Neonate-primed CD8\(^+\) memory cells rival adult-primed memory cells in antigen-driven expansion and anti-viral protection

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Abstract

Immunizations early in life, when the host is most susceptible to infection, allow protective immunological memory to develop. Decreasing the dose of Cas-Br-E murine leukemia virus when priming neonatal mice results in adult-like, Type 1 protective responses, but the resulting memory cell populations are smaller than after adult priming. After secondary challenge, virus-specific CD8\(^+\) memory cell populations expand twice as much in neonate-primed mice as in adult-primed mice. We found that when equivalent numbers of virus-specific cells were transferred into virus-susceptible mice, protection from disease was similar whether donor, immune mice were primed as neonates or adults, and IL-4 did not alter in vivo virus-specific CD8\(^+\) memory cell effector function. Hence, neonate-primed CD8\(^+\) cells develop into memory cells that rival adult-primed cells in proliferation and effector function.

Introduction

Classical experiments demonstrating neonatal tolerance to allogeneic grafts (1) led to the view that neonatal immune systems are less competent than adult systems in generating specific immune responses. However, despite decreased numbers of lymphocytes, compromised antigen-presenting cell (APC) function and Type 2 (IL-4, IL-10)-biased cytokine regulation (2–4), recent evidence has shown that neonatal mice can generate competent Type 1 (promoting cytotoxicity and IFN\(_\gamma\) production) T cell responses (5–10). Notably, the production of adult-like cytokine, proliferative and CTL responses required priming conditions uniquely suited to the neonatal host (6, 7, 11–13).

The effectiveness of neonatal immunity at protecting from pathogens is dependent upon the quality and the magnitude of the immune response. To study the interrelationship between size of the memory cell population and its function in neonate-primed mice, we examined the generation and expansion of CD8\(^+\) memory cells in mice primed as neonates with a natural murine pathogen, Cas-Br-E murine leukemia virus (Cas). When \(\geq 1000\) plaque-forming units (PFUs) of Cas are used to infect newborn mice (1–4 days of age), they become carriers and develop a slow onset (6–8 weeks post-infection) neurodegenerative disease (6, 11, 14). These mice are not immunosuppressed yet fail to generate protective immunity. In contrast, adult mice exposed to the same dose of Cas develop protective CD8\(^+\) CTL activity and IFN\(_\gamma\) production. Decreasing the dose of Cas enables neonates to develop protective, CD8\(^+\)-mediated Type 1 immune responses (6), indicating that the antigen dose is crucial to the development of virus-specific immunity in neonates.

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Anti-viral CD8+ T cell responses after neonatal priming

In adulthood, the number of virus-specific memory T cells is significantly lower in mice primed with Cas as neonates compared with mice first exposed as adults (15–17). We hypothesized that a secondary exposure to virus would yield expansion of CD8+ memory cells in both neonate- and adult-primed mice. We also hypothesized that, on a per-cell basis, the protective quality and effector function of memory CD8+ T cells would be equivalent whether the host was primed in adulthood or as a neonate.

In our previous study, we challenged adult mice that had been primed as neonates or adults with Cas. We found that virus-specific IFNγ-producing CD8+ T cells exhibited greater expansion in neonate-primed than in adult-primed mice (16). In the present study, we found that the greater expansion was consistent with a model in which memory cells compete for resources such as growth factors. We also determined that effector function of memory CD8+ cells in vivo after neonatal priming is equivalent to that seen after adult priming.

**Methods**

**Mice and viruses**

NFS/N mice were obtained from the Charles River/National Cancer Institute Biological Testing Branch (Frederick, MD, USA) and bred in pathogen-free conditions at the Duke University vivarium. NFS/N mice are Fv-1<sup>m</sup>, H-2<sup> AQ</sup>, NK1.1<sup>+</sup> and express no ecotropic murine leukemia virus (MuLV) and only low levels of endogenous xenotropic MuLV. Cas virus was grown on SC-1 fibroblast cells, titered by XC plaque assay and monitored in vivo by paralysis induction (14). A low dose of virus used to prime neonates is equivalent to 1–5 PFU, and a high dose of virus is >1000 PFU (6). Mice were injected intraperitoneally (i.p.) at 2–4 days of age with 0.03 ml of a high dose (carriers) or low dose (neonates) of Cas virus. Naïve mice were left uninfected. Adults (>21 days of age) were injected i.p. with 0.05 ml of a high dose of Cas. Some mice were re-challenged with a 5 PFU (booster dose) of Cas at least 8 weeks after priming. The Duke University Institutional Animal Care and Use Committee approved all protocols for animal studies.

**In vitro stimulation of spleen cells**

Spleens were obtained from mice and stored in complete minimal essential medium (Life Technologies, Grand Island, NY, USA) supplemented with 10% heat-inactivated FCS, 25 mM HEPES buffer, 5 × 10<sup>-5</sup> M 2-mercaptoethanol, 1% non-essential amino acids and 1% penicillin–streptomycin–glutamine (Life Technologies Ltd) (11). Cell cultures were set up as described (16). Splenocytes were co-cultured with irradiated (10,000 rads), Cas-infected cell lines (NS467 or Walter cells at 5 × 10<sup>6</sup> cells per milliliter). NS467 is a Cas-infected pre-B cell line (6), and the Walter cell line was derived from NS467 cells passed once in vivo (phenotype: B220<sup>-</sup>CD3<sup>-</sup>CD11b<sup>-</sup>CD11c<sup>-</sup>CD19<sup>+</sup> and Cas<sup>-</sup> (Hy72)) (11)). Cultures were incubated at 37°C in 5% CO<sub>2</sub> for 5 days. On day 4, supernatants were collected to measure the production of IFNγ. On day 5, effector cells were harvested for use in a standard 51Cr cytotoxicity assay (6, 16).

**ELISA**

Plate modules (Nalgene-Nunc, Rochester, NY, USA) were coated with 0.05 µg per well purified anti-IFNγ (clone RA-6A2) (BD Pharmingen, San Diego, CA, USA) overnight at 4°C and then blocked with carbonate buffer/BSA (18). Next, serial dilutions of supernatant or cytokine standards were added and incubated at 37°C. Plates were then washed and incubated with 0.05 µg ml<sup>-1</sup> of biotinylated anti-IFNγ (clone XMG1.2). After washing, ~2 ng ml<sup>-1</sup> of streptavidin-horse radish peroxidase (SA-HRP) (Southern Biotechnology Associates, Inc., Birmingham, AL, USA) was added to each well and incubated. The plate was developed using a tetramethylbenzidine peroxidase substrate kit (Bio-Rad, Hercules, CA, USA). The reaction was stopped with 1 N H<sub>2</sub>SO<sub>4</sub>. Plates were read and analyzed on a Molecular Devices ELISA spectrophotometer using the Soft Image/Soft Pro software (Molecular Devices, Sunnyvale, CA, USA).

**ELISPOT assay**

ELISPOT assays were performed as described (16, 19). Cells were cultured for 24 h at 37°C in 5% CO<sub>2</sub> with 5 × 10<sup>5</sup> irradiated Cas-infected cells (NS467 or Walter) in HL-1 serum-free media (Bio-Whittaker, Walkersville, MD, USA). Spots were counted using an ELISPOT Imager (Cellular Technology Ltd, Cleveland, OH, USA).

**Flow cytometry and intracellular cytokine staining**

Flow cytometric assays were performed as described using antibodies from BD Pharmingen (16). Cytokine-producing cells were enumerated by culturing freshly isolated splenocytes at 3 × 10<sup>6</sup> ml<sup>-1</sup> in flat-bottom 24-well plates. Cells were left untreated or stimulated with the Walter cell line overnight at 37°C in 5% CO<sub>2</sub>. Monensin (GolgiStop, BD Pharmingen) was added during the last 5–6 h of culture. Cell-surface staining for CD8 (clone 53-6.7) and CD44 (clone IM7) was then performed, followed by intracellular cytokine staining using the Cytotox/Cytoperm kit (BD Pharmingen) and anti-IFNγ (clone XMG1.2). Cell samples were collected on a FACScalibur (Becton-Dickinson) and analyzed using FlowJo software (Tree Star, San Carlos, CA, USA).

**T cell purification for adoptive transfer**

Splenocytes were enriched for CD8<sup>+</sup> sub-populations using T cell subset enrichment columns (R&D Systems, Minneapolis, MN, USA) which provide >95% CD8<sup>+</sup> T cell populations (16). For in vivo proliferation studies, T cells were enriched from spleens using nylon wool columns (Polysciences, Inc., Warrington, PA, USA). Purity of T cells was ~75%.

**Adaptive transfer to determine protection**

Unfractionated (1.5 × 10<sup>6</sup>–5 × 10<sup>6</sup>) or CD8<sup>+</sup> spleen (1 × 10<sup>6</sup>) cells were injected i.p. into 2-day-old recipient mice. Recipients were infected 24 h later with ≥1000 PFU Cas i.p. Examinations for clinical symptoms of neurological disease (tremor, hind limb weakness and paralysis) in the hosts were performed 8–30 weeks after adoptive transfer (14). Transferring 5 × 10<sup>6</sup> naive adult donor cells in three independent experiments provided protection to 8 ± 14% (mean ± SD) of host mice; thus, protection was considered significant if ≥37%
(mean ± 2 SD) of mice did not show symptoms of neurological disease by 30 weeks. To compare protection on a per-cell basis, percent protection was plotted against the number of effector cells transferred as determined by previously reported ELISPOT frequencies (16). Linear regression analysis was performed on the data lines to draw a best-of-fit line using Microsoft Excel software.

In vivo cytotoxicity assay

In vivo cytolytic activity was measured using modified protocols (20, 21). Target cells were prepared from spleens of naive/uninfected mice or infected carrier mice. Cells were re-suspended at 15 × 10^6 ml^-1 in warm PBS/0.1% FCS containing the appropriate concentration of 5(6) carboxyfluorescein diacetate succinimidyl ester (CFSE) (naive spleen cells: 0.3 μM, CFSE<sub>low</sub>; infected spleen cells from carriers: 3.0 μM, CFSE<sub>high</sub>) and incubated at 37°C for 15 min in the dark. Spleen cells from carriers express Cas envelope glycoprotein (14, 18). Cells were washed and re-suspended in PBS at 12.5 × 10^6 ml^-1. Equal numbers of both target cells were mixed, and a total of 5 × 10^6 cells per mouse of the 1:1 mixture were injected via the tail vein. Spleens from recipient mice were harvested 18 h later for flow cytometric analysis (5 × 10^6 CFSE<sup>+</sup> cells were analyzed). Specific lysis was calculated by first determining the following ratio: percentage CFSE<sub>low</sub>/percentage CFSE<sub>high</sub> (=A). The percentage of specific lysis = (1 – A) × 100.

Statistical analysis

Cytotoxicity and ELISPOT values ≥2 SD above that exhibited by naive mice were considered significant. Data from groups were compared using the Student's t-test. Rate of neurological disease in adoptive transfer studies was calculated by Kaplan–Meier method and compared by non-parametric survival analyses using log-rank test. A P-value of ≤ 0.05 was considered statistically significant.

Results

Kinetics of primary and secondary response to Cas

We previously showed that a low number of virus-specific cells was generated after neonatal priming, but the virus-specific population reached adult levels after secondary challenge in adulthood (16). To investigate the mechanism whereby a smaller CD8<sup>+</sup> memory pool reaches adult levels upon adulthood (16). To investigate the mechanism whereby a population reached adult levels after secondary challenge in adulthood was generated after neonatal priming, but the virus-specific We previously showed that a low number of virus-specific cells after primary and secondary challenge of neonate- or adult-primed mice exists. The number of virus-specific CD8<sup>+</sup> cells after primary and secondary challenge of neonate- or adult-primed mice exists. The number of virus-specific CD8<sup>+</sup> cells after primary and secondary challenge of neonate- or adult-primed mice is significantly lower (1.5 × 10^6 and 3 × 10^6 cells) of neonate-primed donor mice fully protected carrier mice from neurodegenerative disease (16). These studies, however, did not quantitate on a per-cell basis the protective ability of memory T cells.

Therefore, we tested whether transferring limiting numbers of splenocytes (1.5 × 10^6 and 3 × 10^6 cells) from neonate- or adult-primed mice provides sufficient protection from Cas-induced neurological disease. Spleen cells from donors were transferred into 2-day-old mice. The following day, neonatal hosts were infected with a high dose of Cas. Mice were then monitored for 7 months for clinical symptoms of neurological disease. Frequencies of virus-specific effectors in unfractionated spleens of neonate and adult-primed mice were determined by ELISPOT (16). Table 1 (and Supplementary Figure 2, available at International Immunology Online) summarizes the protective quality of transferred virus-specific IFNγ-producing cells.

When transferred donor cells came from neonate- or adult-primed mice, protection was gradually lost when <5 × 10^6 splenocytes were transferred and 1.5 × 10^6 spleen cells were not protective (Table 1). Transferring control naive splenocytes at three cell doses (1.5 × 10^6, 3 × 10^6 and 5 × 10^6 cells) did not protect the hosts from disease, and only slightly delayed the onset of disease. This indicated that critical numbers of Cas-specific memory cells are required to maintain protection from disease. Overall, it took more spleen cells from neonate-primed mice than adult-primed mice to provide equivalent protection to carriers (Table 1). Furthermore, we analyzed the
rate of disease onset in hosts that have received either adult-primed or neonate-primed spleen cells generating Kaplan–Meier curves that were compared with each other using a log-rank test. The rate of onset was significantly different between both groups when 3 × 10^6 or 1.5 × 10^6 cells were transferred (P = 0.0131 and 0.0001, respectively) and was delayed in hosts that received adult-primed cells compared with those that received neonate-primed cells (Fig. 2). The lack of protection and delay in disease onset correlated with the lower number of virus-specific neonate-primed memory cells transferred at the lower cell doses (Table 1). Interestingly, the transfer of 5 × 10^6 neonate-primed spleen cells, which would be equivalent in virus-specific cell numbers to transferring 2 × 10^6 adult-primed spleen cells (325 virus-specific effectors based on a frequency of 1/6369), leads to complete protection (100%) (Table 1). Using linear regression analysis, we calculated the number of virus-specific cells needed from each donor group to provide significant protection to neonatal
carriers (protection was considered significant if it is >36%, a value that is 2 SD above protection provided by naive splenocytes, see Methods). Using this analysis, we found that neonate-primed mice provided significant protection when 193 virus effectors were transferred, whereas 371 adult-primed virus-specific effectors were needed to provide the same protection (Table 1).

In vivo protective quality of memory T cells after secondary challenge

After secondary challenge, the frequency of virus-specific IFNy-producing splenocytes was similar between neonate- and adult-primed mice (16). Hence, we predicted that the protective ability of the cells in both groups should be similar. We transferred 1.5 \times 10^6-5 \times 10^6 splenocytes from neonate- or adult-primed mice re-challenged 3 weeks earlier into neonatal hosts. The hosts were infected with a high dose of Cas the following day. Surprisingly, protection decreased from 100% before secondary challenge to 46–61% after secondary challenge when 5 \times 10^6 donor cells were transferred from both donor groups (log-rank test \( P < 0.0001 \) for both neonate- and adult-primed cells); however, the rate of disease onset was not different from before secondary challenge when 3 \times 10^6 neonate- or adult-primed cells were transferred (log-rank test \( P = 0.7417 \) and 0.3404, respectively).

We predicted the number of virus-specific cells needed from each donor group to provide 37% protection by linear regression analysis. We found that after secondary challenge adult- and neonate-primed mice could provide protection when 678 and 727 virus effectors, respectively, were transferred (Table 1). The rate of onset was not different between both groups when 1.5 \times 10^6 cells were transferred (\( P = 0.0654 \)), but disease onset was slightly delayed in hosts that received 3 \times 10^6 adult-primed cells (\( P = 0.0316 \)) (Fig. 2).

The effect of IL-4 during priming of a neonatal host

Adoptively transferred immune cells mediate protection by lowering the viral load in neonatal recipients of Cas (22). Lower viral loads also stimulate endogenous immune responses from neonatal recipients (6), which would ultimately contribute to protection from disease. Therefore, the surprising decrease in protection seen after the transfer of re-challenged donor cells could be a result of decreased responses in the recipient mice, compromised efficacy of donor cells or both.

We previously showed that IL-4, produced by non-CD8+ cells, is increased after secondary challenge of neonate- and adult-primed mice, relative to after primary exposure (16). Furthermore, IL-4 treatment during neonatal exposure to Cas led to fatal neurodegenerative disease (23) and decreased Type 1 function (Supplementary Figure 3, available at International Immunology Online). Therefore, we asked whether, in our adoptive transfer experiments, the non-specific IL-4 produced after secondary challenge impaired the neonatal recipient’s primary response to Cas. CD8+ spleen cells from neonate- and adult-primed mice do not produce any detectable IL-4 (16). Therefore, we predicted that by transferring CD8+ cells alone, we would eliminate the IL-4-producing cells. In addition, the number of IL-4-producing cells is highest 4–6 days after secondary challenge (16), and by 6 days after secondary challenge, virus-specific donor CD8+ cells would have been exposed in vivo to the highest levels of IL-4 measured after secondary challenge. Therefore, we also predicted

Table 1. Donor cells from neonate-primed mice provide the same protection as those from adult-primed mice

<table>
<thead>
<tr>
<th>Number of cells transferreda</th>
<th>Frequency of virus-specific IFNy-producing splenocytesb</th>
<th>Estimated number of virus-specific effectors transferredc</th>
<th>Percent mice protected from disease</th>
<th>Number of effector cells providing 37% protectiond</th>
</tr>
</thead>
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<tr>
<td>Adult primed</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5 \times 10^6</td>
<td>n = 7</td>
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<td>3 \times 10^6</td>
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<td>235 ± 36</td>
<td>14</td>
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<tr>
<td>Neonate primed secondary</td>
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<tr>
<td>5 \times 10^6</td>
<td>n = 8</td>
<td>325 ± 110</td>
<td>100</td>
<td>193</td>
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<tr>
<td>3 \times 10^6</td>
<td>n = 9</td>
<td>195 ± 66</td>
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<tr>
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<td>n = 8</td>
<td>97 ± 33</td>
<td>12</td>
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<td>Adult primed secondary</td>
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<tr>
<td>5 \times 10^6</td>
<td>n = 19</td>
<td>970 ± 160</td>
<td>61</td>
<td>678</td>
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<td>n = 20</td>
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<td>n = 17</td>
<td>459 ± 63</td>
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<tr>
<td>1.5 \times 10^6</td>
<td>n = 9</td>
<td>229 ± 31</td>
<td>0</td>
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</table>

aSplenocytes (1.5 \times 10^6, 3 \times 10^6 and 5 \times 10^6) from adult-primed and neonate-primed, before and after secondary challenge, were transferred into 2-day-old neonates. Hosts were then infected with >1000 PFU of Cas the following day and monitored for 30 weeks for symptoms of neurological disease (hind limb weakness, tremor, and paralysis). bFrequencies of virus-specific effectors were determined in previous studies (16). cThe number of virus-specific effectors transferred was estimated by multiplying the number of spleen cells transferred by the frequency of virus-specific splenocytes. dTo determine the number of effector cells providing significant (37%) protection, the percent protection (previous column) was plotted against the number of effector cells transferred as determined by ELISPOT frequencies (16). Linear regression analysis was performed on the data lines by drawing a best-of-fit line using Microsoft Excel. R^2 values for each line/group follow: 0.99 (adult primary), 0.83 (neonate primary), 0.98 (adult secondary) and 0.82 (neonate secondary).
that if IL-4 altered the function of virus-specific donor CD8+ cells, transferring CD8+ cells 6 days after challenge would not improve protection compared with transferring unfractionated spleen cells alone.

We transferred $5 \times 10^6$ unfractionated or $1 \times 10^6$ CD8+ spleen cells from immune mice 6 days after secondary challenge into neonatal hosts (23). The hosts were then infected with $>1000$ PFU of Cas the following day. Hosts were monitored for 30 weeks for symptoms of neurological disease (hind limb weakness, tremor and paralysis). Kaplan–Meier curves were generated from the results using GraphPad Prism. Log-rank test was used to determine whether the curves differ from each other.

In vivo cytolytic function

Adoptive transfer experiments (Fig. 3) indicated that donor CD8+ cells remained protective when transferred into neonatal hosts. We therefore wanted to test whether the in vivo effector function of virus-specific CD8 cells remained effective in the original donor mice in the presence of non-specific IL-4 produced after secondary challenge. Cytotoxic effector function delayed the onset of disease by 5–7 weeks (neonate primed: 16–21 weeks, adult primed: 17–24 weeks) as compared with transferring whole splenocytes.

Fig. 2. The number of virus-specific memory cells transferred into neonatal hosts determines the rate of disease onset. Splenocytes ($1.5 \times 10^6$ (A and C), $3 \times 10^6$ (B and D) and $5 \times 10^6$ (E)) from adult-primed or neonate-primed mice were transferred into 2-day-old neonates. Hosts were infected with $>1000$ PFU of Cas the following day. Hosts were monitored for 30 weeks for symptoms of neurological disease (hind limb weakness, tremor and paralysis). Kaplan–Meier curves were generated from the results using GraphPad Prism. Log-rank test was used to determine whether the curves differ from each other.
is more sensitive than IFNγ production to the suppressive effect of IL-4 (Supplementary Figure 3, available at International Immunology Online). Thus, we compared memory cell cytotoxic function directly in the donor immune mice utilizing a sensitive in vivo cytolytic assay. Uninfected and Cas-infected syngeneic spleen cells were labeled with a low or high concentration of CFSE, respectively (target cells). Target cells were then co-injected as a 1:1 mixture into naive or immune mice. Mice with virus-specific memory CTL should specifically lyse infected target cells without harming uninfected targets. Specific lysis of Cas-infected cells was determined 18 h post-transfer by measuring the relative levels of infected and uninfected targets remaining in the host spleen. Both infected and uninfected targets were recovered completely in naive adult hosts (Fig. 4). Recovery of infected targets was greatly decreased in all immune mice before secondary challenge, 4–6 days after secondary challenge (when non-specific IL-4 is highest) and >3 weeks after secondary challenge (Fig. 4).

**Discussion**

The susceptibility of murine neonates to infection has been attributed to decreased lymphocyte numbers, reduced APC function and biased cytokine regulation (3). Optimizing conditions for neonatal priming overcomes such deficiencies, leading to qualitatively adult-like T cell responses (6, 9, 10, 13, 16, 17, 24). Despite their ability to generate qualitatively effective responses, neonatal mice are left with fewer virus-specific...
memory T cells compared with adult-primed mice, reflecting a smaller magnitude of the initial clonal burst generated during neonatal priming (15–17, 25, 26).

To investigate the relationship between the numbers of memory cells generated after neonatal priming with the effectiveness of these cells in the host as adults, we analyzed their expansion and protective ability after a secondary challenge with Cas. Even though protection decreased as fewer virus-specific memory cells were transferred, neonate-primed memory cells showed equal ability to protect hosts from Cas infection, on a per-cell basis, compared with their adult-primed counterparts before or after secondary challenge (Table 1).

The numbers of lymphocytes present in the spleens of murine neonates are ~10% of those present in adult spleens (3). One might hypothesize that a proportionately smaller T cell pool (~10%) would be activated in neonates than in adults. We demonstrated, however, that the number of virus-specific IFN-γ-producing CD8+ cells in mice primed with Cas as neonates is 50% of that measured in mice primed as adults (Fig. 1A and C). Our results are similar to those obtained in mice immunized as newborns with influenza virus, which developed virus-specific memory T cells at 40% of adult frequencies (17).

These findings are quite remarkable and may be interpreted as if neonates recruit more of their available CD8+ cells into the response to virus than adult mice. The increased recruitment may be due to permissive activation of cross-reactive CD8+ T cells. Neonatal lymphocytes exhibit a decrease in N-nucleotide addition resulting in shorter CDR3 regions (3) that can lead to promiscuous or cross-reactive antigen recognition by CD8+ T cells (27). The implication of permissive activation in response to neonatal exposure to Cas for the immunity of adult mice to heterologous viruses remains elusive. Nevertheless, the specificity and quality of Cas-specific responses were not compromised (6, 16).

Alternatively, the increased recruitment may be due to increased lymphopenia-induced proliferation of virus-specific cells. The neonatal environment is lymphopenic and hence induces increased homeostatic proliferation of neonatal and adult cells (28, 29). Therefore, it is plausible that activated virus-specific cells are driven to proliferate by antigen and environmental pressures to reach homeostasis. The bimodal CD8 response seen during the adult response to Cas virus (Fig. 1C) is very similar to that seen in response to influenza virus (30). Marshal et al. reported peak frequencies of virus-specific CD8 cells at day 10 post-infection, a drop in frequencies at day 20 and a new peak at day 31 post-infection. The significance of this bimodal profile was not discussed. Since their study, like ours, does not distinguish between effector and central memory populations, it is possible that a contraction occurred in the size of the effector memory population followed by an expansion of the central memory population. It is also conceivable that these populations were not fully developed in neonates following priming.

The smaller magnitude (in absolute number) and the recruitment of twice as many of the available CD8+ cells during the primary neonatal response to Cas made us question whether the protective quality of virus-specific cells on a per-cell basis is compromised in neonate-primed mice. When protective donor immune cells are adoptively transferred into neonatal hosts, viral replication is controlled in the hosts’ target organs, preventing the onset of neurological disease (14, 22). Meanwhile, donor cells could be assisting the development of a host-derived immune response (31, 32). Hence, we assayed the protective function of virus-specific memory cells in neonatal recipients and adult donors. We found that, when directly compared, neonate- and adult-primed CD8+ memory cells equally protected neonatal recipients when transferred after primary or secondary challenge, exhibited effective in vivo cytotoxic function in adult hosts and were not altered by pre-exposure to non-specific IL-4 (Figs 2–4). The data are consistent with reports by other groups showing that committed memory Type 1 CD8+ cells resisted switching to Type 2 cytokine production upon stimulation in vivo and in vitro (33, 34). Within 2 days after secondary challenge, the number of virus-specific cells becomes equal between neonate- and adult-primed mice (Fig. 1C). These experimental results allowed for the development of a mathematical model that can be tested experimentally in the future (Supplementary Material Figure 1).

The model suggests the existence of a growth factor that increases in abundance gradually in the presence of antigen and allows for proliferation of CD8+ memory cells. This insight can be used to design experiments aimed at determining whether such a growth factor exists, and if so, thoroughly characterizing its kinetics. IL-15 is one growth factor having properties that satisfy those required by the model: the stimulation of CD8+ memory cell proliferation (35, 36) and an increase in abundance in response to antigen (37). Cookson and Reen (38) also reported that IL-15 expanded effector T cell populations in cord blood. On the other hand, Saito et al. (39) reported a one-third decrease in the expression of gamma chain expression in cord blood lymphocytes. Thus, to rule out the possibility that intrinsic differences between neonate- and adult-primed virus-specific cells exist, the role of other gamma chain cytokines, e.g. IL-7 (40), should also be the subject of future studies.

In conclusion, we have shown that the protective qualities of CD8+ memory cells are essentially equivalent on a per-cell basis between mice primed as neonates and adults (Table 1, Figs 2–4). If the secondary challenge could represent a natural viral exposure, our studies would indicate that the neonatal vaccine effectively generated CD8+ memory cells that cleared virus-infected cells (Fig. 4) even if they are less in number compared with adult-primed hosts. Their effectiveness in part is due to their enhanced capacity to expand after secondary challenge (Fig. 1). Our data argue that neonatal priming with Cas can lead to virus-specific CD8+ cell responses qualitatively equal, if not more effective on a per-cell basis, to those induced by priming in adulthood.

Supplementary data

Supplementary data are available at International Immunology Online.

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Abbreviations

APC  antigen-presenting cell
Cas  Cas-Br-E murine leukemia virus
i.p.  intraperitoneally
MuLV  murine leukaemia virus
PFU  plaque-forming unit

References


