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Innate Inflammatory Signals Induced by Various Pathogens Differentially Dictate the IFN-I Dependence of CD8 T Cells for Clonal Expansion and Memory Formation[1]

Lucas J. Thompson,[2]* Ganesh A. Kolumam,[2]* Sunil Thomas, and Kaja Murali-Krishna[3]

Type-I IFNs (IFN-I) provide direct survival signals to T cells during Ag-driven proliferation. Because IFN-I production differs depending on the pathogen, we assessed CD8 T cell requirement for direct IFN-I signals during responses to vaccinia virus (VV), vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), and *Listeria* monocytogenes (LM) immunizations in vivo. IFN-I-receptor-deficient (IFN-IR°) CD8 T cells expanded 3- to 5-fold less and formed a diminished memory pool compared with wild-type (WT) CD8 T cells in response to VV, VSV, or LM. WT CD8 T cells expanded more robustly in response to LCMV-encoded Ags than to Ags encoded by the other three pathogens, and under these conditions the lack of direct IFN-I signals inhibited their expansion by ~100-fold. To test whether the high antigenic-load provided by LCMV caused greater expansion and greater IFN-I dependency, we primed WT and IFN-IR° OVA-specific OT-1 CD8 T cells with a fixed-number of OVA-peptide-pulsed dendritic cells along with adjuvant effect provided by LCMV, VV, VSV, or LM. Both WT and IFN-IR° OT-1 cells were recruited, proliferated, and differentiated into effectors in all the four cases. However, WT OT-1 cells expanded similarly in all four cases. IFN-IR° OT-1 cells expanded ~20-fold less than the WT OT-1 CD8 T cells when LCMV was used as adjuvant, whereas their expansion was affected only marginally when VV, VSV, or LM were used as adjuvants. Thus, innate/inflammatory signals induced by different pathogens contribute to CD8 T cell expansion and memory formation via distinct levels of IFN-I dependence. ***The Journal of Immunology,*** **2006, 177: 1746–1754.**

Generation of the adaptive immune response is regulated by innate signals induced by the infectious agent. Professional APCs, such as dendritic cells (DC)[4]and macrophages, play a central role in this process. APCs can sense infectious agents either directly via pattern recognition receptors or indirectly via inflammatory cytokines produced in response to infection. Some of the inflammatory cytokines produced in response to infection, in addition to acting on the APC, also may act directly on the responding T cells (1–4). One prominent example of such cytokines is IFN$\alpha\beta$ (IFN-I), a family of more than a dozen antiviral cytokines that can be produced by virtually all infected cells (5–7). IFN-I have attracted attention due to their pleiotropic actions on various cells of the innate system as well as their promising use as adjuvants, and as therapeutic agents in infection, cancer, and autoimmunity (8–11). We recently found that IFN-I act directly on CD8 and CD4 T cells in LCMV infection and provide survival signals that contribute to clonal expansion (12, 13). Because the amount of IFN-I, the cell types producing IFN-I, and the availability of IFN-I produced in response to infection is highly dependent on the specific pathogen, it remains important to understand whether CD8 T cells require direct IFN-I signals under conditions of immunization with different pathogens.

In this study, we addressed this question by comparing the response of adoptively transferred wild-type (WT) and IFN-I receptor-deficient (IFN-IR°) CD8 T cells to immunization with lymphocytic choriomeningitis virus (LCMV), vaccinia virus (VV), vesicular stomatitis virus (VSV), or *Listeria monocytogenes* (LM). We find that in all four cases the expansion of the CD8 T cells is dependent on direct IFN-I signaling, but the extent of dependence greatly differed depending on the specific pathogens. The greatest expansion of the CD8 T cells, as well as the greatest dependence on IFN-I signals, was found when CD8 T cells were responding to LCMV-immunization. To assess whether the greater IFN-I dependence of LCMV-specific CD8 T cells was due to massive expansion caused by stimulation by high antigenic load provided by LCMV, or to differences in the cell-types presenting Ag, or to the differences in innate/inflammatory cytokines, we compared the response of WT and IFN-IR° OVA-specific CD8 T cells (OT-1) primed with OVA peptide-pulsed DC under the conditions of LCMV, VV, VSV, or LM immunizations as adjuvant (none of which carried the OVA epitope in these experiments). Our results show that the greater IFN-I dependence of CD8 T cells during LCMV immunization is not related to differences in overall primary expansion of CD8 T cells or differences in Ag load but is attributable to the pattern of innate/inflammatory activation caused by LCMV.

Materials and Methods

Mice

B6.SJL-*Ptprc*^{a}*Pep3*^{b}/BoyJ (B6 Ly5.1), C57BL/6 (Ly5.2), and B6.PL-*Thy1*^{a}/CyJ (B6 Thy1.1) mice were purchased from The Jackson Laboratory

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[4] Abbreviations used in this paper: DC, dendritic cell; IFN-I, type I IFN; IFN-IR°, IFN-I receptor deficient; LCMV, lymphocytic choriomeningitis virus; LM, *Listeria monocytogenes*; VSV, vesicular stomatitis virus; VV, vaccinia virus; WT, wild type.

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and either maintained or bred in our colony. IFNAR-deficient B6.J.129S2-Ifnar<tm1Agt> (IFN-IR°) mice (14) bearing LCMV-specific P14 (15) or OVA-specific OT-1 (16) TCR transgenes on the B6 genetic background were generated as described (12). Mice aged 6–12 wk were used for experiments, and donor-recipient pairs were appropriately matched for gender. Mice were maintained under specific pathogen-free conditions at the University of Washington, Seattle, animal care facility under the guidelines of the Institutional Animal Use and Care Committee.

Viral, bacterial, and DC immunizations

LCMV, VV, and VSV were plaque purified and grown in BHK, HeLa, and BHK cells, respectively, and titered on Vero cells. Recombinant VV (17), and LM (18) expressing the LCMV glycoprotein (GP33-41) and recombinant VV, VSV (19), and LM (20) expressing OVA were previously described. LM was grown from single colonies in brain-heart infusion, harvested at log phase, and washed with PBS before inoculation. A total of 2×10^5 PFU of LCMV, 1×10^6 PFU of VV, 1×10^6 PFU of VSV, 2×10^3 CFU of LM or 400 μg of poly(I:C) were injected into indicated mice 20–30 h after adoptive transfer of T cells. Bone marrow-derived DC were grown in GM-CSF (R&D Systems) as described (21). Before injection, DC were incubated for 2–5 h at 37°C in the presence of 4 μg/ml OVA-derived SIINFEKL peptide and washed three times with serum-free medium. A total of 1×10^6 DC was injected i.p. once at the time of infections and again at 24 and 48 h after the first injection. All time points were measured from the first injection of DC.

Adoptive transfers and CFSE labeling

WT and IFN-IR^0 CD8 T cells were mixed at a 1:1 ratio, and an aliquot was analyzed by FACS to validate the donor cell ratios. For CFSE labeling, cells were incubated with 5 μM CFSE for 7 min, quenched with 10% FCS, and washed three times with serum-free medium before injection into recipient host mice.

Abs and staining

All Abs were purchased from BD Biosciences or eBioscience. Intracellular staining was done as described previously (12).

Serum cytokine assays

Serum was collected from mice that had been immunized 24, 48, or 72 h before collection. Serum from noninfected mice was used as a negative control. Detection of TNF-α, IFN-γ, MCP-1, IL-6 was conducted using a cytokine bead array kit (Cytometric; BD Biosciences). Detection of IFN-α was conducted by ELISA using rat anti-mouse IFN-α Ab clone RMMA-1 as the capture Ab and rabbit anti-mouse IFN-α polyclonal Ab for detection (PBL Laboratories).

Results

The role of direct IFN-I signals on CD8 T cells responding to different pathogens

We first performed side-by-side comparison of the expansion of polyclonal CD8 T cells in B6 mice immunized with the four different pathogens. Expansion of CD62L^{low} CD8 T cells in the spleen was greatest in response to LCMV, followed by VV, VSV, and LM on day 7 postimmunization (Fig. 1*A*). This is not unique to spleen, because a similar pattern was seen in other compartments analyzed (Fig. 1*B*). Quantitation of serum cytokines showed that the diversity and kinetics of each cytokine was different between infections at early time points (Fig. 1, *C* and *D*). Using this system, we next assessed the effect of direct IFN-I signaling on CD8 T cells responding to each pathogen. WT and IFN-IR^0 naive P14 TCR-transgenic CD8 T cells specific for the LCMV glycoprotein (GP33-41) were mixed at equal ratios and then adoptively transferred into WT naive mice. Recipient mice were immunized either with LCMV or recombinant VV expressing the LCMV GP33-41 epitope (rVV-GP33) or recombinant LM expressing the LCMV GP33-41 epitope (rLM-GP33). Donor P14 CD8 T cells expanded in response to all the three pathogens (Fig. 2*A*). Note that the expansion was far greater in response to LCMV, consistent with the result that LCMV induces the greatest levels of total CD8 T cell expansion (Fig. 1). In all four immunizations, the ratio of the WT P14 CD8 T cells was higher than the IFN-IR° P14 CD8 T cells, showing that CD8 T cells responding to each of the three pathogens were dependent on direct IFN-I signals to some extent (Fig. 2*B*). However, the extent to which CD8 T cells become dependent on direct IFN-I signals was determined by the specific pathogen encoding the Ag. Lack of direct IFN-IR direct signals inhibited clonal expansion by >100-fold when the donor cells were responding to LCMV-encoded GP33 epitope (Fig. 2, *B* and *C*, *left panel*), whereas this inhibition was only ~3- to 5-fold when the GP33 epitope was encoded by either VV or LM (Fig. 2, *B* and *C*, *middle* and *right panels*).

To further extend the findings to VSV infection, we shifted to OT-1 CD8 T cells as donor cells. WT and IFN-IR° OT-1 CD8 T cells were transferred at equal ratios, and recipient mice were immunized with recombinant VSV, VV, or LM-expressing OVA (rVSV-OVA, rVV-OVA, and rLM-OVA, respectively). Lack of IFN-I direct signals inhibited clonal expansion by ~3- to 5-fold irrespective of whether the donor OT-1 CD8 T cells were responding to rVV-OVA, rLM-OVA, or rVSV-OVA (Fig. 3).

Together, the above results show that the overall primary expansion of the CD8 T cells was less efficient in VV, VSV, or LM immunizations, compared with LCMV immunization, and lack of direct IFN-I signals to CD8 T cells inhibits clonal expansion by ~3- to 5-fold when responding to VV, VSV, or LM-encoded Ags, and ~100-fold when responding to LCMV-encoded Ags.

Lack of direct IFN-I signals affect expansion but not effector differentiation in the four infection models

Although the lack of direct IFN-I signaling differentially affected CD8 T cell expansion in different infections, it did not markedly affect their ability to differentiate into cytokine-producing effector cells. Both WT and IFN-IR° CD8 T cells expanded by each of the pathogens were capable of producing IFN-γ (Fig. 4, *top panels*) and TNF-α (Fig. 4, *bottom panels*) upon in vitro peptide restimulation.

Direct IFN-I signals enhance the memory pool via their positive effect on clonal expansion

To assess whether the lack of IFN-I signals affects the memory pool, we compared the ratio of WT to IFN-IR° donor CD8 T cells at the peak primary response and during memory phase in rLM-GP33 infection. The ratio of WT to IFN-IR° CD8 T cells did not change from that at the peak expansion to the memory phase (Fig. 5). Similar results were obtained in LCMV infection (12). Thus, the effect that direct IFN-I signals have during clonal expansion in different infections has a long-lasting effect on the size of the memory pool formed.

CD8 T cell expansion in the various infections is similar when Ag load and APC are kept constant

The above results show that 1) CD8 T cell expansion is IFN-I-dependent under conditions of immunization with four different pathogens, 2) the IFN-I dependence was substantially higher when the cells were responding to LCMV, and 3) the overall expansion of the WT CD8 T cells was greatest in LCMV immunization. The reason for greater expansion of the WT CD8 T cells in LCMV, compared with the other three pathogens, may be related to the following: 1) abundance of Ag in LCMV infection, either due to differences in cytopathicity of infected cells, cell tropism, or replication efficiency, or due to differences between a naturally processed viral Ag in LCMV vs artificially inserted recombinant protein sequence in rVV, rVSV, or rLM; 2) differences in the pattern of innate/inflammatory activation induced by LCMV vs the other three pathogens (Fig. 1, *C* and *D*); or 3) both. We wondered

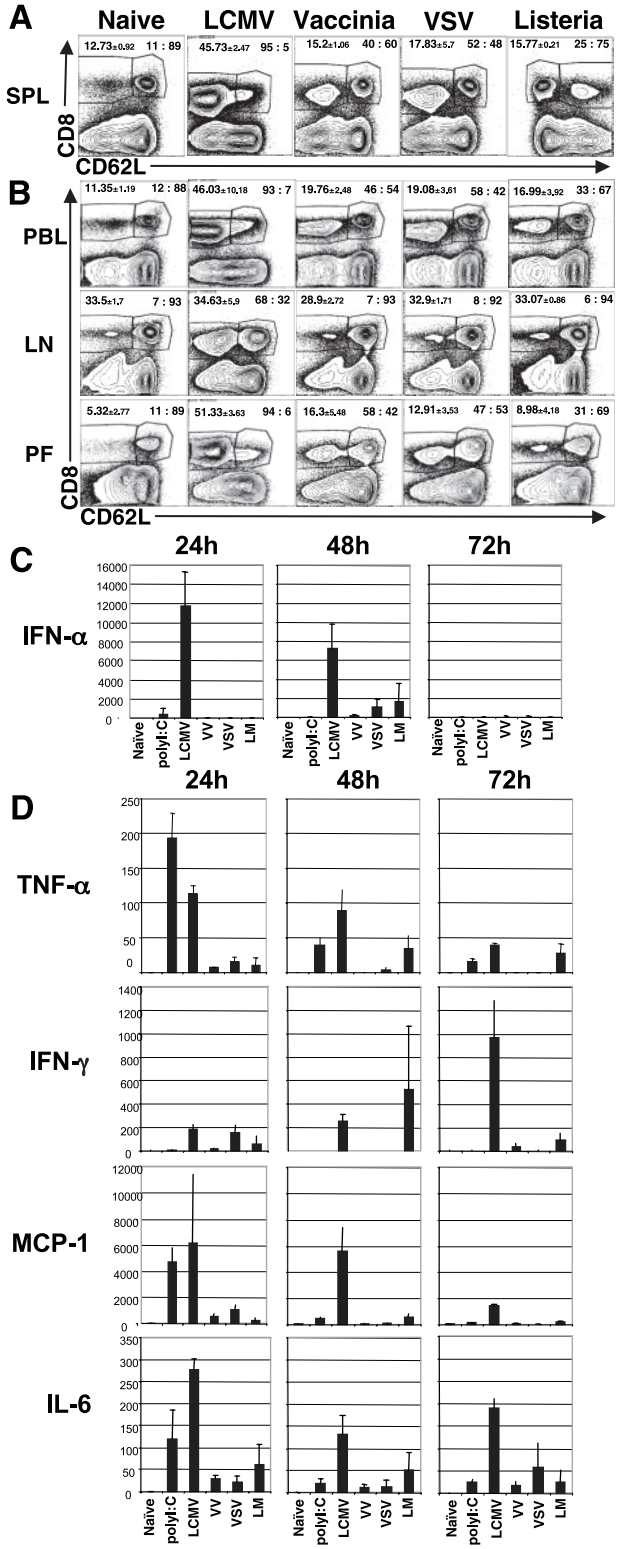

FIGURE 1. Higher levels of CD8 T cell expansion and inflammatory cytokine production in response to LCMV compared with VV, VSV or LM in B6 mice. C57BL/6 mice were infected with indicated pathogens. CD8 T cell expansion and activation at day 7 after immunization was analyzed. Numbers in the left represent average ±SD of CD8 T cell percentages ($n = 3$). Numbers in the right represent ratio of $CD62L^{low}$ vs $CD62L^{high}$ CD8 T cells. Lymphocytes from spleen (*A*) and blood (PBL) (*B*), lymph nodes (LN), and peritoneal fluid (PF) are shown. Serum was harvested from C57BL/6 mice infected 24, 48, or 72 h previously with the indicated pathogens ($n = 3$ per time point). *C*, IFN-α, measured as U/ml serum. Serum IFN-I titers in poly(I:C)-injected mice were higher at time points <20 h postinjection (data not shown). *D*, TNF-α, IFN-γ, MCP-1, and IL-6 pg/ml serum.

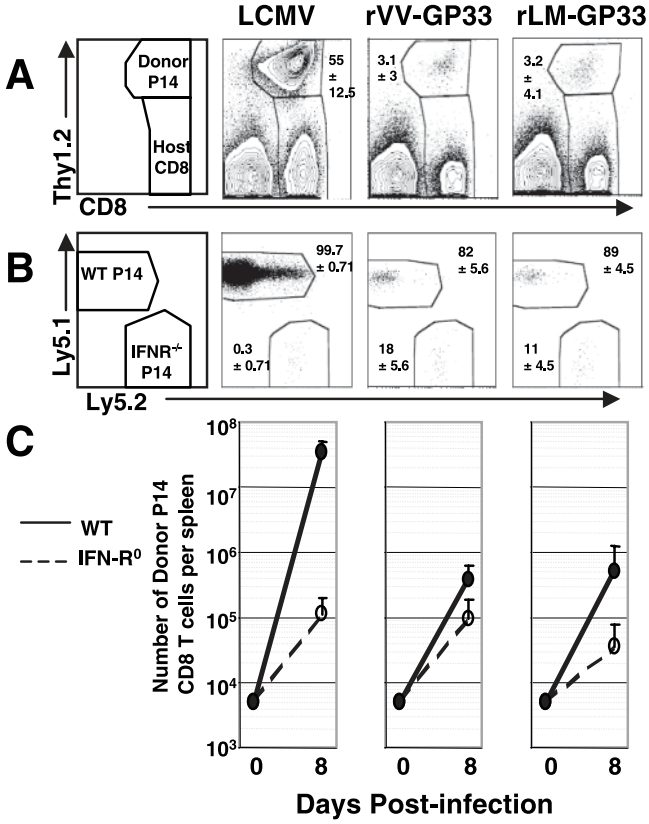

FIGURE 2. CD8 T cells responding to LCMV-encoded GP33 epitope are much more dependent on direct IFN-I signals than CD8 T cells responding rVV or rLM-encoded GP33 epitope. $Ly5.1^+$ WT P14 CD8 T cells and $Ly5.2^+$ IFN-IR° P14 CD8 T cells (both $Thy1.2^+$) were mixed equally and transferred (2×10^4 cells/mouse) into B6 WT $Thy1.1^+$ mice, and then immunized with indicated pathogens. Spleen cells were analyzed on day 8 post immunization. *A*, Expansion of the donor $Thy1.2^+$ cells. Numbers indicate frequency of the donor cells among CD8 T cells ($n = 3$ mice per group). *B*, Ratio of WT and IFN-IR° P14 CD8 T cells among the donor cells. *C*, Recovery of the WT (solid lines, shaded symbols) and IFN-IR° (dotted lines, open symbols) donor CD8 T cells per spleen. Data are representative of three separate experiments.

whether the robust expansion of CD8 T cells in response to high antigenic load provided by LCMV, possibly due to its noncytopathic nature, might be accentuating the differences between WT and IFN-IR° CD8 T cells, possibly by making them highly IFN-I dependent to avoid activation-induced cell death.

To delineate these possibilities, we first asked whether CD8 T cells that were primed under the conditions of innate/inflammatory signals induced by LCMV vs other three pathogens differ in overall expansion if we ensure that they receive similar antigenic load through a defined set of APC. To address this question, the following immunization strategy was developed: OVA peptide-specific WT and $IFN\text{-}IR^0$ OT-1 T cells were adoptively transferred into WT mice, which were then primed by injecting with OVA peptide-pulsed dendritic cells (DC) and concurrently infected with either LCMV, VV, VSV, or LM (none of these microbes carried the OVA epitope). In this way, the donor OT-1 CD8 T cells are stimulated in vivo via a defined set of APC bearing similar levels of Ag in the context of the innate/inflammatory milieu provided by different pathogens. Peptide-pulsed DC injection in the absence of any infection served as controls. In another set, DC-immunized mice were injected with poly(I:C), an inducer of IFN-I and several other cytokines (Fig. 1, *C* and *D*), including IL-12 (22, 23), as a noninfectious inflammatory agent. Data in Fig. 6, *A* and *B*, show

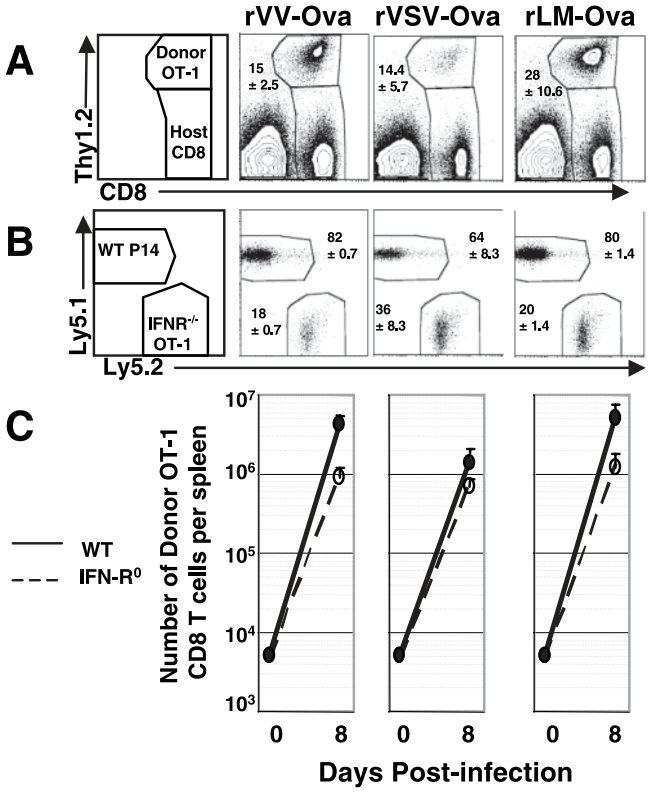

FIGURE 3. Direct IFN-I signals contribute to the expansion of CD8 T cells responding to VV, VSV or LM-encoded Ags, but this contribution is minimal. Ly5.1$^+$ WT OT-1 CD8 T cells and Ly5.2$^+$ IFN-IR° OT-1 CD8 T cells (both Thy1.2$^+$) were mixed equally and transferred (4X10^4 cells/mouse) into B6 WT Thy1.1$^+$ mice, and then immunized with indicated pathogens. Spleen cells were analyzed on day 8 post immunization. *A*, Expansion of the donor Thy1.2$^+$ cells. Numbers indicate frequency of the donor cells among CD8 T cells ($n = 3$ mice per group). *B*, Ratio of WT and IFN-IR° P14 CD8 T cells among the donor CD8 T cells. *C*, Recovery of the WT (solid lines, shaded symbols) and IFN-IR° (dotted lines, open symbols) donor CD8 T cells per spleen. Data are representative of three separate experiments.

that the overall expansion of the donor OT-1 CD8 T cells was not strikingly different irrespective of whether they were primed with DC under the context of LCMV, VV, VSV, LM, or poly(I:C) as adjuvant. Similar results were seen in spleen, blood, lymph nodes, or peritoneal fluid, indicating that these results were not confounded by trafficking issues (Fig. 6*C*). Thus, under the conditions of identical antigenic load provided by a defined set of APC, the overall expansion of the WT CD8 T cells was not drastically affected by the pattern of innate/inflammatory activation caused by each of the pathogens tested. This system allowed us to to ask whether CD8 T cells responding to the same antigenic load and APC differ in their IFN-I dependency based on the innate/inflammatory activation caused by each of the pathogens (see below).

The greater IFN-I dependence of CD8 T cells during LCMV infection is not necessarily related to differences in overall primary expansion of CD8 T cells in different infections or differences in antigenic load, but is attributable to differences in innate/inflammatory activation

Data in Fig. 7*A* (*fourth panel from left*) show that CD8 T cells that were primed by OVA peptide-pulsed DC in the context of LCMV infection are still highly dependent on IFN-I signals, whereas the IFN-I dependence of the CD8 T cells primed in the context of VV, VSV, LM, or poly(I:C) as adjuvants, was minimal (Fig. 7*A*, *third*, *fifth*, *sixth*, and *seventh panels from left*). This pattern of IFN-I dependence was not unique to spleen, because similar trends were observed among donor cells derived from blood, lymph nodes, or peritoneal fluid (Fig. 7*B*).

To assess whether the lack of expansion of OVA-pulsed DC-primed IFN-IR° OT-1 CD8 T cells in the context of LCMV was due to deficiency in recruitment, activation, proliferation, or effector differentiation, we transferred CFSE-labeled WT and IFN-IR° OT-1 cells into WT mice and primed with OVA peptide-pulsed DC concurrently with LCMV, VV, VSV, or LM as adjuvants. In all four groups, both WT and IFN-IR° donor OT-1 CD8 T cells down-regulated CD62L, diluted CFSE (Fig. 8*A*), and differentiated into effector cells capable of producing IFN-γ (Fig. 8*B*). Despite the fact that these cells proliferated and acquired effector function, the DC-primed IFN-IR° OT-1 T cells were not able to accumulate, suggesting that the lack of expansion under these conditions was due to defective survival of Ag-experienced cells. Similar trends were seen when the donor cells derived from different tissues were analyzed (data not shown).

Discussion

In this study, we show that 1) IFN-I direct signals contribute to CD8 T cell clonal expansion in the context of immunization with three different viruses and an intracellular bacterium, and the IFN-I-mediated effect on clonal burst is reflected in the resulting long-lived CD8 T cell memory pool; 2) presence or absence of direct IFN-I signals did not influence the effector differentiation process, as measured by IFN-γ and TNF-α production; and 3) the degree of IFN-I dependence varied among infections, and was most dramatic in LCMV infection. These studies suggest that different pathogens contribute to CD8 T cell expansion and memory formation via distinct overlapping pathways and have implications for understanding the way protective immunity is generated depending on the host–pathogen interaction.

Why are LCMV-specific CD8 T cells more dependent on IFN-I direct signals? We initially considered the following three mutually nonexclusive possibilities: 1) The potentially higher antigenic load provided by LCMV, due to its noncytopathic nature, may cause massive expansion of the CD8 T cells and, under these circumstances, direct IFN-I survival signals may become necessary for rescuing CD8 T cells from activation-induced cell death. This idea is supported by the observations that activation-induced cell death is generally higher under the conditions of intense TCR stimulus (24). 2) An alternate possibility is that the overall increase in the amount and persistence of IFN-I (Fig. 1*C*) during LCMV infection may on one hand cause greater expansion of the CD8 T cells by providing survival signals, while on the other hand serve to accentuate the differences in the expansion of the WT and IFN-IR° cells. This idea is supported by the observations that serum IFN-I levels induced following LCMV infection are generally higher than IFN-I levels induced following VV, VSV, or LM infections. This is further confounded by the fact that LCMV is not known to have evolved any IFN antagonistic mechanisms whereas VV encodes soluble IFN-IR-like molecules to decrease the biologically available IFN-I (8, 25). LM and VSV generally induce IFN-I only in specific cells types (26–28). 3) The third possibility is that IFN-I serves as the most dominantly available survival signal for CD8 T cells under the conditions of LCMV infection, whereas the other three pathogens induce other survival signals, in addition to low levels of IFN-I. This idea is supported by the observations that the high levels of IFN-I induced in response to LCMV actively inhibits production of IL-12 (29), which was proposed to serve as a third signal for CD8 T cell expansion (1).

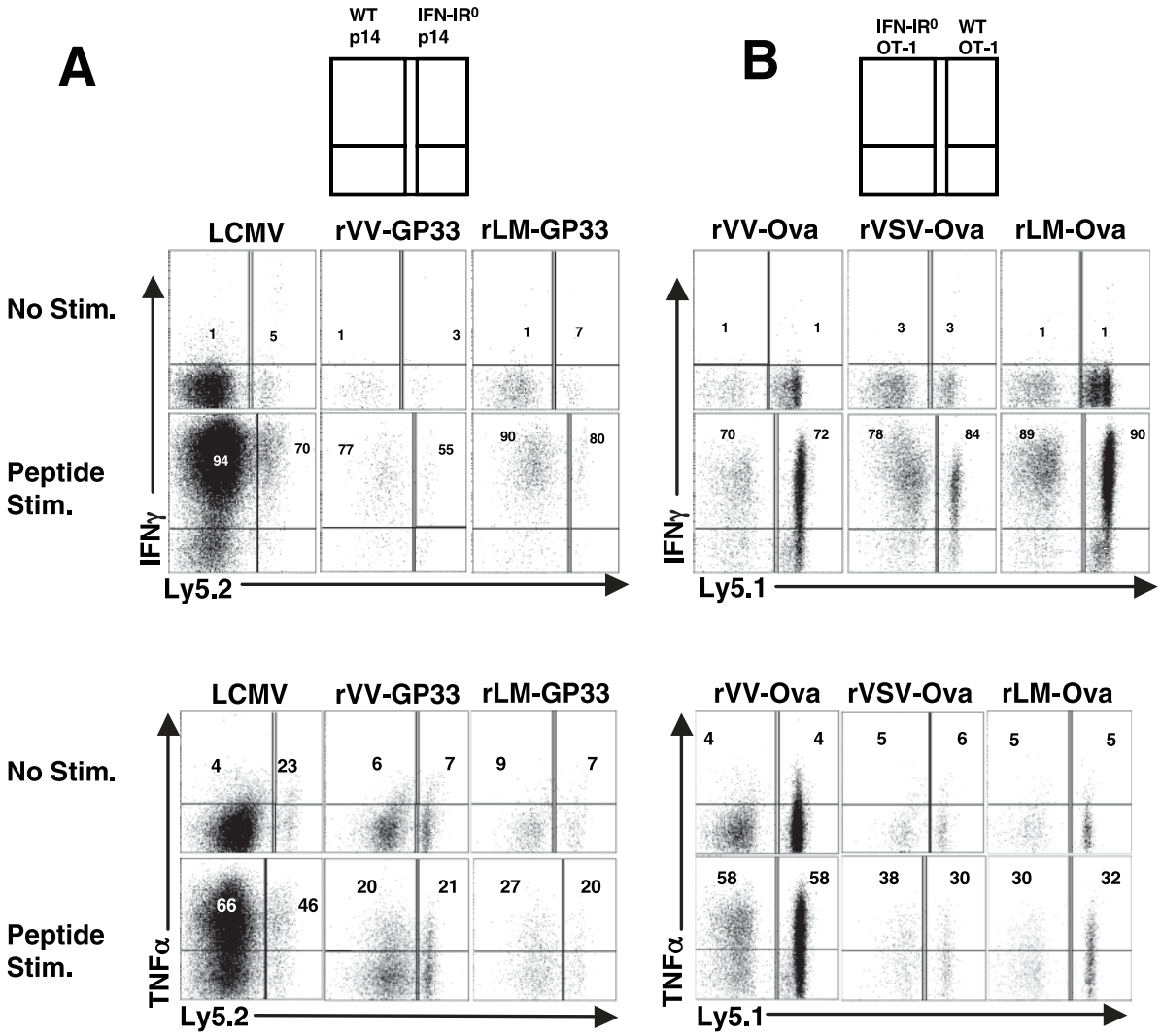

FIGURE 4. Direct IFN-I action on CD8 T cells in different infections does not markedly influence the IFN-γ or TNF-α production. Experimental setup similar to the one described in Fig. 2 (*A*) and Fig. 3 (*B*). Spleen cells were stimulated in vitro for 5 h with cognate peptide and then assessed for intracellular IFN-γ or intracellular TNF-α. Events are gated on donor P14 CD8 T cells (*A*) or donor OT-1 CD8 T cells (*B*). The positions of the WT and IFN-IR° cells indicated at top. Data represent one example of three mice per group.

One way to test the above possibilities is by artificially manipulating IFN-I levels during various infections. This proved to be a problematic approach because of the direct anti-viral affects of the IFN-I-which decreases viral load (5, 14) or due to probacterial affect of the IFN-I which increase LM replication (30). Another way of testing these possibilities is to perform coinfections of LCMV plus other pathogen(s) with one of the infectious agents providing donor T cell specific Ag and the other not. This approach proved difficult due to the combined adverse effects of the pathogens on mutual replication, antigenic load and pathogenicity. Hence, we designed experiments to prime T cells using an identical Ag load via defined set of APC under the conditions of inflammatory signals provided by each of the pathogens. These experiments showed that indeed the massive expansion of the WT CD8 T cells to LCMV-"encoded" Ags may possibly be linked to either the property of LCMV replication, antigenic load, cytopathicity, cell tropism or a combination of these factors. At the same time, these experiments disproved the notion that the higher IFN-I dependence is necessarily linked to the overall level of expansion, antigenic load, or APC, and suggest that innate/inflammatory signals induced by LCMV-infection are most likely be the reason for acute IFN-I dependence of CD8 T cells responding to LCMV infection.

It is notable that, in our later experiments when we primed OT-I CD8 T cells with OVA peptide-pulsed DC in the context of VV, VSV, or LM as adjuvants, the lack of IFN-I signals affected their expansion only by <2-fold. This is not as striking as the 3- to 5-fold effect seen when the OT-1 cells were primed directly by rVV-OVA, rLM-OVA, or rVSV-OVA (compare Figs. 3 and 7). Similarly, lack of IFN-I signals when CD8 T cells primed with OVA peptide-pulsed DC in the context of LCMV as adjuvant affected their expansion only by ~20-fold; whereas the effect was >100-fold when the CD8 T cells were primed with LCMV-encoded Ag. It also should be noted that the overall expansion of the WT CD8 T cells was much higher when primed with pathogen-"encoded" Ags rather than when primed with OVA peptide pulsed DC along with pathogen. These observations, together, raise the following mutually nonexclusive possibilities: 1) The more the CD8 T cells proliferate, the more they will require IFN-I mediated survival signals. Interestingly, data in Fig. 6 show that despite the fact that WT OT-1 cells expand similarly in the four different infection models (under the conditions of similar antigenic load), and

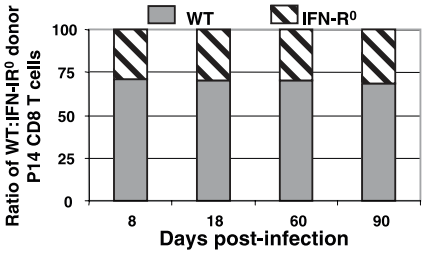

FIGURE 5. Ratio of WT and IFN-IR° donor CD8 T cells was similar in primary expansion and memory phases. Experimental setup is similar to that of Fig. 2. Ratio of the frequency of WT to IFN-IR° donor CD8 T cells in the peripheral blood at days 8, 18, 60, and 90 postimmunization with rLM-GP33.

despite the fact that both WT and IFN-IR° CD8 T cells underwent at least five rounds of proliferation, the IFN-I dependency for survival and accumulation is different between LCMV and the other three infections. This result argues against the possibility of cells becoming more IFN-I dependent within the limited window of expansion and division in this system, and suggests that inflammatory signals induced by LCMV may actually dampen other survival signals provided by DC that sustain clonal expansion, thereby making IFN-I the dominant survival signal available to CD8 T cells proliferating in response to Ag. 2): There are fundamental differences in the costimulatory activity, inflammatory molecules expressed, viability, and diversity of the Ag-expressing cells harboring an actual replicating microbe as opposed to the DC used in our later experiments. Most pathogens, including the ones used in this study, infect a wide variety of nonhemopoietic targets

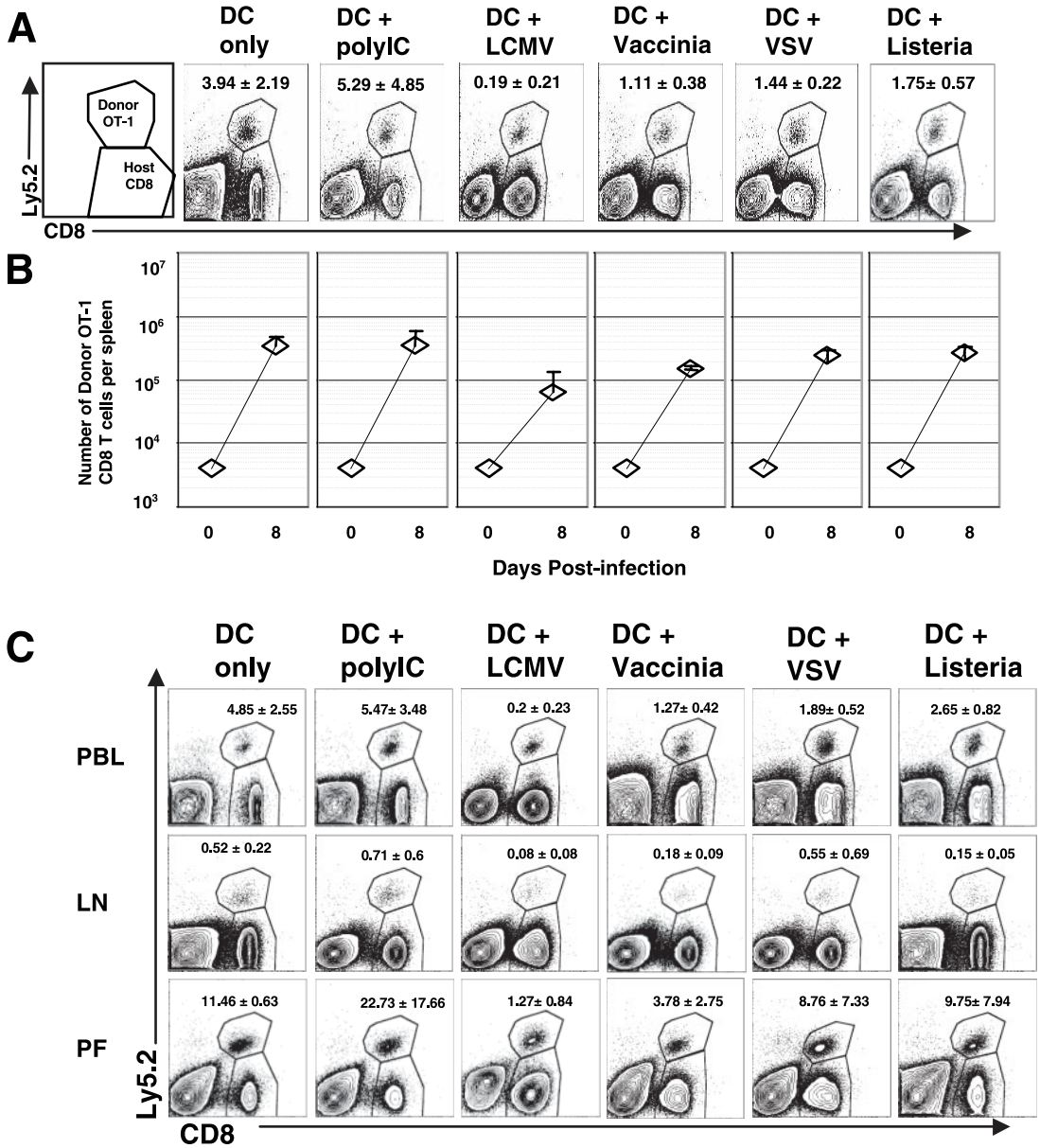

FIGURE 6. Ag-specific CD8 T cell expansion is similar in different infections when equivalent number of DC present Ag: Ly5.2$^+$ Thy1.2$^+$ WT and Ly5.2$^+$ Thy1.1$^+$ IFN-R^0 OT-1 T cells were adoptively transferred into WT Ly5.1$^+$ host mice (4×10^4 total donor CD8 T cells). One day after transfer, bone marrow-derived DC pulsed with OVA peptide were injected into mice alone (DC only), or along with poly(I:C), LCMV, VV, VSV, or LM. *A*, Expansion of the donor OT-1 CD8 T cells at day 8 postimmunization in the spleen. Numbers represent donor cell percentages among CD8 T cells (n = 3 per group). Note that host CD8 T cell expansion varies among infections. *B*, Recovery of the donor OT-1 CD8 T cells per spleen. *C*, Expansion of the donor OT-1 CD8 T cells in the blood (PBL), lymph nodes (LN), and peritoneal fluid (PF). Data are representative of three separate experiments.

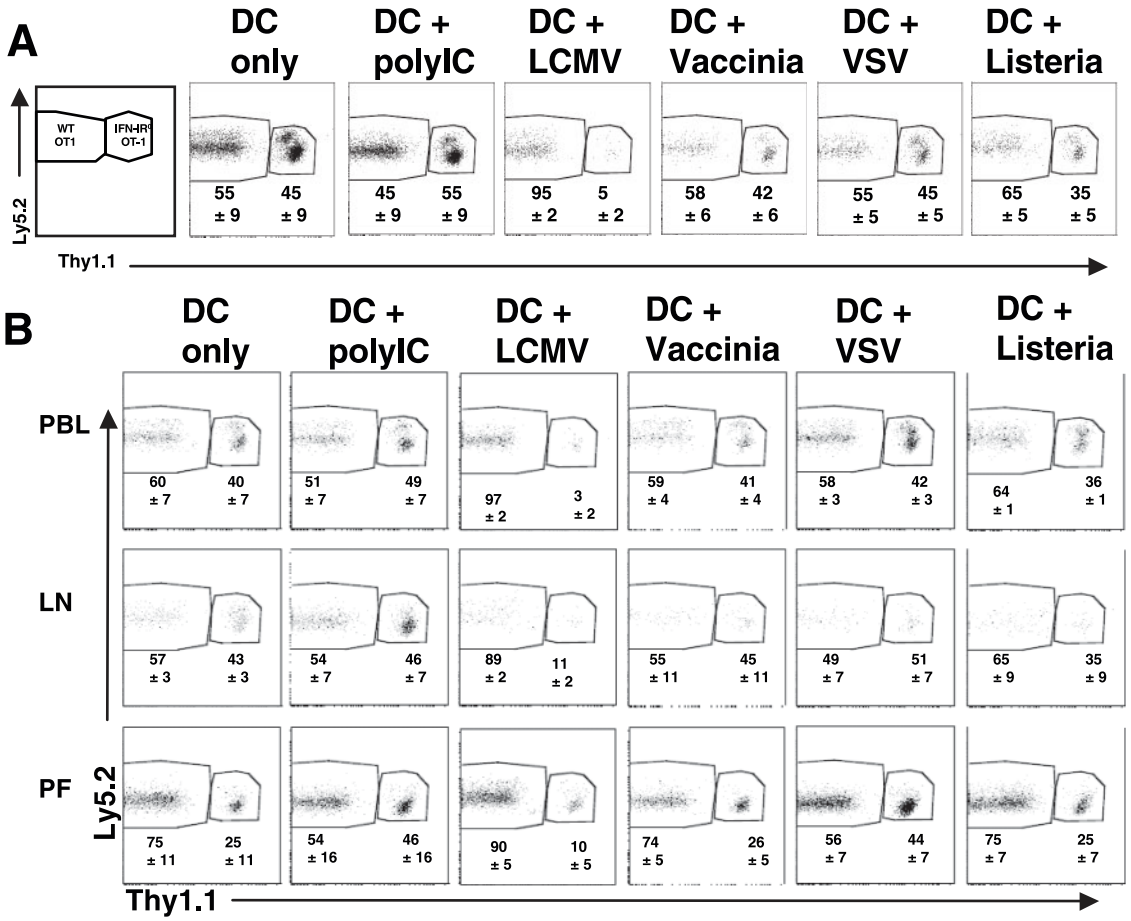

FIGURE 7. Increased dependence of CD8 T cells on direct IFN-I signals in LCMV infection is independent of Ag Load or APC. Ly5.2$^+$ Thy1.2$^+$ WT and Ly5.2$^+$ Thy1.1$^+$ IFN-R° OT-1 T cells were adoptively transferred into WT Ly5.1$^+$ host mice (4×10^4 total donor CD8 T cells). One day after transfer, bone marrow-derived DC pulsed with OVA peptide were injected into mice alone (DC only), or along with poly(I:C), LCMV, VV, VSV, or LM. Ratio of WT to IFN-IR° donor CD8 T cells at day 8 postimmunization in spleen (*A*) and in the blood (PBL) (*B*), lymph nodes (LN), and peritoneal fluid (PF). Data are representative of three separate experiments.

that lack the properties of the professional APC. These infected nonhemopoietic targets are generally deficient in expression of costimulatory molecultes and survival cytokines, such as IL-12, but can potentially produce IFN-I upon infection (depending on the pathogen). Studies from our laboratory show that cognate interaction of the primed effector CD8 T cells with such infected targets causes further expansion, and the contribution by nonhemopoietic targets is much higher in LCMV than the other three pathogens tested here (S. Thomas, G. Kolumam, and K. Murali-Krishna, unpublished data), We think that these factors, in turn, may dictate differential IFN-I dependency of the effector CD8 T cells primed with peptide coated DC$^+$ infection vs those primed directly by infection.

Based on the above findings, we propose the following model, which requires further investigation: 1) Under the conditions of LCMV infection high levels of IFN-I are produced, which seem to persist longer than in other immunizations (Fig. 1*C*). We speculate that either via IFN-I signaling or by some other unknown mechanism, the APC, under the conditions of LCMV, suppresses production of an unknown prosurvival factor. Indeed, the high levels of IFN-I produced in response to LCMV infection have been shown to suppress IL-12 production (29, 31). CD8 T cell expansion under these conditions is mostly dependent on survival signals provided by IFN-I because IFN-I is probably the major survival cytokine produced by these cells. IFN-γ is shown to be another survival factor for T cells during LCMV infection (32), but the severe defect in the IFN-IR° CD8 T cells, despite the fact that they were capable of making IFN-γ, indicates that IFN-γ mediated survival function may be down-stream of IFN-I action (33). 2) However, under the conditions of VV, VSV, or LM immunization, IFN-I is made at low levels only (Fig. 1*C*), and we speculate that, under these conditions, the prosurvival factor is not suppressed. As a result, the CD8 T cells are only partially dependent on IFN-I signals. 3) Not all infected cells are equipped with capacity to produce the unknown prosurvival factor (e.g., if we assume this factor to be costimulatory molecules or IL-12, these are generally produced by professional APC but not every infected cell). As a result, CD8 T cells encountering the Ags on IFN-I$^+$ prosurvival factor-producing cells (as seen in our DC experiments) are likely to become less dependent on IFN-I whereas CD8 T cells encountering Ags on non-prosurvival factor-producing cells (such as infected cells of nonhematopietic origin in all the four infections or DC under the conditions of high IFN-I induced by LCMV) are likely to become more dependent on IFN-I (as seen in our direct pathogen-encoded Ags). The extent of this process is likely to be further influenced by the cytoptahic effect of the pathogen on nonhemopoietic targets, and the ability of nonhematopietic targets to produce IFN-I upon infection. This model underlines the importance of further understanding of the complex factors that differentially regulate IFN-I dependence, clonal expansion, and memory formation, and how these processes are further effected under the

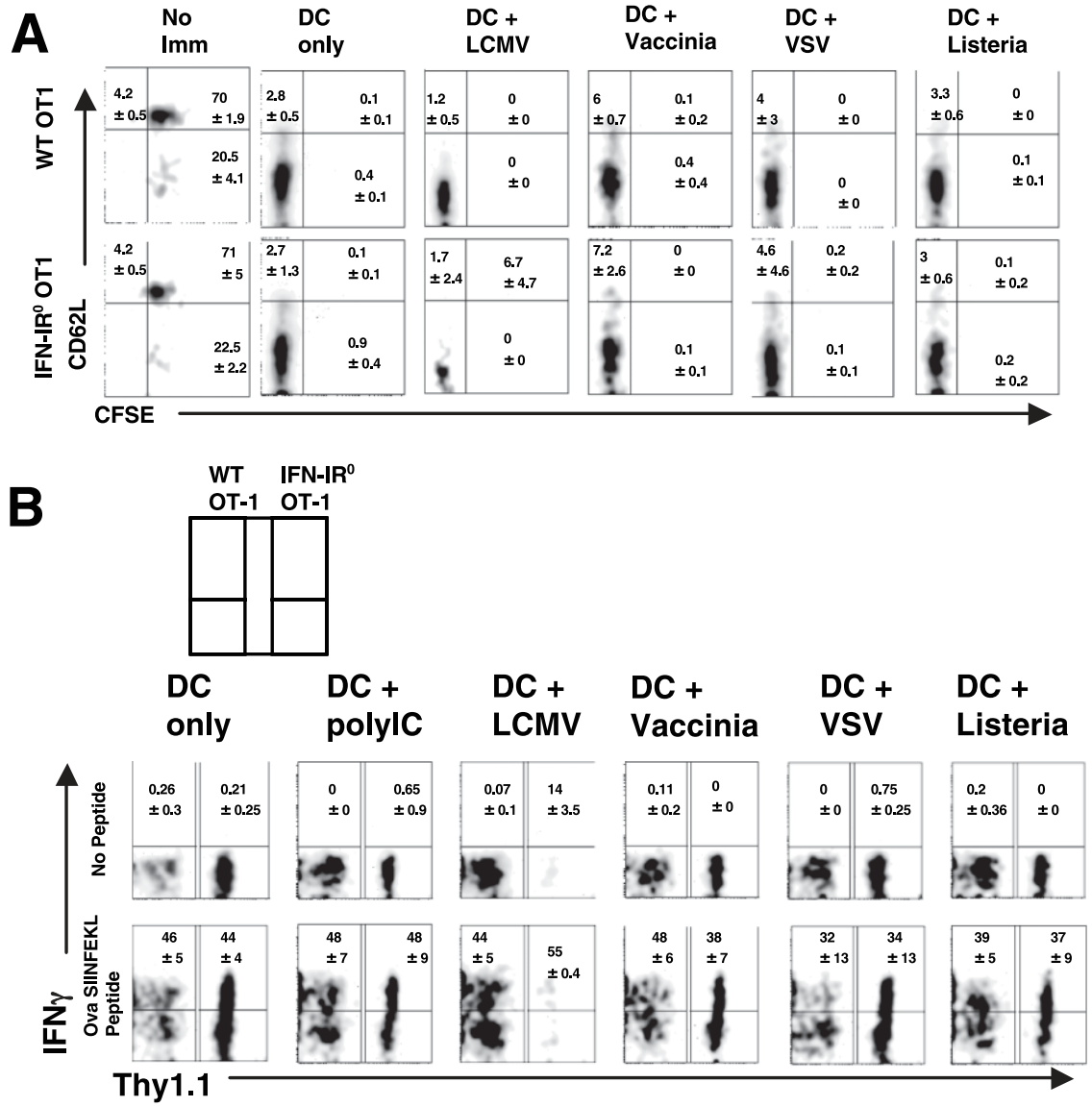

FIGURE 8. DC-primed WT and IFN-IR° OT-1 cells proliferate and acquire effector T cell phenotype: Experimental setup is same as that in Fig. 7, except that the donor cells were labeled with CFSE before transfer. Mice received DCs and infections one day after T cell adoptive transfers. CD62L down-regulation and CFSE dilution (*A*) and intracellular IFN-γ production (*B*) in response to in vitro peptide restimulation in gated WT Thy1.1$^-$ or IFN-IR° Thy1.1$^+$ donor Ly5.2$^+$ CD8 T cells recovered from the spleen of mice immunized as indicated.

conditions of dynamic changes in IFN-I production during transition from acute to chronic phases of infection as seen in HIV and hepatitis viruses. Critical understanding of these factors will have implications in understanding host–pathogen interface, how it influences the generation of protective immunity, and how to design rational therapeutics/vaccines depending on the specific pathogen or vaccine vector.

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Disclosures

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