defines distinct T cell subsets: naive, TEM, TEMRA, and NCM. The purity of the sorted cells is shown on the right. Lower panel, levels of the indicated transcripts were determined by RT-qPCR. Results are reported as fold increase compared with naive cells. Similar results were obtained with three different healthy donors. One representative experiment is shown. B, Upper panel, CD8\(^+\) T cells from human peripheral blood were stained with CD45RA and CD27 Abs. Lower panel, levels of the indicated transcripts were determined by RT-qPCR and reported as fold increase compared with naive cells.

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longitudinal follow-up of T cell response was obtained in CMV-naive individuals transplanted with CMV-positive kidneys. CMV-specific CD8\(^+\) T cells were collected for microarray analysis from renal transplant patients (n = 3) at two stages of the response: 1 mo after transplantation, i.e., during the acute phase (peak, Fig. 5), and 1 year after transplantation (latency, Fig. 5). Since in the acute phase, virus-specific cells presented an activated phenotype, at this stage cells were sorted for...
expression of activation markers HLA-DR and CD38. This population contained all CMV-specific CD8$^+$ T cells, as defined by pp65 CMV peptide tetramer staining. At 1-year latency, tetramer$^+$ cells were isolated from transplanted patients. These cells were resting, had lost CD27 expression, and switched back to CD45RA$^-$, thus they presented the phenotypic profile of fully differentiated effector CD8$^+$ T cells (CD45RA$^-$CD27$^-$CCR7$^-$). To analyze CMV-specific CD8$^+$ T cells in long-term latency, we included CMV seropositive healthy individuals ($n = 3$) who are likely to have been infected during childhood (long-term latency, Fig. 5). The isolated tetramer$^+$ cells presented the fully differentiated effector CD8$^+$ phenotype (CD45RA$^-$CD27$^-$CCR7$^-$) as observed in the 1-year latency. In the two color microarrays from Agilent, naive CD8$^+$ cells (CD45RA$^+$CD27$^+$) from seropositive individuals ($n = 5$) were used as the reference population (30). The CD45RA/CD27 phenotype of the CMV tetramer$^+$ CD8$^+$ T cells used in the microarray study can be found in Fig. 2 of Ref. 29 and in Fig. 1 of Ref. 30.

As shown in Fig. 5, Jakmip1 mRNA was found to be highly up-regulated in all stages of the CMV infection with respect to the naive CD8$^+$ cells. This expression profile was similar to that of effector molecules IFN-γ and granzyme B. Perforin mRNA was also up-regulated, though to a lesser extent, and it weakly decreased in the long-term latency stage. Overall, Jakmip1 was found to be one of the most highly up-regulated transcripts in this longitudinal follow-up of T cell response against CMV infection. This finding suggests that Jakmip1 may play a role in the control of host responses to viral infections in vivo.

**Cytotoxicity is increased in Jakmip1-depleted CD8$^+$ T cells**

The results described above indicated that Jakmip1 induction is part of the coordinated differentiation program that is initiated upon Ag encounter. Moreover, they also suggested that Jakmip1 could play a prominent role at the terminal differentiation stage, when T lymphocytes develop full effector functions. To study the role of Jakmip1 in cytolytic effector function, we used the RNA interference approach and compared the killing ability of Jakmip1-depleted and control cells. A HIV-1-derived lentiviral vector (20) was chosen to stably transduce Jakmip1-shRNA (sh-J1) or control-shRNA (sh-ctrl) in primary total CD8$^+$ T lymphocytes (Fig. 6A). This lentiviral vector coexpresses EGFP, providing a way to track transduced cells. Freshly purified CD8$^+$ T cells were first activated with CD3/CD28 Ab-coated beads to increase the efficiency of transduction (Fig. 6B). One day postactivation, cells were transduced in parallel with the sh-J1 and the sh-ctrl lentivirus. The percentage of EGFP$^+$ cells was monitored daily and found to peak at days 4 to 5 post infection (50–65%, Fig. 6C) and to remain constant thereafter. EGFP$^+$ cells were sorted 4 days after transduction and attained a postsorting purity of 96–98% (Fig. 6C). Silencing of Jakmip1 was evaluated by Western blot at the time when effector functions were assessed and an 80–90% decrease in Jakmip1 was observed (Fig. 6D). The two EGFP$^+$ populations expressed comparable surface levels of CD8, CD3, and CD28 were and were found not to be affected in their ability to secrete IFN-γ upon reactivation (data not shown). The cytotoxic ability of Jakmip1 depleted cells and of control cells was measured around days 8 to 10 post infection by redirected lysis in re-activated cells (Fig. 6B). In four independent experiments performed with cells from different donors, Jakmip1-depleted cells exhibited a statistically significant increase in cytotoxic potential. The average increase was greater than 60–70% with respect to control cells (Fig. 6E). Thus, the augmented cytotoxicity measured in condition of reduced Jakmip1 expression is the sign of a released inhibition and suggests that Jakmip1 participates to a negative feedback loop induced by T cell activation.

**Cytotoxicity is impaired in N-terminal-transduced CD8$^+$ T cells**

To further link Jakmip1 function and cytotoxicity, we investigated the effect of overexpressing full-length Jakmip1 or its N-terminal region on the killing activity of CD8$^+$ T lymphocytes. The choice of the N-terminal protein was motivated by previous evidence showing that this truncated form localizes on the microtubule network-like the full-length protein, but lacks phosphorylation target sites which are located at the C terminus (19). The HIV-1-derived FEIGW lentiviral vector, allowing expression of a transgene and EGFP on a bicistronic transcript, was chosen to stably transduce tagged Jakmip1 or the N-terminal protein in primary CD8$^+$ T lymphocytes (Fig. 7A). Freshly purified CD8$^+$ T cells were transduced with supernatants containing recombinant or control lentiviruses. EGFP$^+$ cells were sorted at day 6 after transduction and expanded in vitro. At day 8 after the third stimulation, CD8$^+$ T cell lines were found to be 70–80% EGFP$^+$. Transgene expression was analyzed by Western blot. The level of the full-length protein was similar to that of endogenous Jakmip1, while the N-terminal level was slightly lower.
higher (Fig. 7B). At day 9, the cytotoxic ability of EGFP+/CD8+/T lymphocytes was tested by redirected lysis. Jakmip1-transduced cells showed no variation of cytotoxicity with respect to cells transduced with the empty vector (Fig. 7C). On the contrary, the N-terminal-transduced cells exhibited a 50% decrease in cytotoxic potential. To rule out a nonspecific effect of the N-terminal protein on cellular fitness, we measured proliferation of the transduced cells by [3H]thymidine incorporation at days 3 and 6 after CD3/PBMC stimulation. As shown in Fig. 7D, the three EGFP+/CD8+/T cell lines did not show substantial difference in their proliferation rate. In conclusion, we showed that an increase in Jakmip1 over the endogenous level does not perturb cytotoxic function. However, expression of a truncated Jakmip1 form, presumably lacking proper regulation, strongly impairs cytotoxicity. These results further implicate Jakmip1 as a negative regulator of cytotoxic activity.
Discussion

This work reports the expression and functional analysis of Jakmip1 in human primary T lymphocytes. It provides evidence of expression of Jakmip1 only upon T cell activation: it is absent in naive T cells from cord blood whereas Jakmip1 transcript and protein are abundantly expressed in primed CD8+ and CD4+ T cells. Moreover, its transcriptional activation upon TCR stimulation parallels induction of effector molecules such as granzyme B, perforin, and IFN-γ. Interestingly, expression of Jakmip1 in CD8+ and CD4+ subsets is regulated according to the state of cell differentiation. It increases from TEM to TEMRA and reaches its maximal level in the terminally differentiated CD8+ TEMRA subset that also exhibits the highest effector potential.

Virus-specific T cells, whether CD8+ or CD4+, exhibit distinct differentiation phenotypes in different chronic infections: hepatitis C virus- and EBV-specific CD8+ T cells are less differentiated than CMV-specific CD8+ T cells (5, 31). Indeed, in chronically CMV-infected donors, the terminally differentiated TEMRA subset was shown to be particularly enriched (26, 32). Interestingly, in CD8+ T cells Jakmip1 was found to be among the most highly up-regulated transcripts in all stages of the CMV infection and its expression profile overlapped with that of granzyme B and IFN-γ. Moreover, up-regulation of Jakmip1 persisted in long latency, i.e., more than 30 years after primary infection. As previously described, these CMV-specific CD8+ T cells exhibit an effector profile and appear to function as “vigilant resting effector cells,” which are in constant equilibrium with the virus (26). Altogether, these expression data hinted at a functional role of Jakmip1 in effector CD8+ T cells and possibly in maintenance of long latency.

To gain insights into the role of Jakmip1 in effector function, we have monitored in vitro the cytotoxic ability of CD8+ T cells depleted of Jakmip1. Importantly, Jakmip1 silenced cells from four independent donors consistently exhibited an increased cytotoxic potential. Moreover, this finding was corroborated by the evidence that expression of the microtubule-interacting N-terminal portion of the protein has an opposite effect, impairing the killing potential. Altogether these data point to an inhibitory function of Jakmip1 in TCR-induced killing. In CD4+ T cells, acquisition of lytic capacity and perforin expression occur when cells reach a highly differentiated stage (TEMRA), as under conditions of strong or chronic activation (6). The increased expression of Jakmip1 that we have observed along CD4+ differentiation would support a role in cytolytic CD4+ T cells whose physiological relevance remains ill defined.

To our knowledge, this is the first description of a non-receptor protein whose silencing in human primary T lymphocytes potentiates cytotoxicity. This finding points to Jakmip1 as a potential player of a negative feedback loop induced by T cell activation. Interestingly, another member of the Jakmip family, Jakmip2, was recently proposed to exert a negative function in the secretory activity of neuroendocrine cells (33). Overall, these independent findings assign to Jakmip family members a specialized role in regulated secretory pathways.

How could Jakmip1 restrain target cell killing? Lysis of virally infected or tumor cells by CTL occurs primarily through the polarized delivery of secretory lysosomes or lytic granules along microtubules to a specialized region of the immunological synapse, the secretory domain (34–36). Description of the various steps of the granule-mediated cytotoxic pathway and identification of critical molecules have come from studies of inherited human disorders with impaired CTL-mediated cytotoxicity. Mutations have been identified in genes essential for granule transport and exocytosis (16, 17). An essential feature of Jakmip1 is its ability to contact microtubules (19), and recent studies in primary neurons showed that Marlin-I/Jakmip1 is highly mobile and is anchored to
microtubules through binding to kinesin-1, a plus-end-directed microtubule motor protein (37). Based on these properties, Jakmip1 could control the movement of secretory granules, which, like other organelles, can move bi-directionally along microtubules by means of kinesin- and dynein-based motors (38). Jakmip1 may, for instance, promote plus-end-directed transport of granules to the cell periphery via its binding to kinesin. This activity may limit clustering of granules at the synapse and consequently reduce polarized secretion. Looking for direct evidence of such a function is an exciting direction for further research.

In summary, Jakmip1 appears to belong to the “effector memory signature” group of genes and to be a new player in the process of regulated secretion in lymphocytes. Its sustained expression in long latency CMV-specific CD8+ T cells with high effector potential, its association through kinesin to the microtubule cytoskeleton, and its ability to restrain cytolitic activity highlight a role in the maintenance of a controlled cytotoxic immune response in humans.

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Disclosures

The authors have no financial conflict of interest.

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