The Small Heat Shock Protein 27 Is a Key Regulator of CD8^+CD57^+ Lymphocyte Survival

Karen L. Wood, Oliver H. Voss, Qin Huang, Arti Parihar, Neeraj Mehta, Sanjay Batra and Andrea I. Doseff

*J Immunol* 2010; 184:5582-5588; Prepublished online 12 April 2010;
doi: 10.4049/jimmunol.0902953
http://www.jimmunol.org/content/184/10/5582

Supplementary Material
http://www.jimmunol.org/content/suppl/2010/04/13/jimmunol.0902953.DC1

References
This article cites 39 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/184/10/5582.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
The Small Heat Shock Protein 27 Is a Key Regulator of CD8+CD57+ Lymphocyte Survival

Karen L. Wood,*1 Oliver H. Voss,‡1 Qin Huang,* Arti Parihar,* Neeraj Mehta,* Sanjay Batra,* and Andrea I. Doseff* ‡ 

Differences in CD8+CD57+ and CD8+CD57+ lymphocyte lifespan have been documented. Lower numbers and shorter lifespan are characteristic of CD8+CD57+ in normal individuals. However, CD8+CD57+ are expanded in certain disease states including T cell large granular leukemia and other hematologic malignancies. The mechanisms responsible for the differences in CD8+CD57+ and CD8+CD57+ lifespan remain elusive. In this study, we demonstrate that the small heat shock protein (Hsp) 27 is a key regulator of CD8+CD57+ lymphocyte lifespan. We found that Hsp27 expression is significantly lower in CD8+CD57+ than in CD8+CD57+ lymphocytes. In contrast, Hsp60 and Hsp70 are expressed at comparable levels. Unlike other antiapoptotic Bcl-2-like molecules, the expression of Hsp27 tightly correlates with CD8+CD57+ and CD8+CD57+ lifespan. We demonstrate that Hsp27 overexpression in CD8+CD57+ lymphocytes to levels found normally in CD8+CD57+ lymphocytes decreased apoptosis. Accordingly, silencing of Hsp27 in CD8+CD57+ lymphocytes increased apoptosis. Collectively these results demonstrate that Hsp27 is a critical regulator of normal CD8+CD57+ lifespan supporting its use as a marker of lifespan in this lineage, and suggest a mechanism responsible for the decreased apoptosis and clonal expansion characteristic of certain disease states. The Journal of Immunology, 2010, 184: 5582–5588.

Lymphocytes are major cells involved in the adaptive immune response. CD8+ cytotoxic T lymphocytes play a fundamental role in the response against foreign pathogens, including virus and also tumor Ags. Usually, CD8+ lymphocytes expand rapidly in response to Ag and die by apoptosis as the pathogen is cleared. In normal individuals, only ~5–20% of CD8+ lymphocytes express the CD57 Ag (CD8+CD57+) (1, 2). CD8+CD57+ lymphocytes are thought to represent clonally expanded cytotoxic T lymphocytes that have suppressive properties (3). The percentage of CD8+ lymphocytes that express CD57+ is expanded in chronic infections including HIV, rheumatoid arthritis, normal aging, and in hematological malignancies (1, 2, 4–12). In fact, T cell large granular leukemia (LGL) is defined by the clonal expansion of CD3+CD8+CD57+ large granular lymphocytes (13, 14). The exact cellular mechanism leading to the expansion of these cells in disease states is not known.

Differences in lifespan between CD8+CD57+ and CD8+CD57+ have been documented. These differences may be due to alterations in apoptosis. For example, it was shown that normal CD8+CD57+ lymphocytes undergo increased apoptosis in comparison with CD8+CD57+ when stimulated in vitro with anti-CD3 Ab, and express increased levels of Fas, FasL, and caspase-3 activity (10, 15, 16). We previously showed an expansion of CD8+CD57+ lymphocytes in HIV-infected subjects probably caused by the failure to regulate apoptosis normally (16). Alterations in apoptosis have been hypothesized to contribute to the expansion of CD8+CD57+ lymphocytes in LGL (17). PBMCs from LGL patients showed resistance to Fas-stimulated apoptosis despite increased levels of Fas and FasL expression (17). However, the exact mechanisms responsible for these differences in lifespan remain unknown.

Recent attention has focused on heat shock proteins (Hsps) as regulators of cell death and survival. Hsps belong to a family of conserved chaperones induced by stress conditions that have been mainly studied for their participation in protein folding (18). Hsps are classified based on their m.w. into large and small. Hsp70 and Hsp60 are members of the large Hsp group, whereas Hsp27 belongs to the small Hsp group. Recently, Hsp27 emerged as a multifunctional regulator of apoptosis (19). Hsp27 inhibits apoptosis by sequestering cytochrome c, resulting in the inhibition of caspase-9 (20, 21), and by directly associating with caspase-3, inhibiting its activation (22). In addition, Hsp27 can inhibit the Fas-induced apoptotic pathway by blocking the interaction of Daxx with Fas (23, 24). High levels of Hsp27 expression were found to be a marker for increased malignancy in breast cancer (25). Interestingly, we found that expression of Hsp27 is constitutive and independent of heat shock in primary human monocytes, suggesting a different regulatory mechanism of expression than its large Hsp family members, such as Hsp70 (22).

Little is known about the mechanisms that lead to alterations in CD8+CD57+ lymphocyte numbers. In this study, we investigated the role of Hsp27 in the regulation of CD8+CD57+ lymphocyte lifespan. We showed that Hsp27 expression is constitutive in this lineage. Unlike Hsp60 and Hsp70, Hsp27 expression is lower in CD8+CD57+ than in longer living CD8+CD57+ lymphocytes. We found that contrary to the Bcl-2 family members, Hsp27 expression is a predictable marker to assess CD57 lifespan. We demonstrated
by overexpressing and silencing of Hsp27 in CD8CD57+ and CD8CD57' primary lymphocytes that Hsp27 is a key regulator of CD8CD57' lymphocyte cell fate. Together, these findings suggest a key role of Hsp27 in the regulation of CD8CD57'/CD8CD57' lymphocyte lifespan.

Materials and Methods

CD57 purification and cell culture

Human lymphocytes were purified from normal donors following The Ohio State University protocols. Blood was diluted 1:1, with PBS and centrifuged through a Histopaque-1077 gradient (Sigma-Aldrich, St. Louis, MO) at 600 × g for 20 min at 4˚C. The mononuclear layer was removed, washed, and further processed using the Dynal CD8 Positive isolation kit (113-33D, Invitrogen, Carlsbad, CA). CD8' cells were isolated, according to the manufacturer’s protocol, and resuspended in MACS buffer (PBS, 0.5% BSA, and 2 mM EDTA). For CD57' isolation, CD8' cells were resuspended in MACS buffer containing an anti–CD57-biotin labeled Ab (347391, BD Biosciences, San Jose, CA) and incubated for 10 min at 4˚C. Cells were washed and resuspended in MACS buffer containing antibody beads (130-090-485, Miltenyi Biotec, Auburn, CA) and incubated for 15 min at 4˚C. The cell/bead suspension was loaded onto a MS/LS column (347391, BD Biosciences, San Jose, CA) and incubated for 10 min at 4˚C. Cells were given an arbitrary value (β-gal negative) and β-gal expression was quantified using the Image Pro Software. The β-gal transfected cells that had a higher value than Hsp27 cells were considered to be β-gal positive. To determine Hsp27 transfection efficiency, 1 × 10⁶ cells were rinsed with PBS-1% FBS, fixed, and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) as recommended by the manufacturers. The cells were stained with either IgG-FITC (Catalogue: 555786; BD Pharmingen) or anti-Hsp27-FITC (SPA-800FI, StressGen Biotechnologies) Abs at a concentration of 1:100 and analyzed by flow cytometry. For detection of Hsp27 protein expression, cells were lysed in NP-40 buffer and lysates were subjected to SDS-PAGE. Western blot analysis was conducted with anti-Hsp27 and β-Tubulin Abs.

Sequestration of Hsp27

CD57' cells were loaded with anti–Hsp27-FITC (StressGen Biotechnologies) or anti-IgG-FITC Abs (BD Biosciences) using the Chariot Transfection Kit, following the manufacturers’ protocol. Briefly, 1 × 10⁶ cells were resuspended in 200 μl MACS containing 10 μg CD57-biotin and 20 μg anti-Hsp27-FITC or anti–IgG-FITC Abs. Cells were incubated with the Chariot-Ab mix for 48 h at 37˚C. Efficiency of Ab delivery was determined after 48 h by counting at least 200 FITC-positive cells from four independent donors. Fluorescence was visualized on an epifluorescence microscope (Olympus, Melville, NY) and digital images were captured using Optronics Imaging System (Goleta, CA) and ImageProPlus software (excitation at 488 nm; emission at 520 nm).

Casparase-3 activity assay

The presence of active caspase-3 was determined using the 7-amino-4-trifluoromethyl coumarin assay (AFC) linked to the tetrapeptide DEVD, as previously described (22, 26). Briefly, 1 × 10⁶ cells were lysed in NP-40 lysis buffer and incubated in a cyto-buffer (10% glycerol, 50 mM Pipes, pH 7.0, 1 mM EDTA, 1 mM DTT) containing 20 μM DEVD-AFC. Release of free AFC was determined using a Cytofluor 4000 fluorometer (Perceptive, Framingham, MA; excitation, 480 nm; emission, 508 nm).

Statistical analysis

Data expressed as mean ± SEM and comparisons using Student t test.

Results

Differential expression of Hsp27 in CD8CD57' and CD8CD57' lymphocytes

To examine the role of Hsp27 in regulating lymphocyte lifespan, we compared the expression of Hsp27 between CD8CD57' and CD8CD57' lymphocytes from normal donors. Analysis of freshly isolated CD8CD57' lymphocytes lysates showed that these cells express significantly lower levels of Hsp27 than CD8CD57', as demonstrated by ELISA (Fig. 1A) and immunoblotting (Fig. 1B).

Transfection of CD57' cells using purified proteins

CD57' cells were transfected with 0.1, 0.5, and 1 μg human purified Hsp27 obtained as previously described (22) or with 1 μg β-galactosidase (β-gal) proteins using the Chariot Transfection Kit (Active Motive, Carlsbad, CA), following the manufacturer’s protocol. Briefly, 0.5 × 10⁶ cells were resuspended in 100 μl PBS containing 10 μg Chariot peptide and recombinant Hsp27 or β-gal. Cells were incubated with the Chariot-protein mix for 4 h at 48 h at 37˚C. Percentage of protein uptake was determined after 4 h of incubation using the β-gal staining assay, following the manufacturers’ specifications. Efficiency of protein loading was determined from 100 cells by microscopy. For this purpose, the values obtained from Hsp27-loaded cells were given an arbitrary value (β-gal negative) and β-gal expression was quantified using the Image Pro Software. The β-gal transfected cells that had a higher value than Hsp27 cells were considered to be β-gal positive. To determine Hsp27 transfection efficiency, 1 × 10⁶ cells were rinsed with PBS-1% FBS, fixed, and permeabilized using the Cytofix/Cytoperm kit (BD Biosciences) as recommended by the manufacturers. The cells were stained with either IgG-FITC (Catalogue: 555786; BD Pharmingen) or anti-Hsp27-FITC (SPA-800FI, StressGen Biotechnologies) Abs at a concentration of 1:100 and analyzed by flow cytometry. For detection of Hsp27 protein expression, cells were lysed in NP-40 buffer and lysates were subjected to SDS-PAGE. Western blot analysis was conducted with anti-Hsp27 and β-Tubulin Abs.

FIGURE 1. Differential expression of heat shock proteins in CD8CD57' and CD8CD57' lymphocytes. A, Hsp27, 60, and 70 expression was determined by ELISAS using CD8CD57' and CD8CD57' lymphocytes from normal donors (mean = SEM, n = 4), p < 0.01. B, Hsp27 protein level in CD8CD57' and CD8CD57' lymphocytes lysates from two normal donors was determined by immunoblotting and equal protein loading was verified using an anti-β-Tubulin Ab.

Downloaded from http://www.jimmunol.org/ on April 30, 2017 by guest
The expression of Hsp27 in these cells is constitutive and did not require heat shock stimulation. In contrast, the expression of other Hsps, such as Hsp60 and Hsp70, were similar in CD8^+CD57^+ and CD8^+CD57^- lymphocytes (Fig. 1A). These results suggest a central role of Hsp27 in the regulation of cell fate in the CD8^+CD57^+/CD8^+CD57^- lineage.

**CD8^+CD57^+ and CD8^+CD57^- lymphocytes have distinct cellular lifespan**

In normal individuals, the numbers of CD8^+CD57^+ correspond to ~15%, but expansion of CD8^+CD57^- lymphocyte subsets have been shown in several conditions (3). Based on these observations, we compared the levels of apoptosis in CD8^+CD57^- and CD8^+CD57^+ lymphocytes from normal donors at baseline (Time 0) and after culturing for 48 h. We found similar numbers of apoptotic cells around 10% at baseline in CD8^+CD57^- and CD8^+CD57^+ cells as determined by Annexin V/7-AAD staining (Fig. 2A). The percentage of CD8^+CD57^- lymphocytes undergoing apoptosis after 48 h in culture was significantly higher than CD8^+CD57^- reaching almost 50% (Fig. 2A). Consistently, we found a similar level of around 10% of basal active caspase-3 in CD8^+CD57^- and CD8^+CD57^+ cells at baseline (Fig. 2B). The percentage of cells with active caspase-3 in CD8^+CD57^- increased to almost 50% when cultured for 48 h (Fig. 2B). This increase was significantly higher than in CD8^+CD57^- cells (Fig. 2B).

To understand the mechanisms responsible for the discrepancy in lifespan of CD8^+CD57^-/CD8^+CD57^+ lymphocytes, next we evaluated the expression of well known pro- and antiapoptotic proteins at baseline and after 48 h in culture. Expression of antiapoptotic molecules is reduced in apoptotic cells, whereas proapoptotic proteins increase as the cells undergo apoptosis (27). Unexpectedly, we found that the expression of proapoptotic proteins remained fairly unchanged in fresh and 48 h cultured CD8^+CD57^-/CD8^+CD57^+ lymphocytes (Fig. 3, Time 0 versus 48 h, a representative of n = 6). BCL-XL and MCL-1 expression were lower at baseline in CD8^+CD57^- lymphocytes, but did not consistently drop as expected during apoptosis (Fig. 3). BCL-XL expression levels remained unchanged over time in both CD8^+CD57^- and CD8^+CD57^+ (p > 0.1). MCL-1 expression significantly decreased in CD8^+CD57^- (p < 0.05) but the level did not change in CD8^+CD57^+ cultured for 48 h. The only antiapoptotic marker that significantly decreased after 48 h in culture in both CD8^+CD57^-/CD8^+CD57^+ lymphocytes was BCL-2. BCL-2 was expressed at higher levels in freshly isolated CD8^+CD57^- and CD8^+CD57^+ lymphocytes and decreased after 48 h (Fig. 3, Time 0 versus 48 h). However, when we compared BCL-2 expression in freshly isolated CD8^+CD57^- and CD8^+CD57^+ lymphocytes, we found that the BCL-2 expression was significantly higher in CD8^+CD57^- than in CD8^+CD57^+ (Fig. 3). Thus, the basal levels of BCL-2 did not correlate with the difference in lifespan observed in CD8^+CD57^- and CD8^+CD57^+ lymphocytes after 48 h. Interestingly, Hsp27 was the only protein that consistently followed the pattern expected for an antiapoptotic marker. Hsp27 expression was higher in freshly isolated CD8^+CD57^- and CD8^+CD57^+ lymphocytes (Fig. 3, Time 0) and decreased significantly as cells underwent apoptosis (Fig. 3, Time 0 versus 48 h n = 6, p < 0.001). In the case of the proapoptotic molecules Bim and Bax, we found no significant difference in the expression levels between baseline and 48 h in CD8^+CD57^- lymphocytes (Fig. 3, n = 6, p > 0.1). Bax expression increased significantly in CD8^+CD57^- cultured for 48 h, but Bim levels were unchanged. Notably, we found that the expression of Hsp27 was lower in CD8^+CD57^- lymphocytes at baseline than in CD8^+CD57^-, correlating with the increase in apoptosis observed in the CD8^+CD57^- subpopulation as compared with CD8^+CD57^- (Fig. 3, n = 6, p < 0.01). These findings suggest a key role of Hsp27 in the regulation of survival of this lineage.

**Overexpression of Hsp27 inhibits apoptosis CD8^+CD57^- lymphocytes**

Studies have shown that Hsp27 is highly expressed in cancer cells and may contribute to chemotherapeutic resistance (25, 28). We showed that increased expression of Hsp27 in primary human macrophages contributes to their prolonged survival (22). Based on these observations, we evaluated the role of Hsp27 in CD8^+CD57^- lifespan. We overexpressed Hsp27 in CD8^+CD57^- cells from normal donors. Unlike cell lines or other primary cells, CD57 cells are extremely fragile and all efforts to transfect DNA or small interfering RNA using standard approaches rendered them necrotic. Thus, we used a lipophilic carrier to load CD8^+CD57^- cells with purified Hsp27 or β-gal as a control (Fig. 4). Cells stained for β-gal showed that the efficiency of the loading is ~50% (Supplemental Fig. 1A). A similar percentage of cells overexpressing Hsp27 was observed by flow cytometry (Supplemental Fig. 1B compared nontreated [NT] versus transfected). CD8^+CD57^- lymphocytes express lower levels of Hsp27 compared with CD8^+CD57^- at Time 0 (freshly isolated) and the expression is further reduced after culturing cells for 48 h (Fig. 4A, NT time 0 and

![Figure 2](https://example.com/figure2.png)  **FIGURE 2.** Differential lifespan in CD8^+CD57^- and CD8^+CD57^- lymphocytes. A. Percentage of apoptosis was determined in CD8^+CD57^- and CD8^+CD57^- cells from normal donors at baseline (Time 0) and after 48 h in culture using Annexin-V/7-AAD staining and (B) active caspase-3 staining (mean ± SEM, n = 6). p < 0.01; p < 0.02, respectively.

![Figure 3](https://example.com/figure3.png)  **FIGURE 3.** Expression of pro- and antiapoptotic proteins in CD8^+CD57^- and CD8^+CD57^- lymphocytes lifespan. Immunoblots of pro- and antiapoptotic proteins were performed using CD8^+CD57^- and CD8^+CD57^- lysates from normal donors; anti-β-Tubulin Abs were used as loading control. Representative of six independent donors.
48 h CD8^+CD57^+ and 48 h CD8^+CD57^-) We overexpressed different amounts of Hsp27 (ranging from 0.1, 0.5, to 1 μg), titrating its level until reaching the expression found on CD8^+CD57^- at 48 h (Fig. 4, Hsp27 Time 0 and 48 h CD8^+CD57^- and 48 h CD8^+CD57^+). Next, we compared the percentage of apoptosis in CD8^+CD57^+ expressing different amounts of Hsp27. We found that CD8^+CD57^+ cells overexpressing Hsp27 showed a significant decrease in apoptosis at 48 h compared with cells loaded with β-gal, and this decrease was inversely correlated with the amount of Hsp27 loaded (Fig. 4B, a representative of n = 4). The reduction of apoptosis in CD8^+CD57^+ cells overexpressing Hsp27 compared with CD8^+CD57^- loaded with β-gal was significant even at the lower concentrations (Fig. 4C, n = 4; *p < 0.05 and #p < 0.001 using 0.1 and 1 μg Hsp27, respectively). These results demonstrate that overexpression of Hsp27 renders CD8^+CD57^- lymphocytes more resistant to apoptosis.

Hsp27 depletion increases apoptosis of CD8^+CD57^- lymphocytes

To investigate whether the decreased ability for CD8^+CD57^- lymphocytes to undergo apoptosis was due to the higher level of expression of Hsp27, we inhibited the expression of Hsp27. CD8^+ lymphocytes to undergo apoptosis was due to the higher level of expression of Hsp27, we inhibited the expression of Hsp27. CD8^+ lymphocytes were treated with 1 μg anti-Hsp27 or 1 μg anti-IgG as a control. Approximately 50% of CD8^+ lymphocytes compared with control anti-IgG CD57^- were CD57^+ from normal donors left untreated (NT) or loaded with Hsp27 or β-gal reaching levels found in NT CD8^+CD57^- Membranes were immunoblotted with anti-Hsp27 and anti-β-Tubulin Abs. B. CD8^+CD57^- cells overexpressing different amounts of Hsp27 or 1 μg β-gal were cultured for 48 h and stained with Annexin-V/7-AAD to determine apoptosis. A representative of four different experiments. C. Percentage of apoptosis as shown in B. Mean ± SEM (n = 4). *p < 0.05; #p < 0.001.

FIGURE 4. Hsp27 expression regulates CD8^+CD57^- lymphocytes lifespan. A. Immunoblot analysis of CD8^+CD57^+ from normal donors left untreated (NT) or loaded with Hsp27 or β-gal reaching levels found in NT CD8^+CD57^- Membranes were immunoblotted with anti-Hsp27 and anti-β-Tubulin Abs. B. CD8^+CD57^- cells overexpressing different amounts of Hsp27 or 1 μg β-gal were cultured for 48 h and stained with Annexin-V/7-AAD to determine apoptosis. A representative of four different experiments. C. Percentage of apoptosis as shown in B. Mean ± SEM (n = 4). *p < 0.05; #p < 0.001.

Discussion

CD8^+ lymphocytes respond to Ags by rapidly expanding to modulate adaptive immunity, but once the stimuli are cleared the majority undergoes spontaneous apoptosis. One marker of clonal expansion of CD8^+ lymphocytes is CD57. In normal individuals, only a small percentage of CD8 lymphocytes express the CD57 Ag, and these CD8^+CD57^- lymphocytes undergo higher levels of apoptosis than CD8^+CD57^+ lymphocytes. The expansion of CD8^+CD57^-expressing lymphocytes in late stage chronic viral infections, autoimmunity, malignancy, and alloimmunity suggests chronic Ag exposure leads to expansion of these cells by dysregulation of the normal apoptotic pathway. However, the mechanisms responsible for the dysregulation of CD8^+CD57^- lifespan remain unknown. The current study demonstrates that Hsp27 is a central regulator of CD8^+CD57^-CD8^+ lifespan.

Hsp27 is emerging as a multifunctional antiapoptotic factor (19). Overexpression of Hsp27 is associated with resistance to chemotherapeutic drugs (25). Elevated expression of Hsp27 was shown to
determine macrophage resistance to apoptosis (22). Increased expression of Hsp27 during monocyte and macrophage apoptosis seems to be responsible for the differences in lifespan between these two cell types originating from the same progenitor (22, 29). Hsp27 modulates the apoptotic cascade by associating with several proteins involved in cell death (20, 22, 30). Recently, we demonstrated that Hsp27 can interact with procaspase-3 to inhibit caspase-3 activity (21, 31). Hsp27 can also inhibit the Fas apoptotic pathway (23) and this can be independent of the caspase system by blocking the interaction of Daxx with Fas (24).

We found that in normal donors, CD8<sup>+</sup>CD57<sup>+</sup> lymphocytes undergo a higher rate of apoptosis at 48 h than CD8<sup>+</sup>CD57<sup>-</sup> lymphocytes and have higher levels of active caspase-3 (Fig. 2). Baseline levels of Hsp27 in CD8<sup>+</sup>CD57<sup>-</sup> are dramatically lower than in CD8<sup>+</sup>CD57<sup>+</sup> and this level drops even further during apoptosis. We found that in contrast to other regulators of apoptosis (Bcl-2, BCL-XL, and MCL-1), Hsp27 expression in both CD57<sup>+</sup> and CD57<sup>-</sup> is modulated consistently with levels of apoptosis, suggesting its key function in cell fate determination of this lineage (Fig. 3). Next, we demonstrated that apoptosis was reduced in CD8<sup>+</sup>CD57<sup>+</sup> overexpressing Hsp27 (Fig. 4). Notably, when Hsp27 was overexpressed in CD8<sup>+</sup>CD57<sup>+</sup>, at equivalent amounts found in CD8<sup>+</sup>CD57<sup>-</sup>, we observed a percentage of apoptotic cells after 48 h similar than in CD8<sup>+</sup>CD57<sup>-</sup> (Fig. 2A black bar versus Fig. 4C). Conversely, we lowered Hsp27 in CD8<sup>+</sup>CD57<sup>-</sup> lymphocytes using anti-Hsp27 Abs (these cells are referred to as anti-Hsp27 CD57<sup>-</sup> cells). We found that sequestration of Hsp27 increased CD8<sup>+</sup>CD57<sup>-</sup> apoptosis by almost 3-fold (Fig. 5A, 5B). This increase in the percentage of apoptotic cells was consistent with the almost 3-fold increase observed in the activity of caspase-3 (Fig. 5B versus 5D).

These findings demonstrate the central role of Hsp27 in CD8<sup>+</sup>CD57<sup>-</sup> and CD8<sup>+</sup>CD57<sup>+</sup> lymphocyte cell fate and highlight the potential use of Hsp27 as a marker of cell fate in this lineage.

Having a molecule as a potential target to alter the rate of apoptosis in this cell population may be important in certain disease states. Certainly in LGL, where the clonal expansion of these cells defines the malignancy, being able to alter the levels of a protein and decrease the expansion of these large granular lymphocytes may be critical. The clinical features of LGL are consistent with a level of immunosuppression, which is also seen in other conditions where these cells are expanded. The expansion of CD57<sup>+</sup> expressing lymphocytes in late-stage chronic viral infections, autoimmunity, malignancy, and alloimmunity suggests a correlation relationship with immunosuppressed states. In addition, many studies have demonstrated immunosuppressive properties of CD8<sup>+</sup>CD57<sup>+</sup> lymphocytes. CD8<sup>+</sup>CD57<sup>+</sup> lymphocytes exhibit regulatory properties, such as the ability to suppress immune responses by an immunosuppressive mechanism. The regulation of normal lymphocyte apoptosis (and alterations of level of Hsp27) may contribute to the expansion of these CD8<sup>+</sup>CD57<sup>+</sup>-expressing lymphocytes.

**FIGURE 5.** Hsp27 depletion induces CD8<sup>+</sup>CD57<sup>-</sup> lymphocyte apoptosis. A, CD8<sup>+</sup>CD57<sup>-</sup> cells loaded with anti-Hsp27-FITC or anti-isotype control (cells are referred as anti-Hsp27 CD57<sup>-</sup> or anti-IgG CD57<sup>-</sup>, respectively) were cultured for 48 h and the percentage of apoptosis determined by Annexin-V/7-AAD staining. B, Percentage of apoptosis as shown in A. Mean ± SEM (n = 6; p < 0.005). C, To evaluate the effect of sequestering Hsp27, lysates from anti-Hsp27 CD57<sup>-</sup> or anti-IgG CD57<sup>-</sup> cells were immunoprecipitated with anti-caspase-3 Abs, resolved by SDS-PAGE, and transferred to a membrane. Membranes were immunoblotted with anti–Hsp27-FITC or anti-isotype control (cells are referred as anti-Hsp27 CD57<sup>-</sup>). Lysates loaded with anti-Hsp27 sequestered of Hsp27 showed a decreased association of Hsp27 with caspase-3. Representative of n = 6. D, Caspase-3 activity was determined using the DEVDS-AFC assay in anti-Hsp27 CD57<sup>-</sup> or control anti-IgG CD57<sup>-</sup> (black and white bars respectively) and in CD8<sup>+</sup>CD57<sup>-</sup> (gray bar). Mean ± SEM (n = 6). ∗p < 0.005; or #p > 0.1.

**FIGURE 6.** Proposed mechanism for CTL maturation and the relationship to CD57 and HSP expression. Naive CD8 cells do not express CD57, but can develop into CD57 expressing lymphocytes after Ag exposure. CD57<sup>+</sup> cells are sensitive to apoptosis, and have decreased Hsp27. We have shown Hsp27 is a regulator of apoptosis in this setting. We hypothesize that in conditions with continued antigenic exposures, dysregulation of normal lymphocyte apoptosis (and alterations of level of Hsp27) may contribute to the expansion of these CD8<sup>+</sup>CD57<sup>+</sup>-expressing lymphocytes.
lymphocytes have been reported to inhibit PWM-induced Ig production (6), inhibit lymphocyte proliferation (7), and secrete a soluble inhibitor of cytolytic function (32). In bone marrow transplantation patients, the CD8+/CD57+ cell population inhibits alloreactive CTL killing (33). In addition, others have shown the CD8+/CD57+ population suppressed the generation of CMV-specific CTL (34). Therefore, if the presence of these cells is responsible for the disease manifestations, it is important to better define the cellular pathway leading to their apoptosis or survival in certain conditions. In the current study, we propose Hsp27 is a critical regulator of CD8+/CD57+ and CD8+/CD57− lifespan.

Many studies have shown alterations in apoptosis in cells expressing the CD57+ marker. Several studies have shown that CD3 triggered apoptosis is increased in CD8+/CD57+ cells (14, 15, 35) and functions through the caspase-3 pathway. CD8+/CD57+ cells express less survivin, an antiapoptotic molecule (15). However, in disease states, studies are at times conflicting. In multiple myeloma, decreased expression of Fas receptor was suggested to contribute to the reduced rate of cell death observed in CD8+/CD57+ cells (8). It has been suggested that a decrease in apoptosis may explain T cell dysfunction in multiple myeloma (7). However, contrary to this model, in patients with hematologic malignancies, CD8+/CD57+ cells had increased tendencies to undergo apoptosis in culture (10).

Alterations in apoptosis have been used to explain the expansion of CD8+/CD57+ lymphocytes in LGL (36). Studies using whole PBMCs from patients with LGL have found cells resistant to Fas-stimulated apoptosis despite increased levels of Fas and FasL expression (17). The PI3K and STAT3 pathways have been found to be upregulated in CD8+ cells and PBMCs from patients with LGL and play a role in this Fas resistance (37, 38). These studies looked at PBMCs in general, and did not look at differences between different lymphocyte subsets. Larger studies will need to be performed looking at the role of Hsp27 in hematologic malignancies.

Apoptosis has also been examined during HIV infection, and CD8+/CD57+ cells undergo Ag-activated apoptosis (16, 39). However, contrary to this model, in patients with hematologic malignancies, CD8+/CD57+ cells had increased tendencies to undergo apoptosis in culture (10).

References


