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Type 1 interferon licenses naïve CD8 T cells to mediate anti-viral cytotoxicity

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ABSTRACT

Naïve T cells, unlike memory T cells, exhibit very limited effector function in response to cognate antigen, but exposure to type 1 interferon (IFN) prior to cognate antigen allows for rapid manifestation of effector functions. A full assessment of the functions of these IFN-sensitized otherwise naïve T cells has not been made, nor has their capacity to be effector cells *in vivo*. We describe here that IFN-sensitized naïve T cells in the absence of cognate antigen adopt a partial activated phenotype distinguished by the upregulation of the surface activation marker CD69, effector-associated transcription factors Eomes and IRF4, and cytotoxicity effector molecule granzyme B. IFN-sensitized naive T cells lysed target cells *in vivo* and responded to low concentrations and affinities of cognate ligands. We suggest that this rapid and sensitive effector function of IFN-conditioned naïve CD8 T cells may play a role in pathogen control and help ward off superinfections.

1. Introduction

High levels of type 1 interferons (IFN) are induced during infections with viruses and other pathogens, and they control infections by directly inhibiting viral replication and by modulating immune system functions (Hervas-Stubbs et al., 2011; Welsh et al., 2012; McNab et al., 2015). CD8 T cells are efficient regulators of viral infection, and type 1 IFN can contribute to the activation, differentiation, and proliferation of these cells by being a signal 3 cytokine (Pham et al., 2011; Curtsinger et al., 2005; Kolumam et al., 2005; Mescher et al., 2006; Thompson et al., 2006; Curtsinger and Mescher, 2010; Obar and Lefrançois, 2010; Keppler et al., 2012). This contribution by IFN normally occurs after a naïve T cell encounters its MHC-expressed cognate peptide ligand (pMHC; signal 1) and co-stimulation by molecules such as B7.1 and 2 (signal 2). These T cells then bathe in the signal 3 inflammatory environment, which over 2-3 days drives naïve T cell proliferation and differentiation into effector cells. This requirement for cell division prior to effector function has now been challenged by some recent studies (Hosking et al., 2014; Curtsinger et al., 2012), and we have shown that prior, or out-of-sequence, exposure to type 1 IFN before exposure of naïve T cells to signals 1 and 2 can drive T cells down an alternative differentiation pathway (Urban and Welsh, 2014). Although the degree of clonal expansion is impaired in out-of-sequence CD8 T cells, they quickly become effector cells in regards to the synthesis of the transcription factor (TF) eomesodermin (Eomes) and the ability to produce IFNγ after exposure to their cognate ligand, much like the rapid activation of effector function seen with memory T cells (Urban and Welsh, 2014; Marshall et al., 2010, 2011). This may mean that only the T cells that get pMHC-engaged in the first one or two days of a viral infection would be expected to undergo the canonical differentiation pathway, whereas others, whether they be latecomer virus-specific T cells or T cells responding to a super-infecting pathogen, would be sensitized by type 1 IFN to become instant effector cells. Presumably, T cells derived from persistently infected or autoimmune hosts chronically producing type 1 IFN would be similarly affected and be activated through this non-canonical pathway. A full assessment of the functions of these IFN-sensitized otherwise naïve T cells has not been made, nor has their capacity to be effector cells *in vivo*. We define here the activation parameters of this type 1 IFN-induced sensitization and show that these sensitized naïve T cells elicit effector functions and lyse target cells *in vivo*. Further, these sensitized T cells can respond to low concentrations and affinities of cognate ligands, suggesting

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that sufficient numbers of non-proliferating naïve T cells may become functional *in vivo* to contribute to viral control.

2. Materials and methods

2.1. Mice

C57BL/6J (WT B6) male mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Congenic (Ly5.1 or Thy1.1) P14 and OT-1 TCR-transgenic, B7.1/B7.2 double KO (B7 KO), and TAP1/TAP2 double KO (TAP KO), were bred in the Department of Animal Medicine at the University of Massachusetts Medical School (UMMS). All mice were maintained in accordance with the guidelines of the Institutional Animal Care and Use Committee of the UMMS.

2.2. Peptides and poly(I:C)

Poly(I:C), purchased from InvivoGen (San Diego, CA), was diluted in HBSS for a concentration of 1 μg/μl. Mice were inoculated with 200 μl HBSS or 200 μg poly(I:C) i.p. and spleens were harvested 1 day (18–22 h) after treatment. Peptides used for intracellular cytokine staining and labeling splenocytes for *in vivo* cytotoxicity assay include GP33 (KAVYNFATC), K3L (YSLPNAGDVI), OVA (SIINFEKL) and altered peptide ligands Y3, T4, V4, and G4 (SIYNFEKL, SIITFEKL, SIIVFEKL, and SIIGFEKL) (Zehn et al., 2009). Cells were incubated at a concentration of 1 μM unless otherwise noted.

2.3. Adoptive transfers of splenocytes

Ly5.1 OT-1 or P14 splenocytes were isolated, removed of red blood cells by lysis with 0.84% ammonium chloride, and washed with HBSS. A total of $1–3 \times 10^7$ transgenic splenocytes were resuspended in HBSS and transferred i.v. into Ly5.2 congenic mouse recipients.

2.4. In vivo cytotoxicity assay

Congenic P14 CD8 T cells were adoptively transferred into B6 mice as described in materials and methods. Mice were either inoculated with HBSS or poly(I:C) for ~1 day, followed by target cell transfer. Leukocytes from B6 mice were pulsed with 1 μM peptide at 37 °C, 5% CO_2 for 1 h. After peptide labeling, cells were dual labeled with 1 μM CellTrace Far Red DDAO (Molecular Probes), and various concentrations of CellTrace Violet (Molecular probes) to differentiate cells labeled with different peptides. Peptide pulsed target cells were adoptively transferred into recipient mice, and splenocytes were harvested ~20 h post transfer. Specific lysis was calculated by the following formula:

$$\%\ \text{specific lysis} = 100 - ((\text{Experimental}/\text{Control})/\text{Control x } 100)$$

2.5. Surface, transcription factor, and intracellular cytokine staining

Splenocytes were stained with a combination of fluorescently labeled monoclonal antibodies (MAb) specific for CD8α (53–6.7), CD8β (YTS156.7.7), Vα2 TCR (B20.1), Ly5.1 (A20), Thy1.1 (HIS51), CD44 (IM7), CD127 (A7R34), CD62L (MEL-14), CD69 (H1.2F3), IFNAR1 (MAR1-5A3), and CD86 (GL1) for 20 min at 4 °C. Cells were fixed with BD Cytofix for 5 min at RT and then resuspended in FACS buffer for collection or permeablized for intracellular transcription factor staining. Cells were permeablized for at least 1 h at 4 °C using the Foxp3 staining buffer kit (eBioscience) followed by intracellular staining for IRF4 (3E4), Eomes (Dan11mag), Tbet (eBio4B10), and granzyme B (GB11).

Intracellular cytokine staining was performed as described previously (Urban and Welsh, 2014). Cells were stained with a combination of fluorescently labeled MAbs specific for TNF (MP6-XT22), IFNγ (XMG1.2), and granzyme B (GB11, Invitrogen). Stimulating in presence of CD107a (1D4B) and CD107b (ABL-93) identified cells undergoing antigen-induced degranulation. All MAbs were purchased from eBioscience (San Diego, CA), BioLegend (San Diego, CA), or BD Bioscience (San Diego, CA) unless otherwise noted.

All samples, freshly stained or previously fixed, were acquired using a BD Bioscience LSR II flow cytometer with FACS Diva software. Data were analyzed with FlowJo software (Tree Star Inc., Ashland, OR).

2.6. Statistical analysis

Where appropriate, Student's *t* test and linear regression were calculated using GraphPad InSt software. Significance was set at a p value of 0.05; * indicates a p of < 0.05, ** a p of < 0.01, *** a p of < 0.001, and **** a p of < 0.0001. Results are expressed as means +/− standard deviations.

3. Results and discussion

3.1. Naïve T cells acquire an early activated phenotype associated with immediate effector function after poly(I:C) inoculation

To study the response of naïve CD8 T cells pre-exposed to signal 3 cytokine activation signals, antigen-specific congenic OT-1 and P14 transgenic CD8 T cells were adoptively transferred into B6 mice, which were then inoculated with HBSS or poly(I:C), as an inducer of type 1 IFN. These transgenic T cells remained small, as judged by flow cytometry, and our previous studies using the dye marker CFSE, which reduces in intensity when cells divide, have shown that P14 cells remain small and non-diving after poly(I:C) treatment or even after 12 days of infection by a non-crossreactive virus (Marshall et al., 2010, 2011). Representative histograms gated on host CD44lo (naïve) WT B6 and IFN alpha receptor 1 (AR1) KO, or on donor CD44lo OT-1 and P14 CD8 T cells from HBSS (shaded histograms)- or poly(I:C) (red open histograms)- treated mice are shown (Fig. 1A and B). Following poly(I:C) treatment, CD44lo WT B6 and donor transgenic CD8 T cells upregulated the early activation markers CD69 and CD86 and downregulated IFNAR1 and CD127, with a small population downregulating CD62L (Fig. 1A). This poly(I:C)-induced activation phenotype in the absence of cognate antigen was seen for naïve transgenic T cells and also in naïve polyclonal CD8 T cell populations in WT B6 mice, but not in IFNAR KO polyclonal CD44lo CD8 T cells, indicating a role for type 1 IFN in poly(I:C)-induced early activation. The upregulation of CD69 was seen at the earliest time point tested, 12 h after poly(I:C) inoculation, but by day 3 post treatment, CD44lo CD8 T cells were phenotypically similar to control-treated counterparts (data not shown), indicating that poly(I:C) transiently induces naïve CD8 T cells to acquire a partial activation phenotype. Poly(I:C) also induced changes in TFs in naïve T cells. The TF IRF4, which is normally upregulated through TCR activation (Nayar et al., 2014), and the TF Eomes, normally associated with CD8 T cell memory (Intlekofer et al., 2005), were upregulated in CD44lo CD8 T cells after poly(I:C) treatment in the absence of cognate antigen (Fig. 1B). However, the CD8 T cell effector-associated T-box TF Tbet remained largely unchanged (Joshi et al., 2007; Joshi and Kaech, 2008). The upregulation of TFs also required type 1 IFN signals, as IFNAR KO CD44lo T cells did not induce IRF4 or Eomes expression.