IL-1 enhances expansion, effector function, tissue localization, and memory response of antigen-specific CD8 T cells

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Here, we show that interleukin-1 (IL-1) enhances antigen-driven CD8 T cell responses. When administered to recipients of OT-I T cell receptor transgenic CD8 T cells specific for an ovalbumin (OVA) peptide, IL-1 results in an increase in the numbers of wild-type but not IL1R1−/− OT-I cells, particularly in spleen, liver, and lung, upon immunization with OVA and lipopolysaccharide. IL-1 administration also results in an enhancement in the frequency of antigen-specific cells that are granzyme B+, have cytotoxic activity, and/or produce interferon γ (IFN-γ). Cells primed in the presence of IL-1 display enhanced expression of granzyme B and increased capacity to produce IFN-γ when rechallenged 2 mo after priming. In three in vivo models, IL-1 enhances the protective value of weak immunogens. Thus, IL-1 has a marked enhancing effect on antigen-specific CD8 T cell expansion, differentiation, migration to the periphery, and memory.

A major goal in developing protective immune responses is to obtain robust CD8 T cell expansion, effector differentiation, and memory generation using simple protein antigens as immunogens. We have previously reported that IL-1 strikingly enhances CD4 T cell responses when administered to mice during the period immediately after priming (Ben-Sasson et al., 2009), and thus wished to determine whether it would have a comparable effect on CD8 cells.

IL-1’s effect on CD4 T cells was observed in vivo, was direct, and largely reflected enhanced survival rather than increased proliferative rate. Furthermore, when wild-type and IL1R1−/− CD4 TCR transgenic T cells specific for an OVA peptide were jointly transferred to IL1R1−/− recipients, only the wild-type cells responded to IL-1 with enhanced antigen-driven expansion (Ben-Sasson et al., 2009). This result indicates that IL-1 acts directly on the antigen-responding CD4 cell. Of a wide range of cytokines, including IL-2, -4, -6, -7, -9, -15, -18, -21, and -33, as well as TNF, only IL-1α and IL-1β showed such profound enhancement activity (Ben-Sasson et al., 2009). The IL-1 effect was observed in both IL-6− and in CD28-deficient recipients. Neutralizing IL-1 diminished responses to protein plus LPS by ~60%, implying that endogenous IL-1 enhances antigen-specific CD4 T cell responses.

IL-1 strikingly enhanced antigen-driven expansion in vivo and enhances in vitro expansion of Th17 cells, which express large amounts of IL-1R1 (Guo et al., 2009; Lee et al., 2010), but it had no detectable effect on in vitro expansion of Th1 or Th2 cells. However, administering IL-1 in vivo during CD4 T cell priming, while increasing the proportion of Th17 cells among responders, also causes striking expansion of both IFN-γ and IL-4–producing cells (Ben-Sasson et al., 2009).
The role of IL-1 in regulating CD8 T cell responses has not been clear. Some have reported that IL-1 enhances in vitro expansion of CD8 cells responding to polyclonal stimulants (Mizuochi et al., 1988; Hope et al., 2001). Where studied, it appears that the in vitro effects of IL-1 have been limited to cells expressing large amounts of IL-1R1 (Klarnet et al., 1989; Nagoya et al., 1994). In one instance, enhanced capacity to produce IFN-γ was observed (Fischer et al., 1990). However, others have failed to observe IL-1–mediated enhancement of in vitro TCR–driven CD8 T cell expansion (Halvorsen et al., 1987; Panzer et al., 1990; Curtisnger et al., 1999).

IL-1 acts on CD8 T cells to enhance antigen-driven expansion
To test the effect of IL-1 on antigen-driven expansion of CD8 T cells, we transferred 10⁴ lymph node cells from B6 OT-I TCR transgenic Rag1−/− donors to C57BL/6 mice that express GFP under the direction of the ubiquitin C promoter (UBC-GFP mice). The recipients were immunized subcutaneously with 1 mg OVA together with 25 µg LPS.

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IL-1 enhances effector function of CD8 T cells in response to antigen stimulation

Immunization of mice that received OT-I cells alone or OT-I cells plus OT-II cells with OVA plus LPS caused relatively few cells to express granzyme B or to produce IFN-γ when stimulated with PMA plus ionomycin. Addition of IL-1 caused a striking increase in the proportion of granzyme B–expressing cells and in the fraction of OT-I cells that produced IFN-γ in response to stimulation (Fig. 4 A). In spleen, and particularly in liver and lung, the addition of IL-1 strikingly increases the proportion of granzyme B–expressing cells and in the fraction of OT-I cells that produced IFN-γ in response to stimulation (Fig. 4 A). In spleen, and particularly in liver and lung, the addition of IL-1 strikingly increases the proportion of granzyme B–expressing cells and in the fraction of OT-I cells that produced IFN-γ in response to stimulation (Fig. 4 A). 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We also immunized normal B6 mice with OVA plus LPS with or without IL-1. The addition of IL-1 increased the frequency of CD8 T cells that bound a SIINFEKL tetramer (Fig. 5 B) in both lymph node and spleen, indicating that IL-1 is active on conventional CD8 T cells as well as on transgenic cells. Although mice immunized with OVA plus LPS displayed very limited cytotoxic activity for SIINFEKL-sensitized cells as determined by injection of mixture of sensitized and non-sensitized target cells into these animals, those that had received IL-1 in the immunization protocol showed striking cytotoxic activity in both lymph nodes and spleen (Fig. 5 C).

Mice primed with OVA, LPS, and IL-1 show striking secondary responses

Mice that had received OT-I cells were immunized with OVA and LPS with or without IL-1. 8 wk later, these mice were challenged subcutaneously with OVA plus LPS, without IL-1. 3 d after the boost, there was a trend for more OT-I cells in lymph node, spleen, liver, and lung in mice that had been originally primed in the presence of IL-1 (Fig. 6 A and B). The IL-1–primed cells from each organ showed high frequencies of granzyme B+ cells and cells that produced IFN-γ in response to PMA and ionomycin, whereas cells from these organs derived from mice primed without IL-1 showed substantially less granzyme B or IFN-γ (Fig. 6 C). The relatively high proportion of IFN-γ producers in the lung of mice that
had not been immunized with IL-1 may reflect the difficulty in phenotyping the very small number of such cells in the lung. An example of the difference in liver and lung between mice primed with or without IL-1 is shown in Fig. 6 A. In the IL–1–primed groups, the majority of spleen, liver, and lung OT-I cells were granzyme B+, and the frequency of IFN–γ producers in these organs was ~40% (Fig. 6 C). Thus, the striking phenotypic properties of OT-I cells observed 7 d after priming with IL-1 were also observed when these mice were boosted 8 wk later, even though IL-1 was not administered in the booster immunization.

**IL-1 enhances protectivity of weak or ineffective vaccines**

We wished to determine whether the striking IL–1–mediated enhancement in both CD4 and CD8 T cell expansion and the accompanying increase in effector phenotype would allow greater efficacy of weak vaccines if IL-1 was included in the vaccination protocol. We chose three model systems for analysis, heat-killed *Listeria monocytogenes* (HKLM) as a vaccine for challenge with live *L. monocytogenes*, an HIV peptide as a vaccine for recombinant vaccinia, a human papilloma virus (HPV)–derived peptide as a therapeutic vaccine for TC1 HPV-transformed lung epithelial cells.

Figure 3. IL-1 increases the frequency of OT-I cells in liver and lung. (A) 10⁴ OT-I cells were injected into UBC-GFP C57BL/6 mice. Mice were immunized with OVA and LPS with or without IL-1. 7 d after priming, lymphocyte-enriched fractions were isolated from liver and lung and cells were analyzed for proportion of OT-I cells. This experiment was performed six times with similar results. (B) 10⁴ OT-I cells were injected into UBC-GFP C57BL/6 mice that were then immunized with OVA and LPS with or without IL-1. 7 d after priming, lymphocyte-enriched fractions were isolated from liver and lung and number of OT-I cells was measured. This experiment was performed four times with similar results. (C) 1–3 × 10⁴ OT-I cells were injected into UBC-GFP CD45.1, CD90.1 C57BL/6, CD45.1 C57BL/6, or IL-1R1−/− C57BL/6 mice. Recipients were immunized with OVA plus LPS. 7 d later, the percentage of transferred OT-I cells among the CD8 population in lymph nodes, spleen, liver, and lung was determined. The mean ratio of the percentage of OT-I in CD8 in liver, lung, and spleen compared with that of the lymph nodes from five experiments in WT mice (red) and from three experiments in IL-1R1−/− recipients (green) were calculated. In addition, in one experiment (blue), 10⁴ OT-I cells were transferred without immunization and the ratio of the percentage of OT-I in CD8 in liver, lung, and spleen compared with that of the lymph nodes was determined. (D) 10⁴ CD45.1 OT-I and 10⁴ OT-II cells were injected into CD45.2 IL-1R1−/− and wild-type C57BL/6 mice. Mice were immunized with OVA and LPS with or without IL-1. 7 d after priming, single-cell suspensions from the lymph nodes, spleen, liver, and lung were analyzed by FACS for the content of OT-I cells. The ratio of the number of OT-I cells in the presence of IL-1 to that in its absence is plotted. This experiment was performed twice with similar results.
Figure 4. IL-1 enhances CD8 T cell differentiation. (A) $10^4$ OT-I and $10^4$ OT-II cells were injected into UBC-GFP C57BL/6 mice. Mice were immunized with OVA and LPS with or without IL-1. 7 d after priming, cells from the lymph nodes, spleen, liver, and lung were stimulated for 4 h with PMA and ionomycin, and the percentage of granzyme B$^+$ cells and IFN-$\gamma$ cells among the OT-I cells was determined. This experiment was performed four times with similar results. (B) $10^4$ OT-I and $10^4$ OT-II cells were injected into UBC-GFP C57BL/6 mice. 6 d later, the mice were immunized with OVA and LPS with or without IL-1. 7 d after priming, cells from the lymph nodes, spleen, liver, and lung were stimulated for 4 h with PMA and ionomycin. The percentage of granzyme B$^+$ cells and IFN-$\gamma$ cells among the CD8$^+$ GFP$^-$ OT-I cells in the various organs was determined. This experiment was performed five times with similar results. (C) The MFI of granzyme B and IFN-$\gamma$ among positive cells in the various organs was determined by FACS analysis. This experiment was performed four times with similar results. (D) $10^4$ CD45.1 OT-I and $10^4$ CD45.1 OT-II cells were injected into CD45.2 IL-1R1$^-/$C57BL/6 mice. Mice were immunized with OVA and LPS with or without IL-1. 7 d after immunization, cells from the lymph nodes, spleen, liver, and lung were stimulated for 4 h with PMA and ionomycin. The percentage of granzyme B$^+$ cells and IFN-$\gamma$ cells among the OT-I cells was measured. There were no statistically significant differences in values obtained in mice that did and did not receive IL-1. This experiment was performed twice with similar results.
Fig. 7 A shows that unvaccinated mice infected with $4 \times 10^4$ live *L. monocytogenes* display $5.85 \log_{10}$ CFU of bacteria in the liver 3 d after challenge. If they had been immunized with 500 live *L. monocytogenes* 30 d earlier, they had $2.6 \log_{10}$ fewer CFU. Immunization with 500 HKLM provided no benefit, but the addition of IL-1 to HKLM immunization protocol yielded a $2.0 \log_{10}$ reduction in liver CFU compared with unimmunized mice. We repeated this experiment using 50,000 HKLM as the immunogen with or without IL-1 and again observed no protection in the HKLM group and significant protection in the group that received HKLM and IL-1 (unpublished data). In a separate experiment, we measured survival of mice infected with live *Listeria*. All mice that were unimmunized, received 50,000 HKLM alone or IL-1 alone had either died by day 4 or had to be euthanized because of excessive weight loss. In contrast, three of five mice that received HKLM plus IL-1 survived as did all the mice that had been immunized with live *Listeria* (data not shown).

Challenging BALB/c mice with $2 \times 10^7$ PFU of recombinant vaccinia virus expressing HIV gp160IIIB resulted in $7.3 \times 10^5$ PFU in the ovaries 6 d after infection. Immunization with peptide containing the immunodominant CTL epitope of the vaccinia-expressed gp160IIIB HIV protein (Takahashi et al. 1988; Takeshita et al., 1995) 7 d earlier resulted in a modest degree of protection, to $4.0 \times 10^6$ PFU. Including TLR ligands (MALP2, poly[IC], and CpG ODN 1555 and 1466; Zhu et al., 2010) in the immunization protocol resulted in $8.5 \times 10^6$ PFU upon challenge. Using IL-1 rather than the TLR ligand mixture provided greater protection, reducing the PFU to $4.0 \times 10^6$, a statistically significant improvement over the TLR ligand mixture (Fig. 7 B).

$5.0 \times 10^4$ HPV-transformed lung epithelial TC1 cells were injected into the right flank of C57BL/6 mice. 14 d later, when the tumor could be palpated, the animals were either not immunized or immunized with 100 µg HPV E749-57 peptide plus 25 µg LPS or LPS + IL-1 (4 doses of 2 µg on day 0, 1, 2, and 4 after immunization). Tumor sizes were measured until day 18 after immunization. The group immunized with IL-1 had statistically significantly smaller tumors from day 4 onward (Fig. 7 C).

DISCUSSION

In this study, we have shown that the expansion of naive TCR transgenic CD8 T cells in response to immunization with their cognate antigen plus LPS is enhanced by >60-fold in the spleen and ~20-fold in lymph nodes by administration of IL-1 over the 5 d after immunization. The effect of IL-1 on CD8 T cell expansion in lymph node and spleen does not require that cells other than the CD8 T cells express IL-1R1. The IL-1 effect does not depend on the presence of CD4 T cells although IL-1 does enhance the antigen-driven response of these cells (Ben-Sasson et al., 2009).

Although the mechanism of the IL-1–induced enhancement in lymph node and spleen is not known, it is unlikely to be a result of an “overriding” of a possible inhibitory effect.
of regulatory T cells because only CD8 cells need to express IL-1R1 for the response to IL-1 to occur. For the same reason, the principal effect is not on accessory cells, including DCs. A possible explanation for the IL-1–induced enhancement in cell number is IL-1–mediated suppression of apoptosis in the antigen-stimulated CD8 cells (McConkey et al., 1990; McAleer et al., 2007; von Rossum et al., 2011), which appears to be the major mechanism for IL-1 enhancement of CD4 responses (Ben-Sasson et al., 2009).

The enhanced expansion of OT-I cells is particularly dramatic in the liver and lung where, even with transfer of 10,000 cells, the responding OT-I cells constitute the great majority of all the CD8 T cells in the organ. Interestingly, although the IL-1 effect on expansion in spleen and lymph node does not require IL-1R1 expression on cells other than the OT-I cells, this is not the case for cells trafficking to liver or lung. If the recipient is an IL-1R1–deficient mouse, OT-I cells fail to accumulate in liver or lung, implying that entry into, or retention in, these tissues is enhanced by the action of IL-1 on a tissue cell. Indeed, even the accumulation of OT-I cells in liver and lung in mice immunized with OVA plus LPS without exogenous IL-1 is diminished in IL1R1−/− recipients.

This implies that IL-1 action, possibly on vascular endothelial cells, is important to the robust entry of memory/effector or regulatory T cells.
effector cells into liver and lung, and possibly other tissues, or to retention in these tissues. Indeed, IL-1 has been reported to regulate adhesion and/or transmigration of CD8 effector lymphocytes into various tissues by controlling chemokine production and possibly adhesion molecule expression (Pietschmann et al., 1992; Sikorski et al., 1993; Wang et al., 1993; Kanda et al., 1995; Freedman et al., 1996; Muehlhofer et al., 2000; Stocker et al., 2000; Mohamadzadeh et al., 1998; Ding et al., 2000; McCandless et al., 2009).

Priming in the presence of IL-1 markedly induces granzyme B expression, antigen–specific cytotoxic T cell activity, and IFN-γ–producing capacity of the antigen–specific responding CD8 T cells. What was quite interesting and somewhat perplexing was that the increased expression of granzyme B and the enhanced capacity to produce IFN-γ required expression of IL-1R1 by cells other than the responding CD8 T cells. This implies that factors other than IL-1, possibly produced by dendritic cells that have received an IL-1 signal, are required to cooperate with those generated by IL-1 in the responding T cells to yield robust differentiation in the expanding cells (Wesa and Galy, 2002; Sheng et al., 2008; Thompson et al., 2012).

Not only did recently primed cells display a greater degree of differentiation when IL-1 was part of the immunization regimen, CD8 cells from these mice showed greater granzyme B expression and IFN-γ–producing capacity when challenged 8 wk after priming without readministration of IL-1. Whether this secondary response reflects the expansion of highly differentiated cells in the tissues upon rechallenge or, more likely, the response of cells in spleen and/or lymph node followed by traffic to tissues immediately after the boost is still uncertain. In challenge with live influenza, the process of expansion of CD8 memory cells has been reported to occur largely in the secondary lymphoid organs, with the activated cells then migrating to the tissues (Kohlmeier et al., 2010).

The capacity of IL-1 to enhance both the expansion and differentiation of responding cells and the retention of the enhanced effector capacity of the differentiated cells upon boosting 8 wk late implies that IL-1 as an “adjuvant” could increase the “competence” of weak vaccines to provide protective activity. Indeed, we observed that IL-1 given together with HKLM resulted in a reduction by $10^{-2}$ of the CFU titer in liver compared with immunization with HKLM alone even when the number of HKLM was very low. When IL-1 was used with an HIV peptide, $10^{-1}$ log of increased protection was obtained against a recombinant vaccinia expressing the gp160IIIB HIV protein. IL-1 also enhanced the efficacy of a peptide vaccine against an established tumor. In unpublished work, we have observed that IL-1 enhances the role of both subunit and attenuated Herpes simplex vaccines to offer protection (unpublished data) and increases the protection
obtained in priming with heat killed Blastomyces dermatitidis (unpublished data).

The molecular mechanisms underlying IL-1 actions are still unresolved. IL-1 signals through the Myd88 pathway and has been reported to activate both NF-κB and MAP kinases (Weber et al., 2010). This would allow both the expansion and differentiation processes that have been observed. Recently, it has been reported that IL-1–driven cytokine production by CD4 T cells is largely mediated through the activation of PI-3 kinase and mTOR (Gulen et al., 2010; Chi, 2012). In addition, it has been shown that IL-33 signaling in CD8 T cells augments both the proliferative and the cytolytic activity of cells responding to vaccinia virus (Bonilla et al., 2012). Because IL-1 and IL-33 share IL-1Rα as a co-receptor for signaling and activate similar, if not identical, pathways (Weber et al., 2010; Palmer and Gabay, 2011), it may well be that both molecules perform the same function in antigen-stimulated CD8 cells, depending on expression of the appropriate cytokine receptor. Interestingly, the in vivo generalized expansion of antigen-stimulated CD4 cells is augmented by IL-1 but not by IL-33 (Ben-Sasson et al., 2009), possibly because of little or no expression of the IL-33 binding chain (T1–ST2) on naive CD4 T cells.

IL-1 displays properties that would seem to be of great value in enhancing primary responses and readiness to deal with reintroduction of pathogens. Thus, it strikingly enhances the magnitude of primary expansion, increases the degree of differentiation, allows the cells to enter tissues, and induces the capacity to develop an enhanced secondary response with reintroduction of pathogens. Thus, it strikingly enhances the magnitude of primary expansion, increases the degree of differentiation, allows the cells to enter tissues, and induces the capacity to develop an enhanced secondary response.

### MATERIALS AND METHODS

**Mice.** Female mice of the following strains were obtained from Taconic Farms: TCR Tg OT-I/RAG1+/− C57BL/6; TCR Tg OT-I/RAG1−/− CD45.1 C57BL/6; TCR Tg OT-I/RAG1−/− IL-1R1−/− C57BL/6; TCR Tg OT-II/RAG1−/− C57BL/6; TCR Tg OT-II/RAG1+/− CD45.1 C57BL/6; CD45.1 C57BL/6; RAG1−/− C57BL/6; IL-1R1−/− C57BL/6; and CD90.1 C57BL/6. UBC-GFP C57BL/6 and the appropriate C57BL/6 control female mice were obtained from The Jackson Laboratory. WT BALB/c and C57BL/6 mice for the vaccinia and tumor experiments were used at 6–8 wk of age. Mice was cared for under protocols approved by the NIAID Animal Care and Use Committee.

**Cytokines, peptides, and antibodies.** Recombinant mouse IL-1β was prepared as previously described (Wingfield et al., 1986). Fluorochrome-conjugated anti-Ve2 (B20.1) and anti-Vβ5 (MR9–4) antibodies were purchased from BD. Anti-CD45.1 (A20), anti-CD45.2 (104), anti-CD90.1 (H5S1), anti-CD90.2 (53–2.1), anti-CD4 (L3T4), anti-CD8 (53–6.7), anti–IFN-γ (XMG1.2), anti–granzeyme B (16G.6) fluorochrome-conjugated antibodies were purchased from eBioscience. SIINFEKL peptide was purchased from American Peptide Co. APC-H-2Kb restricted SIINFEKL tetramer was obtained from the National Institutes of Health Tetramer Core Facility. HIV envelope peptide RLPGPRAPYTI and HPV E7 peptide RAYNYIVTF were obtained from the Polypeptide Group.

**Tetramer staining.** Lymph node or spleen cells (2–5 × 10⁶/ml) from lymph nodes, spleen, liver, or lung were stimulated with ionomycin and phorbol 12-myristate 13-acetate (PMA), fixed, permeabilized, and stained as previously described (Ben-Sasson et al., 2009). For cytokine staining, cells were blocked with anti-FcyRII (2.4G2, 50 µg/ml) for 5 min and stained for 30 min at room temperature with anti-PerCP Cy5.5 anti-CD8 and eFluor 450 anti–IFN-γ, PE anti–granzeyme B. The stained cells were analyzed with a BD LSRII, using FlowJo software (Tree Star).

**Intracellular cytokine staining.** Single-cell suspensions (1–5 × 10⁶ cells/ml) from lymph nodes, spleen, liver, or lung were stimulated with ionomycin and phorbol 12-myristate 13-acetate (PMA), fixed, permeabilized, and stained as previously described (Ben-Sasson et al., 2009). For cytokine staining, cells were blocked with anti-FcyRII (2.4G2, 50 µg/ml) for 5 min and stained for 30 min at room temperature with anti-PerCP Cy5.5 anti-CD8 and eFluor 450 anti–IFN-γ, PE anti–granzeyme B. The stained cells were analyzed with a BD LSRII, using FlowJo software (Tree Star).

**In vivo priming of adoptively transferred transgenic cells.** Unless otherwise specified, cells (0.1–3 × 10⁶/ml) from lymph nodes of donor mice were injected i.p. or retroorbitally (RO) into normal recipients (3–5 mice for each experimental group). Mice were immunized s.c. by injection of 1 mg of OVA (Sigma–Aldrich) with 25 µg of LPS (Escherichia coli 0111:B4; InvivoGen). IL-1β was injected daily (2 µg/injection) s.c. for 5 d starting at 24 h after immunization, unless otherwise stated.

**Preparation of single-cell suspensions from lymph nodes and spleen.** Single-cell suspensions were prepared from the lymph nodes and spleens by mechanical dispersion of the organs with a syringe top over a 40-µm sieve on a 50-µm tolu in RPMI 1640 containing 5% FCS.

**Preparation of lymphocyte-enriched populations from liver and lung.** The lymphocyte-enriched fraction was isolated from the liver by mechanical dispersion of the organs, followed by Percoll gradient centrifugation. In brief, the liver was cut into small pieces that were transferred to a 70-µm sieve on a 50-µm tolu and pushed through the sieve with a syringe top. The cells were washed with RPMI 1640 containing 1% FCS, and the pellet was resuspended in 40% isotonic Percoll solution that was underlayed by 60% isotonic Percoll and spun at 2,000 RPM for 20 min at room temperature. Cells at the 40–60% Percoll interface were collected and washed with RPMI 1640 containing 5% FCS. The pellet was resuspended in the same medium.

The lymphocyte-enriched fraction was isolated from the lung after digestion with collagenase/DNase and further purification by Percoll gradient centrifugation. In brief, washed, perfused lung tissue was cut into small pieces and digested by incubation in Petri dishes for 30 min at 37°C in huminidized CO₂ incubator with collagenase/DNase (50 µg/ml collagenase-Liberase; Roche) and 8 U/ml DNase type I (Roche) in RPMI. The lung digest was then treated in the same manner as the liver fragments.

**Monitoring donor CD8 transgenic cells.** The percentage of donor cells among the single-cell suspensions of the lymph nodes, spleen, liver, and lung of the recipient mice was determined by staining for 30 min at 4°C with a cocktail of fluorochrome-conjugated anti-cell surface antibodies in the presence of 50 µg/ml of blocking anti-FcyRII/III Abs (2.4G2), followed by flow cytometric analysis with a BD LSRII, using FlowJo software (Tree Star) for the identification of the OT-1–transferred cells. In C57BL/6 recipients, FITC–anti-Vβ5, PE–anti-Vα2, and APC–anti-CD8 antibodies were used for the identification of the CD8+/Vα2+/Vβ5+ OT-1 cells. In GFP C57BL/6 mice, the FITC conjugated antibody was omitted for the identification of the CD8+/Vα2+/GFP− OT-1 cells.

In CD45.1 C57BL/6 recipients, FITC–anti-CD45.1, PE–anti-Vα2, APC–anti-CD45.2, and PE–Cy7 anti-CD8 antibodies were used for the
identifications of the CD8+ /Vα2+ /CD45.1+ /CD45.2- OT-1 cells. In CD90.1 CS7BL/6 recipients, PE-anti-Vα2, APC-anti-Vα2, FITC-anti-CD45.1, PerCP Cy5.5-anti-CD45.2, eFlour 450-anti-CD90.1, and PE-Cy7-anti-CD90.2 were used to identify the CD8+/Vα2+/CD90.1+/CD90.2-/CD45.1- /CD45.2- OT-1 cells or the CD8+/Vα2+/CD90.1+ /CD90.2+ /CD45.1+ /CD45.2+ OT-1 cells. The number of OT-1 cells in the examined organs was determined by multiplying the cell number in the organ by the percentage of OT-1 Tg cells in that organ.

In vivo cytotoxicity assay. Target cell populations were prepared from naive B6 mice using RBC-lysed splenocytes. The residual white cells (2 × 10^7 cells/ml PBS) were labeled with either 2.5 or 0.25 µM carboxyfluorescein succinimidyl ester (CFSE), (Molecular Probes) for 8 min at RT followed by quenching with 50% FCS for 2 min at RT. The CFSEhigh and CFSElow labeled cells were washed 3x with RPMI 1640 containing 10% FCS. The CFSEhigh target cells were pulsed with SIN1FEKL peptide for 90 min at 37°C and the CFSElow cells were incubated under the same conditions as an internal control. The cells were mixed at a ratio of 2:1 CFSEhigh /CFSElow and injected retroorbitally into mice. Lymph nodes and spleens were removed 18 h later and single-cell suspensions were analyzed for the percentage of CFSElow among total CFSE-labeled cells. A reduction to less than the starting percentage of 67% implies cytotoxicity of peptide-pulsed target cells.

Preparation of heat killed L. monocytogenes. L. monocytogenes (Lm) 10403S was grown at 30°C overnight in BHI broth. Before infection, bacteria were washed in Ringer’s lactate solution twice. Heat-killed L. monocytogenes (HKLM) were generated by heating the Listeria in Ringer’s lactate solution at 65°C for 30 min. All HKLM preparations were tested for residual bacterial viability by plating 100 µl of HKLM on BHI agar.

L. monocytogenes vaccination. Mice were inoculated via tail vein with either no bacteria (PBS), with 500 CFU of live L. monocytogenes, or equivalent of HKLM or with HKLM and IL-1 (10 µg) which was delivered by 7-d miniosmotic pump as previously described (Ben-Sasson et al., 2009). 29 d after priming, mice were challenged with 5 × 10^6 CFU of live L. monocytogenes, and then sacrificed 3 d after the challenge. Bacteria in liver were enumerated using serial dilution and colony counts on either BHI or TSA 5% blood agar plates. At least 5 mice were used in each experimental group.

Immunization with virus-expressed peptide and challenge with virus. BALB/c mice were immunized subcutaneously with 100 µg of the P18-110 (RGPGRAFVFT) peptide from the V3 loop of the IIIB strain of HIV-1 (Takahashi et al., 1988) complexed in DOTAP liposomal transfection reagent (20 µg/Roche). Some mice were also given IL-1 (2 µg on days 0, 1, 2, and 4) or a single dose of 0.1 µg MALP2, 25 µg poly (I:C), and 2 µg CpG ODN 1826 at 30°C overnight in BHI broth. Before infection, bacteria were washed in Ringer’s lactate solution twice. Heat-killed L. monocytogenes 10403S was grown at 30°C overnight in BHI broth. Before infection, bacteria were washed in Ringer’s lactate solution twice. Heat-killed L. monocytogenes L. monocytogenes (Lm) 10403S was grown at 30°C overnight in BHI broth. Before infection, bacteria were washed in Ringer’s lactate solution twice. Heat-killed L. monocytogenes (HKLM) were generated by heating the Listeria in Ringer’s lactate solution at 65°C for 30 min. All HKLM preparations were tested for residual bacterial viability by plating 100 µl of HKLM on BHI agar.

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