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Serine Protease Inhibitor 6 Is Required To Protect Dendritic Cells from the Kiss of Death

Elena Lovo, Manling Zhang, Lihui Wang, and Philip G. Ashton-Rickardt

How dendritic cells (DC) present Ag to cytotoxic T cells (CTL) without themselves being killed through contact-mediated cytotoxicity (so-called kiss of death) has proved to be controversial. Using mice deficient in serine protease inhibitor 6 (Spi6), we show that Spi6 protects DC from the kiss of death by inhibiting granzyme B (GrB) delivered by CTL. Infection of Spi6 knockout mice with lymphocytic choriomeningitis virus revealed impaired survival of CD8+ DC. The impaired survival of Spi6 knockout CD8α+ DC resulted in impaired priming and expansion of both primary and memory lymphocytic choriomeningitis virus-specific CTL, which could be corrected by GrB deficiency. The rescue in the clonal burst obtained by GrB elimination demonstrated that GrB was the physiological target through which Spi6 protected DC from CTL. We conclude that the negative regulation of DC priming of CD8+ T lymphocyte immunity by CTL killing is mitigated by the physiological inhibition of GrB by Spi6. The Journal of Immunology, 2012, 188: 1057–1063.

The killing of infected cells by CD8+ cytotoxic T cells (CTL) is critical for immunity to intracellular pathogens such as viruses (1). After the resolution of an acute viral infection, a subpopulation of CTL differentiates into memory CD8+ T cells (2). Memory cells exhibit long-lived protection from subsequent infections by the same virus through robust secondary responses involving immediate cytotoxic function from effector memory (Teffm) cells (3) and rapid expansion of central memory into new CTL effectors (3, 4). Dendritic cells (DC) are the physiological APCs that stimulate both naive CD8+ T cells and memory CD8+ T cells to differentiate and proliferate into CTL (5, 6). In mice, the CD11c+ CD8α+ CD205+ DC population can best acquire a wide variety of cellular Ags (including viral proteins) from infected and apoptotic cells, and present them on self class I MHC to cognate CD8+ T cells in a process known as cross-presentation (7, 8). CD8α+DC are also the subset specifically responsible for cross-presentation of lymphocytic choriomeningitis virus (LCMV) Ags to CD8+ T cells (7).

Contact-dependent, lymphocyte-mediated cytotoxicity proceeds through two pathways. The first pathway is triggered by members of the TNF receptor family, of which Fas is the most important (1). The second involves the exocytosis of proteins present in CTL and NK cell granules (1). Exocytosis of perforin (1) facilitates the entry of serine proteases called granzymes, which trigger apoptosis in target cells (9). Granzymes A and B (GrB) are the most abundant granzymes in mice and humans, and are the best characterized (1). GrB activates the caspase-dependent pathways of apoptosis and like caspases, cleaves after aspartic-acid residues.

Given the effectiveness of the granule exocytosis pathway to deliver a kiss of death (1), it is not surprising that Ag-presenting DC are themselves killed by cognate CTL. Experiments with perforin-deficient CTL show that primary CTL eliminate DC that express cognate peptide-Ag/MHC (pMHC) as part of a negative-feedback mechanism that limits the expansion of the immune response to tumor (10) or virus (11). This is consistent with observations that the maximum primary clonal burst requires the continual presentation of Ag by DC to differentiated CTL over the course of several days in addition to the initial presentation for a few hours to cytotoxically inert naive CD8+ T cells (12, 13). A negative regulatory loop working through the killing of DC has also been observed in the memory CD8+ T cell response (14) to allow the containment and efficient resolution of CTL expansion from cytotoxic Teffm cells (15). However, the fact that DC are still highly effective at priming CTL expansion implies that they have mechanisms that protect them from the kiss of death.

The mouse serine protease inhibitor (serpin), serine protease inhibitor 6 (Spi6), is a potent inhibitor of GrB (16). Spi6 lacks signal secretory sequences, and thus it has been suggested that this endogenous inhibitor protects cells from CTL-induced damage by inhibiting GrB in the cytoplasm (1). The upregulation of Spi6 in DC upon maturation or through transgene expression results in the protection of DC from granule-mediated programmed cell death (PCD) in vitro (17). However, increased Spi6 expression in bone marrow-derived DC (BMDDC) is not sufficient to protect from direct killing by CTL in vivo (18). Therefore, whether protection of DC from GrB-mediated killing is a physiological mechanism for the control of CTL immunity remains to be determined. We show that Spi6 when upregulated in mature BMDDC is required to protect from GrB-mediated CTL killing. Importantly, we show a similar requirement for protection from GrB by Spi6 in the in vivo survival of CD8α+DC after LCMV infection. The impaired
Spi6 protects DC from CTL

Materials and Methods

**Mice**

Thy1.2+ and Thy1.1+ C57BL/6J wild-type and Grb KO mice (9) were purchased from The Jackson Laboratory, C57BL/6J Spi6 KO mice and P14 mice were bred inhouse (19). Grb KO P14 Thy1.1 mice were generated by intercrossing and screening by PCR for the Grb allele (9) and flow cytometry for the P14 TCR (Vα2+) and CD90.1 in the blood (19). All animals were housed in a pathogen-free environment at Imperial College London, and all experiments were conducted in accordance to Home Office (U.K.) regulations.

**Flow cytometry**

The following fluorescently labeled mAbs were purchased from eBiosciences: anti-CD86 (allophycocyanin-labeled), anti-I-Aα (FITC-labeled), anti–CD11c (R-PE–labeled), anti–CD8-allophycocyanin, anti–CD90.1-Allexa Fluor 450. The following fluorescently labeled Abs were purchased from BD Pharmingen: Ly-6C-PE-Cy7, CD4-FITC. The gp33 peptide (KAVYNFATM/H-2Dα–allophycocyanin tetramer was purchased from Becton Dickinson (19). Lymph node (LN) cells, splenocytes, and BMDC were prepared and stained with tetrathers and mAb as described previously (19). Intracellular staining (ICS) with anti–5-ethynyl-2'-deoxyuridine (EdU–Alexa Flour 488 to detect EdU incorporation in dividing P14 CD8 cells was performed according the manufacturer’s protocol (Invitrogen). DC were subjected to ICS with rabbit anti-Spi6 antiserm (1/1000 dilution) or rabbit preimmune serum (1/1000 dilution) (22), then goat anti-rabbit IgG-allophycocyanin (1/100 dilution; Jackson Immunoresearch). Stained cells were acquired on a Cyan ADP machine (Beckman Coulter) and analyzed with FlowJo (Tree Star).

**CTL assays**

Bone marrow was flushed out of femoral bones taken from 6- to 8-wk-old mice and immature BMDC generated by 8–10 d of culture as described in Lutz et al. (21). BMDC were matured by culture for 24 h with LPS (1 µg/ml; Sigma). Spleen cells (106/ml) from wild-type or Grb KO P14 mice were cultured with LCMV gp33 peptide [KAVYNFATM] (10-6 M) and IL-2 (2 U/ml) for 2 d to generate CTLs (19). BMDC targets were pulsed with gp33 (10-7 M) for 1 h, genomic DNA labeled with [H]thymidine, and then incubated with P14 CTL over a range of ratios in 12 replicates, to measure CTL-induced apoptosis. The percentage apoptosis was determined after 4 h as follows: % apoptosis = (3H-labeled DNA retained in target without CTL) / (H-labeled DNA retained in target with CTL/H-labeled DNA retained in target without CTL) × 100 (22). Apoptosis was induced by treatment with anti-mouse Fas mAb (clone Jo2) and cycloheximide (5 µg/ml). BMDC targets were pulsed with targets (104/ml) for 1 h and labeled with [32P]Orthophosphate, then incubated with P14 CTL over a range of ratios in quadruplicate, to measure CTL-induced lysis. The percentage specific lysis was determined after 4 h as follows: % specific release = (spontaneous release − spontaneous release/m maximum release − spontaneous release) × 100. The percentage specific lysis of RMA cells in the absence of gp33 was <10%, and the spontaneous release was <10% of the maximum release.

**Adoptive transfer and LCMV infection**

Naive CD8+ cells were purified (>90%) from the spleens of wild-type or Grb KO P14 mice (Thy1.1+) by positively sorting with anti-CD8 magnetic beads (Miltenyi Biotec) and adoptively transferred (5 × 107) by i.v. injection into wild-type or Spi6 KO (Thy1.2+) mice, and then transferred to infected mice (21). For memory cell experiments, LCMV Armstrong recipients were reinfected after 40 d with 105 PFU i.v.. A quantitative PCR method was used to measure the level of LCMV Armstrong from infected mice exactly as described by McCauslan and Crotty (23). In brief, total spleens were weighed, then a known amount was homogenized and total RNA recovered. First-strand cDNA synthesis was performed, then cDNA was used as the template for real-time PCR using primers specific for the glycprotein (GP forward and reverse primers) and nucleoprotein (NP forward and reverse primers) of LCMV. A standard curve with linearized plasmids encoding LCMV GP and NP genes was used to calculate the number of copies of LCMV genome per milligram spleen.

**Ex vivo DC analysis**

Spleens were cut into small fragments and digested with collagenase D grade II (1 mg/ml) and DNase grade II (20 µg/ml) (both from Roche) in RPMI 1640 medium + 10% FCS for 25 min at room temperature. EDTA (0.2 mM) was successively added for another 5 min to disrupt DC–T cell interaction. Spleen fragments were filtered through mesh (70 µm) and centrifuged at 2000 × g for 5 min at room temperature (7). Cells were resuspended in MACS buffer (2% BSA + 0.5 M EDTA in PBS) and magnetically sorted with anti-CD11c beads (according to Milleniy Biotech protocol). The number of CD8αC DC in each spleen was determined by measuring the percentage of CD11c+ Ly6C− CD8αC CD4− cells, then multiplying by the number of enriched CD11c+ cells. The number of plasmacytoid DC (pDC) in each spleen was determined by multiplying the percentage of CD11c+ Ly6C− cells by the number of enriched CD11c+ cells. For ex vivo priming and expansion experiments, CD8αC DC from the spleens of infected mice were FACS sorted after Ab staining (MoFlo; Dakocytomation). In brief, CD11c+–enriched cells (protocol as described earlier) were stained for surface markers, and CD11c+/CD8α+/CD4− cells (8% CD11c+–enriched cells in wild-type, 3% in Spi6 KO) were purified by FACs (>98% pure). FACs-purified CD8αC DC (0.5 × 106 per 96 U-bottom well/0.2 ml) were incubated with bead-sorted CD8+ T cells from wild-type P14 mice (5 × 106 cells/well). Positive control cultures that were pulsed with gp33 peptide gave 55% EdU+ cells, and negative control cultures (5 × 106 P14 CD8 T cells alone/well) gave 0.7% EdU+ cells. Cells were pulsed with EdU (5 µM) for 2.5 d, and the percentage of EdU+ cells was then determined by ICS with anti–EdU-Alexa Flour 488 and flow cytometry according to manufacturer instructions (Click-iT EdU; Invitrogen).
This conclusion is supported by Fig. 1E, which shows that BMDDC from Spi6 KO mice are equally susceptible to PCD induced by anti-Fas Ab. Finally, we used P14 CTL from GrB KO mice (9, 19) to address the specific role of GrB in the susceptibility of Spi6 KO BMDDC to CTL killing. GrB KO P14 CTLs did not induce the lysis of gp33-pulsed DC after either 4 (Fig. 1G) or 8 h (Fig. 1H), indicating that the increased killing of Spi6 KO BMDDC was due to increased sensitivity to GrB. We conclude that Spi6 upregulation is required to protect BMDDC from GrB-mediated killing by CTL.

**Protection from GrB by Spi6 determines the survival of CD8α+ DC in vivo**

Expression correlation studies have given contradictory findings on the role of Spi6 in controls the negative feedback loop of CTL expansion based on the killing of DC (17, 18). To address this issue, we determined whether Spi6 was required for the protection of endogenous splenic DC from GrB delivered by CTL. Congenically marked (Thy1.1+) wild-type P14 CD8 T cells were adoptively transferred to either wild-type or Spi6 KO mice (both Thy1.2+), which were then infected with LCMV (Fig. 2A). After the infection of mice with LCMV, CD8α+ DC acquire LCMV proteins from infected cells and cross-present them with class I MHC to cognate CD8 T cells (7). CD11c+ DC are purified with magnetic beads from the spleens of infected mice; then CD8α+ DC (CD11c+ CD8α+ CD4−) and pDC (CD11c+ Ly-6C+) populations were identified by Ab staining and flow cytometry (Fig. 2B). We measured the expression of Spi6 in wild-type compared with Spi6 KO negative control DC by ICS with anti-Spi6 Ab (20). We observed the significant upregulation of Spi6 in wild-type CD8α+ DC on days 2 (mean fluorescence intensity [MFI]: wild-type 3.3-fold higher than Spi6 KO; p = 0.050), 4 (MFI: wild-type 2-fold higher than Spi6 KO; p = 0.047), and 8 (MFI: wild-type 2-fold higher than Spi6 KO; p = 0.017) after LCMV infection (Fig. 2C, 2D). However, we did not observe any upregulation of Spi6 in wild-type pDC over the level of Spi6 KO cells (Fig. 2E).

We then measured the absolute number of CD8α+ DC and pDC in recipient mice after LCMV infection. On day 4, we observed a 75% reduction (p = 0.040) in the number of CD8α+ DC in Spi6 KO mice compared with wild-type recipients (Fig. 2E) but did not observe any difference in the number of pDC at any time point (Fig. 2F). The deficit in the level of CD8α+ DC in Spi6 KO mice is consistent with the expression of Spi6 from day 2 onward. Wild-type pDC do not express Spi6 and are present in wild-type levels in Spi6 KO mice; however, the strong cross-reactivity with Spi6 antiserum (Fig. 2E) suggests that other serpins may play a role in pDC survival and function (1).

Staining for congenic markers revealed that >95% of the clonal burst of anti-gp33 CTL in Spi6 KO recipients was derived from adoptively transferred P14 CD8 T cells, resulting in a 20-fold excess of the number of donor over endogenous primary CTL (Supplemental Fig. 2). Therefore the replacement of wild-type donor with GrB KO cells in Spi6 KO recipients was an effective means to reduce the potential for GrB-mediated killing of targets by CTL (Fig. 2A).

We next determined whether the decrease in the level of viable CD8α+ DC in Spi6 KO mice was due to increased CTL killing by donor P14 CD8 T cells. The adoptive transfer of GrB KO P14 CD8 T cells resulted in the rescue of the number of Spi6 KO CD8α+ DC up to wild-type levels on day 4 after LCMV infection (Fig. 2G). Therefore, we conclude that Spi6 is required for the survival of CD8α+ DC through protection from GrB delivered by CTL. The specific upregulation of Spi6 and corresponding requirement for survival in CD8α+ DC compared with pDC is consistent with the role of CD8α+ DC in cross-presentation of LCMV to CD8 T cells (7).

**Functionality of Spi6 KO CD8α DC**

We next determined whether Spi6 was required for CD8α DC function. Equal numbers of FACs-purified CD8α+ DC from recipient wild-type or Spi6 KO mice (Thy1.2+) on day 4 after LCMV infection were cocultured with naive P14 CD8 responder...
T cells (Fig. 3A). P14 CD8 T cell division was measured by EdU incorporation into genomic DNA (25) (Fig. 3B). We found that the number of CD8α+ DC from LCMV-infected mice determined the extent of proliferation of wild-type P14 CD8 T cells, as evidenced by the titration of percentage EdU+ P14 CD8 T cell with the ratio of P14 CD8 T cell to CD8α+ DC (Fig. 3B). However, we observed no difference in the percentage of EdU+ P14 CD8 T cells after culture with Spi6 KO compared with wild-type CD8α+ DC (Fig. 3B,3C). Therefore, ex vivo Spi6 KO DC are not qualitatively impaired in their ability to prime the proliferation of cognate CD8 T cells. We conclude that although Spi6 is required for the survival of CD8α DC, it is not directly required for function.

**Spi6 is required for the priming of primary CTL responses in vivo**

Given that the number of viable LCMV+ CD8α DC determines the proliferation of P14 CD8 T cells (Fig. 3B), we determined whether the impaired survival of Spi6 KO CD8α DC resulted in impaired expansion of primary CTL in vivo. Wild-type P14 CD8 T cells (Thy1.1+) were adoptively transferred to wild-type or Spi6 KO recipients (Thy1.2+), and the number of donor-derived gp33+ CD8+ cells was determined in the clonal burst on day 8 by flow cytometry (Fig. 4A). We observed a 2-fold (p = 0.01) decrease in the level of donor-derived gp33+ CD8+ cells in the spleen and an 8-fold (p = 0.003) decrease in the LNs of Spi6 KO compared with wild-type recipients (Fig. 4B). Therefore, the decrease in the number of Spi6 KO CD8α DC compared with wild-type resulted in impaired priming and expansion of anti-LCMV CTL. This defective expansion could be rescued when GrB KO P14 CD8 T cells were adoptively transferred, demonstrating that the GrB-dependent deficit in Spi6 KO CD8α DC survival (Fig. 2G) also results in impaired CTL expansion (Fig. 4C). The defective expansion of wild-type anti-LCMV CTL in Spi6 KO compared with wild-type recipients led to a corresponding 100-fold increase in the level of LCMV in the spleen on day 6 postinfection (Supplemental Fig. 3). Therefore, Spi6 is required for the priming of functionally relevant CTL responses by DC in vivo.

**FIGURE 2.** Spi6 upregulation in CD8α DC is required for survival. A, Experimental plan. B, Flow cytometry plots of staining for markers identifying DC subsets from CD11c+ magnetic bead-sorted splenocytes. Left plot shows conventional DC (cDC) and pDC; right plot shows the markers for CD8α DC of gated cDC. C, Histograms for staining with anti-Spi6 antisera on CD8α DC and pDC from the spleen after LCMV infection over time. Numbers represent MFI WT/Spi6 KO. Mean MFI values for ICS anti-Spi6 serum in CD8α DC (C) and pDC (D). Mean absolute number of CD8α DC (E) and pDC (F). G, Mean absolute number of CD8α DC in WT mice or Spi6 KO after adoptive transfer of either WT or GrB KO P14 CD8 T cells. All mean values are ±SEM (n = 3–6) and are representative of three independent experiments; *p < 0.05. n.s., Not significant.

**FIGURE 3.** Functionality of Spi6 KO CD8α DC. A, Experimental plan. B, Flow cytometry plots for ICS with anti-EdU mAb on P14 CTL after 60 h of culture with CD8α DC FACS purified from LCMV-infected mice. Percentage EdU+ is indicated next to gates. C, Mean percentage EdU+ P14 CD8 T cells. Broken line indicates the background level of proliferation. All mean values are ±SEM (n = 3–4) and are representative of three independent experiments; *p < 0.05.
**Spi6 is required for the priming of memory CTL responses in vivo**

The elimination of DC by cytotoxic T\textsubscript{em} cells also is thought to provide a negative feedback mechanism for the control of secondary effectors (6, 14, 15, 26). Therefore, we determined whether Spi6 was required to protect CD8\textsuperscript{a} DC from cytotoxic T\textsubscript{em} cells. First, we established the level of memory CD8 T cells in wild-type and Spi6 KO mice. On day 40 after primary LCMV infection, the level of donor memory gp33 CD8 T cells in the spleen of Spi6 KO mice was \(\sim\)2-fold higher than wild-type, and in the LN the same as wild-type (Supplemental Fig. 4). This is consistent with previous observations in the spleens of intact Spi6 KO mice that homeostatic mechanisms correct diminished clonal burst by increasing development in the memory phase (20).

We then examined the expansion of secondary CTL from anti-LCMV memory CD8 T cells. Wild-type and Spi6 KO recipient mice were infected with LCMV, then on day 40 were reinfected with a high dose of the clone 13 variant of LCMV (10\textsuperscript{6} PFU i.v.), and the re-expansion of gp33\textsuperscript{+} CD8\textsuperscript{+} cells was measured after 5 d (Fig. 5A) (4). We show in Fig. 5B that the number of donor

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**FIGURE 4.** Defective expansion of primary CTL in Spi6 KO mice. A. Experimental plan. B, Mean absolute number of donor WT P14 CTL (gp33\textsuperscript{+}Thy1.1\textsuperscript{+}CD8\textsuperscript{+}) at day 8 after LCMV infection in the spleen and LNs of recipients. C, Mean absolute number of donor P14 CTL (gp33\textsuperscript{+}Thy1.1\textsuperscript{+}CD8\textsuperscript{+}) at day 8 after LCMV infection. All mean values (horizontal line) are ±SEM (vertical lines with bars; \(n = 3–6\)) and are representative of three independent experiments; \(*p < 0.05, **p < 0.005, ***p < 0.0005. n.s., Not significant.**

**FIGURE 5.** Defective expansion of secondary CTL in Spi6 KO mice. A. Experimental plan. B, Mean absolute number of donor WT P14 CTL (gp33\textsuperscript{+}Thy1.1\textsuperscript{+}CD8\textsuperscript{+}) at day 5 after LCMV clone 13 reinfection in the spleen and LNs of recipients. C, Mean absolute number of donor P14 CTL. All mean values (horizontal line) are ±SEM (vertical lines with bars; \(n = 3–6\)) and are representative of three independent experiments; \(*p < 0.05, **p < 0.005, ***p < 0.0005. n.s., Not significant.**
secondary effectors was 2-fold lower in the spleen (p = 0.0014) and 4-fold lower in the LN (p = 0.0006) of Spi6 KO compared with wild-type mice. This corresponds to defective expansion in number of anti-LCMV CTL in Spi6 KO mice upon reinfection over the level observed in day 40 memory (Supplemental Fig. 4), such that the 4-fold expansion in the number of donor gp33+ CD8+ cells observed in the spleens of wild-type mice upon reinfection was completely abolished in Spi6 KO mice (Fig. 5B, Supplemental Fig. 4). The defect in memory CTL expansion in Spi6 KO recipients could be corrected by GrB deficiency of the donor P14 CD8 T cells (Fig. 5C). We conclude that Spi6 is required for the priming of secondary effectors from memory CD8 T cells, and thus dampens the negative feedback control of effector cell expansion exerted by DC elimination.

**Discussion**

Understanding the mechanisms that initiate and terminate CTL responses is central to our understanding immunity to intracellular pathogens and tumor cells. A negative feedback mechanism in which priming DC expressing pMHC are killed by the CTL they prime has been proposed for the control of the expansion of naive and memory CD8 T cells levels (11, 15). This model predicts that factors that control the viability of DC in the face of CTL killing will exert control over the expansion of CTL. However, expression correlation (17, 18) gives contradictory findings on the role of Spi6 in controlling this negative feedback loop at the level of DC viability. Collectively, our findings resolve the controversy on the role of Spi6 in protecting DC from CTL activity in vivo (17, 18). Using Spi6 KO mice, we demonstrate a nonredundant role for Spi6 in facilitating the priming and expansion of CTL through the protection of DC from the kiss of death.

Our in vitro finding that Spi6 KO BMDDC are susceptible to CTL killing is in agreement with previous reports of increased expression of Spi6 upon maturation by LPS activation correlating with resistance to CTL killing (17, 18). However, our in vivo findings differ from those of Andrew et al. (18), who showed that LPS-treated BMDDC (Spi6hi) were no more resistant to killing by cognate CTL than non–LPS-treated BMDDC (Spi6lo). However, an important point to consider is that Andrew et al.’s study (18) correlated the expression of Spi6 mRNA to in vivo survival, whereas our study directly interrogated the role of Spi6 in DC by using Spi6-deficient cells. Furthermore, whether LPS-treated BMDDC continue to express Spi6 after adoptive transfer was not tested, and it is well-known that LPS induces PCD in DC (8, 21), so this may have counteracted any cytoprotection afforded by initial Spi6 upregulation.

A negative feedback loop in which priming DC expressing pMHC are killed by cognate CTL has been proposed for the control of the expansion of primary CTL (11, 15). Our results indicate that Spi6 is a physiological factor that controls the viability of DC, and thus regulates the negative feedback of CTL expansion. Spi6 was upregulated in CD8αDC, but not pDC, after LCMV infection, and in Spi6 KO mice, only the CD8αDC were diminished. CD8α+ DC are responsible for cross-presentation to anti-LCMV CD8 T cells, and so are in direct contact with GrB+ cells, in contrast with pDC, which although they drive inflammation and support indirect CTL activation by secreting type I IFNs (8), are less efficient than CD8α+ DC at cross-presentation to CTL (27). In addition, our findings are consistent with reports that CD8αDC priming requires several days to generate the clonal burst of CTL in addition to the initial presentation for a few hours to noncytolytic naive CD8 T cells (12, 13).

The elimination of DC by cytotoxic Tem cells also is thought to provide a negative feedback mechanism for the control of secondary effectors (6, 14, 15, 26). We observed that the expansion of wild-type, donor-derived secondary effectors was also impaired in Spi6 KO mice. Furthermore, the deficit could be corrected when GrB KO cells replaced wild-type donor CD8 T cells. We conclude that Spi6 is required for the priming of secondary effectors from memory CD8 T cells, and thus dampens the negative feedback control of effector cell expansion exerted by DC elimination by GrB.

The cytoprotective function we describe for Spi6 in DC can be placed in a wider context of protection from GrB by the intracellular serpin (1). Spi6 KO mice have revealed that Spi6 is also required to protect CTL (19) and invariant NK T cells (28) from their own GrB, as well as mesenchymal stem cells from CTL-delivered GrB (29). Spi6 and the human homolog Proteasine Inhibitor 9 (30, 31) also inhibit other proteases. For example, Spi6 can inhibit neutrophil elastase (20), and proteasine inhibitor 9 inhibits caspase 1 (32). However, the complete rescue of DC survival and expansion of anti-LCMV CTL by GrB deficiency argues that at least in this context GrB is the physiological target of Spi6.

GrB was first characterized as an effector molecule in the granule exocytosis pathway of killing (9). The discovery that CTL can be killed by their own GrB (19) or when it is delivered by a T regulatory cell (33) has led to the suggestion that perhaps GrB should also be viewed as a negative immunomodulator (1). The survival of DC during the expansion phase is required for potent T cell responses; therefore, our current findings would predict that preservation of DC by pharmacological inhibition of GrB would enhance not only priming of naive CD8 T cells but also the boosting of vaccine responses by presentation to Tem cells.

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**Disclosures**

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**References**


