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In vivo Conversion of Bone Marrow Plasmacytoid Dendritic Cells into CD11b⁺ Conventional Dendritic Cells during Virus Infection

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Abstract

Dendritic cells (DCs) are a highly heterogeneous population that plays a critical role in host defense. We previously demonstrated that virus infection induces bone marrow (BM) plasmacytoid (p)DCs differentiation into CD11b⁺ conventional (c)DCs upon *in vitro* culture with Fms-like tyrosine kinase 3 ligand (Flt3L). Here we use immunoglobulin D-J rearrangements and pDC adoptive transfer to provide definitive proof supporting BM pDC conversion into CD11b⁺ cDCs during *in vivo* viral infection. We show that *in vivo* BM pDC conversion into CD11b⁺ cDCs relates to enhanced ability to prime virus specific T cells. Furthermore, we demonstrate that *in vivo* pDC conversion does not rely on viral infection of BM pDCs, but instead, is mediated by type I interferon (IFN-I) signaling. Finally, by exploiting recently identified pDC specific Abs, we provide further characterizations of the BM pDC fraction that exhibits this broader developmental plasticity. Collectively, these data indicate that BM pDCs actively contribute to the CD11b⁺ cDC pool during *in vivo* viral infection and delineates molecular, functional, and phenotypic features of this novel developmental pathway.

Keywords

Plasmacytoid dendritic cells; CD11b⁺ conventional dendritic cells; Type I interferon; Virus infection

Introduction

Dendritic cells (DCs) play a central role in innate and adaptive immunity [1-3] and are composed by a large variety of subsets differing in tissue locations, surface phenotypes, and immunological functions [4,5]. In mouse spleen, three major DC subsets have been identified including CD11b⁺ DC (originally named myeloid DCs), CD8α⁺ DCs (originally named lymphoid DCs), and plasmacytoid DCs (pDCs) [6-8]. Typically, CD11b⁺ DCs and CD8α⁺ DCs are categorized as conventional DCs (cDCs) that are set apart from pDCs in phenotypic and functional features. pDCs, a unique DC subset with plasma cell-like morphology defined as CD11c⁺B220⁺CD11b[−] in mice, produce large quantities of type I

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IFN (IFN-I) upon viral infection and represent a crucial element in antiviral immunity (reviewed in [9-11]).

The development of distinct DC subsets and their lineage relationship comprise a complex process that could be influenced by microenvironment changes such as pathogen infection or inflammation (reviewed in [12]). To gain more insight into the impact of virus infection on DC differentiation and the distribution of DC precursors, we used lymphocytic choriomeningitis virus (LCMV) infection in its natural host, the mouse, as a model system. In an earlier study, we demonstrated that BM pDCs isolated from LCMV-infected mice can convert into CD11b⁺ cDCs *in vitro* [13]. Herein we provide direct evidence that conversion of BM pDCs into CD11b⁺ cDCs is an ongoing process during *in vivo* viral infection. Moreover, we show that the reprogramming of pDCs *in vivo* is independent of direct infection of pDCs, but instead requires IFN-I signaling. Finally, we describe a CD11c⁺B220⁺CD11b⁺CD3⁺NK1.1⁺CD19⁺Siglec-H⁺ BM fraction (hereafter referred as Siglec-H⁺ pDCs) that increases during LCMV infection in a IFN-I dependent manner and exhibits high capacity to generate CD11b⁺ cDCs. Altogether, our studies reveal that the BM pDCs serve as an alternative source to generate CD11b⁺ cDCs during *in vivo* viral infection and this process is mainly mediated by IFN-I signaling.

Results and discussions

Bone marrow pDCs convert into CD11b⁺ cDCs during *in vivo* viral infection

Earlier we showed that BM, but not spleen, pDCs isolated from LCMV-infected mice can differentiate into CD11b⁺ cDCs *in vitro* in the presence of Flt3L [13]. The same phenomenon was observed when challenging mice with poly IC [13] and MCMV (data not shown), indicating that BM pDC conversion is a general event after virus infection. However, the essential biological question raised from this finding was whether the differentiation of pDCs into CD11b⁺ cDCs occurs *in vivo*. Given that the phenotypic and functional properties of pDCs have been changed after conversion, we used an intrinsic permanent marker, the D-J rearrangements of the IgH, as an indicator to monitor pDC-derived CD11b⁺ cDCs *in vivo* [14,15]. For this, we isolated splenic CD11b⁺ cDCs from uninfected and LCMV CI 13 infected mice and the IgH D-J rearrangements were analyzed by a PCR-based approach (Fig. 1A). Importantly, the purity of sorted CD11b⁺ cDCs was over 98% and the percentage of contaminating B cells, T cells or NK cells was less than 1 % (Suppl. Fig. 1A). As expected, large amounts of D-J rearrangements were detected in B cells but no visible signal could be observed in granulocytes. In line with the findings by others [14,15], the rearrangements of IgH were detected in splenic pDCs but not in CD11b⁺ cDCs isolated from naïve mice. Remarkably, a significant increase of D-J rearrangements were detected in CD11b⁺ cDCs from LCMV CI 13 infected mice, while the V-DJ rearrangements, a feature of B cell lineage, were undetectable (Fig. 1A and Suppl. Fig. 1B, respectively). Although indubitably detected, the intensity of the IgH D-J rearrangements in the CD11b⁺ cDCs was lower than the bands observed in pDCs and B cells. This could result from the intrinsic heterogeneity of the CD11b⁺ cDC population during infection. Indeed, other CD11b⁺ cDCs precursors, such as monocytes, have been demonstrated to contribute to the CD11b⁺ cDC pool during inflammation [16]. Thus, pDC-derived-CD11b⁺ cDCs containing IgH D-J rearrangements likely represent only a fraction of the CD11b⁺ cDCs generated during the infection, in agreement with the lower intensity of the IgH D-J signal in this cell population. Altogether, these data suggest that a significant proportion of CD11b⁺ cDCs is derived from pDCs *in vivo* after viral infection.

To further prove the reprogramming of pDC *in vivo*, we transferred FACS-purified BM pDCs from naïve or LCMV CI 13 infected C57BL/6 mice (CD45.2) into congenic (CD45.1) mice. Four days after transfer, BM and spleens from recipient mice were obtained and the

donor cells (CD45.2⁺) were identified by flow cytometry (Fig. 1B). Given that a fraction of BM pDCs are infected with LCMV Cl 13, the recipient mice become infected upon cell transfer (data not shown). When transferring pDCs from naive mice, none or marginal levels of CD11b⁺ cDCs derived from donor pDC were detected in BM and spleen (Fig. 1C). In contrast, donor pDCs from LCMV Cl 13 infected mice gave rise to approximately 15-20% of CD11b⁺ cDCs in spleen and a ~7% in BM. These CD11b⁺ cDCs did not result from contamination with BM proliferating progenitors as indicated by minimal cell division after cell transfer (Suppl. Fig. 2). Collectively, these results reveal that BM pDCs actively differentiate into CD11b⁺ cDCs during *in vivo* viral infection and provide new insights into the interrelationship of different DC subsets upon microbial invasion. Our data suggest that the addition of pDC-derived CD11b⁺ cDCs increases the heterogeneity of the cDC pool during viral infection.

pDC conversion relates to enhanced antigen presenting capacity *in vivo*

To gain more insight on the phenotypic and functional properties of *in vivo* generated pDC-derived CD11b⁺ cDCs, we transferred BM pDCs into congenic recipients as described in Fig. 1B. We first investigated the morphology and expression of antigen presenting molecules on pDC-derived CD11b⁺ cDCs in comparison to their counterparts existent in the recipient mice (Suppl. Fig 3). We found that the pDC-derived CD11b⁺ cDCs exhibit similar morphology, expression of CD11c, costimulatory molecule B7.2 and MHC class II molecules as the CD11b⁺ cDCs from recipient origin.

To evaluate the ability of pDC-derived CD11b⁺ cDCs to prime LCMV-specific T cells *in vivo*, we utilized a system in which Thy1.1⁺, TCR transgenic CD8 T cells (P14-Thy1.1) specific for the LCMV GP₃₃₋₄₁ epitope presented in the context of H-2D^b were labeled with CFSE and transferred into C57BL/6 D^{b-/-} (Thy1.2⁺) mice. Twenty-four hours later, DCs from LCMV infected wild type (WT) mice, were transferred into the same C57BL/6 D^{b-/-} mice. The extent of CFSE dilution (i.e. proliferation) of P14-Thy1.1 cells was analyzed by FACS at day 9 post-DC-transfer (Fig. 1D). In this system the donor DCs, expressing H-2D^b molecules, are the only cells that could prime LCMV-specific-P14 T cells, as indicated by the lack of P14 division when D^{b-/-} mice were infected with LCMV without receiving WT-DCs. Transfer of BM pDC from LCMV infected mice (which differentiate into CD11b⁺ cDCs *in vivo*; Fig. 1C) induced significant proliferation of P14-Thy1.1 cells to a similar extent as spleen CD11b⁺ cDCs from LCMV infected mice. In contrast, transfer of spleen pDCs from the same LCMV infected mice (which do not generate CD11b⁺ cDCs; [13]), induce background levels of T cell proliferation. These data indicate that in the described experimental conditions pDCs themselves are poor antigen presenting cells and are limited in priming virus specific T cells during LCMV infection. Therefore, the T cell-expansion observed in D^{b-/-} mice transferred with BM-pDCs is most likely triggered by the pDC-derived-CD11b⁺ cDCs generated *in vivo* after cell transfer. These findings support the idea that CD11b⁺ cDCs derived from BM pDCs can prime virus-specific T cells during LCMV infection to similar extents as endogenous CD11b⁺ cDCs existent in the spleen. These data are in agreement with our previous *in vitro* observations that pDC-derived CD11b⁺ cDCs are *bona fide* cDCs and emphasize the role of pDC conversion in enhancing antigen presentation during viral infection *in vivo*.

BM pDC conversion is dependent on IFN-I and does not require virus infection of pDCs

We next asked whether direct viral infection of pDCs was required for their differentiation into CD11b⁺ cDCs and if there was any association between pDC conversion and the ability of the virus to establish chronic infection. To this end, we exploited the fact that while LCMV Cl 13 robustly replicates in DCs and persists in most tissues ~60 days post-infection (dpi), its parental strain Armstrong 53b (ARM) exhibits minimal DC infection and is cleared

within 7-10 dpi [17]. We hypothesized that if infection of pDCs was required for this differentiation process, BM pDCs from ARM infected mice should be unable to generate CD11b⁺ cDCs. On the contrary, we found that pDCs isolated from both ARM and CI 13 infected mice gave rise to a significant amount of CD11b⁺ cDCs after culture with Flt3L for 4 days (Fig. 2A). Moreover, when we analyzed the expression of LCMV NP in cultures of BM pDCs from CI 13-infected mice, we observed that less than 10% of pDC-derived CD11b⁺ cDCs express LCMV NP versus 25-30% of the remaining pDCs (Fig. 2B), indicating that infected pDCs do not exhibit preferential conversion into CD11b⁺ cDC over uninfected pDCs in the same culture. Finally, we evaluated the presence of pDC-derived-CD11b⁺ cDC *in vivo* during ARM and CI 13 infection by examining IgH rearrangements in splenic CD11b⁺ cDCs at different time points post-infection. In accordance with our *in vitro* results, we detected a substantial amount of IgH rearrangements in CD11b⁺ cDCs from both LCMV ARM and CI 13 infected mice (Fig. 2C), suggesting that the differentiation of pDCs into CD11b⁺ cDCs *in vivo* occurs during infection with both acute (ARM) and persistent (CI 13) viruses.

We next sought to address whether IFN-I was involved during the *in vivo* conversion of pDCs into CD11b⁺ cDCs. To this end, we analyzed IgH rearrangements in splenic CD11b⁺ cDCs from wild-type (WT) and IFN- α and IFN- β receptor deficient (IFN- $\alpha\beta$ R^{-/-}) mice either uninfected or infected with LCMV. While significant D-J rearrangements were detected in splenic CD11b⁺ cDCs isolated from LCMV infected WT mice, only background levels were found in their counterparts from IFN- $\alpha\beta$ R^{-/-} mice (Fig. 2D), indicating that the lack of IFN-I signaling hampers the reprogramming of BM pDCs *in vivo*.

Taken together, these data indicate that reprogramming of BM pDCs during *in vivo* viral infection does not rely on direct infection of pDCs and is instead the IFN-I signal that determines their differentiation into CD11b⁺ cDCs.

Differential capacity of BM pDC subtypes to give rise to CD11b⁺ cDCs

Recently, several molecules have been identified as pDC specific markers including Ly49Q [18,19], bone marrow stromal cell antigen 2 (BST2, mAb 120G8 and PDCA-1) [20], and Siglec-H (mAb 440c) [21]. Among them, Ly49Q was defined to divide BM pDCs into two sub-populations [18,21]. We next investigated whether these two pDC subtypes have differential capability to convert into CD11b⁺ cDCs.

When we stained Ly49Q on BM pDCs, we observed similar proportions of Ly49Q⁺ and Ly49Q⁻ cells in BM from uninfected and LCMV-infected mice. Interestingly, after 4 days of culture with Flt3L, Ly49Q⁺ pDCs, as well as Ly49Q⁻ pDCs gave rise to similar proportions of pDC-derived CD11b⁺ cDCs (Fig. 3A), indicating that both Ly49Q⁺ and Ly49Q⁻ pDCs possess similar plasticity to differentiate into CD11b⁺ cDCs regardless of their distinct differentiation states. Our results differ from a recent report showing that Ly49Q⁺ and Ly49Q⁻ BM pDCs isolated from poly(I:C) treated BALB/c mice have differential capacity to reprogram into CD11b⁺ cDCs [22]. This dissimilarity could be due to variations between stimuli, mouse strains, or technical differences.

Siglec-H plays an inhibitory effect on IFN-I production from activated pDCs under cross-linking conditions [21,23]. When we used the 440c mAb to detect Siglec-H, over 98% of BM pDCs from uninfected mice were Siglec-H⁺ (Fig. 3B). Notably, after LCMV infection, 20-30% of BM pDCs were Siglec-H⁻. BST2 antigen was expressed at comparably high levels in both Siglec-H⁺ and Siglec-H⁻ pDCs (Suppl. Fig 4A). Moreover, even when BST2 was also up-regulated in other BM leukocytes after LCMV infection, their mean fluorescence intensity was much lower compared with that of pDCs from infected mice (Suppl. Fig 4 B and C). We further sorted Siglec-H⁺ and Siglec-H⁻ BM pDCs from LCMV-

infected mice and cultured them in the presence of Flt3L. Interestingly, the Siglec-H⁻ pDCs from LCMV infected mice gave rise to a great proportion of CD11b⁺ cDCs, whereas Siglec-H⁺ pDCs only generated ~6% of CD11b⁺ cDCs (Fig. 3B). It should be noted that the reduced conversion in the Siglec-H⁺ pDC cultures could be related to the inhibitory properties of the 440c Ab previously described [21,23] and not necessarily indicate lack of potential to generate CD11b⁺ cDCs. Interestingly, Siglec-H⁻ pDCs isolated from LCMV-infected mice derived into Siglec-H⁺ pDCs, as well as into CD11b⁺ cDCs, after culture in the presence of Flt3L (Fig. 3C), suggesting that Siglec H⁻ pDCs could be either an earlier DC precursor or the resultant of internal redistribution of the heterogeneous BM pDCs. Since the Siglec-H⁻ pDC subset that exhibit high developmental plasticity is detected only after viral infection and the reprogramming of pDCs is mediated by IFN-I, we hypothesized that IFN-I could be critical to generate the Siglec-H⁻ pDC subset which can subsequently differentiate into CD11b⁺ cDCs. To test this hypothesis, we infected IFN- $\alpha\beta$ R^{-/-} mice with LCMV Cl 13 and analyzed the expression of Siglec-H in BM pDCs at 3 dpi. Remarkably, only a minimal percentage of Siglec-H⁻ pDCs could be detected in BM from infected IFN- $\alpha\beta$ R^{-/-} mice compared to the WT controls (Fig. 3D). Together with Fig. 2D, these results suggest that one of the mechanisms by which IFN-I promotes pDC conversion is the induction of Siglec-H⁻ pDCs which exhibit high plasticity to differentiate into CD11b⁺ cDCs.

Concluding remarks

With the notion that CD11b⁺ cDCs serve as a better antigen presenting cell (APC) to prime T cells [24,25], the reprogramming of pDCs into CD11b⁺ cDCs may result in the increase of APCs and thus facilitate the switch from innate into adaptive immunity. Indeed, our data support the idea that while pDCs themselves are limited at priming antiviral T cells, pDC-derived-CD11b⁺ cDCs contribute to antigen presentation and expansion of virus-specific T cells during LCMV infection. Previous studies have demonstrated that pDCs are potent producers of IFN-I during LCMV infection [26,27]. Therefore, pDC conversion may have evolved as a mean to switch from a poor antigen presenting interferon producing DC subset (pDC) to a more potent antigen presenting DC subpopulation (CD11b⁺ cDC). The host would benefit from this cell conversion by maximizing antigen presentation and T cell priming to fight the infection while preventing sustained IFN-I production that could cause immunopathology. On the other hand, re-direction of BM pDCs into CD11b⁺ cDCs may result in reduced pDC numbers and subsequently compromise the production of IFN-I after secondary infections. Finally, it is possible that pDC-derived CD11b⁺ cDCs would play a unique role in anti-viral defense different from other CD11b⁺ cDCs (e.g. monocyte-derived-CD11b⁺ cDCs) that account for their emergence upon virus infection.

Our present work demonstrate that BM pDC conversion into CD11b⁺ cDCs is an ongoing process during *in vivo* viral infection, and better characterizes the mechanisms, the populations involved and biological significance of this cellular event. This novel pathway of DC differentiation is turned on during viral infection and actively contributes to the ensuing DC pool to fight the infection. These findings further highlight the flexibility and the dynamics of the DC subsets in response to the micro-environmental changes, which may be critical to mount the most appropriate type of immune response against the invading pathogens.

Materials and Methods

Viruses and Abs

LCMV Armstrong clone 53b (ARM) and Clone 13 (Cl 13) stock were generated, genotyped and quantified by plaque assay as described previously [28]. The following Abs (Abs) were

purchased from either eBioscience (San Diego, CA) or BD Biosciences (San Jose, CA): anti-CD3, anti-CD19, anti-Ter119, anti-Ly6G (clone 1/A8), PE or APC anti-CD11c, PE or PerCp-Cy5.5 anti-CD3, CD19, and NK1.1, PE-Cy7 anti-CD11b, PE anti-B7.2 (CD86), Alexa-700 anti-MHC class II, APC-Cy7 anti-B220, Pacific Blue anti-CD8, and APC streptavidin. Anti-120G8 mAb was kindly provided by Dr. Giorgio Trinchieri (National Institutes of Health, Frederick, USA) and conjugated with Alexa-488 (Molecular Probes) in our laboratory. Biotin-conjugated Siglec-H Ab clone 440c was provided by Dr. Marco Colonna. Biotin-conjugated Ly49Q Ab was obtained from Dr. Noriko Toyama-Sorimachi (Tokyo Medical and Dental University Graduate School, Tokyo, Japan). Anti LCMV-Nucleoprotein (NP) 113 mAb was produced in our laboratory.

Mice

C57BL/6 (CD45.2, Thy1.2⁺), C57BL/6 CD45.1, C57BL/6 Thy1.1⁺D^bGP₃₃₋₄₁ TCR transgenic, C57BL/6 IFN α R^{-/-}, and C57BL/6 D^b^{-/-} mice were obtained from the Rodent Breeding Colony at The Scripps Research Institute (TSRI). All mice were handled according to the NIH and TSRI Animal Research Guidance. Mice were infected with 2×10^6 pfu of LCMV ARM or CI 13 intravenously (i.v.).

Cell isolation, flow cytometry and FACS purification

BM and spleen cell isolation, flow cytometry and FACS-purification were performed and analyzed as described previously (18). pDCs were defined as CD11c⁺B220⁺120G8^{high}CD11b⁻CD3⁻NK1.1⁻CD19⁻ cells and CD11b⁺ cDCs as CD11c⁺CD11b⁺B220⁻CD3⁻NK1.1⁻CD19⁻ cells. Cell purity was exceeded 98%.

Adoptive transference

To transfer pDC into congenic mice, sorted pDCs (1×10^6 cells) from C57B6-CD45.2 mice were i.v. injected into congenic C57BL/6-CD45.1 female mice after a sub-lethal irradiation dose (600 rad). Four days after transfer, CD11c⁺ cells were enriched from BM or spleen with CD11c MACS beads according to the manufacturer's instructions (Miltenyi Biotec, Auburn, CA). Positive selected cells were incubated with fluorochrome conjugated specific Abs and analyzed by flow cytometry (LSRII, BD Biosciences, San Jose, CA).

Rearrangements of immunoglobulin heavy chain

The preparation of DNA and the PCR assays for detecting D-J and V-DJ rearrangements of the immunoglobulin heavy chain (IgH) were described previously [29,30]. It is important to note that all PCRs including the control OCT-2 have been tested in the linear phase of amplification. Therefore, the OCT-2 signal represents the equal amount of input DNA for PCR.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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FIG. 1. Reprogramming pDCs into CD11b⁺ cDCs *in vivo* after LCMV infection

(A) FACS-purified splenic B cells, granulocytes, pDCs, and CD11b⁺ cDCs from naïve or LCMV CI 13 infected mice (at 3 dpi) were processed for PCR-based detection of IgH rearrangements. OCT-2 was measured as internal control. Lanes 6-8 show isolated CD11b⁺ cDCs from three individual experiments. (B) and (C) Adoptive transference of FACS-purified BM pDCs from uninfected or LCMV-infected mice (CD45.2) into congenic mice (CD45.1). Numbers in each region indicate the frequency of CD45.2⁺ donor cells (B) and the frequency of CD11c⁺CD11b⁺B220[−] cDCs derived from donor pDCs (C) in BM and spleen of recipient mice as indicated. (D) 2×10^6 CFSE labeled Thy1.1⁺D^bGP₃₃₋₄₁ specific CD8⁺ T cells (P14-Thy1.1) were transferred into MHC class I D^{b-/-} mice. The following day, recipient mice were either untreated, infected with LCMV ARM or transferred with sorted spleen CD11b⁺ cDCs, spleen or BM pDCs from LCMV-CI 13 infected mice as indicated. Nine days after DC were transferred, CFSE dilution of P14-Thy1.1 was analyzed.

FIG. 2. Conversion of pDCs into CD11b⁺ cDCs is independent of pDC infection

(A) FACS-purified BM pDCs from LCMV ARM or CI 13 infected mice at 3 dpi were cultured with Flt3L for 4 days. The frequency of resulting CD11b⁺ cDCs is shown. (B) BM pDCs were sorted from uninfected and LCMV CI 13 infected mice and cultured as in A. LCMV-NP in pDCs and pDCs-derived CD11b⁺ cDCs was detected by FACS and the frequency of NP-expressing cells is indicated. (C) FACS-purified splenic CD11b⁺ cDCs from uninfected, LCMV ARM, and CI 13 infected mice at the indicated times p.i. were processed for PCR-based detection of IgH D-J rearrangements. A representative result from 2-3 independent experiments is shown. (D) WT and IFN- $\alpha\beta$ R^{-/-} mice were infected with LCMV CI 13 for 5 days. IgH D-J rearrangements in FACS-purified splenic pDCs and CD11b⁺ cDCs were detected by PCR-based method. Results are representative of at least 3 independent experiments.

FIG. 3. Siglec-H⁻ pDCs exhibit high plasticity to differentiate into CD11b⁺ cDCs

BM pDCs were isolated from uninfected or LCMV Cl 13-infected mice and further divided into two subpopulations based on their expression of Ly49Q (A) or Siglec-H (B). Upper panels, pre-sort gating for Ly49Q and Siglec-H staining. Middle panels, purity of FACS-purified populations from LCMV-infected mice. Lower panels, frequency of pDC-derived CD11b⁺ cDCs 4 days after Flt3L-culture. (C) Siglec-H⁻ pDCs from LCMV Cl 13 infected mice were isolated and cultured for 4 days in the presence of Flt3L. Numbers within regions indicate the frequency of CD11b⁺ cDCs and Siglec-H⁺ DCs derived in the culture. (D) Siglec-H expression on BM pDCs from WT and IFN- $\alpha\beta$ R^{-/-} mice at day 3 p.i. with LCMV Cl 13. Each representative data set was obtained from at least 3 independent experiments.