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IL-10 is an immunomodulatory cytokine that acts to antagonize T cell responses elicited during acute and chronic infections. Thus, the IL-10R signaling pathway provides a potential therapeutic target in strategies aimed at combating infectious diseases. In this study, we set out to investigate whether IL-10 expression had an effect on NK cells. Murine CMV infection provides the best characterized in vivo system to evaluate the NK cell response, with NK cells being critical in the early control of acute infection. Blockade of IL-10R during acute murine CMV infection markedly reduced the accumulation of cytotoxic NK cells in the spleen and lung, a phenotype associated with a transient elevation of virus DNA load. Impaired NK cell responsiveness after IL-10R blockade was attributed to elevated levels of apoptosis observed in NK cells exhibiting an activated phenotype. Therefore, we conclude that IL-10 contributes to antiviral innate immunity during acute infection by restricting activation-induced death in NK cells. The Journal of Immunology, 2011, 187: 2944–2952.

Natural killer cells are critical mediators of immunity to viruses (1) including human and murine CMVs (MCMVs) (2–5). Therefore, understanding the immunological mechanisms that regulate these responses in vivo may afford important insight for the design of vaccination and immunotherapeutic strategies to counter viral pathogens.

The cytokine IL-10 is expressed by innate and adaptive immune cells (6). The majority of in vitro and in vivo studies to date suggest a suppressive function for IL-10, most of which have focused on IL-10-mediated effects on T cells, predominantly indirectly via regulation of APC function (6, 7). In contrast, the role that IL-10 plays in regulating NK cell responses requires better understanding. In vitro, IL-10 directly enhances NK cell cytotoxicity (8, 9), proliferation (10), and IFN-γ production (9, 11, 12), whereas limited in vivo studies indicate that IL-10 inhibits IFN-γ expression by NK cells during inflammation (13, 14).

In the context of infections, IL-10 restricts T cell-mediated pathologies, often at the expense of pathogen clearance (15). IL-10 limits T cell responses during acute viral infections (16–21) and also antagonizes T cell immunity during persistent/chronic MCMV (22, 23) and lymphocytic choriomeningitis virus infections (24, 25). Importantly, human herpesviruses human CMV (26) and EBV (27) have acquired functional IL-10 orthologs. Collectively, these studies imply that IL-10 is profoundly important in the regulation of antiviral immunity and that manipulation of the IL-10R signaling pathway may be therapeutically exploited to counter viruses and associated diseases. Critically, however, the impact that IL-10 has on innate immunity, in particular NK cell responses, during viral infections requires further investigation.

The MCMV infection model is a well-characterized experimental system for studying in vivo NK cell responses to acute and persistent viral infection. NK cells in C57BL/6 mice express the activating receptor Ly49H (28) that binds to the MCMV-encoded cell surface glycoprotein m157 (29) and controls acute viral infection (30) via direct killing and secretion of IFN-γ (31, 32). Aside from activating receptors, the cytokine milieu elicited during MCMV infection also regulates NK cells. Proinflammatory cytokines IL-12 and IL-18 induce IFN-γ expression and NK cell accumulation (33–35), whereas type I IFN promotes cytotoxicity (36, 37). TNF also enhances IFN-γ expression yet concurrently impairs cytotoxicity (38). Moreover, IL-15 promotes expansion of the NK cell response (37). These studies demonstrate that cytokines can promote NK cell responses during MCMV infection. However, the role that immune-suppressive cytokines play in regulating these responses requires a better understanding.

In this article, we report that IL-10 promotes NK cell responses during acute MCMV infection. Blockade of IL-10R in the first days of infection with an antagonist Ab reduced NK cell numbers despite elevated levels of proinflammatory cytokines known to promote NK cell responsiveness. Treatment elicited a loss of cytotoxic NK cells and a concurrent transient elevation of viral genome load. Finally, we observed that anti–IL-10R blockade increased the frequency of NK cell apoptosis with cells exhibiting a phenotype indicative of activation-induced cell death. Collectively, these results show that IL-10 participates in early antiviral innate immunity via regulation of activation-induced death of NK cells.

Materials and Methods

Mice, viral infections, and treatments

All experiments were conducted according to U.K. Home Office guidelines. Specifically, these experiments were performed under a Home Office project license (PPL 30/2442, granted to I. R. Humphreys) at the Home Office-designated facility at Heath Park, Cardiff University. Wild-type (wt)
C57BL/6 mice were purchased from Harlan U.K. (Blackthorn, U.K.). The MCMV strain (American Type Culture Collection, Manassas, VA) was prepared in BALB/c salivary glands and purified over a sorbital gradient. Virus stocks and virus from homogenized organs excised from infected mice were serially diluted and titered for 6 d on 3T3 cells with a carboxymethyl-
ycellulose overlay. Mice were infected i.p. with 5 × 10^7 PFU MCMV and, in some experiments, also infected on day 0 with 250 μg of either rat IgG (Chemicon International, Temecula, CA) or rat IgG1 (clone 12ABP, Bio- Xcell) as control. IL-10R blockade (clone 1B1.3A; eBioscience). In some experiments, mice were also administrated anti-NK1.1 on −2, 0, and +2 d postinfection (clone PK136; BioXcell).

Leukocyte isolation, intracellular cytokine staining, and flow cytometry

Mice were perfused with 10 ml sterile PBS, and lungs were surgically excised, cut into small pieces, and incubated in RPMI 1640 supplemented with 5 mM CaCl2, 5% FBS, 1 mg/ml collagenase D (Roche Diagnostics, Basel, Switzerland), and 10 μg/ml DNase (USB, Cleveland, OH) at 37°C with mild agitation for 45 min. Cells were then passed through a 70-μm nylon cell strainer, RBCs were lysed, and extruded lung leukocytes were washed with PBS prior to analysis. Spleens were also excised and cells isolated by passage through a 70-μm nylon cell strainer prior to RBC lysis and washing with PBS.

Leukocytes (1 × 10^6 cells/well) were then incubated with Fc receptor blocking reagent (eBioscience, San Diego, CA) and stained with anti-CD3-PerCP-Cy5.5 and anti-NK1.1-allophycocyanin (BD Pharmingen, San Diego, CA). In some experiments, leukocytes were also stained with anti-Ly49H-FITC (BD Pharmingen) and anti–IL-10R-PE (clone 1B1.3A; BD Pharmingen) or anti–CD96-PE-Cy7 and anti–CD27-PE or anti–CD25-PE (all eBioscience). Following surface staining, some cells were permeabilized with saponin buffer (PBS, 0.5% FCS, 0.05% sodium azide, and 0.5% saponin) and stained with anti-Ki67 FITC (BD Pharmingen). To assess early NK cell apoptosis, isolated leukocytes were incubated with Live/Dead Fixable Aqua (Invitrogen, Carlsbad, CA) and then stained with anti-CD3-PerCP-Cy5.5, anti–NK1.1-allophycocyanin, and, either Annexin V-FITC or anti–caseapse-3-PE (active form), according to the manufacturer’s instructions (all BD Pharmingen).

To assess ex vivo cytokine production by NK cells, leukocytes were incubated at 5 × 10^6 cells/ml for 4 h at 37°C in the presence of 2 μg/ml brefeldin A (Sigma-Aldrich, St. Louis, MO). Cells were then washed and incubated with Fc block (eBioscience) and surface stained with anti-CD3-PerCP-Cy5.5 and anti–NK1.1-allophycocyanin prior to fixation with 3% formalin. Cells were then permeabilized with saponin buffer and stained with anti–IFN-γ-FITC (BD Pharmingen). To assess NK cell cytotoxicity, cells were incubated with 0.7 μg/ml monomeric and anti–CD107a-FITC (both BD Pharmingen) and then surface stained with anti-CD3 and anti-NK1.1, as described above.

For detection of IL-10 expression, splenocytes and lung leukocytes were stimulated for 5 h at 37°C with either 50 ng/ml PMA and 500 ng/ml ionomycin (T cells and NK cells), 10 μg/ml LPS, 50 ng/ml FMA, and 500 ng/ml ionomycin (B cells) or 10 μg/ml LPS (macrophages; all reagents from Sigma-Aldrich). Cells were stained with Fc block and surface stained with anti-CD4-Pacific Blue, anti-CD8-allophycocyanin-H7, anti–CD3-PerCP-Cy5.5, anti–NK1.1-allophycocyanin, and anti–γδTCR-FITC, anti–CD11b-PE-Cy7 (all from BD Pharmingen), or anti–B220-PE-Cy7 (BioLegend) prior to fixation, permeabilization, and intracellular staining with anti–IL-10-allophycocyanin (eBioscience). To assess APC accumulation, undiluted supernatants were then assayed for cytokines (with the exception of IL-12) by cytokometric bead array (Bender MedSystems, Vienna, Austria) using a FACSCanto II, and data were analyzed using FlowCytomix Pro 2.3 software. IL-12 was detected by ELISA (PeproTech, Rocky Hill, NJ) and analyzed on a FLUOstar OPTIMA plate reader.

Viral genome detection

Genomic DNA was isolated from spleen and lung tissue using a DNAeasy tissue kit (Qiagen, Valencia, CA). MCMV glycoprotein B (gB) was then assayed by quantitative PCR using a Mini Opticon (Bio-Rad, Hercules, CA) and Platinum SYBR Green MasterMix reagent (Invitrogen). Aliquots (10 ng) of DNA were used as templates for each reaction. The primer sequences used for detection of β-actin were 5’-GATGTCACGACATTTCCG-3’ and 5’-GGGCTAGTGTCTCCCTCAC-3’; primers used for detection of gB were 5’-GAAGATCGGAGTCTTCTCAG-3’ and 5’-AATCCGTG-CAACATCTTGTGC-3’. Genome copy numbers were calculated using a standard curve generated with the pARK25 MCMV plasmid (a gift from A. Redwood, University of Western Australia, Perth, WA, Australia). The limit of detection was 10 genome copies/100 ng DNA.

Statistics

Statistical significance was determined using the Mann–Whitney U test (viral load analysis – two groups), one-way ANOVA using logarithmic transformation (viral load analysis – multiple groups), or the two-tailed Student t test (flow cytometry data): *p < 0.05, **p < 0.01, and ***p < 0.005.

Results

IL-10 expression is induced during acute MCMV infection

Acute MCMV infection is established in a number of visceral organs including the lung and spleen. IL-10 protein expression was significantly upregulated upon infection in both organs (Fig. 1A). B cells are a significant source of IL-10 during acute MCMV infection (20), and in accordance with published work using IL-10 reporter mice (20), we observed that B cells derived from the lung (Fig. 1B) and spleen (Fig. 1C) 4 d postinfection were the predominant cell type capable of expressing IL-10 in response to polyclonal stimulation ex vivo, although inflammatory macrophages (Fig. 1B, 1C) and splenic dendritic cells (Fig. 1C) also represent significant populations of IL-10–expressing cells. More generally, MCMV infection promoted the accumulation of IL-10 expressing cells within the lung.

IL-10R blockade transiently increases viral genome load during acute MCMV infection

We hypothesized that virus-induced IL-10 suppressed protective immunity during infection. To test this, MCMV-infected mice were administered an antagonist nondepleting anti–IL-10R Ab on the day of infection, and virus load was measured. Contrary to our initial hypothesis, early viral DNA load in the lungs and spleen at day 2 postinfection were not influenced by anti–IL-10R blockade. Interestingly, however, although anti–IL-10R treatment did not significantly influence the low levels of replicating virus detectable in the lungs (IgG: 2 × 10^3 versus anti–IL-10R: 3.4 × 10^3 PFU/g) or spleen (IgG: 1.4 × 10^3 PFU/g versus anti–IL-10R: 1.9 × 10^3) at 4 d postinfection, virus genome content in these organs was actually increased following IL-10R blockade (Fig. 2A, 2B). By day 7, however, viral DNA load was comparable in both groups (Fig. 2A, 2B). Thus, these data suggested that IL-10R blockade transiently increased MCMV genome load.

IL-10R blockade impedes the accumulation of NK cells during MCMV infection

NK cells are critical for early control of MCMV in our model (30–32), and depletion of this cell subset increased virus DNA load at day 4 postinfection irrespective of anti–IL-10R treatment (Fig. 2C, 2D). Interestingly, however, IL-10R blockade in NK1.1-depleted mice did not further elevate viral genome copies, implying a possible NK cell defect following Ab treatment. During infection,
large numbers of NK1.1<sup>+</sup>CD3<sup>+</sup> cells at day 0 to 80–85% by day 7 postinfection. These data suggest that IL-10R blockade did not preferentially inhibit Ly49H<sup>+</sup> NK cells during infection.

**Cytotoxic but not IFN-γ-mediated NK cell responses are abrogated by IL-10R blockade**

In view of the recognized immunosuppressive properties of IL-10, it was clearly important to evaluate whether IL-10 influenced NK cell function during infection. We first quantified recent degranulation, based on CD107a mobilization and found reduced numbers of degranulating NK cells in the lung (Fig. 4A) and spleen (Fig. 4B) following IL-10R blockade. Moreover, accumulation of granzyme B-expressing NK cells was also reduced (data not shown). The percentage of NK cells that were degranulating (CD107<sup>+</sup>) or expressing granzyme B were comparable in IgG and anti–IL-10R–treated mice (data not shown). Therefore, our data suggest that the loss of cytotoxic NK cell numbers following IL-10R blockade reflected an overall reduction in NK cells rather than an IL-10–dependent qualitative regulation of cytotoxicity.

Although anti–IL-10R treatment increased the percentages of pulmonary (Fig. 4C) and splenic (Fig. 4D) NK cells capable of spontaneously expressing IFN-γ in the absence of ex vivo stimulation, total numbers of IFN-γ–expressing cells were comparable in control and anti–IL-10R–treated mice because of loss of NK cell numbers after IL-10R blockade (Fig. 4E, 4F). NK cells from anti–IL-10R–treated mice expressed more IFN-γ on a per cell basis (as indicated by mean fluorescence intensity [MFI]; Fig. 4C, 4D), and higher concentrations of IFN-γ were detected in lung and spleen supernatants isolated from anti–IL-10R–treated mice (Fig. 4G, 4H). Collectively, these data demonstrate that IL-10R signaling promotes the accumulation of cytotoxic but not IFN-γ–expressing NK cells during acute MCMV infection.

**IL-10R blockade enhances NK cell activation-induced cell death**

Cytokines including IL-12 can promote NK cell accumulation during MCMV infection (34). However, concentrations of IL-12, IL-6, and TNF were all elevated following IL-10R blockade (Fig. 4G, 4H). Moreover, IL-10R blockade did not influence Ki-67 expression by either pulmonary or splenic NK cells at any time point during acute infection (data not shown), suggesting that impaired NK cell responsiveness following IL-10R blockade was not due to reduced proliferation.

Next, apoptosis was measured over a time course. Interestingly, we observed a high proportion of apoptotic NK cells in the lungs (Fig. 5A, 5C) and spleens (Fig. 5B, 5D) during the first 4 d of infection, demonstrating that the contraction of the NK cell response during this period (Fig. 3C, 3D) was due to cellular apoptosis. When NK cell apoptosis in control and anti–IL-10R–treated mice was examined, we observed that, in line with comparable contraction of NK cell numbers in the first 2 d of infection (Fig. 3C, 3D) both groups displayed high frequencies of apoptotic NK cells at this time (Fig. 5C, 5D). Importantly, however, the frequency of apoptotic NK cells increased further in anti–IL-10R–treated mice at 4 d postinfection in the lung (Fig. 5A, 5C) and
spleen (Fig. 5B, 5D) as compared with IgG-treated controls; the time point at which elevated viral DNA load was observed in anti–IL-10R–treated mice. In accordance with the selective role for IL-10R blockade in reducing NK cell accumulation (Fig. 3C, 3D), annexin V binding by NK1.1+ cells in the lungs and spleen 4 and 7 d postinfection was not significantly increased following IL-10R blockade (data not shown).

The prosurvival factor Bcl2 is expressed by NK cells but is substantially lost at late (day 7) but not early (day 3) times after MCMV infection (40). Elevated NK cell apoptosis at day 4 (defined by annexin V binding) following IL-10R blockade was not associated with an accelerated loss of Bcl2 expression, implying that IL-10 does not influence this antiapoptotic pathway (data not shown). Importantly, however, elevated frequencies of annexin V-binding NK cells in anti–IL-10R–treated mice were accompanied by increased frequencies of pulmonary NK cells positive for intracellular active caspase-3 (Fig. 5E). These data therefore suggest that impaired NK cell responses following IL-10R blockade was a consequence of increased apoptosis.

We next performed phenotypic analysis of apoptotic NK cells in anti–IL-10R–treated mice. Expression of CD11b and CD27 defines the maturation status of murine NK cells, with CD27 expression associated with cytotoxic effector function (41). Splenic CD11bhighCD27high NK cells, which exhibit high cytotoxic

FIGURE 2. IL-10R blockade increases virus load during acute MCMV infection. A and B, MCMV-infected mice were treated with IgG (closed symbols) or anti–IL-10R (open symbols) and on days 2, 4, and 7, and MCMV gB was detected by quantitative PCR and normalized to β-actin. Data are expressed as genome copy number per 100 ng genomic DNA. Median of 4–6 mice/group ± interquartile range is shown. Data represent two (day 2), five (day 4), or three (day 7) experiments in total. C and D, Mice were infected with MCMV, treated with combinations of IgG or anti–IL-10R ± anti-NK1.1, and at 4 d postinfection, genomic DNA was measured in the lungs (C) and spleen (D). Horizontal dashed lines depict the lower limit of detection. Data from two to three experiments are shown. ○, IgG; △, anti–IL-10R, △, IgG and NK depletion; ▼, anti–IL-10R and NK depletion. *p < 0.05, **p < 0.01, ***p < 0.005.

FIGURE 3. IL-10R blockade impedes the accumulation of NK cells during acute MCMV infection. MCMV-infected mice were treated with IgG or anti–IL-10R on day 0 and cellular accumulation assessed 2, 4, and 7 d later. A and B, Representative bivariate flow cytometry plots showing NK1.1+CD3+ cell accumulation 4 d postinfection. C and D, Numbers of NK1.1+CD3+ cells in the lungs (C) and spleens (D) of IgG (○) and anti–IL-10R (△)–treated mice analyzed over 7 d. Results are expressed as the mean ± SEM of 6–10 mice/group and are representative of five independent experiments. *p < 0.05, **p < 0.01, ***p < 0.005.
capacity ex vivo (42), were the predominant NK cell subset binding annexin V (Fig. 6B), whereas high frequencies of apoptosis were observed in CD27^high and CD27^low pulmonary NK cell populations (Fig. 6A). In accordance with loss of cytotoxic NK cells following IL-10R blockade (Fig. 4A, 4B), anti–IL-10R further increased the frequency of apoptotic splenic and pulmonary CD11b^highCD27^high NK cells, as well as the less mature CD11b^lowCD27^high NK cells (Fig. 6A, 6B).

Finally, the activation status of NK cells was assessed. Strikingly, NK cell expression of activation markers CD25 (lung and spleen) and CD69 (lung only) was associated with increased annexin V binding (data not shown) or active intracellular caspase-3 (Fig. 6H, 6I). These data collectively demonstrate that impaired NK cell accumulation following IL-10R blockade was the consequence of enhanced activation-induced death of NK cells.

**Discussion**

We report that IL-10 limits activation-induced death of NK cells during acute virus infection. IL-10 was expressed at high levels in sites of MCMV infection. Using Ab blockade of IL-10R, we demonstrated that IL-10R signaling promotes the accumulation of NK cells, including cytotoxic cells, during infection despite concurrently suppressing production of proinflammatory cytokines. We demonstrated that this impaired NK cell response following IL-10R blockade in vivo was due to NK cells preferentially undergoing activation-induced death. Collectively, these data show that IL-10 participates in the innate antiviral immune response by promoting NK cell survival.

NK cells decline during a number of viral infections, and this is linked to disease susceptibility (43–46). In agreement with previous studies (21, 39, 40), we observed that NK cell numbers decline during the first days of MCMV infection. A high fre-
Importantly, NK cell apoptosis following IL-10R blockade was accompanied by elevated expression of IFN-γ, TNF, and IL-12. NK cells were identified as a significant source of elevated IFN-γ production. In contrast, inhibition of IL-10R signaling did not upregulate expression of TNF by NK cells (data not shown), with APC populations representing probable sources of both TNF and IL-12 (7). Critically, although these proinflammatory cytokines are required for optimal NK cell responses to MCMV and associated control of viral infection (32–34, 38), overexpression of IFN-γ inhibits NK cell development (47), and intriguingly, TNF and IL-12 enhance apoptosis of IL-2–stimulated human NK cells (48). Our data therefore imply that IL-10 may indirectly regulate NK cell survival by limiting the expression of proinflammatory cytokines and also suggest that elevated proinflammatory cytokine production at day 4 postinfection could not, in terms of limiting virus DNA load, compensate for reduced cytotoxic NK cell accumulation following IL-10R blockade. Of interest, hypercystimulation drives activation-associated NK cell death in vivo (49, 50). IL-10 is a potent inhibitor of costimulatory ligand expression (7), and it is therefore conceivable that inhibition of these ligands may contribute to indirect regulation of NK cell survival.

IL-10 protein was detected at high concentrations in both the lung and spleen. Under certain conditions, NK cells produce IL-10 (21, 51). However, on the basis of ex vivo cytokine production, we observed B cells to be the predominant source of IL-10, as previously described in a study using MCMV-infected IL-10-GFP reporter mice (20). NK cells are critical mediators of innate protection from pulmonary infections (52) and under homeostatic conditions (~10% of all murine pulmonary leukocytes are NK cells (53, 54). Although NK cells are rapidly recruited into the lung during inflammation (55), our data suggest that IL-10 may regulate pulmonary NK cells independently of the splenic NK cell response.

IL-10R blockade reduced numbers of cytotoxic NK cells while concurrently increasing apoptosis of CD27+ NK cells; the NK cell subset reported to preferentially exhibit ex vivo cytotoxicity (41, 42). IFN-γ production by NK cells also contributes to protective immunity (31, 32), although this is less important in our model (56). Interestingly, IL-10R blockade did not influence numbers of IFN-γ–expressing NK cells and actually increased abundance of this cytokine. Activated NK cells often exhibit dual functionality of IFN-γ expression and degranulation (57), and CD27 expression is associated with both functions (42). Increased expression of the IFN-γ–inducing cytokine IL-12 (33, 35) was observed following IL-10R blockade. Therefore, although we cannot rule out differential regulation of mono-(cytotoxic) and dual-functional (cytotoxic and IFN-γ+) NK cells by IL-10, our data suggest that IL-10 limits apoptosis of activated (including cytotoxic) NK cells while concurrently inhibiting IFN-γ production by NK cells via suppression of IL-12. Irrespective of the exact mechanisms, our data suggest that IL-10 promotes antiviral NK cell responses by promoting the survival and subsequent expansion of cytotoxic, not IFN-γ expressing, NK cells. IL-10R blockade led to an elevation in MCMV DNA load. Although a slight increase in virus replication in anti–IL-10R–treated mice was also observed, numbers of plaques detected in our assay was low, and results did not achieve statistical significance (data not shown). Therefore, our conclusion that IL-10R blockade results in increased virus replication is guarded. Indeed, virus DNA load in NK-depleted mice was 100-fold higher than viral DNA content after IL-10R blockade. Although elevated antiviral cytokine production in treated mice may in part compensate for reduced NK cell responsiveness, these data suggest

**FIGURE 5.** IL-10R blockade enhances NK cell apoptosis during acute MCMV infection. Mice were infected with MCMV and treated with IgG or anti–IL-10R. A and B, Apoptosis of NK cells was assessed by annexin V binding. Representative overlay histograms of annexin V binding of NK cells in the lungs (A) and spleens (B) of mice following treatment with anti–IL-10R (solid line) or IgG (dashed line) 4 d postinfection (shaded histogram = fluorescence minus one [FMO] control). Annexin V binding by NK cells in the lungs (C) and spleens (D) of mice treated with IgG (●) or anti–IL-10R (○) shown as percentage of NK+CD3+ cells over a 7-d time course. Results are expressed as the mean ± SEM of four to six mice per group and are representative of two to three independent experiments. E, Intracellular expression of active caspase-3 by NK+CD3+ cells in the lungs and spleens of mice 4 d postinfection, treated with IgG (●) or anti–IL-10R (○) directly ex vivo. Results are expressed as the mean ± SEM of 10 mice/group and are representative of three independent experiments. *p < 0.05, **p < 0.01.
that surviving Ly49H⁺ NK cells in anti–IL-10R–treated mice contribute to protective immunity. We speculate that the loss of cytotoxic NK cells following IL-10R blockade may impact on the number of cells infected (and therefore viral DNA content), but a sufficient number may remain to contribute to the limitation of infectious virion secretion.

Interestingly, IL-10 gene deletion reduces virus replication in the spleens during acute MCMV infection (19). Unfortunately, the absence of NK cell characterization and different kinetics of virus clearance in wt controls in the study by Oakley et al. (19) precludes direct comparison with our own experiments. Differences in methodology for quantification of virus load may in part explain differences obtained from the two studies. However, repletion of IL-10 into IL-10⁻/⁻ mice does not influence splenic viral DNA load (58). Although a comparison of viral load between wt and IL-10⁻/⁻ mice was not performed in the Tang-Feldman study (58), the data collectively imply that subtle differences exist between the two systems. Our experimental approach of inhibiting IL-10R signaling in wt mice with antagonistic Ab precludes the possibility of developmental factors associated with gene-deficient mice influencing our experimental readouts. Whether such factors associated with IL-10⁻/⁻ mice influences early viral replication and possibly alter the threshold of NK cell numbers required to transiently limit viral DNA load in the spleen, is unclear.

Reduced NK cell accumulation 7 d after IL-10R blockade was not associated with significantly increased viral DNA burden. IL-10R blockade substantially amplifies the acute virus-specific CD4 T cell response (22). Although we observed no comparable increase in CD8 T cells (data not shown), these data suggest that enhanced T cell immunity may compensate for impaired NK cell responses at this time. Importantly, IL-10R blockade exacerbated weight loss (data not shown) suggesting that, as previously demonstrated in this model (19), IL-10 limits immune-driven pathology during acute viral infection. The data presented in this article demonstrate that IL-10 also promotes NK cell survival during acute viral infection through the limitation of activation-induced cell death.

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Disclosures

The authors have no financial conflicts of interest.

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