Studying Jakmip1, a protein that is expressed upon T cell differentiation and has an inhibitory function in cytotoxic T lymphocytes. This research, by Valentina Libri, Dörte Schulte, Amber van Stijn, Josiane Ragimbeau, Lars Rogge, and Sandra Pellegrini, is published in *The Journal of Immunology* 2008; 181:5847-5856; doi: 10.4049/jimmunol.181.9.5847.

References: This article cites 38 articles, 18 of which you can access for free at: http://www.jimmunol.org/content/181/9/5847.full#ref-list-1

Subscription: Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions: Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts: Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
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/CCL28 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. The Journal of Immunology, 2008, 181: 5847–5856.

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 the peripheral inflamed tissues (3, 4), sequentially losing the lymph node homing receptors CCR7 and CD62L, which allow them to enter high endothelial venules and home to secondary lymphoid tissues (10, 11). These cells have a higher proliferative potential but do not exhibit prompt effector function upon Ag re-encounter. In contrast, cells of effector memory subsets (TEM and TEMRA) express low levels of CCR7 and CD62L and tend to localize predominantly in peripheral tissues (10, 12). These cells have limited proliferative capacity but exhibit immediate effector functions, such as cytotoxicity and cytokine production, in response to susceptible targets. The TCM and the TEM/TEMRA subsets are thought to play distinct roles in the memory response: TCM may represent memory cells involved in long term protection, whereas TEM/TEMRA are involved in immediate protection (10).

Despite recent progress, a clear understanding of the cellular and molecular mechanisms by which diversity and plasticity of immune memory is generated and maintained is still lacking. Several groups have performed gene expression profiling to better define the functional properties of memory subsets and their lineage relationships (9, 13–15). The group of Callan (14) investigated the gene expression profiles of the three CD8+ memory subsets and identified genes with an “effector memory signature,” whose expression is low in naive and increases from TCM > TEM > TEMRA. Among genes highly expressed in the two effector memory subsets (TEM/TEMRA) are genes encoding cytolytic molecules as well as genes involved in protein sorting to specialized lytic granules and in granule exocytosis. Importantly, mutations in some of these genes (HPS3, RAB27A, and RABGGTA) have been found in patients with impaired T cell cytotoxicity (16, 17).

In the present study, we have focused on Jakmip1, a recently identified protein that is expressed predominantly in neuronal and lymphoid cells. Jakmip1 was independently cloned by two groups using the yeast two-hybrid approach. In a neuronal screen, Couve et al. (18) identified Marlin-1, alias Jakmip1, as a binding partner of the GABA<sub>B</sub>R1 subunit of the G protein-coupled GABA<sub>B</sub>
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 microbeads (Miltenyi Biotec) according to the manufacturer’s instructions. Purity was >98% as determined by flow cytometry. Naïve CD4+ and CD8+ T cells from cord blood were stimulated with 10 µg/ml plate-bound anti-CD3 (clone TR66) and 0.5 µg/ml anti-CD28 Abs (BD Pharmingen; clone CD28.2). For lentiviral transduction, CD8+ T cells were stimulated using Dynabeads CD3/CD28 T Cell Expander using a cell to bead ratio of 1:2.

Generation of lentiviral plasmids

Short interfering RNA (siRNA) was designed to target Jakmip1 coding sequence. The sense strand of the targeted sequence corresponds to: 5'-CGAAGCTTGGATACATCGA-3' (nt 1771–1791 from ATG). The control siRNA corresponding to the no-silencing Qiagen control: 5'-TCTTCT CGAAGCTTGGATACATCG-3'. To construct the hairpin siRNA (shRNA) expression cassette, two complementary DNA oligonucleotides were synthesized, annealed, and inserted in pSUPER plasmid between BglII and HindIII sites downstream of the H1 promoter. Oligonucleotides: forward: 5'-GATTCGCC(N)19TTCAGAGA(N)19TTTTTGAA-3' and reverse 5'-GATTCGCC(N)19TTCAGAGA(N)19GGGCTG-3'. The H1 promoter with the shRNA cassette was then subcloned in the FG12 lentiviral vector (20) between the XhoI and Xhol sites. Lentiviral vector pFEIGW was derived from pFU GW (21). The Ubic promoter was replaced with the EF1α promoter and the GFP-cDNA was replaced with an IRES-eGFP cassette from pIRESE2GFP (BD Clontech). Full-length Jakmip1 (626 aa) and the N-terminal (363 aa) encoding fragments were PCR amplified from the original pCDNA plasmids (19) to add the XhoI and BamHI restriction sites. The oligonucleotides used for amplification are: forward 5'-TCAATCCAAATTGATGCTCAGAAGGACC-3' and reverse 5'-TCAATCCAAATTGATGCTCAGAAGGACC-3'. The amplified fragments were cloned upstream of the IRES sequence into the EcoRI and BamHI-digested pFEIGW vector.

Lentiviral vector production and transduction

For lentivirus generation, 2.5 × 106 HEK293T cells were plated in a 10-mm dish 1 day before transfection. Plasmids carrying the shRNA expression cassettes, the Jakmip1 and the N-terminal coding sequences were all packaged by cotransfection with pRSVREV and pMDLg/pRRE helper plasmids and the VSV-G expression plasmid pHMVCYG by calcium phosphate precipitation. Two days post-transfection, supernatant was harvested and concentrated 40-fold using Amicon filter tubes. Concentrated virus was analyzed by Western blot, Protein analysis.

Quantification of mRNA levels. Total RNA was purified with RNeasy columns (Qiagen). Reverse transcriptions 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 reaction was run in triplicate. The mRNA levels were normalized to 18S RNA amplification levels in each sample and calculated relative to expression of the target gene in unstimulated (naïve) T cells.

Protein analysis. For Western blot, ~1 × 106 of cells were lysed in 50 µl of RIPA buffer and protease inhibitors. Blots were incubated first with...
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 at day 3, and augmented 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 CD45RO proteins by FACs and left at 37°C. Cytotoxicity was calculated with the equation: Percent cytotoxicity = (experimental − effector spontaneous − target spontaneous/target maximum − target spontaneous) × 100.

**Statistical analyses.** To determine the significance of variance in cytotoxicity assays, data were analyzed using the Student’s t test. Significance was accepted if p < 0.05. For microarray analysis, trendplotting was performed across the different cell populations. For microarray analysis, trendplotting was performed across the different cell populations.
CCR7 (Fig. 3A, upper panel). Percentages of TN (CD45RA+/CCR7+/H11001), TCM (CD45RA–/CCR7+/H11001), and TEMRA (CD45RA+/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+ 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<sup>+</sup> T cells

Detailed studies of virus-specific CD8<sup>+</sup> 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<sup>−</sup>/CCR7<sup>−</sup>CD27<sup>+</sup>) (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. Primary CMV infection can be monitored in renal transplantation (27, 29), so a detailed

**FIGURE 3.** Differential expression of Jakmip1 in CD8<sup>+</sup> memory subsets. A, Upper panel, CD8<sup>+</sup> T cells purified from human peripheral blood and stained with CD45RA and CCR7 Abs. The labeling profile defines distinct T cell subsets: naive, T<sub>CM</sub>, T<sub>EM</sub>, and T<sub>EMRA</sub>. 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<sup>+</sup> 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.
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+. These cells were naive CD8+ T lymphocytes. The choice of these cells was dictated by the need to exclude CD8+ T cells from long-term latency (long-term latency, Fig. 5). The isolated tetramer+ cells presented the 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 T cell 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 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 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 than the full-length protein level.
higher (Fig. 7B). At day 9, the cytotoxic ability of EGFP⁺ CD8⁺ T cells 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⁺ 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.
exhibits the highest effector potential. As previously described, these CMV-specific CD8 T cells persist more than 30 years after primary infection. We have monitored in vitro the cytotoxic ability of CD8 T cells that were activated 1 day prior to assaying cytotoxicity. Total lysates from 5 × 10⁵ cells were run on a 4–12% gradient SDS-PAGE transferred to membrane and analyzed by Western blot with anti-Jakmip1 (left panel) as well as anti-tag V5 Abs (right panel). C, Killing potential of EGFP⁺ CD8⁺ T cell lines. EGFP⁺ CD8⁺ T cells transduced with empty vector, full length or N-terminal were incubated with P815 target cells at 10:1, 5:1, and 2.5:1 E:T ratios in the presence of CD3 mAb (UCHT1 0.5 µg/ml). Each sample was in triplicate. The same result was obtained in three different experiments. One representative experiment is shown. D, Proliferation potential of EGFP⁺ CD8⁺ T cell lines. EGFP⁺ CD8⁺ T cells expressing empty vector, full length, or N-terminal were stimulated with plate bound CD3 mAb (OKT3 0.5 µg/ml) and irradiated PBMC or left unstimulated. At days 3 and 6, cells were pulsed with [³H]thymidine for 7 h. Proliferation is expressed as the mean [³H]thymidine incorporation (cpm) of quadruplicate wells.

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 T_em to T_em and reaches its maximal level in the terminally differentiated CD8⁺ T EMRA 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 T_EMRA 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 (T_EMRA), 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-1/Jakmip1 is highly mobile and is anchored to
microtubules through binding to kinesin-I, 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 cytolytic activity highlight a role in the maintenance of a controlled cytotoxic immune response in humans.

Acknowledgments
We thank Drs. C. Steindler for providing reagents, D. Baltimore for generously providing lentiviral vectors, and Anna Sinemus for constructing pFEIG7; A. Akbar, S. Garcia, M. Gakovic, and R. Mallone for helpful discussions; R. van Liere for insightful advice and manuscript reviewing; and A. Freitas, J. Chilton, F. Michel, and G. Uézé for critical reading of the manuscript.

Disclosures
The authors have no financial conflict of interest.

References