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The Low Affinity Fc Receptor for IgG Functions as an Effective Cytolytic Receptor for Self-Specific CD8 T Cells¹

Salim Dhanji, Kathy Tse, and Hung-Sia Teh²

We have recently described a population of self-Ag-specific murine CD8⁺ T cells with a memory phenotype that use receptors of both the adaptive and innate immune systems in the detection of transformed and infected cells. In this study we show that upon activation with IL-2 with or without Ag, between 10 and 20% of the activated self-specific CD8⁺ T cells express the low affinity FcR for IgG. By contrast, all IL-2-activated NK cells express high levels of this FcR. The FcR comprises the FcγRIIα and FcγR subunits. However, the FcγR subunit also associates with the CD3 complex, and this association probably contributes to the low expression of FcR in activated cells. Although the FcR is expressed at a low level on activated self-specific CD8⁺ T cells, it functions very efficiently as a cytolytic receptor in ADCC. FcR-dependent killing occurred in the absence of TCR stimulation, but could be augmented by concurrent stimulation of the TCR. In addition to mediating ADCC, engagement of the FcR on self-specific CD8⁺ T cells results in the production of both IFN-γ and TNF-α. This is the first report of an activating FcR on self-specific murine CD8⁺αβ TCR⁺ T cells and establishes the importance of innate immune system receptors in the function of these self-specific CD8⁺ T cells. The Journal of Immunology, 2005, 174: 1253–1258.

Natural killer cells represent a highly specialized lymphoid population characterized by potent cytolytic activity against tumor or virally infected cells. Their function is finely regulated by a series of inhibitory or activating receptors (1). The inhibitory receptors, specific for MHC class I molecules, allow NK cells to discriminate between normal cells and cells that have lost the expression of MHC class I (e.g., tumor cells). Inhibitory receptors, such as those belonging to the Ly49 family in mice and the killer Ig-like receptors in humans, contain ITIM motifs in their cytoplasmic domains (2). Engagement of these receptors results in the recruitment of inhibitory phosphatases that prevent NK cell activation. The activating receptors responsible for NK cell triggering include Nkp46, Nkp30, Nkp44, and NKGD2 in humans and Nkp46 and NKGD2 in mice (3, 4). The activating receptors, some of which belong to the same families, do not contain activation motifs, but, rather, associate with adaptor molecules or signaling partners important for signal transduction (5).

One of the first activating receptors described on NK cells is FcγRIIIα or CD16. CD16 is a low affinity FcR that binds to IgG and is involved in Ab-dependent cell-mediated cytotoxicity (ADCC),³ in which an Ab-coated target cell is destroyed by NK cells (6). Stimulation of CD16 on NK cells also results in the production of cytokines, such as IFN-γ, TNF-α, and GM-CSF (7). FcγRIIIα associates mainly with ITAM-containing homo- or heterodimers of CD3ζ and FcεRIγ (FcγRy) in humans (8) or solely with FcγR homodimers in mice (9). The binding of IgG to CD16 results in the phosphorylation of ITAMs in the signaling chains, leading to the recruitment of kinases such as ZAP-70 and Syk (10). These kinases initiate a signaling cascade resulting in the lysis of Ab-coated target cells. T cells also use ITAM-containing receptors and Syk and ZAP-70 for their signal transduction (reviewed in Ref. 11). Thus, the signals transduced by the engagement of CD16 on NK cells are very similar to those transduced by the engagement of TCR on T cells.

CD16 expression is not limited to NK cells; other cell types have been described that also express this receptor. γδ T cells have been shown to express CD16, as have a population of large granular lymphocytes (mostly αβ TCR⁺CD4⁻CD8⁻ T cells) in humans (12, 13). In addition, some memory phenotype αβ TCR⁺CD8⁺ T cells in humans have been shown to express CD16 (14). We have previously described a population of αβ TCR⁺CD8αβ T cells in normal B6 mice that exhibit a memory phenotype characterized by the expression of high levels of CD44, IL-2Rβ, and Ly6C (15). These cells can be activated by cytokines such as IL-2 and IL-15, and upon activation they express several functional NK receptors, including 2B4, CD94, and NKG2D as well as the NK adaptor protein DAP12. Using an H-Y TCR transgenic model, we have shown that the development of CD8⁺CD44high T cells is driven by the high affinity interaction of the αβ TCR with cognate self-Ag (16). The H-Y TCR is specific for a male Ag (H-Y) presented by H-2Db, and in H-2b H-Y female mice, the lack of male Ag results in the development of H-Y CD8 T cells with a naive phenotype (CD44low). In H-2b H-Y male mice, in contrast, the presence of the cognate (H-Y) Ag results in the development of a population of CD8⁺ T cells that is virtually identical in cell surface and functional phenotypes with the memory phenotype CD8⁺CD44high cells in normal B6 mice (16). Because memory phenotype CD8⁺ T cells in normal and H-Y TCR transgenic mice are specific for self-Ags, we will refer to these cells as self-specific CD8⁺ T cells to distinguish them from conventional memory CD8⁺ T cells. Self-specific CD8⁺ T cells from

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² Abbreviations used in this paper: ADCC, Ab-dependent cell-mediated cytotoxicity.
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both H-Y TCR transgenic (16) and nontransgenic (15) mice preferentially kill syngeneic tumor cells. This killing of syngeneic tumors involves the MHC-restricted αβ TCR as well as the activating NK receptor, NKG2D, which results in non-MHC-restricted lysis of target cells that express the NKG2D ligand, Rae-1 (16).

In this report we have described the expression and function of CD16 in self-specific CD8+ cells from B6 and male H-Y TCR transgenic mice. We showed that this FcR, comprising the FcRI/IIIa/FcRγ subunits, is similar in composition to the NK FcR. Although this FcR is expressed at a low level in self-Ag-specific CD8+ T cells, it is particularly efficient at initiating the destruction of Ab-coated target cells and can induce the production of two key inflammatory cytokines, IFN-γ and TNF-α. These observations underscore the importance of innate immune system receptors in the function of these self-specific CD8+ T cells.

Materials and Methods

Mice

Breeders for C57BL/6 (B6) mice were obtained from The Jackson Laboratory. The H-Y TCR transgenic mice (17) were bred to the B6 background. Mice, 6–12 wk of age, were used for the experiments described.

Abs and flow cytometry

The following Abs were used: anti-CD4 (PK136), anti-CD8α (53.67), anti-CD8β (53.38), anti-NK.1.1 (PK136), anti-CD3ε (2C11), anti-CD45 (24, Rat IgG) (18), anti-CD16/32 (2.4G2, Rat IgG2b), anti-CD44 (PGP1), anti-FcγR, anti-CD3γ (19), and anti-H-Y TCRα (T3.70) (17). All Abs were purchased from BD Pharmingen, except anti-FcγR (Upstate Biotechnology), anti-CD16/32 (American Type Culture Collection), and anti-H-Y TCRα (eBioscience). The CellQuest software program (BD Biosciences) was used for data acquisition and analysis.

Cell lines

The cell lines used were the RMA lymphoma (H-2b+, Rae-1+), and TAP-deficient RMAS (H-2b+, Rae-1−). The cell lines were cultured in IMEM (Invitrogen Life Technologies) supplemented with 10% (v/v) FBS (Invitrogen Life Technologies), 5 mM HEPES (Invitrogen Life Technologies), 5 × 10−5 M 2-ME, and antibiotics (I-medium).

Cell purification and activation

CD8+ CD44low T cells from B6 mice were purified and cultured in IL-2 (200 U/ml) for 5 days as previously described (15). NK cells were enriched by depletion of CD4+ CD8+ CD3+ IgG cells using Dynabeads (Dynal Biotech) and then cultured in IL-2 for 5 days, resulting in a pure population of activated NK cells (15). Purified naive CD8+ (CD44low) cells do not respond to IL-2 alone and were activated for 5 days on plate-bound anti-CD3ε (10 μg/ml) and IL-2 (20 U/ml). Purified H-Y TCRα CD8+ T cells (1 × 105) were activated by culture with 1 × 106 B6 splenocytes, 1 μM H-Y, peptide, and 20 U/ml IL-2 for 5–6 days.

RT-PCR

RNA was extracted from activated cells and reverse transcribed as previously described (15). PCR was performed using previously described primers and reaction conditions (20).

CTL assays

CTL assays against RMA and RMAS target cells were performed as previously described (15). For ADCC, the target cells were pretreated with anti-CD90 mAB (10 μg/ml) for 15 min at room temperature before use. For the FcR-blocking experiment, anti-FcR mAB (2.4G2 used at 15 μg/ml) was added to the effector cells 15 min before the addition of targets and was added to the effector cells 15 min before the addition of targets and was present throughout the assay. Spontaneous release varied from 8–15% of the maximum. All assays were performed in triplicate. The percent specific lysis was calculated as 100% × [cpm (experimental well) – cpm (spontaneous release)]/[cpm (maximum release) – cpm (spontaneous release)].

Immunoprecipitation and immunoblot analysis

Cells were activated as described above and pelleted/dissolved in 10 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and protease and phosphatase inhibitors. The lysates were separated on a 4–15% Tris-HCl polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. Blots were developed using an ECL system (Amersham Biosciences). CD3 immunoprecipitation was performed by treating lysates with preconjugated anti-CD3e-protein G-Sepharose beads for 2 h at 4°C, followed by several washes. Complexes were then removed from the beads by resuspension in 2X protein sample buffer, followed by boiling for 5 min. The samples were then run in a 4–15% Tris-HCl gel and immunoblotted as described above.

Results

Expression of FcγRIIIa/FcγRγ on activated self-specific CD8+ cells

We have previously shown that self-specific CD8+ T cells express an activated/memory phenotype (15, 16). Initially, ex vivo self-specific CD8+ cells from B6 and male H-2b H-Y mice did not express significant levels of CD16 (Fig. 1a and data not shown). However, upon activation with IL-2 alone (B6) or Ag and IL-2 (H-Y male) for 5–6 days, between 10 and 15% of the activated self-specific CD8+ cells from B6 (CD8+ CD44low) and male H-2b H-Y mice expressed CD16 (Fig. 1a). In contrast, all IL-2-activated NK cells expressed high levels of CD16 (Fig. 1a). Because naive (CD8+ CD44low) cells from B6 and female H-Y TCR transgenic mice did not proliferate in response to stimulation with IL-2 alone, these cells were activated with anti-CD3 and IL-2 (B6) or Ag plus IL-2 (H-Y female) to induce activation and proliferation. It is clear that activated CD8+ CD44low cells from B6 and female H-Y TCR transgenic mice do not express CD16 (Fig. 1a), consistent with the conclusion that conventional CD8 T cells do not express CD16 upon activation.

We have previously described the bystander expansion and activation of self-specific CD8 T cells in response to infection with the bacterial pathogen Listeria monocytogenes (15, 16). Therefore, we wanted to determine whether the frequency of CD16+ self-specific CD8 T cells were activated with Ag and IL-2 for 6 days. The activated cells were stained for the expression of CD16/C57D2. a, CD16/C57D2 profile of the indicated cell type; b, unstained controls. b, Naive B6 mice were infected with 10,000 CFU of L. monocytogenes. On day 5 the mice were killed, and the spleens were removed and stained. The histograms depict the expression of CD8 and CD16 on gated CD8 T cells from infected (right) or uninfected (left) mice, and the percentages of CD16+ CD8+ T cells are indicated. Three-color analysis revealed that the CD8+ CD16+ cells also expressed high levels of CD44 (data not shown).
In uninfected B6 mice, there was a small percentage of CD8\(^+\) T

cells that expressed CD16, but this number increased by ~2-fold upon infection with *Listeria* (Fig. 1b). In addition, all CD8\(^+\) CD44\(^{high}\) cells in both infected and uninfected mice expressed high levels of CD44.

The Ab used to detect CD16 expression also bound to CD32, and it was important to determine which FcR subunits were actually expressed by the self-specific CD8\(^+\) cells. RT-PCR with primers specific for various FcR subunits was used to determine the composition of the expressed FcR. It is clear from this analysis that activated self-specific CD8\(^+\) cells from B6 and male H-2\(^b\) H-Y mice as well as NK cells from B6 mice expressed the mRNA for only FcyRIIa (CD16) and FcγRy, not FcyRI or FcyRIIB (Fig. 2a).

A macrophage cell line was used as a positive control for the expression of FcyRI and FcγRIIB (Fig. 2a).

In murine NK cells, CD16 can only pair with FcγR homodimers (9). Furthermore, atopic expression of CD3ζ in murine NK cells actually interfered with the surface expression and function of CD16 through the formation of CD3ζ/FcγR heterodimers, which cannot associate with CD16 (21). This finding suggests that the low cell surface expression of CD16 on self-specific CD8\(^+\) cells may be due to the high expression of CD3ζ in these cells. To address this possibility, we compared the total amount of CD3ζ and FcγRy in activated NK and self-specific CD8\(^+\) cells by Western blot. Fig. 2b shows that activated NK cells express an undetectable level of CD3ζ and the most FcγRy compared with self-specific CD8\(^+\) cells from B6 (CD8\(^+\) CD44\(^{high}\)) and H-Y male mice. By contrast, self-specific CD8\(^+\) cells from B6 and H-Y male mice express both CD3ζ and FcγRy (Fig. 2b). As expected, conventional CD8 (CD8\(^+\) CD44\(^{low}\)) cells only express CD3ζ. These findings suggest that the low levels of CD16 surface expression in self-specific CD8\(^+\) cells are probably due to the inhibition of binding of FcγR homodimers to CD16 by CD3ζ/FcγRy heterodimers in these cells.

In conventional T cells, the αβ TCR pairs with the CD3 family of signaling chains, including CD3ζ and CD3γ (22). Because FcγRy is expressed in self-specific CD8\(^+\) cells, we determined whether this signaling molecule could associate with the TCR/CD3 complex in these cells. To determine whether FcγRy associates with the CD3 complex, we immunoprecipitated the CD3 complex from IL-2-activated self-specific CD8\(^+\) cells from B6 and male H-Y mice using an anti-CD3ε Ab and immunoblotted with either an anti-CD3ζ or anti-FcγRy Ab. Fig. 2c clearly shows that anti-CD3ζ precipitates both CD3ζ and FcγRy in self-specific CD8\(^+\) cells from B6 and male H-Y mice. As expected, anti-CD3ε precipitates only CD3ζ in conventional CD8\(^+\) CD44\(^{low}\) cells. This association of FcγRy with the CD3 complex in self-specific CD8\(^+\) cells probably interferes with the association of CD16 with FcγRy, resulting in low expression of CD16 in these cells.

**FIGURE 2.** Activated self-specific CD8 T cells express a low affinity FcR similar to NK cells. Cells were activated as described in Fig. 1. a, RT-PCR analysis of FcγRIIa, FcγRy, FcyRI, and FcyRIIB transcripts present in activated cells. RNA from the J774 macrophage cell line was used as a positive control for FcyRI and FcyRIIB. b, Whole cell lysates of the activated cells were subjected to immunoblot analysis for the detection of FcγRy and CD3ζ protein. Blots were stripped and reprobed with anti-ERK2 as a loading control. c, Activated cells were lysed, and the lysates were immunoprecipitated (IP) with anti-CD3ζ Ab. The immunoprecipitates were then immunoblotted (IB) with anti-CD3ζ or anti-FcγRy mAbs. N.D., not determined.

**IL-2-activated self-specific CD8\(^+\) cells can mediate ADCC**

After observing the expression of CD16 on IL-2-activated self-specific CD8\(^+\) cells, we determined whether this receptor could mediate ADCC. To this end, we activated self-specific CD8\(^+\) cells and NK cells from B6 mice with IL-2, then tested their ability to kill Ab-coated RMAS targets cells. Anti-CD3- and IL-2-activated naïve CD8 (CD8\(^+\) CD44\(^{low}\)) cells were included as a negative control. TAP-deficient RMAS cells were used as target cells to rule out contribution by MHC class I molecules in the killing reaction. The RMAS cells were left untreated or were pretreated with anti-
CD90 (clone T24; 10 μg/ml) mAb before use as target cells. Fig. 3a clearly shows that IL-2-activated self-specific CD8\(^+\) and NK cells can efficiently kill Ab-coated RMAS targets, whereas anti-CD3- and IL-2-activated conventional CD8 (CD8\(^+\) CD44\(^{low}\)) cells show absolutely no activity. We noted that anti-CD90 was more efficient in promoting the killing of RMAS targets by self-specific CD8\(^+\) cells compared with NK cells. This finding is remarkable considering that only a small fraction of the self-specific CD8\(^+\) cells express CD16, and the level of CD16 expressed per cell is significantly lower than that for NK cells (Fig. 1a). Because RMAS cells are killed efficiently by activated NK cells, the lack of killing of untreated RMAS cells by activated self-specific CD8\(^+\) cells also indicates the lack of contaminating NK cells in the killing assay.

After observing that self-specific CD8\(^+\) cells from B6 mice could efficiently lyse RMAS targets, we determined whether the presence of MHC class I had any effect on lysis by using TAP-sufficient RMA targets. In addition, we determined whether blocking the CD16 receptor with an anti-CD16 mAb on the activated self-specific CD8\(^+\) cells could block killing of Ab-coated target cells. Fig. 3b demonstrates that self-specific CD8\(^+\) cells efficiently killed Ab-coated RMA cells. Furthermore, the killing of Ab-coated target cells was greatly reduced by blocking the CD16 receptor on self-specific CD8\(^+\) cells before culture with Ab-coated RMA targets. For NK cells, the lysis of Ab-coated RMA cells was only partially inhibited by blocking CD16 on NK cells. This was probably due to the high expression of CD16 on NK cells, which could not be blocked completely by the anti-CD16 Ab treatment. An alternative explanation for the inefficient blocking of killing of Ab-coated target cells by NK cells is that the anti-CD16 mAb
functions as an agonistic mAb. However, this is unlikely, because the treatment of NK cells with anti-CD16 mAb did not have any effect on the lysis of untreated RMA target cells (data not shown). These results clearly demonstrate that self-specific CD8⁺ cells express a functional FcR, which can mediate ADCC. Furthermore, the expression of MHC class I molecules on the target cells does not affect FcR-mediated killing.

**FcR on self-specific CD8⁺ cells from H-Y male mice functions independently of the TCR**

Because self-specific CD8⁺ cells from male H-Y mice express only the male-specific H-Y TCR, we used these cells to determine whether the FcR can function independently of the TCR. Self-specific CD8⁺ cells from male H-Y mice and conventional CD8⁺ cells from female H-Y mice were activated with Ag and IL-2 for 6 days. The activated cells were then assessed for cytolytic activity against untreated or anti-CD90-treated RMA (H-2b) target cells. The killing of anti-CD90-coated target cells in the absence of exogenous H-Y peptide was used as a measure for the contribution of the FcR in the killing reaction. Inclusion of the H-Y peptide in the assay allows an estimation of the contribution of H-Y TCR in the killing reaction. It is clear from the data presented in Fig. 4 that self-specific CD8⁺ cells from male H-Y mice killed Ab-coated RMA targets very efficiently even in the absence of the H-Y peptide. The killing of anti-CD90-coated target cells was slightly enhanced by the addition of H-Y peptide (Fig. 4). The activated self-specific CD8⁺ cells from male H-Y mice required almost 10 nM exogenous H-Y peptide to attain the same level of killing as that seen with anti-CD90-coated target cells in the absence of H-Y peptide. This is remarkable because the entire population of H-Y male cells expressed the H-Y TCR, whereas at most 20% of the cells expressed CD16. By contrast, activated conventional CD8⁺ T cells from female H-Y mice could only kill peptide-loaded target cells, and the presence of Ab on the targets had no effect on this killing. These results suggest that CD16 functions independently of the TCR as an effective cytolytic receptor on self-specific CD8⁺ cells. Furthermore, the FcR and the TCR can act in an additive manner in the killing reaction.

**Engagement of CD16 on self-specific CD8⁺ cells induces cytokine production**

CD16 engagement on NK cells has been shown to induce the expression of several cytokines in addition to being able to induce ADCC (7). To test whether CD16 engagement on self-specific CD8 T cells could also mediate the production of cytokines, we cultured Ag-activated H-Y male CD8 cells with RMA targets either pretreated with anti-CD90 mAb (10 μg/ml) or left untreated. We found that these cells showed a significant increase in IFN-γ production over untreated RMA targets (1.3%). Furthermore, this increase in IFN-γ production was reduced to near basal levels by the inclusion of soluble anti-CD16 mAb in this assay (Fig. 5a). This result suggests that the Fc portion of the bound anti-CD90 mAb on RMA cells induces the production of IFN-γ by H-Y male CD8 cells.

To obtain more direct evidence for FcR-mediated cytokine production by H-Y male CD8 cells, we used Ab cross-linking to stimulate CD16 directly. We cultured day 6 Ag-activated H-Y male CD8 T cells in wells coated with no Ab (Fig. 5b, upper row), anti-CD16 (middle row), or anti-CD3ε (bottom row). After a 5-h
incubation period, we fixed and stained the cells for CD8 and IFN-γ (left column), TNF-α (middle column) or GM-CSF (right column). CD16 engagement resulted in a large increase in both IFN-γ and TNF-α production, with the percentage of cytokine-positive cells being similar to the percentage of CD16+ cells in the sample; CD16 engagement did not induce the production of GM-CSF (Fig. 5b). We also found that soluble anti-CD16 is inefficient in inducing IFN-γ production by H-Y male CD8 cells (data not shown). It is likely that soluble anti-CD16 mAb is less efficient than plate-bound anti-CD16 mAb in aggregating FcRs, and that receptor aggregation is required for efficient activation. By contrast than plate-bound anti-CD16 mAb in aggregating FcRs, and that shown). It is likely that soluble anti-CD16 mAb is less efficient column). CD16 engagement resulted in a large increase in both CD8+ cells in the majority of the cells expressing IFN-γ and TNF-α. In addition, anti-CD3-stimulated cells produced significant amounts of GM-CSF (Fig. 5b). These results indicate that FcR and αβ TCR function as directly activating receptors, which transduce qualitatively and quantitatively distinct signals upon activation.

Discussion
In this study we showed that upon activation with IL-2 or Ag plus IL-2, self-specific CD8+ T cells express an FcR similar in composition to the low affinity FcR for IgG that is found on NK cells. Even though these cells express only a relatively low level of this receptor compared with NK cells, it functions very efficiently in the lysis of Ab-coated target cells. FcR-mediated killing is independent of Ag expression on the target cells. Furthermore, the expression of MHC class I molecules on target cells did not affect the efficiency of FcR-mediated killing. The FcR can also act in an additive manner with the TCR in the lysis of susceptible target cells. This FcR not only mediates efficient lysis of susceptible targets, but it also induces the production of both IFN-γ and TNF-α. The combination of these properties would enable self-specific CD8+ T cells to detect infected or transformed target cells, which might not be detected by NK cells.

It is interesting to note that even though the H-Y male cells represent a clonal population in which all cells should have been activated equivalently, only a fraction of the activated cells express CD16. The low cell surface expression of CD16 on self-specific CD8+ cells is probably due to the high expression of CD3ζ in these cells. In murine NK cells, CD16 can only pair with FcRγ homodimers (9). Furthermore, the atomic expression of CD3ζ in murine NK cells interferes with the surface expression and function of CD16 through the formation of CD3ζ/FcRγ heterodimers, which cannot associate with CD16 (21). We found that the FcRγ-chain in self-specific CD8+ T cells is coprecipitated with CD3ζ by the anti-CD3ζ mAb. It is likely that the resulting CD3ζ/FcRγ heterodimers interfere with the expression of the FcR on IL-2-activated self-specific CD8+ T cells. The association of the FcRγ-chain with CD3ζ is not unique to self-specific CD8+ T cells, because in large granular lymphocytes and in T cells from tumour-bearing mice, the CD3 complex has been shown to associate with FcRγ (23, 24). The association of CD3ζ with the FcRγ-chain also provides a potential explanation for the high efficiency of lytic activity of the FcR in these cells. We speculate that engagement of the FcR causes conformational changes in CD3ζ, resulting in activation of signaling pathways associated with the αβ TCR. A second, nonmutually exclusive explanation is that the differences in the lytic activity of CD16 in these two cell types may be cell intrinsic, which reflects differences in their physiology and/or developmental pathway.

We have previously shown that self-specific CD8+ T cells undergo bystander expansion in vivo in response to Listeria infection, probably as a consequence of the high expression of IL-2Rβ by these cells, which enabled these cells to proliferate in response to IL-2 or IL-15 (16). Furthermore, self-specific CD8+ T cells that proliferate in response to bacterial infection exhibit a heightened ability to produce IFN-γ (16). These properties of self-specific CD8+ T cells would enable them to detect infected cells and provide an early source of IFN-γ. The expression of a self-specific TCR and NKG2D on these cells would allow them to focus on host cells that expressed ligands induced by infection or transformation. In this study we have provided evidence for the expression and functional significance of another activating NK receptor that adds to the arsenal of self-specific CD8+ cells. We have shown that CD16 is expressed on a significant fraction of CD8+CD44high T cells upon Listeria infection in vivo or upon activation with IL-2 or Ag and IL-2 in vitro. Furthermore, engagement of CD16 on self-specific CD8+ T cells results in efficient lysis of Ab-coated targets as well as in the production of inflammatory cytokines. The possession of activating receptors of the innate as well as the adaptive immune system distinguished these cells from NK cells and suggests that this interesting cell type may be particularly adept in providing an early response to infected and transformed cells. These cells will also provide an early source of cytokines, such as IFN-γ and TNF-α, which would prime the adaptive immune system for the elimination of infected and transformed cells.

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